

**Epidemiology of schistosomiasis in The Gambia: from human infections to
intermediate snail host distribution and genetic diversity**

by

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**Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy**

March 2022



**University of
Nottingham**
UK | CHINA | MALAYSIA



Acknowledgements

First of all, I thank my supervisor Dr. Chris Wade for his relentless support, patience, guidance and stewardship of this PhD research, especially during the Covid-19 restriction period. I also thank my second supervisor Dr. Angus Davison and my internal assessor Dr. Sara Goodacre for their scientific advice and roles in my PhD.

This PhD research would not have been possible without the financial benevolence of the Islamic Development Bank (IsDB). I therefore thank IsDB for funding my PhD studentship.

I am grateful to the National Public Health Laboratories (NPHL) and Epidemiology and Disease Control (EDC) Unit of the Ministry of Health, The Gambia for providing logistic and material support for both the human epidemiological and malacological fieldworks. I must thank Mr. Bakary Sanneh, Deputy Director of NPHL for his advice, support and encouragement right from the inception of this journey. A special mention of Yusupha Badjie, Alagie Saine, Ousman Jallow and Ebrima Daffeh for their support in the fieldwork. I am not forgetting Abou Kebbeh, Tumbul Drammeh, Abdoulie Sanyang, Saran Colley, Yusupha Sonko and all other colleagues from NPHL Kotu, Bansang and Basse for their roles in my PhD.

Special appreciation to Ahmed J. Saadi and Kehinde Sowunmi for their guidance on molecular techniques and allowing me access to their materials for PCR optimisation during my early days. I thank my colleagues Victor Magero and Peter Andrus, and the Technicians in lab A100, especially Liaque Latif for their support throughout this PhD.

Finally, I dedicate this PhD to my Wife Haja Seckan, kids Muhammed Joof and Abubacarr Joof, and my entire family for their support, patience and sacrifice during the course of my PhD studies.

Abstract

Background

Urogenital schistosomiasis is endemic in the eastern regions (Central River and Upper River Regions) of The Gambia, with occasional reports of intestinal schistosomiasis. Following the London Declaration for schistosomiasis control/elimination in 2012, The Gambia initiated a national schistosomiasis control programme. However, a lack of comprehensive and current information on the epidemiology of schistosomiasis in the country presents a major challenge for the implementation of the control programme. This study investigated the epidemiology of schistosomiasis in The Gambia, from human infections to intermediate snail host distribution and genetic diversity, to generate novel information required for the control of schistosomiasis in the country.

Methods/Principal findings

To assess prevalence and risk factors of schistosomiasis, we used data from a previous study involving 40 primary schools from 4 regions of The Gambia: North Bank Region (NBR), Lower River Region (LRR), Central River Region (CRR) and Upper River Region (URR). We extracted unpublished data generated by this previous study and utilised it in this PhD research for epidemiological analysis of schistosomiasis in The Gambia. Urogenital schistosomiasis had an overall prevalence of 10.2% while intestinal schistosomiasis had a prevalence of 0.3% among the sampled school children. Prevalence of urogenital schistosomiasis was highest in CRR (27.6%), followed by URR (12.0%), then LRR (0.6%), and NBR (0.0%). Intestinal schistosomiasis was observed in only CRR and URR. Blood in urine (haematuria) was significantly associated with presence of *S. haematobium* eggs in urine. Boys had higher infection rate for *S. haematobium* compared to girls and being a male was a risk factor of *S. haematobium* infection.

Malacological surveys were conducted in all 5 regions of The Gambia: Western Region (WR), North Bank Region (NBR), Lower River Region (LRR), Central River Region (CRR) and Upper River Region (URR). Field collected snails were identified morphologically and screened for schistosome infections using molecular techniques. While three species of *Bulinus* were collected, no *Biomphalaria* snails were found in any of the sites sampled. *Bulinus senegalensis* was the most common species, found mostly in seasonal pools while *Bulinus forskalii* and *Bulinus truncatus* were observed to thrive in permanent habitats. CRR had the highest abundance of *Bulinus* snails, followed by URR among the regions. *Bulinus senegalensis* was found infected with both *S. haematobium* and *S. bovis*, while *B. forskalii* and *B. truncatus* had only *S. bovis* infection. While the human parasite *S. haematobium* was restricted to just four sites, the livestock parasite *S. bovis* had a much more widespread geographical distribution across both CRR and URR.

We characterise the genetic structure of field-collected *Bulinus* species in relation to schistosome infection using the mitochondrial cytochrome oxidase subunit 1 (cox1) and the nuclear internal transcribed spacer 2 (ITS-2) genes. *B. senegalensis* had a relatively low genetic diversity. There was a pattern in the distribution of *B. senegalensis* haplotypes with some shared haplotypes restricted to only certain parts and regions of the country while some were found broadly across multiple regions of the country. High genetic variation with a clear haplotype structuring was observed in *B. forskalii*. More haplotype sharing and homogeneity was observed in *B. forskalii* from URR compared to *B. forskalii* from CRR. *B. truncatus* had a relatively low genetic diversity with less haplotype sharing between populations. No association was observed between genetic diversity and schistosome infection for any of the three *Bulinus* species. We however found a significant association between two *B. senegalensis* genotypes (haplotypes) and *S. bovis* infection.

Conclusions

This new information on the epidemiology of schistosomiasis in humans and the distribution of intermediate snail hosts in The Gambia will be vital in establishing and guiding an integrated control approach for schistosomiasis in the country. *B. senegalensis* genotypes associated with *S. bovis* infection might pose an increased risk of transmission in areas they occur. Future studies should genetically characterise schistosome parasites from humans and snails, and investigate *S. haematobium* hybrids in The Gambia.

Preface

This thesis comprises seven chapters: chapters 1 – 7.

Chapter 1 entitled “Introduction” entails an introductory background on schistosomiasis and intermediate snails (*Bulinus* and *Biomphalaria*).

Chapter 2 entitled “Materials and methods” includes a detailed description of the general methods used in the study.

Chapter 3 entitled “Prevalence and risk factors of schistosomiasis among primary school children in four selected regions of The Gambia” is published in PLOS Neglected Tropical Diseases, 2021.

Chapter 4 entitled “Species diversity and distribution of schistosome intermediate snail hosts in The Gambia” is published in PLOS Neglected Tropical Diseases, 2021.

Chapter 5 entitled “Genetic variation of *Bulinus senegalensis* in relation to *Schistosoma haematobium* and *Schistosoma bovis* infections in The Gambia.”

Chapter 6 entitled “Genetic variation of the *Schistosoma* snail hosts *Bulinus forskalii* and *Bulinus truncatus* in relation to *Schistosoma bovis* infection in The Gambia.”

Chapter 7 entitled “General discussion” includes a general discussion of the study main findings and conclusions.

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1 Introduction

1.1 Schistosomiasis

1.1.1 Distribution and burden

Schistosomiasis (also known as bilharzia) is a parasitic disease caused by trematode worms (blood flukes) of the genus *Schistosoma*. The disease is reported to exist in 78 countries globally, and more than 229 million people were estimated to require preventive chemotherapy in 2018 (WHO, 2020a). Sub-Saharan Africa carries the largest burden of the disease with at least 90% of people requiring treatment living in the region (WHO, 2020a, see Figure 1.1). Nearly 192 million people in over 40 countries in Sub-Saharan Africa have schistosomiasis (Adenowo et al., 2015). Nigeria is the most affected country in the region with 29 million cases, followed by Tanzania with 19 million cases, then Ghana and Congo DR each with 15 million cases (Adenowo et al., 2015). Other regions of the world where schistosomiasis is endemic include the Eastern Mediterranean Region, the Americas, the Western Pacific Regions and Southeast Asia (WHO, 2013a). Although Turkey is the only country affected in the European Region (WHO, 2013a), schistosomiasis infection has occasionally been documented in European travelers from endemic regions. In 2013, there was an outbreak of urogenital schistosomiasis in the French Mediterranean region of Corsica (Boissier et al., 2016). Active schistosomiasis infections are increasingly being detected in migrants and returning travelers in mainland Europe including Belgium, Italy and France (Soentjens et al., 2016; Infurnari et al., 2017; Gillardie et al., 2021).

Schistosomiasis as one the most prevalent tropical diseases, is a leading cause of morbidity in many parts of the globe (Chitsulo et al., 2000). Its complications can range from less obvious disabling symptoms of anaemia, stunted growth, cognitive impairment, and decreased aerobic

capacity, to more severe morbidities such as periportal fibrosis for intestinal schistosomiasis and bladder deformity and hydronephrosis for urogenital schistosomiasis (Colley *et al.* 2014). Female genital schistosomiasis resulting from *S. haematobium* infection is an emerging form of schistosomiasis that is strongly linked with infertility and other reproductive health complications in affected women (Colley *et al.* 2014). King (2010) estimated the disability-adjusted life years (DALYs) lost due to schistosomiasis to be 24 – 29 million, and some assessments estimated it to even exceed DALYs loss from malaria or tuberculosis and almost equaling that of HIV/AIDS (King and Dangerfield, 2008).

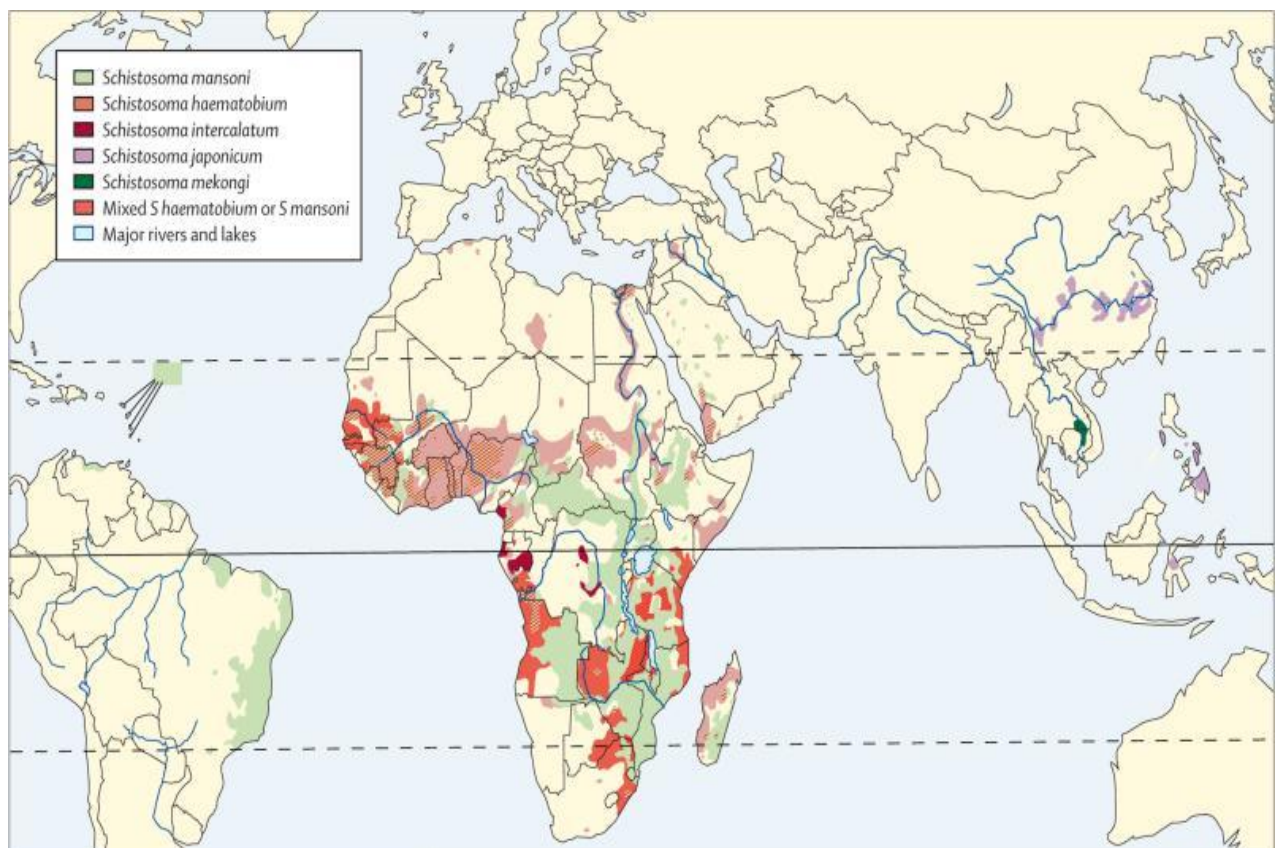


Figure 1.1. Global distribution of human schistosomiasis. From Gryseels *et al.* (2006)

1.1.2 Causative agents: schistosome parasites

Schistosomiasis is caused by a group of parasitic flatworms (trematodes) belonging to the genus *Schistosoma*. Globally, several species of *Schistosoma* cause schistosomiasis, but the human form of the disease is caused by six species: *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. guineensis* and *S. mekongi* (Colley *et al.* 2014). Most human infections result from three species, *S. haematobium*, *S. mansoni* and *S. japonicum*. *S. haematobium* and *S. mansoni* are both found in Africa and the Middle East, with the former recently introduced in Corsica, France (Boissier *et al.*, 2016). *S. mansoni* alone occurs in the Americas, whilst *S. japonicum* is confined to Asia. The other three species have more localised distributions and cause infections in Southeast Asia (*S. mekongi*), and in Central West and Central Africa (*S. intercalatum* and *S. guineensis*) (WHO, 2020a). The two most widely distributed species in Africa are *S. haematobium*, the causative agent of urogenital schistosomiasis and *S. mansoni*, which causes the intestinal form of the disease (Colley *et al.* 2014). *Schistosoma bovis*, *S. curassoni*, *S. mattheei* are notable *Schistosoma* species known to cause intestinal schistosomiasis in livestock in Africa and the Mediterranean (You, *et al.*, 2018). An emerging public health concern is that hybrids between *S. haematobium* and *S. bovis* or *S. curassoni* or *S. mattheei* are increasingly being found causing human infections in many African countries (Webster *et al.*, 2013; Sene-Wade *et al.*, 2018, Webster *et al.*, 2019). Hybrids of *S. haematobium* and *S. bovis* have also been isolated in human samples in some European territories such as in France (Corsica) (Mone *et al.*, 2015) and Belgium (Soentjens *et al.*, 2016). Hybrids of livestock schistosomes *Schistosoma bovis* and *Schistosoma curassoni* previously found only in livestock (Webster *et al.*, 2013) have also been observed to cause human infection (Leger *et al.*, 2016).

Figure 1.2 shows the life history of schistosome parasites. Schistosome eggs are released into freshwater with urine (*S. haematobium*) and faeces (*S. mansoni*). As soon as the eggs come in contact with water, they hatch releasing free-living larvae, called miracidia, which use their cilia to swim to suitable snail hosts (Colley *et al.* 2014). The miracidium penetrates the snail host and develops into a mother sporocyst, which produces daughter sporocysts by asexual multiplication. They migrate and develop in the hepatic and gonadal tissue of the snail. These daughter sporocysts eventually give rise to cercariae which are released into the water where they swim freely. This period from the time miracidia penetrate the snail host to the time before cercariae are released takes 4 – 6 weeks, and is termed prepatent period. The fork-tailed cercariae swim through the water until they meet human skin then penetrate (Innobaya *et al.* 2014). The cercariae lose their tail after penetration and transform into schistosomulae before finding their way to the blood or lymphatic vessels via the epidermis and dermis. They move to the heart and lungs, then to the portal or vestibule circulation where they mature into male and female adult worms (Innobaya *et al.* 2014). The adult worms live in the perivesicular (*S. haematobium*) or mesenteric venules (*S. mansoni*, *S. japonicum*, and others), where sexual reproduction (mating) takes place as the female is carried in the gynecophoric canal of the male. They can live in the human host for an average of 3 to 10 years and up to a maximum of 40 years (Colley *et al.* 2014).

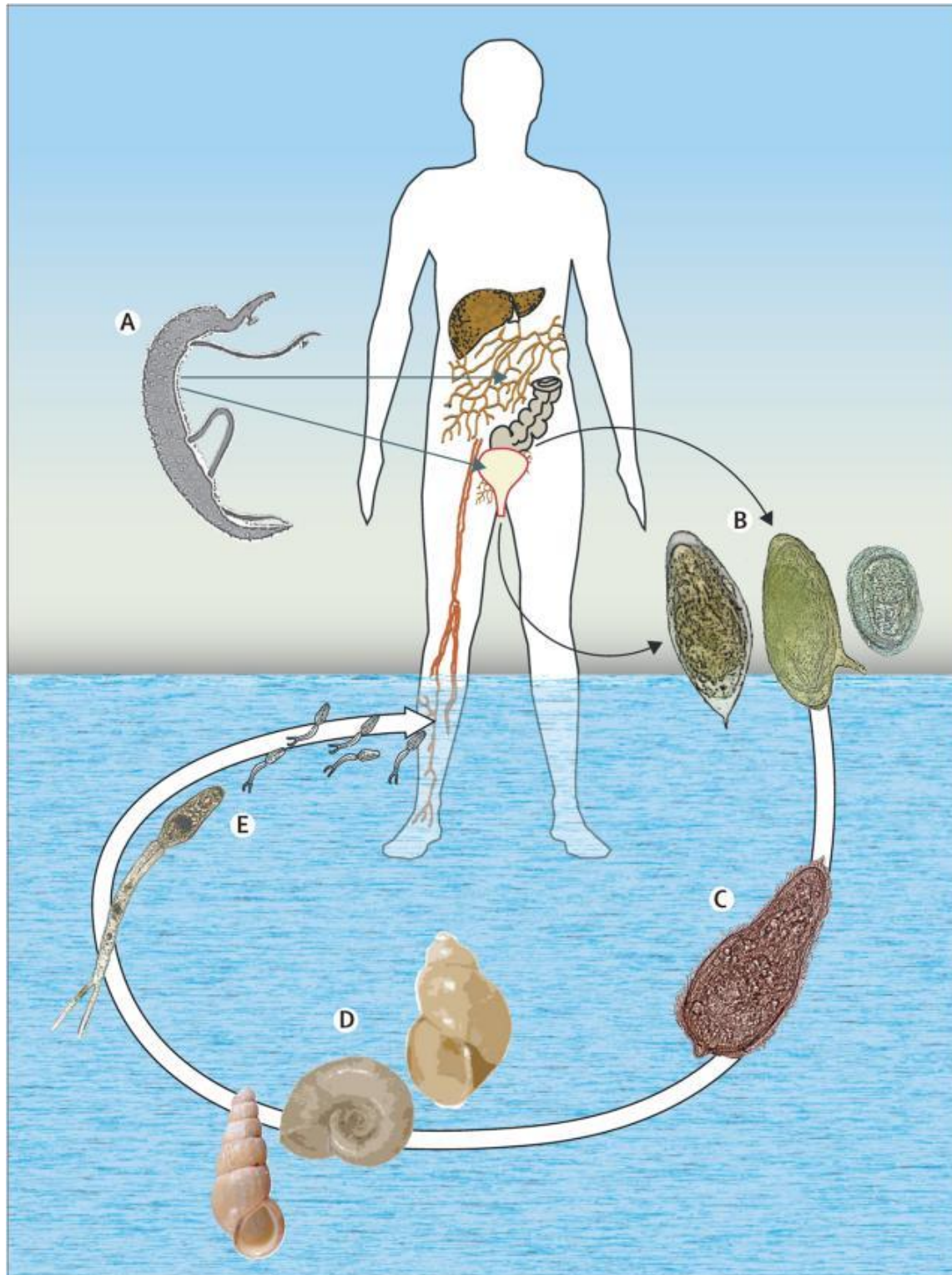


Figure 1.2. Life cycle of schistosomes. A – large adult schistosome male carrying the smaller female in its gynecophoric canal; B – from left to right, *S. haematobium*, *S. mansoni*, *S. japonicum* eggs; C – miracidium; D – from left to right, *Oncomelania*, *Biomphalaria*, *Bulinus* snails; E – free-swimming cercariae. Adapted from Colley et al. (2014)

1.1.3 Clinical manifestation

The clinical presentation of schistosomiasis can range from mild dermatitis to severe complications such as bladder carcinoma and periportal fibrosis (Inobaya *et al.*, 2014; Colley *et al.*, 2014). The first symptom of infection with schistosomiasis in humans may show as cercarial dermatitis also known as “swimmer’s itch,” especially if this is the first episode of exposure. It is characterised by mild prickling sensations with rash at the site of cercarial penetration on the skin within an hour of freshwater contact and is caused by immune response to antigens on the cercarial outer coat (Boros, 1989; Gryseels and Strickland, 2013). In those with previous exposures, the cercariae that penetrated the skin provoke a protective immune response involving specific immunoglobulin E antibodies, eosinophils, and macrophages against the invading schistosomulae. Schistosomulae killed by antibody response remain in the skin and become engulfed in swellings which lead to a more severe state of schistosomal dermatitis expressed as papules, erythema, edema, and pruritus (Maguire, 2010). A hypersensitive reaction termed Katayama fever is triggered by the movement of schistosomulae in the blood or lymphatic system and the deposition of eggs by adult worms. This acute syndrome is characterised by flu-like symptoms such as fever, fatigue, myalgia, headache and dry coughing (Ross *et al.*, 2007; Gryseels, 2012). Acute schistosomiasis (Katayama fever) is less common among people in endemic areas, probably due to early age and congenital exposure to schistosome antigens (Ross *et al.*, 2007; Gryseels, 2012).

Chronic schistosomiasis is manifested in a series of clinical signs and symptoms caused primarily by immune reactions against *Schistosoma* eggs trapped in the tissues during migration. The proteolytic enzyme producing eggs elicit an immune reaction in humans in the form of eosinophilic inflammation and subsequent granuloma formation (Inobaya *et al.*, 2014). These large granulomas and fibrosis produced as a defense by the host’s immune system

against the deposited eggs are the major cause of sickness in chronic schistosomal infections (Maguire, 2010).

Urogenital schistosomiasis is often caused by the deposition of *S. haematobium* eggs in the vesical and ureteral walls leading to granulomatous inflammation. This form of schistosomiasis is manifested in the form of bloody urine (hematuria), dysuria, proteinuria, calcification in the bladder, obstruction of the ureter, renal colic, dark-colored urine, and frequent and painful micturition. Further complications from chronic infection may include obstructive uropathy, renal failure, hydroureter, hydronephrosis, and bladder cancer (Gryseels, 2012; Gryseels and Strickland, 2013). When *S. haematobium* eggs lodge in the genital tract, they cause a gynecological manifestation/complication called genital schistosomiasis. In females, this is called female genital schistosomiasis (FGS) and in males, it is termed male genital schistosomiasis (MGS). Clinical manifestations of FGS and MGS include hematuria, abdominal and pelvic pain, dyspareunia, post-coital and contact bleeding, vaginal discharge, inflammation of the cervix, epididymitis and hematospermia. Complications include miscarriage, ectopic pregnancy and infertility (Christinet et al., 2016).

Intestinal schistosomiasis is caused by *S. mansoni*, eggs which become trapped in the wall and mesenterium of the large intestine and rectum (Inobaya *et al.*, 2014). The symptoms may comprise of abdominal pain, diarrhea with constipation, and inapparent blood in the feces. (Ross *et al.*, 2002; Gryseels and Strickland, 2013). It may cause obstruction in the stomach, appendicitis, and ruptured bowel. Granuloma formed around *S. mansoni* or *S. japonicum* eggs leads to presinusoidal inflammation and periportal fibrosis (Ross et al., 2002). Ascites, enlarged spleen and liver, varices, and variceal bleeding may ensue because of portal hypertension (Ross *et al.*, 2002; Li *et al.*, 2011).

Although the mechanism is not yet understood, deposition of eggs in the central nervous system has been reported, with the presence of *S. haematobium* and *S. mansoni* in the CNS associated with symptoms such as pain in the lumbosacral area and the lower limbs, paraplegia, pain or loss of sensation, and loss of bladder and bowel sphincter control (Nascimento-Carvalho and Moreno-Carvalho, 2005; Gryseels and Strickland, 2013). Epilepsy, paralysis, and meningoencephalitis resulting from granulomatous lesions is often linked to *S. japonicum* infection (Gryseels and Strickland, 2013). Some complications of schistosomiasis may involve the lungs and genital area, and in children, the infection may cause anaemia, stunted growth and cognitive abnormalities (Ross *et al.*, 2002).

1.1.4 Diagnosis of schistosomiasis

The standard diagnostic method for schistosomiasis in humans is the detection of species-specific eggs in urine (for *S. haematobium*) and in stool (for *S. mansoni* and *S. japonicum*). Egg detection methods are highly reliant on identification of characteristic features in the structure of schistosome eggs. Microscopic egg detection is the gold standard approach, although they have some limitations owing to their low sensitivity, especially in individuals with low infection intensity such as travelers and people living in low endemic regions or areas where control interventions are being implemented (De Vlas *et al.*, 1997; Ross *et al.*, 2002; Doenhoff *et al.*, 2004).

Schistosoma haematobium infection is diagnosed by microscopic examination of a urine sample concentrated by sedimentation, centrifugation or filtration (Ross *et al.*, 2002; Gryseels *et al.*, 2006). The WHO recommended technique for diagnosing *S. haematobium* in prevalence assessment or mapping studies is the urine filtration method which has polycarbonate filters that trap eggs as urine is passed through the filter (WHO,1991). This is often used in

conjunction with a reagent strip (urine dipstick) which indicates the presence of blood in urine – haematuria (WHO, 2006). Urine samples for urogenital schistosomiasis diagnosis are ideally collected between 10 am and 2 pm to coincide with the maximum egg excretion period (Gray *et al.*, 2011).

Schistosoma mansoni and other intestinal schistosomes are diagnosed by examining eggs in thick smears of stool specimens (Gray *et al.*, 2011). The Kato-Katz thick smear faecal examination (Katz *et al.*, 1972) that requires 40 – 50 mg of stool is recommended by WHO for intestinal schistosomiasis diagnosis during mapping surveys.

More sensitive tools are required for accurate and rapid diagnosis of schistosomiasis in both health facility and field settings. This is particularly important for the detection of low intensity infections in areas where mass drug administration (MDA) has been implemented, as control interventions gain momentum following the 2012 London declaration which advocates for the control/elimination of the diseases in some countries (WHO, 2012a; WHO, 2012b).

Various tools for improved diagnosis of schistosomiasis have been developed over the years. These include serological assays ranging from ELISA formats that detect antibodies against schistosome parasites (Stothard *et al.*, 2009; El Aswad *et al.*, 2011; Smith *et al.*, 2012) to rapid diagnostic cassette tests that can be used at point of care (Coulibaly *et al.*, 2013; Nausch *et al.*, 2014). An efficient test for schistosomiasis, the point-of-care circulating cathodic antigen (POC-CCA) test, has also been developed and evaluated for *Schistosoma mansoni* and *S. haematobium* diagnosis in various schistosomiasis transmission settings (Stothard *et al.*, 2009; Standley *et al.*, 2010; Coulibaly *et al.*, 2011; Shane *et al.*, 2011; Tchuem Tchuente *et al.*, 2012; Sanneh *et al.*, 2017). The circulating anodic antigen (CAA) assay is a method that detects a *Schistosoma* genus-specific, adult worm gut-derived antigen in both urine and serum samples. It is reported to be more sensitive than both urine filtration and kato-Katz methods in detecting

S. haematobium and *S. mansoni* infections (Corstjens *et al.*, 2020). More sensitive diagnosis has been achieved with the use of molecular techniques that can detect schistosome infection in urine, stool and blood samples (Sandoval *et al.*, 2006; ten Hove *et al.*, 2008; Ibironke *et al.*, 2011; Wichmann *et al.*, 2013). Additionally, new molecular techniques that can detect both *S. haematobium* and *S. mansoni* DNA in a single urine sample have also been developed (Lodh *et al.*, 2013; Lodh *et al.*, 2014).

1.1.5 Risk-factors associated with schistosomiasis

Transmission and distribution of schistosomiasis is highly focal and is often associated with diverse factors that involve human and environmental conditions. More than 90% of global schistosomiasis cases are concentrated in Sub-Saharan Africa (Steinmann *et al.*, 2006; Utzinger *et al.*, 2009; WHO, 2020a). The disease is strongly linked to the presence of water bodies that are habitats to susceptible snail species (WHO, 2002). Prevalence of schistosomiasis in humans is age-dependent and populations that engage in certain occupational and behavioural practices are at higher risks. Individuals that engage in agriculture and fishing activities are particularly at risk of the infection (WHO, 2012c). Women doing domestic chores and children playing in infested waters are all high at-risk populations. Schistosomiasis is strongly associated with poverty and is prevalent in communities situated around water bodies without improved sanitation (WHO, 2002). The infection together with other neglected tropical diseases (NTDs) are considered to be both an effect and cause of poverty in many rural and some disadvantaged urban populations in tropical regions of the world (Hotez *et al.*, 2007).

School-aged children between the years of around 7 -14 are the most at-risk group for schistosomiasis infection (WHO, 2002; Montresor *et al.*, 2002). Prevalence of the disease among this age group may differ from one area to another (Oluwasogo and Fagbemi, 2013;

Ismail *et al.*, 2014; Amuta and Houmsou, 2014; Geleta *et al.*, 2015). Frequent water contact behaviour among members of this age category (Chandiwana and Woolhouse, 1991) predisposes them to schistosomiasis infections. Studies found frequent water contact behaviour through activities like swimming, playing, bathing and washing clothes in freshwater bodies to be a high-risk factor (Oluwasogo and Fagbemi, 2013; Ismail *et al.*, 2014; Couto *et al.*, 2014), with one study reporting “having father as a farmer” as a predisposition for schistosomiasis infection (Geleta *et al.*, 2015). Getting schistosomiasis infection is also associated with gender, as most studies found higher prevalence among the male children cohort (Senghore *et al.*, 2014; Amuta and Houmsou, 2014; Couto *et al.*, 2014). This is likely attributed to the lifestyle of male children and some family tasks assigned to them based on socio-cultural norms.

Because of these epidemiological realities, the World Health Organisation (WHO) recommended primary school children as a target group for schistosomiasis baseline studies and control programmes monitoring (Chandiwana and Woolhouse, 1991; Montresor *et al.*, 1999). The benefit of this strategy is not limited to capturing the most important at-risk population, but has some logistical advantages, as school children are confined in one place during school days, hence become easily accessible and can be targeted.

1.2 *Bulinus* and *Biomphalaria* Snails

1.2.1 Classification and evolutionary history

Bulinus and *Biomphalaria* snails are freshwater pulmonates belonging to the Phylum Mollusca and Class Gastropoda. Gastropods are one of the most diverse animal groups in terms of species, behaviour and geographic distribution. Some members of the Hygrophila, the principal group of *Bulinus* and *Biomphalaria* snails, act as intermediate hosts of many parasitic

schistosome species. Bouchet et al. (2017) divided the Hygrophila into two superfamilies: the Chilinoidea and the Lymnaeoidea. Lymnaeoidea is divided into six families including Bulinidae which comprise *Bulinus*, and Planorbidae which contains *Biomphalaria* (Bouchet et al., 2017). A fine scale taxonomic analysis that utilised mitochondrial cytochrome c oxidase subunit 1 gene (cox1) and 18S ribosomal RNA molecular markers in planorbids recognised at least three family level taxa: Bulinidae, Planorbidae and a group referred to as the A-Clad (Albrecht *et al.*, 2007). In this classification, Bulinidae consist of only one sub-family, Bulinae which contains *Bulinus* (Albrecht *et al.*, 2007). Planorbidae consist of at least four subfamily level clades including Ancylineae and a new subfamily (named the C-Clade) which comprised of the North American planorbids and probably the African *Biomphalaria* (Albrecht *et al.*, 2007). The taxonomic level and arrangements of bulinids have been controversial. In the most recent taxonomic work on Hygrophila by Saadi et al. (2020), Bulinidae cluster within Planorbidae (see Figure 1.3). Based on their findings, they proposed two taxonomic revision options for the Hygrophila: (a) retention of the Bulinidae as a family and reclassification of the Ancylineae as a family outside Planorbidae; or (b) lowering of the Bulinidae to subfamilial rank alongside Ancylineae within the family Planorbidae (Saadi et al., 2020). *Bulinus* belong to the tribe Bulinini and its type species is *Bulinus senegalensis* Muller, 1781 and locality is Podor, Senegal (Muller, 1781), while *Biomphalaria* belong to the tribe Biomphalariini (Bouchet and Rocroi, 2005).

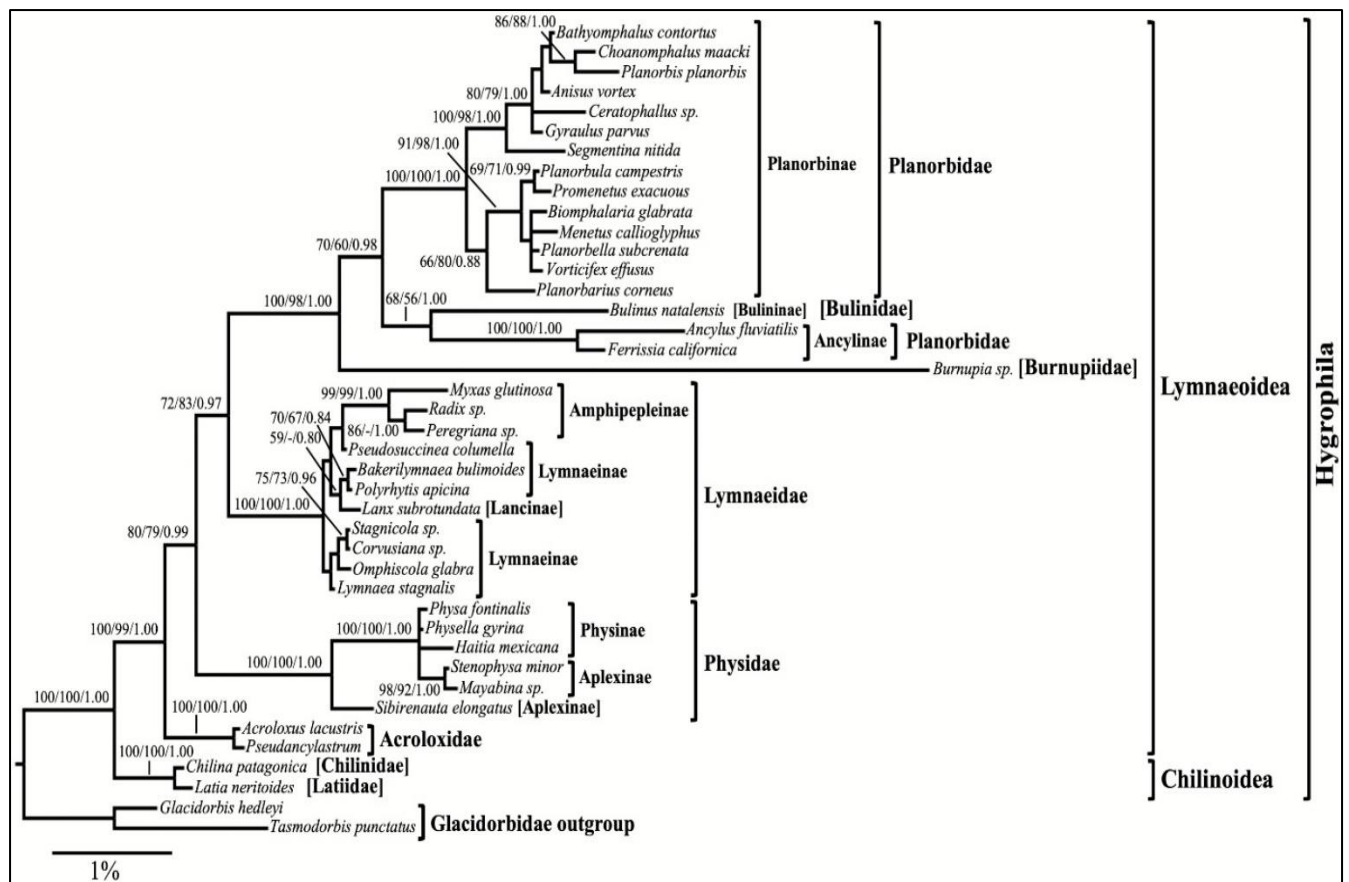


Figure 1.3. Bayesian phylogenetic tree of the Hygrophila based on the nuclear LSU (and 5.8S) gene, depicting the classification of *Bulinus* and *Biomphalaria*. From Saadi et al. (2020).

Bulinus snails have been long documented in history and date as far as 1757 when Michel Adanson collected small snails, containing a bubble of air and floating on the water surface, in Senegal, and named them “Le Bulin.” (Brown, 1994). After 24 years, “Le Bulin” became the type species of the Genus *Bulinus* described by Muller in 1781 as *Bulinus senegalensis* (Brown, 1994). Many new *Bulinus* species were later described in the nineteenth century and placed in the Genus *Physa*, and a significant number of new species, initially placed in *Physa*, were documented towards the end of the century. Extensive research on *Schistosoma haematobium*, the causative agent of urogenital schistosomiasis led to the discovery of several new *Bulinus* species based on shell morphology. George Mandahl-Barth’s monograph published in 1957

was a significant advancement in the taxonomy of *Bulinus*, as he utilised shell morphology, soft tissue and radula variations extensively in classification for the first time (Mandahl-Barth, 1957). Based on morphological characteristics, Brown (1994) classified *Bulinus* into four species groups comprising a total of 37 species. The four groups are the *B. forskalii* group, *B. truncatus/tropicus* group, *B. africanus* group, and the *B. reticulatus* group (see Table 1.1). There is an additional species of *Bulinus*, *B. productus*, not currently recognised (Kane et al., 2008). It was previously considered a form of *B. nasutus* and was called *B. nasutus productus* (Kane et al., 2008).

Table 1.1. The species groups of *Bulinus* (Brown, 1994)

<i>Bulinus forskalii</i> group (11 species)	<i>Bulinus africanus</i> group (10 species)	<i>Bulinus</i> <i>truncatus/tropicus</i> Complex (14 species)	<i>Bulinus</i> <i>reticulatus</i> Group (2 species)
<i>B. barthi</i> (Jelnes, 1979)	<i>B. abyssinicus</i> (Martens, 1866)	<i>B. angolensis</i> (Morelet, 1866)	<i>S. reticulatus</i> Mandahl-Barth, 1954
<i>B. bavayi</i> (Dautzenberg, 1894)	<i>B. africanus</i> (Krauss, 1848)	<i>B. depressus</i> Haas, 1936	<i>B. wrighti</i> Mandahl-Barth, 1965
<i>B. beccarii</i> (Paladilhe, 1872)	<i>B. globosus</i> (Morelet, 1866)	<i>B. hexaploidus</i> Burch, 1972	
<i>B. browni</i> Jelnes, 1979	<i>B. hightoni</i> Brown & Wright, 1978	<i>B. liratus</i> (Tristram, 1863)	
<i>B. camerunensis</i> Mandahl-Barth, 1957	<i>B. jousseaumei</i> (Dautzenberg, 1890)	<i>B. natalensis</i> (Küster, 1841)	
<i>B. canescens</i> (Morelet, 1868)	<i>B. nasutus</i> (Martens, 1879)	<i>B. nyassanus</i> (E. A. Smith, 1877)	
<i>B. cernicus</i> (Morelet, 1867)	<i>B. obtusispira</i> (Smith, 1882)	<i>B. octoploidus</i> Burch, 1972	
<i>B. crystallinus</i> (Morelet, 1868)	<i>B. obtusus</i> Mandahl-Barth, 1973	<i>B. permembranaceus</i> (Preston, 1912)	
<i>B. forskalii</i> (Ehrenberg, 1831)		<i>B. succinoides</i> (E. A. Smith, 1877)	
<i>B. scalaris</i> (Dunker, 1845)			

<i>B. senegalensis</i> (Müller, 1781)	<i>B. ugandae</i> Mandahl-Barth, 1954 <i>B. umbilicatus</i> Mandahl-Barth, 1973	<i>B. transversalis</i> (Martens, 1897) <i>B. trigonus</i> (Martens, 1892) <i>B. tropicus</i> (Krauss, 1848) <i>B. truncatus</i> (Audouin, 1827) <i>B. yemenensis</i> Paggi et al., 1978	
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For *Biomphalaria* there are 34 recognised species, most of which are found in Latin America, with about just 12 species occurring in Africa, Asia and Europe. Based on morphology, Mandahl-Barth (1957) classified African *Biomphalaria* into four species groups, namely: The *B. choanomphala* group, the *B. sudanica* group, *B. pfeifferi* group and the *B. alexandrina* group.

1.2.2 Ecology and life cycle

The occurrence and distribution of schistosomiasis is largely determined by the presence or absence of the intermediate snail hosts. Depending on the species, *Bulinus* snails colonise a variety of habitats ranging from small seasonal pools to streams and rivers. They do not occur in high numbers in rapid flowing waters and deep lakes. When present in big lakes, they inhabit shallow edges (Brown, 1994). They can live in stagnant water and withstand long periods of drought. African bulinids are known for their ability to aestivate and survive in dried habitats during long dry periods (Goll and Wilkins, 1984; Brown, 1994; Senghore *et al.*, 2015). Snails such as *B. senegalensis*, *B. umbilicatus* and *B. reticulatus* are seasonal pool species and may require some period of long dormancy to survive for the next season (Senghore *et al.*, 2015).

In Senegal, *B. senegalensis* and *B. umbilicatus* were observed to survive for 6 to 8 months after the seasonal ponds were dry (Diaw *et al.*, 1989a). *B. umbilicatus* was also reported to emerge from aestivation with schistosome infection after 7 months of dormancy (Senghore *et al.* 2015). This capacity of retaining infection during estivation might have some epidemiological implications as the infection can be carried to the next transmission season, and humans might acquire infection right from the beginning of the rainy season. This widens the transmission window of schistosomiasis in areas where seasonal transmission exists. Habitat preference of *Bulinus* snails is influenced by environmental and ecological factors. There is a strong association between *Bulinus* snail abundance and temperature, and *Bulinus* tend to grow well in areas of optimal elevated temperatures (Opisa *et al.*, 2011). Although snails can inhabit water bodies with pH ranging from 6.7 to more than 11, water pH has been found to be less significant in determining abundance of *Bulinus* snails (Opisa *et al.*, 2011). However, few studies associate low water pH with high snail abundance (Levitz *et al.*, 2013). There is also some association between snail abundance and aquatic vegetation (Mutuku *et al.*, 2019).

Just like *Bulinus*, ecological or environmental preferences of *Biomphalaria* depend on the species of the snail. For instance, *Biomphalaria pfeifferi* prefer to breed in small ponds and streams, while other species such as *B. choanomphala* are reported to be found in larger lakes and deep waters (Brown, 1994). *Biomphalaria* has been observed to breed well in temperatures between 22 – 27 °C, and to extremes of 18 - 32°C (Appleton, 1976, Kristensen *et al.*, 2001). *Biomphalaria* has been found to show preference for aquatic vegetation in some instances, where aquatic microphytes are used as habitat substrate (Kazibwe *et al.*, 2006). However, in some cases, *Biomphalaria* can be found inhabiting exposed surfaces of rocks located on sandy shorelines of lakes (Magendantz, 1972).

All species of *Bulinus* and *Biomphalaria* are hermaphrodite, possessing both male and female organs and being capable of self- or cross-fertilization (Jarne *et al.*, 1991). A single snail is

capable of invading and colonising a new habitat. The eggs are laid at intervals in batches of 5–40, each batch being enclosed in a mass of jelly-like material. The young snails hatch after 6–8 days and reach maturity in 4–7 weeks, depending on the species and environmental conditions. Temperature and food availability are among the most important limiting factors. A snail can lay up to 1000 eggs during its life, which may last more than a year.

1.2.3 Distribution of *Bulinus* and *Biomphalaria* in Africa

Bulinus snails occur across almost the whole of the African continent. Their high tolerance for various climatic conditions enabled them to colonise habitats ranging from low-land areas around the equator to high altitude waterbodies in the mountains of East Africa and the coldest Southern temperate regions (Brown, 1994). *Bulinus globosus*, arguably the most important vector for *S. haematobium* in Africa, is the most common and widely distributed species of the genus *Bulinus* (Mandahl-Barth, 1965; Brown, 1994). It was reported from The Gambia to all of Africa south of the Sahara, except Ethiopia, Somalia and most of the Sudan (Mandahl-Barth, 1965). Temperature and other abiotic factors have a profound influence on the distribution of *Bulinus*. *B. globosus* is present across almost the whole of tropical Africa in contrast to its close relative, *B. africanus*, which occurs mostly in Eastern Africa. However, distribution of *B. globosus* was observed to be less common than that of *B. africanus* in cooler climatic areas of South-eastern Africa (Brown, 1994).

In the past, there have been conflicting reports regarding the identification and thus the distribution of some species of the *Bulinus africanus* group in West Africa, as *B. jousseaumei* (a separate species) was regarded as a sub-species of the bigger *B. globosus*. From size variation observation of snails collected from Angola to The Gambia, Wright (1961) regarded *B. jousseaumei* as a smaller and regional form of *B. globosus* inhabiting the northern limit of its

range. Mandahl-Barth (1965) challenged this hypothesis and argued that smaller *B. jousseaumei* is a distinct species of its own, and he reported the occurrence of both the true *B. globosus* of typical form and normal large size as well as the smaller *B. jousseaumei* in The Gambia. He further retreated that “*B. globosus* from the northern limit (Gambia, Sierra Leone, Liberia, northern Ghana and Nigeria) attain the same shell length as do specimens from Congo (Leopoldville) and Angola” (Mandahl-Barth, 1965). *B. jousseaumei* was reported to breed in permanent streams in The Gambia and Senegal where it acts as an intermediate host of *S. haematobium* (Smithers, 1956).

The geographic range of *B. forskalii* extends to many parts of Africa including Madagascar and Sao Tome Island (Brown, 1994). Its close relative, *B. senegalensis* was earlier known from only The Gambia and Senegal where it inhabits seasonal pools (Mandahl-Barth, 1965). The geographic range of this species subsequently extended eastwards to Nigeria, Niger and Cameroun (Betterton *et al.*, 1983; Vera *et al.*, 1992; Zein-Eddine *et al.*, 2014). *B. senegalensis* was initially described as being restricted to seasonal laterite pools in The Gambia by Smithers (1956). It was later revealed that it also breeds in alluvial pools and irrigated rice fields in The Gambia in sympatry with its close relative, *B. forskalii* (Goll, 1981). *Bulinus senegalensis* was also reported in the South Chad irrigation project area of Northern Nigeria, as it was documented for the first time outside the Senegambia Region by Betterton *et al.* (1983). *B. jousseaumei* and *B. guernei* (now referred to as *B. truncatus*) were also found in streams in The Gambia (Smithers, 1956). *B. senegalensis* remained to be one of the few *Bulinus* species that breed primarily in temporal rain-fed pools where they aestivate in the mud as the pools dry out (Goll and Wilkins, 1984; Diaw *et al.*, 1989a; Senghore *et al.*, 2015).

Like *Bulinus*, the distribution of *Biomphalaria* is influenced by climatic conditions such as temperature. They occur in the tropics, subtropics, Africa, America, Middle East, East Asia and Europe. They are found in all parts of Africa, except the Sahara Desert and some high-

altitude regions where the extreme low temperatures do not support their growth. *Biomphalaria* have been recorded at altitudes of greater than 1500m close to the equator in East Africa (Rubaihayo *et al.*, 2008).

Environmental manipulations through human activities like irrigation and large-scale water management projects have led to large breeding of *Biomphalaria* species in areas where they were few or never existed before. Classical examples of such anthropogenic influence on the distribution of *Biomphalaria* snails are the construction of the Diama Dam in Senegal and the Aswan Dam in Egypt. Both water projects are implicated in the increase of snail population and the coincidental incidences of schistosomiasis among the human populations in the two countries (Southgate *et al.*, 2001).

Biomphalaria snail inhabit various forms of permanent water bodies in areas they are found. *B. sudanica* and *B. pfeifferi* were observed along both the shorelines of Lake Victoria and in dams and rivers around the informal settlements of Kisumu City, in Kenya (Opisa *et al.*, 2011). *B. choanomphala* is distributed in all three countries (Kenya, Uganda and Tanzania), along Lake Victoria (Standley *et al.*, 2014).

Unlike *Bulinus*, *Biomphalaria* is not common in Gambia, and the only species ever reported in the country was *Biomphalaria pfeifferi gaudi* (Mandahl-Barth, 1957; Smithers, 1957). This snail was also reported to occur in Senegal, Casamance River area, Liberia and Ghana (Mandahl-Barth, 1957). Since there is uncertainty about the occurrence of *S. mansoni* in The Gambia, it is worthwhile investigating the presence of its intermediate host in the country.

1.2.4 Snail population genetics

Three groups of factors are believed to drive genetic diversity and variation in freshwater snails (Jarne and Delay, 1991). The first is ecological conditions. As snails inhabit isolated habitats,

this ecological isolation gives rise to reproductive isolation and limit the gene flow, thereby limiting genetic diversity (Zein-Eddine *et al.*, 2014). Additionally, genetic drift is encouraged by the behaviour of some snail species like *B. senegalensis* that primarily breed in isolated seasonal pools which dry out after the rains. Secondly, *Bulinus* and *Biomphalaria* are hermaphrodites and can reproduce by both selfing (self-fertilisation) and outcrossing (cross-fertilisation), and each of these behaviours has different genetic consequences. For instance, high selfing behaviour and population bottlenecks decrease genetic diversity within snail populations and increase variation between populations (Gow *et al.*, 2004; Zein-Eddine *et al.*, 2017). Conversely, high intrapopulation genetic differentiation and low interpopulation genetic differentiation were observed in outcrossing snail species such as *B. globosus* (Mukaratirwa *et al.*, 1996). Thirdly, co-adaptation with parasites, for which many *Bulinus* and *Biomphalaria* snails act as intermediate hosts have been reported to have an influence on the genetic structure of their hosts or vice-versa (Webster *et al.*, 2007). High genetic variation in *Biomphalaria* is said to reduce the parasite transmission potential and a decrease in snail genetic diversity seems to be favourable to an overall increase in parasite transmission rate (Hoffman *et al.*, 1998; Sandland *et al.*, 2007). The co-adaptation and relationships between intermediate snail hosts and their parasites is believed to be a driver for genetic diversity in local snail populations (Webster *et al.*, 2007). Since the genetic diversity and structure of intermediate snail hosts influences parasite infection to this extent, understanding the genetic variations of natural snail populations can help to explain transmission dynamics and may be useful in designing new control approaches.

Mitochondrial DNA sequences revealed high haplotype and nucleotide diversity among seven *Bulinus* species (including *B. senegalensis*, *B. forskalii* and *B. truncatus*) from three African countries, with approximately one out of three to four snail specimens having a different haplotype within the same locality (Zein-Eddine *et al.*, 2014). In contrast, *Bulinus truncatus*

collected from different locations in Egypt were found to have low haplotype diversity and low nucleotide diversity (Abe et al., 2018). *Biomphalaria pfeifferi* collected from several sites in Ivory Coast was found to have low genetic variation within populations and no isolation of populations by distance was observed (Tian-Bi et al., 2013). *Biomphalaria choanomphala* specimens collected from the three countries along Lake Victoria were characterized as having high intrapopulation diversity and elevated levels of population structure, according to cytochrome oxidase subunit 1 (cox1) and 16S molecular analyses (Standley et al., 2014). However, none of these studies investigated the relationship between these genetic variations and schistosome infection. Several studies have been conducted on *Bulinus* in the Senegambia region (Smithers, 1956; Goll, 1981; Goll and Wilkins, 1984; Diaw, 1989a; Diaw et al., 1989b; Diaw et al., 1992; Sene et al., 2004; Zein-Eddine et al., 2014; Senghore et al., 2015), but few have focused on population genetic analysis (Zein-Eddine et al., 2014). Moreover, the primary species (*B. senegalensis*) involved in the transmission of schistosomiasis in The Gambia (Smithers, 1956) is known for the rare behaviour of breeding in isolated seasonal pools that dry out after the rains and resurface at the onset of new rainy seasons. This geographical isolation and seasonality over time might be influencing some unknown genetic consequences in local snail populations, as these factors among other conditions are deemed to be predictors of population genetic variations (Jarne and Delay, 1991).

1.2.5 Molecular markers for population genetics of snails

Population genetic studies have contributed immensely to our understanding of evolutionary processes, species and population history of snails. These studies utilise genetic information contained in both the mitochondrial and nuclear DNA of snails (Standley et al., 2014; Zein-Eddine et al., 2014). Several mitochondrial and nuclear markers have been developed for inferring genealogical and evolutionary relationships of organisms (Folmer et al., 1994; Kane

and Rollinson, 1994; Fontanilla et al., 2017). The mitochondrial genome of molluscs is circular, non-recombinant and is usually maternally inherited. Because the mitochondrial genome mutates faster, sequence variation within the mitochondrial DNA provides excellent criteria for use in population genetic studies (Rollinson et al., 1998). The mitochondrial cytochrome oxidase subunit I (cox1) gene is a popular phylogenetic marker due to its high variation within and between species (Rollinson et al., 1998). In addition, certain regions within this gene are highly conserved enabling portions to be amplified by universal PCR primers (Folmer et al., 1994; Rollinson et al., 1998). Because the cox1 gene is maternally inherited and non-recombinant, it allows for simplified analyses. An additional mitochondrial marker, ribosomal 16S, which is a gene coding for the mitochondrial large ribosomal RNA subunit, is often used in molluscan phylogenies alongside the cox1 marker to provide useful comparison, particularly in terms of elucidating the phylogenetic and population structure of snails (Standley et al., 2014). Other mitochondrial markers include the NADH dehydrogenase subunit 1 (NAD1) gene and the 12S ribosomal gene.

Nuclear markers are recombinant biparentally inherited genes that are used in phylogenetic and population genetic analyses. As nuclear genes contain highly conserved regions, they show homology with a wide variety of organisms, but still show variation for some organisms (Rollinson et al., 1998, Jorgensen et al., 2010). For instance, analysis of the nuclear ITS region revealed no difference between samples of *B. tropicus* and *B. truncatus* but intraspecific variation was observed between samples of *B. forskalii* from São Tomé and Angola (Rollinson & Kane 1991). For a deeper understanding of the evolutionary history of organisms at the population level, analysis of genealogical data from nuclear markers is essential (Zhang and Hewitt, 2003). Despite the variation and usefulness of mitochondrial markers such as the cox1, they are not useful in providing certain genealogical information such as allelic diversity, thus nuclear markers may be required to provide additional information in population genetic

studies. Nuclear microsatellite markers, one of the most revealing genetic markers available for inferring population genetic structure and dynamics (Zhang and Hewitt, 2003), allow for investigation of more detailed demographical factors, such as Hardy-Weinberg equilibrium, levels of inbreeding and the history of past bottlenecks (Standley et al., 2014). However, comparison of results among laboratories is often difficult and standardisation of methods such as size-based allele determination is required. In addition to microsatellite markers, other nuclear markers such as the ribosomal 18S, 28S and the internal transcribed spacer (ITS) region are often used in tandem with mitochondrial markers (e.g., *cox1*) to infer detailed evolutionary interspecific and intraspecific relationships for several planorbid species, e.g., *Bulinus* and *Biomphalaria* (Stothard et al., 1996; Stothard and Rollinson, 1997; Zein-Eddine et al., 2014; Standley et al., 2014). Ribosomal 18S and 28S are highly conserved genes, with 28S slightly more variable than 18S (Jorgensen et al., 2010; Zein-Eddine et al., 2014). The ribosomal ITS is more variable than both 18S and 28S (Stothard and Rollinson, 1997; Zein-Eddine et al., 2014) which makes it a suitable nuclear marker for population genetic analysis. Intraspecific variation has been observed in the ITS fragment of several *Bulinus* species (Stothard et al., 1996; Stothard and Rollinson, 1997; Zein-Eddine et al., 2014). Some of the limitations of nuclear markers include low divergence levels, intraspecific multifurcation evolution and difficulty in data analysis due to recombination (Zhang and Hewitt, 2003).

1.2.6 Schistosome infection and its detection in snails

In addition to infecting humans and other mammals, schistosomes infect a wide range of snails which serve as the intermediate hosts for the asexual reproductive phase of their life cycle. For example, *S. haematobium* infects snails of the genus *Bulinus*, *S. mansoni* infects snails of the genus *Biomphalaria* and *S. japonicum* infects snails of the genus *Oncomelania* (Colley et al., 2014). Closely related species of *S. haematobium* such as *S. bovis*, *S. mattheei*, *S. curassoni*, *S. intercalatum*, *S. guineensis*, *S. margrebowiei* and *S. leiperi* also infect *Bulinus* snails (Rollinson

et al., 2001). These species together with *S. haematobium* are referred to as *S. haematobium* group species. However, infection with schistosome parasites has significant impact and effect on the snail host. Parasite infection elicit some physiological and behavioural changes in the snail which determine the progression of the parasitic infection. Invasion of snails by parasites triggers immunological defence responses (Coustau *et al.*, 2015), such as the surrounding and killing of parasites by hemocytes (Hahn *et al.*, 2001). The subsequent development of schistosome sporocysts within the snail in successful infections greatly affects immunity and metabolism of the snail host, and can impact their reproductive capacity by suppressing the maturation of gonads (Wang *et al.*, 2017).

Assessment of infection rates in snails is increasingly becoming an important component of studies on schistosomiasis epidemiology, with the identification of cercarial shedding in patent snails the traditional method for detecting schistosome infection in field populations (Webbe, 1965). This long-standing method has proven to be useful but not without limitations. Prepatent infection can prolong for many weeks among field snails (Joubert *et al.*, 1991). This coupled with the high mortality rate of infected snails (Woolhouse, 1989) and the delayed sporocyst development in cold periods (Shiff *et al.*, 1975), might lead to prepatent infection rates overshadowing patent infection rates over time (Hamburger *et al.*, 2004). Maintaining non-shedding snails for longer periods in the laboratory for protracted shedding and examination may improve the accuracy of cercarial shedding methods. However, this is time consuming and can be unreliable if snail mortality is high, making it unsuitable for large scale infection screening of field snails. An alternative technique involving searching for the development of cercariae and sporocysts in crushed snails under the microscope (Chu and Dawood, 1970) is also inaccurate in early prepatency and not ideal for screening field snails on a large scale. However, for certain parasitological examination approaches such as schistosome individual isolation and hybrid detection, cercarial shedding method remains indispensable.

The need for more accurate, rapid and specific approaches for identifying schistosome infection in snails has led to the development of various novel techniques over the years ranging from antibody assays to molecular probes. Monoclonal antibodies and a simple ELISA test procedure that can detect antigens of *S. mansoni* in the haemolymph of *Biomphalaria* as early as the second week of infection have previously been developed (Hamburger *et al.*, 1989a; Hamburger *et al.*, 1989b). Development of more novel techniques for schistosomiasis detection in *Biomphalaria* followed such as DNA dot hybridisation, loop-mediated isothermal amplification (LAMP) assay and PCR amplification techniques, which can detect infection from early pre-patency (Hamburger *et al.*, 1987; Janotti-Passos *et al.*, 1997; Hamburger *et al.*, 1998; Abbasi *et al.*, 2010; Lu *et al.*, 2016).

Unlike *S. mansoni*, *S. haematobium* can live in sympatry with closely related species and can sometimes infect the same snail hosts. The differential identification of *S. haematobium* from its closely related species in snails warranted the development of various molecular methods, including isoenzyme profiling, Southern blot using rDNA gene probes, randomly amplified DNA and several polymerase chain reaction techniques (Ross *et al.*, 1978; Walker *et al.*, 1986; Dias *et al.*, 1993; Abbasi *et al.*, 2007; Webster *et al.*, 2010; Abbasi *et al.*, 2012; Akinwale *et al.*, 2014; Schols *et al.*, 2019; Pennance *et al.*, 2020b). Notable among these is the recent development of a molecular xenomonitoring PCR-based technique that can detect and differentially identify *S. haematobium* and *S. bovis* infections in *Bulinus* snails (Pennance *et al.*, 2020b).

As intervention programmes for schistosomiasis are being implemented and ramped up in many endemic countries (WHO, 2013b), intensity of infection among human populations is declining (Carabin *et al.*, 2005). The low sensitivity of standard schistosomiasis detection methods due to reduced egg output in humans is increasing the reliance on alternative and complementary approaches such as screening for infection in snails (Amarir *et al.*, 2014). As a

decrease in infection intensity among the human population may correspond to a reduce rate of infection in snails, infection detection methods in snails would also require an upgrade. As such cercarial shedding and other conventional methods for screening infection in snails may not be sufficient on their own (Woolhouse and Chandiwana, 1989; Kariuki *et al.*, 2004). This has undoubtedly necessitated the need for more rapid, sensitive and specific approaches such as PCR (Amarir *et al.*, 2014; Akinwale *et al.*, 2014; Pennance *et al.*, 2020b; Joof *et al.*, 2020).

PCR techniques have shown some potential for use as a technique in schistosomiasis control and surveillance programmes for monitoring transmission (Amarir *et al.*, 2014). In addition to their feasibility in mass screening of field snails (pooled snail DNA extracts), their high sensitivity enables detection of a single miracidial infection as early as one-day post exposure (Hamburger *et al.*, 1998; Lu *et al.*, 2016). The specificity of the technique addresses the problems encountered in the differential identification of schistosome species that simultaneously infect a single snail (Abbasi *et al.*, 2007; Akinwale *et al.*, 2014; Pennance *et al.*, 2020b). This is even more important now considering hybridisation of *S. haematobium* and related species has increasingly been observed in different parts of the world (Johnston *et al.*, 1993; Huyse *et al.*, 2009; Mone *et al.*, 2015; Webster *et al.*, 2019).

Some logistical challenges may exist in establishing and providing facilities required for PCR analysis in certain remote settings of some endemic countries. However, as snail samples can be preserved in ethanol, samples can easily be transported from collection sites to strategic laboratories with PCR facilities for infection screening. Lu *et al.* (2016) estimated that: “once the basic thermocycler and electrophoresis equipment are available, one sample costs \$ 0.06 to prepare for a 10µL PCR reaction, including the cost of PCR tubes, pipet tips, polymerase, primers, dNTPs and agarose.” Despite the high sensitivity of PCR and its ability to detect infection from early pre-patency, it is important to acknowledge that not all pre-patently infected snails go on to shed cercariae.

While many studies have showed that PCR techniques hold enormous potential as a tool for detecting schistosomiasis infection in snails both in the laboratory and in field settings (Hamburger *et al.*, 2001; Abbasi *et al.*, 2007; Webster *et al.*, 2010; Akinwale *et al.*, 2014), few studies have utilized this promising approach in large-scale field studies for schistosomiasis transmission monitoring (Hamburger *et al.*, 2004; Amarir *et al.*, 2014, Pennance *et al.*, 2020b). For the method to gain global accreditation and possible recommendation as a control programme method, more large-scale field-based studies using the technique should be conducted in different schistosomiasis endemic areas.

1.3 Global strategies for schistosomiasis control

Schistosomiasis control was mainly reliant on behavioural and sanitation measures, prior to the description and understanding of its mode of transmission in 1913. The discovery of treatment agents during the World War I era resulted to a shift from behavioural and hygiene approaches to treatment campaigns using agents of high toxicity (Jordan, 2000). Behavioural approaches were neglected even more when interest shifted to eliminating intermediate snail hosts. One of the earliest mass drug administration campaigns was conducted in Egypt through the administration of intravenous tartar emetic to adults and children in the 1920s (Inobaya *et al.*, 2014). Subsequently, a national control programme was implemented through chemotherapy and snail control among other approaches (Inobaya *et al.*, 2014). About the same period, use of copper sulphate as a molluscicide was intensified in various control programmes, after the realisation of its usefulness in killing snails (Sturrock and Barnish, 1973). Discovery of safe drugs, like niridazole, metrifonate and oxamniquine for treating human infections redirected focus on chemotherapy as a control strategy. Use of chemotherapy as a control strategy was consolidated by the discovery of praziquantel (PZQ), an anthelmintic drug that has an

unprecedented efficacy against adult schistosome worms and little serious side effects when given at the normal dosage (Katz *et al.*, 1979).

In 1984, the World Health Organisation formally advocated for chemotherapy as an approach to reduce the burden of schistosomiasis in endemic countries and regions (Magnussen, 2003). Consequently, many countries responded with the development of national control strategies resulting to a reduction of disease burden in some of these countries. In 2001, the World Health Assembly drafted resolution 54.19 that recommended chemotherapy as the main strategy for control of schistosomiasis, leading to a global shift from a more integrated approach to a treatment-based strategy (WHO, 2002). Chemotherapy through mass drug administration aims to reduce morbidity and mortality due to schistosomiasis and prevent new infection by limiting transmission through reduction of the overall prevalence in the population (Humphries *et al.*, 2012).

In 2007, efforts to combat schistosomiasis and other neglected tropical diseases were redirected to shared commitment to support WHO's strategies, goals and targets after the first Global Partners Meeting (WHO, 2007). These efforts were consolidated in 2012, when a roadmap for implementation of neglected tropical diseases (NTDs) control was developed. The roadmap aimed to guide and accelerate efforts in the elimination or control of NTDs including schistosomiasis to levels at which they will no longer be considered as public health problems by 2020 (WHO, 2012b). The strategies of the roadmap included: (1) Mapping surveys, through prevalence assessment studies; (2) Preventive chemotherapy, through mass drug administration; (3) Intensified case-detection and management; (4) Vector and intermediate host control; and (5) Provision of safe water, sanitation and hygiene (WHO, 2012a). A new road map was endorsed by the WHO's Seventy-third World Assembly in November 2020 (WHO, 2020b). This new road map sets new global targets for the prevention, control,

elimination or eradication of 20 diseases and disease groups including schistosomiasis by 2030 (WHO, 2020b).

1.3.1 The Gambia neglected tropical diseases (NTDs) control programme

To meet the global objective of combating neglected tropical diseases, The Gambia started the process of developing a national control programme for these diseases in 2014. A situation analysis was conducted from May to June 2014 to assess the status of NTDs in the country. This review, that used published data and routine hospital reports revealed the endemicity of schistosomiasis and soil-transmitted helminthiasis (STH), and probable sporadic distribution of lymphatic filariasis in the country. It further revealed knowledge gaps on the status of the infections in the country (MOH, 2014). A subsequent transmission assessment study conducted in February 2015 for lymphatic filariasis revealed likely elimination of the disease in the country, after no infection was detected among 4,500 adults and children from 15 sentinel sites historically known for the disease (Rebollo *et al.*, 2015). Subsequently in June 2015, a nationwide mapping survey of schistosomiasis and soil-transmitted helminthiasis revealed schistosomiasis as the most important neglected tropical disease followed by soil-transmitted helminthiasis in the country (MOH, 2016). The observations of this survey underpinned the establishment of a national control programme for schistosomiasis and soil-transmitted helminthiasis in The Gambia.

The next interventions that would follow according to WHO strategies for the control of schistosomiasis should include intermediate snail host, and behavioural, water, sanitation and hygiene approaches. Given the fact that the latest malacological studies conducted in The Gambia were in the 1980s (Goll *et al.*, 1984), there was a need for a current and reliable knowledge on the distribution of intermediate snail hosts and the role they play in the transmission of schistosomiasis.

1.4 The study site: The Gambia

1.4.1 Geographic, ecological and anthropological characteristics

The Gambia is located on the West African coast between latitudes 13°N and 14°N and longitudes 13°W and 17°W. The River Gambia, from which the country got its name meanders along the entire length of the country leaving a narrow band of land on either side. The country is surrounded by the Republic of Senegal on the north, south and eastern parts, and by the Atlantic Ocean on the Western side. The Gambia has an average width of 24 km (48 km at its widest point) and extends for about 487 km in length, with a total area of 11,295 km² (Haffner, 1981). The land topography is generally flat at highest about 90m above sea level. Approximately 1,300 km² (500 sq mi) (11.5%) of the land area is covered by water (River Gambia). The River Gambia is brackish for about 220 km from the West where it is bordered by mangrove swamps. In the eastern part of the country, flat plains lie along the river forming freshwater swamps in the rainy season. The River Gambia and its tributaries (streams), locally called “bolongs,” are fresh in the eastern part of the country. Schistosomiasis transmission occurs in these streams (Smithers, 1956) during the dry season as the water level and current recede. Further away from the river, the landscape is comprised of a sandstone plateau which has a rocky, lateritic iron pan (cuiasse) in the eastern part of the country termed a laterite plateau (Hanrion, 1971; Goll and Wilkins, 1984). Depressions formed in the plateau collect water during the rainy season giving rise to seasonal pools that sustain schistosomiasis transmission in the eastern part of the country.

The country has a tropical climate characterized by two seasons: rainy season (June – October) and dry season (November – May). Annual rainfall ranges between 900mm and 1100mm (35 and 43 inches), and more rain falls in July, August and September.

According to the population and housing census conducted in 2013, The Gambia has a population of 1,882,450 people (GBoS, 2013). About 60% of the population live in rural areas.

More females than men live in the rural areas, as many men migrated to the coastal region in search of economic opportunities. The Gambia has a very youthful population structure due to the high birth rate, with nearly 44% of the population below the age of 15 years and 19% between the ages of 15 to 24 years. The implication of this as far as schistosomiasis is considered is that a great proportion of the population is within the risk groups for infection.

The Gambia is administratively divided into 5 regions: Western Region, Central River Region, Upper River Region, Lower River Region, North Bank Region (See Figure 1.4).

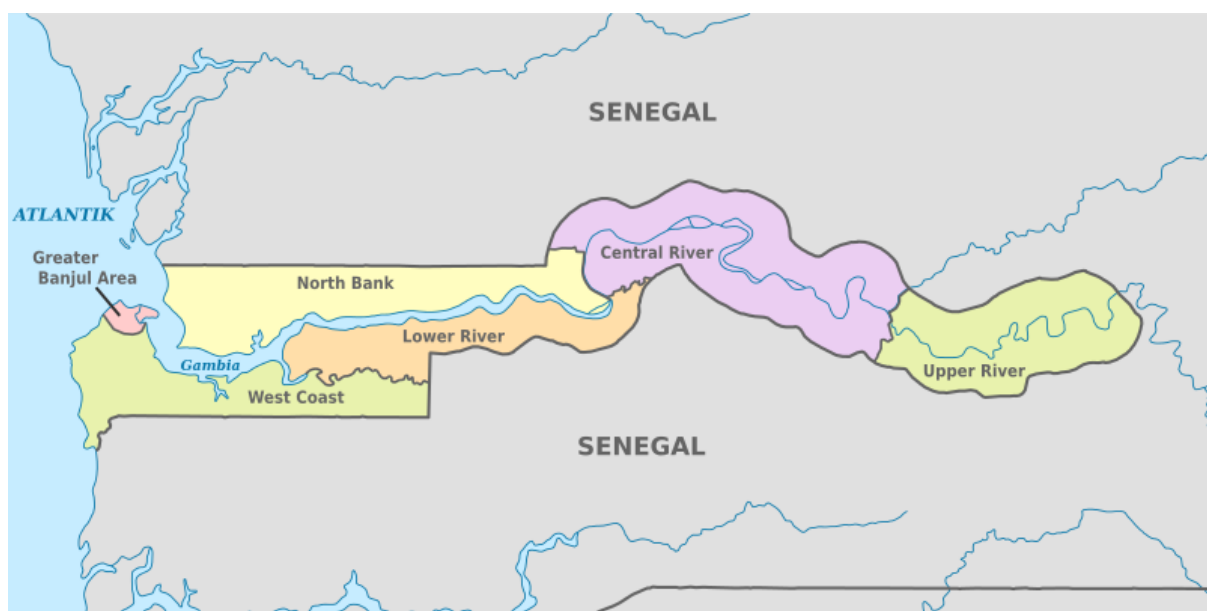


Figure 1.4. Map of The Gambia showing the 5 administrative regions of the country. *Source: File:Gambia, administrative divisions - de - colored.svg - Wikimedia Commons*

The Gambia has a liberal, market-based economy characterised by traditional subsistence agriculture, a historic reliance on groundnuts (peanuts) for export earnings, a re-export trade built around its ocean port, low import duties, minimal administrative procedures, a fluctuating exchange rate with no exchange controls, and a significant tourism industry. Agriculture which accounts for about 30% of the country's GDP includes activities such as production of peanut and other crops, livestock rearing, fishing and forestry. A significant proportion of the

population (especially women) also engage in subsistence rice farming in the rural areas. These rice farming activities are done in irrigated fields, which pose a risk for schistosomiasis infection.

The ethnic groups (tribes) found in The Gambia include mandingo, wolof, fula (Fulani), Sarahule, Jola, Serer, manjago, etc.

1.4.2 Study regions

The study was conducted in all regions of The Gambia namely: Central River Region (CRR), Upper River Region (URR), Lower River Region (LRR), North Bank Region (NBR) and Western Region (WR). CRR and URR formed the eastern part of The Gambia where the laterite plains that support seasonal pools exist, and the waters of the River Gambia are fresh. The fula tribe is common in these areas where they own large herds of cattle and other livestock. These two regions are historically known to be endemic for urogenital schistosomiasis (Duke and McCullough, 1954). The ecological features of the regions favour the breeding of freshwater intermediate snail hosts of schistosomiasis. Additionally, CRR has a major irrigated rice growing project (Jahaly and Pacharr Irrigation Project) which covers a vast area along the length of the region. Women and some men cultivate rice in the irrigated fields, and fishing is done in the canals. Some transmission occurs in the irrigation area but the majority of infections with schistosomiasis are acquired in the temporal seasonal pools (McCullough and Duke, 1954; Smithers, 1956), which are used for bathing, playing, domestic chores such as laundry and watering cattle. CRR also had a host of flood plains that form swamps. For URR, in addition to occurrence of seasonal pools, there are several streams that serve as sources of infection during the dry season when the water current lowers and snail populations are established (Smithers, 1956).

LRR, NBR and WR are not historically known to have schistosomiasis, as the River Gambia along these regions is brackish and laterite plains are not common. They were included in this study to ensure a comprehensive and complete country profile for schistosome snail host mapping.

1.4.3 Epidemiology of schistosomiasis in The Gambia

The first studies on schistosomiasis in The Gambia revealed that urogenital schistosomiasis is endemic in the eastern part (CRR and URR) of the country (Jones and Thomas, 1945; Ross, 1947; Duke and McCullough, 1954). A study conducted between communities in freshwater swampy areas close to the River Gambia and human settlements located on the laterite plateau away from the river in CRR (formerly McCarthy Island Division) found a significantly higher prevalence of *S. haematobium* infection in the laterite plateau settlements where seasonal pools exist (McCullough and Duke, 1954). This led to a suggestion that transmission was more intense in small seasonal pools where water contact area is cramped as opposed to the larger swampy areas. Snails implicated in the transmission of *S. haematobium* in these seasonal pools were initially identified as *Bulinus forskalii* (McCullough and Duke, 1954), but their species status was later confirmed as *Bulinus senegalensis* by Smithers (1956). Smithers (1956) also found *B. senegalensis* from seasonal pools, *B. jousseaumei* and *B. guernei* (*B. truncatus*) from streams shedding *S. haematobium* cercariae. *B. senegalensis* was also observed to shed *Schistosoma bovis* cercariae. *B. forskalii* from swamps did not naturally shed mammalian schistosome cercariae but was receptive to both *S. haematobium* and *S. bovis* miracidiae when exposed in the laboratory. *B. globosus* was also presumed to be a potential intermediate host (Smithers, 1956). Identification of snails and schistosomes in these past studies was based on morphological methods (McCullough and Duke, 1954; Smithers, 1956)

Prevalence and intensity of infection in communities around laterite pools were found to be highest in children, and to decrease with an increase in age (Wilkins, 1977). Increase in

infection intensity after the age of two years was observed to correlate with a rise in water contact behaviour, and low infection intensity in adults was associated with a decrease in water use. In contrast, women over 30 years of age who had more frequent infested water contacts than men surprisingly had less infection intensity. This led to the hypothesis that contact with infested water may not be the only factor influencing infection intensity in adults (Wilkins, 1977). Wilkins and Capron (1977) subsequently found a link between elevated antibody levels and fall in egg counts and suggested immunity may play a role in determining infection intensity of schistosomiasis.

In the 1980s studies on schistosomiasis progressed from observational to interventional approaches when mollusciciding (with niclosamide) was used to control *B. senegalensis* populations in seasonal pools (Goll *et al*, 1984). *B. senegalensis* populations were progressively reduced to almost 1% of the numbers in untreated pools and there was no evidence of new infection in children from the intervention area for a period of three years (Goll *et al.*, 1984). Niclosamide is highly effective against snails, their egg masses, schistosome cercariae and miracidia at very low concentrations. It is generally safe for humans and can be used at the recommended concentrations in the presence of livestock and poultry but is toxic to fish and amphibia (frogs and toads). Application of niclosamide is more beneficial in confined habitats such as seasonal pools with no permanent biodiversity as opposed to open water habitats where it kills fishes and is less effective due to over dilution.

Few studies have been reported on the epidemiology of intestinal schistosomiasis in The Gambia. Smithers (Smithers, 1956) reported *Biomphalaria pfeifferi gaudi* to be a potential vector for transmission of *S. mansoni* in The Gambia. Subsequently, in 1957, *Biomphalaria pfeifferi gaudi* snails collected from a stream (Allahein Bolong) were observed shedding *S. mansoni* cercariae and intestinal schistosomiasis infections were confirmed in inhabitants of an adjacent village, Jiboroh in Western Division (Smithers, 1957).

Over the years, schistosomiasis prevalence in The Gambia seems to be on the decline probably due to increased awareness of people on its mode of transmission, increased access to safe water supply in rural communities and the availability of praziquantel in health facilities. In the absence of comprehensive study reports, it is difficult to establish the true status of the disease in the country. Routine hospital reports and frequent presentation of urogenital schistosomiasis cases at outpatient departments indicate that the disease is still common in CRR and URR (MOH, 2014). On the contrary, cases of intestinal schistosomiasis are not seen in health facilities leading to the believe that *S. mansoni* is not found in The Gambia.

The nation-wide survey conducted in 2015 among 10,434 school children in 209 primary schools showed a national prevalence of 4.2% for urogenital schistosomiasis (*S. haematobium*) (MOH, 2016). This study showed a regional prevalence of 14.2% and 9.4% for CRR and URR, respectively. Intestinal schistosomiasis (*S. mansoni*) had a national prevalence of. 0.05% (MOH, 2016). These rare cases of intestinal schistosomiasis were thought to be imported cases from neighbouring countries like Senegal and Guinea Conakry.

1.5 Study aims

The main aim of this study was to gain an up to date understanding of the epidemiology of schistosomiasis in The Gambia with special focus on human infection dynamics and intermediate snail host incidence, distribution, infection status and genetic diversity. Additional emphasis was made on snail genetic variation and structure to better understand its relationships with schistosome transmission dynamics.

The objectives of the study were as follows:

1. to assess the prevalence, intensity and risk factors associated with schistosomiasis in school children in CRR, URR, LRR and NBR.

2. to assess the current incidence, species diversity and distribution of potential intermediate snail hosts of schistosomes in The Gambia.
3. To determine the infection status (with schistosome parasites) of potential intermediate snail hosts collected from the field.
4. To characterise the population genetic diversity and structure of intermediate snail hosts in relation to schistosome infection.

These objectives were designed to provide much-needed information that is required to undertake an integrated control approach for schistosomiasis in The Gambia; and to add knowledge to our broad understanding of the epidemiology of schistosomiasis.

2 Materials and methods

2.1 Human epidemiological study

For the human epidemiological study involving school children, we utilised data collected during the following study: ‘Field evaluation of a schistosome circulating cathodic antigen rapid test kit at point-of-care for mapping of schistosomiasis endemic districts in The Gambia’ (Sanneh et al., 2017), which was conducted to evaluate a rapid schistosomiasis diagnostic tool. During the fieldwork, participants were sensitised, and informed consent obtained before being enrolled in the study (Figure 2.1). Enrolled participants were students (aged 7 – 14 years) who lived in the selected districts and attended the selected schools (Sanneh et al., 2017).

Participants who gave consent were given two screw-capped containers to voluntarily provide their urine and stool samples. All samples were collected between the hours of 8:00 hrs and 12:00 hrs, which coincides with the maximum egg output period (Sanneh et al., 2017).

Demographic information of study participants was collected using a smart phone with an in-built questionnaire called the LINKS system. The LINKS system is an electronic questionnaire programmed in Android smart phones and designed for use in transmission assessment surveys for NTDs such as schistosomiasis, soil transmitted helminthiasis, lymphatic filariasis and onchocerciasis in the WHO Afro-region. Demographic data of participants collected included age, gender and school/community name (Appendix 2.1). The electronic questionnaire also captured water contact, sanitation and hygiene behaviour of participants for schistosomiasis risk assessment (Appendix 2.1; Sanneh et al., 2017). We extracted unpublished data generated by this previous study (Sanneh et al., 2017) and utilised it in this PhD research for epidemiological analysis of schistosomiasis in The Gambia. The previous study (Sanneh et al., 2017) analysed infection prevalence and intensity data for only participants with matching data

set for urine filtration, dipstick, Kato-Katz and POC-CCA methods, thus 1954 participants were included in the final analysis. In this PhD study, we conducted a new analysis for infection prevalence and intensity and have included all the 2,018 study participants. We also analysed infection prevalence data by school/community, which was missing in the previous study (Sanneh et al., 2017). Additionally, in this PhD study, we conducted analysis for risk factors of schistosomiasis which was not done in the previous study.



Figure 2.1. Recruitment process of school children into the study: A – sensitisation and selection; B – enrolment of study participants using the LINKS system

All processing of samples were carried out in the previous study (Sanneh et al., 2017). Urine samples of school children were processed in the field. Each urine sample upon collection from the participant, was observed for visible blood in urine (macrohaematuria). Each urine sample was then tested for presence of non-visible blood (microhaematuria) using a urine dipstick (Hemastix) (Figure 2.2A). Following the test for haematuria, each urine sample was well shaken, and 10 ml of urine drawn in a syringe and passed through the filter of the urine filtration kit. The filter was then transferred to a microscopic slide for the examination of *S. haematobium* eggs using field microscopes (Figure 2.2B). *S. haematobium* eggs have a characteristic terminal spine. Infection intensity of *S. haematobium* was classified as light (1–49 eggs/10 ml of urine)

or heavy (≥ 50 eggs/10 ml of urine), as defined by the WHO (WHO, 2002). The stool samples were transported back to the laboratory for processing and examination. Two Kato-Katz slides were prepared from each stool sample and then examined for *S. mansoni* eggs using compound microscopes (Figure 2.2C). *S. mansoni* eggs are characterised by the presence of a later spine. Infection intensity of *S. mansoni* was classified as light (< 400 eggs/gram of faeces) or heavy (> 400 eggs/gram of faeces) (WHO, 2002).



Figure 2.2. Assessment of human schistosome infection in the field and laboratory; A – urine dipstick method, B – urine filtration technique, C – microscopic examination of kato-katz stool slides

2.2 Ethics for the human study

The parent study (Sanneh et al., 2017) which generated the data used in the human epidemiological analyses carried out in this PhD research was reviewed and approved by the Gambia Government/Medical Research Council (MRC) Joint Ethical Committee (Ethics No: SCC1415). Informed consent was obtained from students and their parents or guardians. Written consent forms were provided and signed by heads of schools on behalf of all students whose parents, guardians or carers gave consent, after being contacted by the heads of schools prior to the study exercise. Furthermore, information about the study was provided by the data collection teams to each of the participants—after which informed verbal consent was obtained from each student as they were enrolled in the study. Students that were found infected were referred to the nearest health facility for treatment.

2.3 Data processing and analysis for the human study

Chi-square test was used to statistically compare infection prevalence among demographic variables (e.g., region, gender and age categories) of participants (See Appendix 2.8 for chi-square test outputs). Non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) were used to assess significant difference in infection intensity among demographic variables. Relationships between presence of *S. haematobium* eggs and haematuria were investigated using chi-square test. Associations of water contact practices with infection status and intensity were tested using logistic and linear regressions. All statistical analyses were conducted using SPSS statistical software for windows, version 24 (SPSS, Chicago, IL, USA), and infection distribution maps were created using QGIS version 3.0 (Girona Open-Source Geospatial Foundation).

2.4 Malacological surveys

During snail collections, each collection site was searched by two field collectors for 30 minutes over a length of approximately 15m along the bank of streams, rivers and irrigation

canals, and an area of approximately 5m² for seasonal pools, rice fields and swamps. Snails found were collected with a scoop, and those attached to vegetation and sticks were handpicked (Figure 2.3A & B), as some snails attach to the underside of aquatic vegetation. The habitat type, site location, date and time of sampling and geographical coordinates (using a GARMIN Oregon 550 GPS device) of each site were recorded (Figure 2.3C). Collected snails were placed in separate containers according to site and species. The containers were labelled with site name, species name and date of collection and transported to the Microbiology Laboratory of Bansang General Hospital in CRR for sample processing.

For each collection site, presence or absence of aquatic vegetation and algae were recorded. The water temperature and water pH of the sites were taken using a digital probe thermometer 31/162/0 (Brannan Thermometers, Cumbria, England) and a Mettler Toledo EL20 pH meter (Fisher Scientific, Loughborough, UK). Figure 2.4 shows the recording of water temperature at one of the sites.

The malacological study proposal was technically reviewed by the MRC Scientific Coordinating Committee and was deemed to not require an ethical review (Review No: SCC1561).



Figure 2.3. Field collections; A – scooping of snails, B – snail sampling by handpicking, and C – recording of site details



Figure 2.4. Recording of the water temperature of a site using a digital probe thermometer

2.5 Morphological identification and sorting of snails

In the laboratory, the field collected snails were sorted and identified based on morphological characteristics of their shells (Figure 2.5; see Appendix 2.2 for more snail shell pictures). The morphological identifications were aided with guides by Mandahl-Barth, 1957 and Brown, 1994. *Bulinus forskalii* was differentiated from *Bulinus senegalensis* by the presence of a distinctive shoulder angle on the upper whorls (as opposed to more round and smooth upper whorls in *B. senegalensis*) (Mandahl-Barth, 1957). However, this feature is not easily identifiable in some snails. Such snails were putatively identified by morphology and then

stored for molecular identification.



Figure 2.5. Processing and identification of snails in the laboratory, with pictures of some identified specimens

2.6 Cercarial shedding in snails

About 200 *B. senegalensis* snails from 4 different temporal pools were washed and individually placed in glass test tubes containing 10ml of distilled water and exposed to a light source from a lamp for about 5-6 hours. Cercariae macroscopically seen moving in tubes were placed on a slide and viewed with a compound microscope to confirm if they were bifurcated mammalian cercariae (Figure 2.6). Snails observed to be shedding cercariae were noted and individually placed in labelled screw-cap containers containing absolute ethanol. These infected snails were used as positive controls for the infection detection (molecular xenomonitoring) PCR. The cercariae were individually collected with about 0.2ml of water using disposable pipettes and placed in 2.0ml tubes. The tubes were topped with absolute ethanol and then stored in -20 freezers until shipment to the UK. This was done so that the cercariae can be molecularly

identified and used as controls for the infection detection PCR. All field collected snails, after identification and cercarial shedding, were placed in separate tubes containing absolute ethanol and labelled with site name, species name and date of collection for shipment to the United Kingdom.

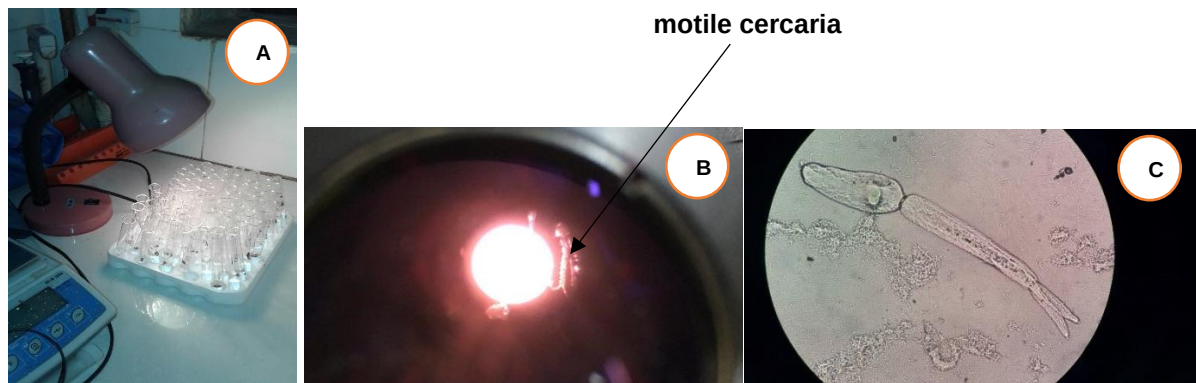


Figure 2.6. Cercarial shedding in field collected snails; A – exposure of snails to artificial light, B – a motile bifurcated *Schistosoma* cercaria (shed by a *B. senegalensis* snail) under a compound microscope (100x magnification), C – an ethanol fixed cercaria under a compound microscope (400x magnification)

2.7 Molecular processing of snails

2.7.1 DNA extraction

DNA was extracted from snails using the CTAB (hexadecyltrimethylammonium bromide) method (Goodacre and Wade, 2001; Joof et al., 2020). The whole head and foot region (or the whole organism in small specimens) of each snail was cut into pieces and placed in 1.5ml eppendorf tubes containing 500ul CTAB buffer (100 mM Tris base, 20 mM EDTA, 1.4 M NaCl, 2% CTAB). Glass beads were added to the tubes and the tissue grinded with plastic pestles. This was followed by addition of 20ul proteinase K (10g/ml concentration) and 1ul of 2-mercaptoethanol. The tubes were incubated at 55 °C for 2 – 3 hours until all tissues dissolved.

500ul of chloroform-isoamyl alcohol (24:1) were then added, the tubes mix-inverted for 5 minutes before being spun at 13000 rpm for 5 minutes. The top aqueous layer containing the DNA was collected and placed into pre-labelled 1.5ml tubes containing 1ml of absolute ethanol and 4ul of sodium acetate was added to each mixture and left at -80 °C overnight (about 12 hours) to precipitate. The mixtures were spun at 13000 rpm before the removal of ethanol leaving the DNA pellets. The DNA pellets were washed with 70% ethanol, and then allowed to air-dry. The DNA pellets were then re-suspended in 200ul Tris buffer and stored under -20 °C till use.

The cercariae preserved in ethanol were centrifuged at 13000 rpm for 10 minutes, after which the top layer was discarded. The samples were air dried and genomic DNA extracted by CTAB method using the same volumes of extraction reagents as described above.

2.7.2 PCR amplification and sequence processing for infection detection in snails

For schistosome infection detection (molecular xenomonitoring) in *Bulinus* snails, we used a three-step PCR approach. In the primary PCR, 3 primers (ETTS2, ETTS1 and ITS2_Schisto_F; see Table 2.1) were used as described in Pennance et al. (2020b). This primary PCR is a rapid non-specific screening method that amplifies a 538bp *Schistosoma* amplicon. It additionally amplifies a 1005bp trematode amplicon and ~1200bp snail amplicon (Figure 2.7), with the snail amplicon serving as a control for sample or PCR failure. Samples that produced the *Schistosoma* amplicon (538bp) in the primary PCR were further tested in a secondary PCR using primers ITS2_Schisto_F and ETTS1 (Table 2.1; Pennance et al., 2020b). These two primers amplify a single 538bp *Schistosoma* amplicon (Figure 2.7; see Appendix 2.4 for gel images) which can be directly sequenced to differentially identify *S. haematobium* and *S. bovis* infections in *Bulinus* snails. Samples that match *S. bovis* upon sequencing were tested in a third

PCR using primers ETTS2 and ITS2_Schisto_R (Table 2.1; Pennance et al., 2020b). These two primers amplify a single 835bp *Schistosoma* amplicon (Figure 2.7), which can be used to differentially identify *S. bovis* and *S. curassoni*. All PCR reactions were carried out in a final volume of 25µl containing 1x reaction buffer, 2.5mM MgCl₂, 0.2mM of each dNTP, 0.2µM of each primer (in their various combinations), 1 unit of Taq polymerase (BioTaq, Bioline), and 1 µl of template DNA. Infected snail DNA and cercariae DNA were used as positive control and water as negative control. The PCR cycling conditions were as follows: pre-heating at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 30 seconds, 58 °C for 30 seconds and 72 °C for 90 seconds, then a final extension at 72 °C for 10 minutes (Pennance et al., 2020b).

Table 2.1. Details of the primers used, including the different primer combinations and what they were used for. Schisto, ID and popgen denotes schistosome, identification and population genetics, respectively.

Primer Name (Direction)	Sequence (5' – 3')	Reference	Target Genomic Region	Purpose/ Use
ETTS2 (forward) ETTS1 (Reverse) ITS2_Schisto_F (Forward)	TAACAAGGTTTCCGTAGGTGA TGCTTAAGTTCAGCGGG GGAAACCAATGTATGGGATTATTG	Kane & Rollinson (1994) Kane & Rollinson (1994) Schols et al., 2019	Complete ITS rDNA	schisto ID, Snail PCR control
ITS2_Schisto_F (Forward) ETTS1 (Reverse)	GGAAACCAATGTATGGGATTATTG TGCTTAAGTTCAGCGGG	Schols et al., 2019 Kane & Rollinson (1994)	ITS2	Schisto ID
ETTS2 (forward) ITS2_Schisto_R (Reverse)	TAACAAGGTTTCCGTAGGTGA ATTAAGCCACGACTCGAGCA	Kane & Rollinson (1994) Schols et al., 2019	ITS1	Schisto ID
LCO (Forward) HCO (Reverse)	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994) Folmer et al. (1994)	Cox1 gene	Snail popgen/ID
LSU-1iii (Forward) LSU-3iii (Reverse)	TGCGAGAATTAATGTGAATTGC ACGGTACTTGTCGCTATCG	Fontanilla et al. (2017) Fontanilla et al. (2017)	LSU rRNA	Snail popgen

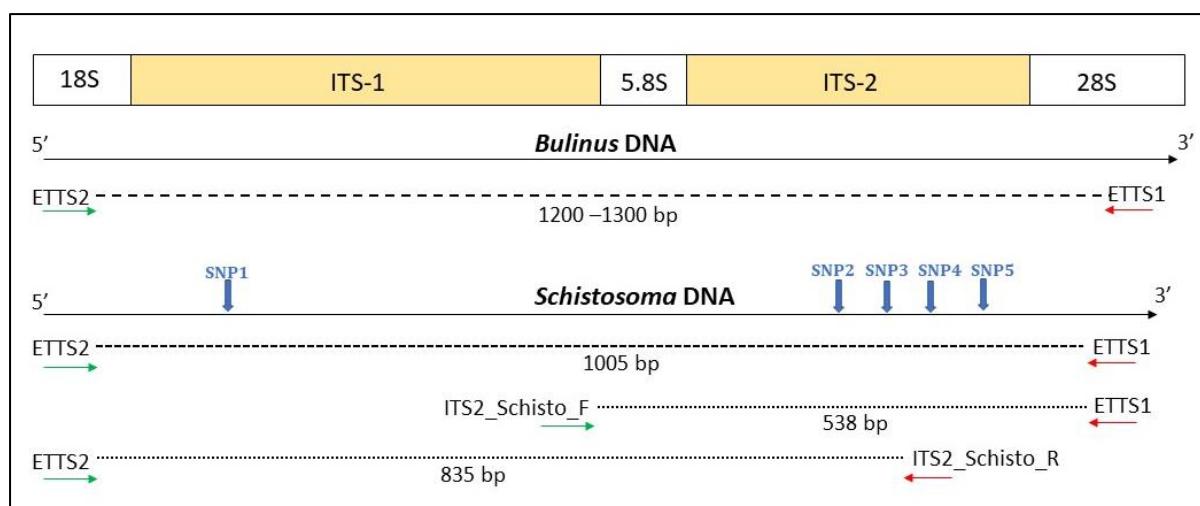


Figure 2.7. Schematic diagram of the fragments and primer annealing positions of the ITS1 + 2 rDNA targets of *Schistosoma* and *Bulinus* used for schistosome infection detection in snails (molecular xenomonitoring). The primer combination (ETTS2, ETTS1 and ITS2_Schisto_F) used in the primary PCR produced three amplicons (~1200bp snail fragment, 1005bp trematode fragment and 538bp *Schistosoma* fragment). The Secondary PCR (ITS2_Schisto_F and ETTS1) produced a single 538bp *Schistosoma* fragment and the third PCR (ETTS2 and ITS2_Schisto_R) produced a single 835bp *Schistosoma* fragment. The four single nucleotide polymorphism (SNP) sites (positions 90, 145, 195 and 265) in the ITS2 rDNA region allow differential identification of *S. haematobium* and *S. bovis*, while the single SNP site (position 52) in the ITS1 rDNA region allows differentiation of *S. bovis* from *S. curassoni*. Diagram redrawn from Pennance et al. (2020b).

To visualise the PCR amplicons, 5µL of each PCR product was run on a 1.5% agarose gel (stained with ethidium bromide) for 1 hour at 100 V. Band sizes of amplicons were viewed using NuGenius gel documentation system (Syngene, Cambridge, UK). The forward strands were Sanger sequenced using the MacroGen Eco-sequencing service (MacroGen B.V, Amsterdam, Netherlands). The sequence data were managed (trimmed, chromatograms viewed and manually edited and aligned) using the STADEN package v.1.5.3 (Staden et al., 2000) and the Genetic Data Environment (GDE) package v.2.2 (Smith et al., 1994). *S. haematobium* and *S. bovis* differ at four polymorphic sites in the ITS2 gene, and *S. bovis* and *S. curassoni* differ at a single polymorphic site in the ITS1 gene (Table 2.2). The species identification of the

sequences was determined by comparing them to *S. haematobium* and *S. bovis* reference sequences using the four polymorphism sites in the ITS2 gene (Table 2.2; Appendix 2.3). The samples that matched *S. bovis* reference sequences were further assessed using the single polymorphic site in the ITS1 gene (Table 2.2) to confirm if they were *S. bovis* or *S. curassoni*. We screened for *S. curassoni* because it uses the same *Bulinus* intermediate snail hosts as *S. haematobium* and *S. bovis*, and it occurs in neighbouring Senegal, even though it has never been reported in The Gambia.

Table 2.2. *Schistosoma* species-specific single nucleotide polymorphism (SNP) positions in the internal transcribed spacer (ITS) 1 and 2 regions. Adapted from Pennance et al. (2020b).

<i>Schistosoma</i> species	ITS Schistosome Species-Specific SNP Positions (bp)				
	ITS1	ITS2			
	SNP1 (52)	SNP2 (90)	SNP 3 (145)	SNP4 (195)	SNP5 (265)
<i>Schistosoma haematobium</i>	A	G	C	G	C
<i>Schistosoma bovis</i>	G	A	T	A	T
<i>Schistosoma curassoni</i>	A	A	T	A	T

2.7.3 PCR amplification and sequence processing for population genetics and species identification of snails

For snail population genetic analysis, we amplified both the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene. For cox1, we amplified an approximately ~700bp fragment (Appendix 2.5) using primers LCO and HCO (Table 2.1; Folmer et al., 1994). For the ITS-2 gene we utilised the primers LSU1iii and LSU3iii (Table 2.1; Fontanilla et al., 2017); these primers amplify an approximately ~800bp region of the ribosomal (r)RNA gene complex including part of the 5.8S rRNA gene, the complete internal transcribed spacer 2 (ITS-2) region and part of the large subunit rRNA gene

(Figure 2.8; see gel image in Appendix 2.6). We utilised only the ITS-2 region from this rRNA gene complex.

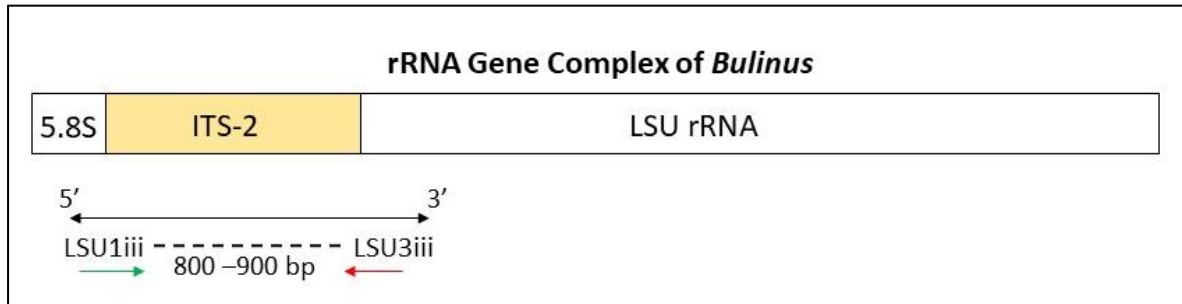


Figure 2.8. Schematic diagram of the ribosomal RNA (rRNA) gene complex of *Bulinus* showing the fragment and annealing positions of primers LSU1iii and LSU3iii used for the amplification of the rRNA gene fragment. Only the ITS-2 region from the gene complex was used for population genetic analysis of *Bulinus* snails in this study.

PCR reaction and cycling conditions were the same for both the *cox1* and ITS-2 genes. PCR reactions were carried out in a final volume of 25µl containing 1x reaction buffer, 2.5mM MgCl₂, 0.2mM of each dNTP, 0.2µM of each primer, 1 unit of Taq polymerase (BioTaq, Bioline), and 1 µl of template DNA. Occasionally, some samples fail to amplify with BioTaq and we used a different Taq polymerase (MyTaq DNA Polymerase, Bioline) for such samples with the following reaction set-up: 1x reaction buffer (Buffer + dNTPs + MgCl₂), 0.2µM of each primer, 1 unit of Taq polymerase, and 1 µl of template DNA in a 25µl final volume. The PCR cycling conditions were as follows: pre-heating at 96 °C for 2 minutes, followed by 40 cycles at 94 °C for 30 seconds, 50 °C for 1 minute and 72 °C for 2 minutes (Saadi et al., 2020). To view the PCR results, 5µL of each PCR product was run on a 1.5% agarose gel (stained with ethidium bromide) for 1 hour at 100 V with the PCR products visualised using the NuGenius gel documentation system (Syngene, Cambridge, UK). For the ITS-2 gene, some amplicons produce a non-target band in addition to the target snail band (see Appendix 2.6). For such samples, the target amplified bands were gel extracted using the QIAquick gel

extraction kit (Qiagen) according to the manufacturer's instructions (See Appendix 2.7). For each gene, the forward strands were directly sequenced using an Applied Biosystems 3730XL DNA sequencer at Macrogen Europe (Amsterdam, the Netherlands). All PCR products were purified at Macrogen Europe before sequencing. Snail DNA sequences were processed (trimmed, chromatograms viewed and edited) using the STADEN package v1.5.3 (Staden et al., 2000) and manually aligned using the Genetic Data Environment (GDE) package v2.2 (Smith et al., 1994). The *cox1* sequences were also used for the species identification (molecular barcoding) of the snails by performing BLAST searches via the National Centre for Biotechnology Information (NCBI) GenBank.

2.8 Snail data analysis

Non-parametric tests (Kruskal-Wallis and Wilcoxon Rank Sum tests) were used to test for difference in snail abundance among *Bulinus* species, regions and habitat types. To test for relationships between *Bulinus* snail abundance and environmental/physicochemical parameters, we employed negative binomial generalised linear mixed models (GLMMs) with the R package 'glmmTMB' (Brooks et al., 2017). Models were fitted separately for seasonal sites and permanent sites (Appendix 2.9). All statistical analyses were carried out in R Version 4.1.0 (R Core Team, 2021). Snail abundance and distribution maps were created using QGIS version 3.0 (Girona Open-Source Geospatial Foundation).

For the snail population genetic analysis, phylogenetic trees were constructed using the maximum likelihood (ML) method with 1000 bootstrap replicates in PhyML version 3.0 (Guindon et al., 2010). The General time reversible model incorporating gamma correction (GTR+ Γ) was used to correct for multiple substitutions. Additionally, median-joining haplotype networks were generated in PopART (Leigh and Bryant, 2015) using the algorithm described by Bandelt et al (1999). Haplotype diversity (H_d) and nucleotide diversity (π) were estimated in DnaSP v6.12.03 (Rozas et al., 2017) as a measure of Genetic diversity of snail

populations. Evidence of selection or recent population size changes were evaluated using Tajima's test of neutrality (Tajima, 1989) in DnaSP. A non-significant test means there is no violations of the mutation-drift equilibrium while a significant test implies violations of the mutation-drift equilibrium and thus evidence of selection or recent population size changes. Genetic distance (average evolutionary divergence over sequence pairs) within and between snail populations was estimated in MEGA v11 (Tamura et al., 2021) using the Maximum Composite Likelihood and GTR+G method. Pairwise fixation index (F_{st}) was estimated in Arlequin v3.5 (Excoffier and Lischer, 2010) as a measure of genetic differentiation and gene flow between snail populations. The analysis was conducted with 10,000 permutations and at significance level of $p < 0.05$. P-values were corrected with the Bonferroni method using the "p.adjust" function of the "stats" package in R v4.1.0 (R Core Team, 2021).

Phylogeographic analyses involving our novel Gambian *Bulinus* species cox1 sequences generated in this study and published cox1 GenBank *Bulinus* sequences from several African countries were conducted. Reference GenBank sequences were aligned with the novel Gambian sequences for each *Bulinus* species in Genetic Data Environment (GDE) v2.2 (Smith et al., 1994). Phylogenetic trees were constructed using the maximum likelihood (ML) method (with 1000 bootstrap replicates) and the GTR+ Γ model in PhyML version 3.0 (Guindon et al., 2010).

Relationships between snail genetic diversity and schistosome infections in snails were investigated through logistic regression analysis. Associations between snail genotype (haplotypes) and schistosome infections were also investigated using the Fisher's exact test at significance level of $p < 0.05$ in GraphPad Prism v8.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Appendix 2.10 shows outputs of Fisher's exact tests in GraphPad Prism.

3 Prevalence and risk factors of schistosomiasis among primary school children in four selected regions of The Gambia¹

¹This chapter is published as “Joof E, Sanyang AM, Camara Y, Sey AP, Baldeh I, Jah SL, Ceesay, SJ, Sambou, SM, Sanyang S, Wade, CM, Sanneh, B (2021) Prevalence and risk factors of schistosomiasis among primary school children in four selected regions of The Gambia. *PLoS Negl Trop Dis* 15(5): e0009380.”

Abstract

The Gambia initiated a control programme for schistosomiasis in 2015. In light of this, recent and comprehensive data on schistosomiasis is required to effectively guide the control programme. This study aimed to evaluate the prevalence and associated risk factors of schistosomiasis among primary school children in The Gambia.

We utilised data from a previous study conducted in 2015 in 4 regions of The Gambia: North Bank Region (NBR), Lower River Region (LRR), Central River Region (CRR) and Upper River Region (URR). In the parent study, ten schools were selected randomly from each region. Urine and stool samples collected from 25 boys and 25 girls (7 – 14 years) in each school were examined for urogenital schistosomiasis (*Schistosoma haematobium* infection) and intestinal schistosomiasis (*Schistosoma mansoni* infection) using urine filtration, dipstick and Kato-Katz methods.

Urogenital schistosomiasis had an overall prevalence of 10.2% while intestinal schistosomiasis had a prevalence of 0.3% among the sampled school children. Prevalence of urogenital schistosomiasis was significantly different among regions ($\chi^2 = 279.958$, $df = 3$, $p < 0.001$), with CRR (27.6%) being the most endemic region, followed by URR (12.0%), then LRR (0.6%), and NBR (0.0%). Prevalence of intestinal schistosomiasis was also significantly variable among regions, with 4 of the 5 positive cases detected in CRR and 1 case in URR. Every school sampled in CRR had at least one student infected with *S. haematobium*, 50% of schools in URR had *S. haematobium* infection, and just one school in LRR had *S. haematobium*

infection. While *S. haematobium* infection was significantly higher in boys ($\chi^2 = 4.440$, $df = 1$, $p = 0.035$), no significant difference in infection rate was observed among age groups ($\chi^2 = 0.882$, $df = 2$, $p = 0.643$). Two of the 5 students infected with *S. mansoni* were boys and 3 were girls. Four of these 5 students were in the 10 – 12 years age group and 1 was in the 7 – 9 years age group. Macrohaematuria and microhaematuria were found to be statistically associated with presence of *S. haematobium* eggs in urine. Being a male was a risk factor of *S. haematobium* infection. Bathing, playing and swimming in water bodies were found to pose less risk for *S. haematobium* infection, indicating that the true water contact behaviour of children was possibly underrepresented.

The findings of this study provide invaluable information on the epidemiology of schistosomiasis in The Gambia which will be useful for the schistosomiasis control efforts of the country. More studies on *S. mansoni* and its intermediate snail hosts are required to establish its true status in The Gambia. As children sometimes tend to provide responses that potentially please the research or their teacher, data collection frameworks and approaches that ensure true responses in studies involving children should be devised and used.

3.1 Introduction

Schistosomiasis is a parasitic neglected tropical disease that affects nearly 192 million people in over 40 countries in Sub-Saharan Africa (Adenowo et al., 2015). It is caused by flatworms of the genus *Schistosoma*. Of the six *Schistosoma* species known to cause disease in humans (Colley et al., 2014), two are common in Sub-Saharan Africa: *Schistosoma haematobium*, the causative agent of urogenital schistosomiasis and *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis (WHO, 2010). Approximately two-thirds of all schistosomiasis cases in Sub-Saharan Africa are caused by *S. haematobium* (Van der Werf et al., 2003; Brindley and

Hotez, 2013). Both *S. haematobium* and *S. mansoni* use molluscan snails as intermediate hosts to reach the infective larval stage in freshwater bodies, which become sources of infection to humans that come in contact with these sites (Couto et al, 2014; Campbell et al., 2017).

Water contact activities such as playing, bathing, swimming, and irrigation farming have been found to predispose people to schistosome infections, and male children are reported to be at higher risk of being infected than female children (Amuta and Houmsou, 2014; Ismail et al., 2014; Senghor et al., 2014; Geleta et al., 2015). As school age children are more likely to make contact with these freshwater bodies, they have an increased risk of getting infected with schistosomiasis (Gryseels et al., 2006; Zida et al., 2016); hence most studies target this age group. The WHO recommended methods for mapping and field diagnosis of schistosomiasis are the urine filtration technique for detecting *S. haematobium* eggs in urine (WHO, 1991; WHO, 2006) and the Kato-Katz thick smear method (Katz et al., 1972) for detecting *S. mansoni* eggs in stool (Colley et al., 2014). Urine dipstick is also often used alongside the filtration technique to detect blood in urine (haematuria), which is a good indicator of *S. haematobium* infection (Zida et al., 2016; Hessler et al., 2017).

Since the London Declaration, made on the 30th January 2012, which called for the control and elimination of schistosomiasis (WHO, 2012a; WHO, 2012b), The Gambia has initiated a neglected tropical diseases (NTDs) control programme for schistosomiasis. However, there was a lack of current and comprehensive information on the prevalence and epidemiology of schistosomiasis in the country to effectively guide this control programme. Some early studies on schistosomiasis in The Gambia focused on prevalence and distribution of the disease in some communities in the country (Duke and McCullough, 1954; Wilkins, 1977) while others looked at its transmission and vectors (McCullough and Duke, 1954; Smithers, 1956). The later studies shifted from observational to more interventional approaches with one study (Goll et al., 1984) using mollusciciding to drastically reduce *Bulinus senegalensis* snail populations in

seasonal pools, such that transmission to humans was halted for three years in the intervention communities. As the latest of these studies were over three decades ago, and the current clinical data on schistosomiasis from hospitals was neither reliable nor adequate (MOH, 2014), there is a need for more up to date and inclusive information on the epidemiology of schistosomiasis to effectively guide control strategies. In light of the above, this study evaluated the prevalence and associated risk factors of schistosomiasis in four selected regions of The Gambia.

3.2 Methods

3.2.1 Ethics statement

Ethical approval was obtained for the parent study (Sanneh et al., 2017) and informed consent was obtained from students and their parents/guardians. Written consent forms were provided and signed by heads of schools on behalf of all students whose parents, guardians or carers gave consent, after being contacted by the heads of schools prior to the study exercise. Furthermore, information about the study was provided by the data collection teams to each of the participants - after which informed verbal consent was obtained from each student as they were enrolled in the study.

3.2.2 Data source

In this study, we extracted and utilised data from the following study: Field evaluation of a schistosome circulating cathodic antigen rapid test kit at point-of-care for mapping of schistosomiasis endemic districts in The Gambia (Sanneh et al., 2017), which was conducted to evaluate a schistosomiasis rapid diagnostic test. The study (Sanneh et al., 2017) was prospective and cross-sectional in design. The investigations were carried out in 4 regions of The Gambia in 2015: North Bank Region (NBR), Lower River Region (LRR), Central River Region (CRR), and Upper River Region (URR). Ten schools were selected at random from

each region for the study (Figure 3.1). Following the WHO guidelines for participant selection in schistosomiasis mapping studies, 50 students (25 boys and 25 girls) between the ages of 7 and 14 years were randomly selected from each school. However, in some schools more than 50 students were sampled to make up for lost samples. The total number of students sampled was 2,018 students distributed as follows: 501 in NBR, 500 in LRR, 515 in CRR, and 502 in URR. The sample size determination followed the WHO recommended guidelines (with modifications) for NTD mapping in the WHO AFRO Region (WHO, 2014).

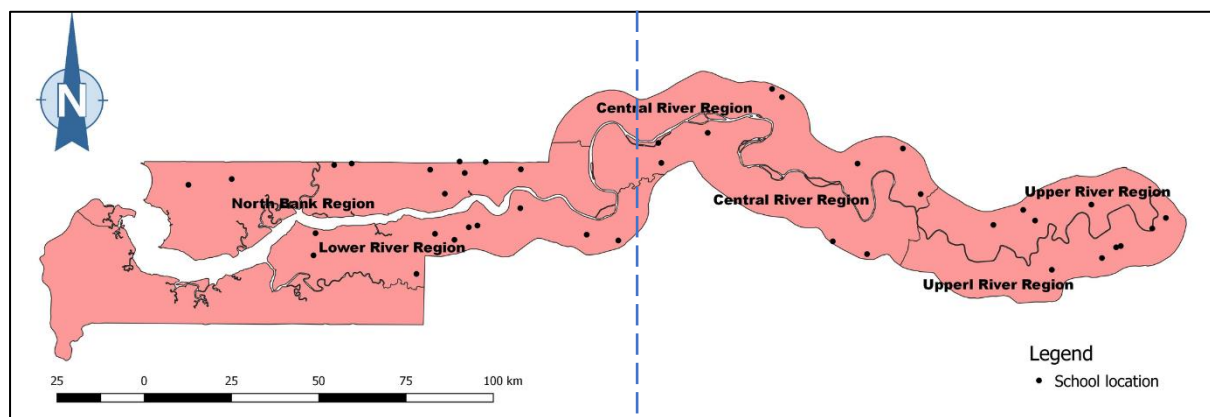


Figure 3.1. A map of The Gambia showing the locations of the 40 schools sampled across the 4 regions of the country. Link to map base layer used in creating this figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>. The vertical broken line shows where the River Gambia change from brackish to fresh water.

All the data collection and sample processing were done in the previous study (Sanneh et al., 2017) and is detailed in Section 2.1. Demographic information and water contact behaviour were recorded for each participant. In the field, each urine sample upon collection from the participant, was observed for visible blood in urine (macrohaematuria). A urine dipstick (Hemastix) was then dip in the samples to investigate microhaematuria levels. After the haematuria testing, each urine sample was well shaken, and 10 ml of urine passed through the urine filtration kit and the filter transferred to a slide for the examination of terminally spined

S. haematobium eggs, using field microscopes. Back in the laboratory, two Kato-Katz slides were prepared from each stool sample and were microscopically examined for laterally spined *S. mansoni* eggs.

We extracted the infection, demographic and water contact data of all the 2,018 participants and included them in the analysis of the current study.

3.2.3 Data analysis

SPSS statistical software for windows, version 24 (SPSS, Chicago, IL, USA) was used for all statistical analysis and computation. Descriptive statistics were used to show the distribution of demographic variables, and cross-tabulation was used to demonstrate infection prevalence, with 95% confidence intervals estimated using the Wilson Score Method. Chi-square test was used to compare infection rates among regions, gender, and age categories. Since the egg count data were not normally distributed, non-parametric tests were used to compare infection intensity means for gender (Mann-Whitney Test) and age categories (Kruskal-Wallis Test). Chi-square was further used to test for association between haematuria (both macrohaematuria and microhaematuria) and *S. haematobium* infection while logistic regression was employed to assess relationships between *S. haematobium* infection status and presence of water bodies around schools. Logistic regression was further used to assess relationships between *S. haematobium* infection status and water contact behaviours of participants. A generalised linear model with negative binomial log link function was used to assess association between presence of water bodies around schools and egg intensity. Spearman's correlation was employed to assess correlations between prevalence of schistosomiasis and egg intensity in schools. All maps were created using QGIS version 3.0 (Girona Open-Source Geospatial Foundation).

3.3 Results

3.3.1 Demography

The study enrolled 2,018 primary school children, of which 1,010 were male, and 1,008 were female. Their ages ranged from 7 to 14 years. The median age (interquartile range) of the participants was 10 years (8 to 12 years). Figure 3.2 is a box/whisker plot showing the age distribution of the students enrolled in the study.

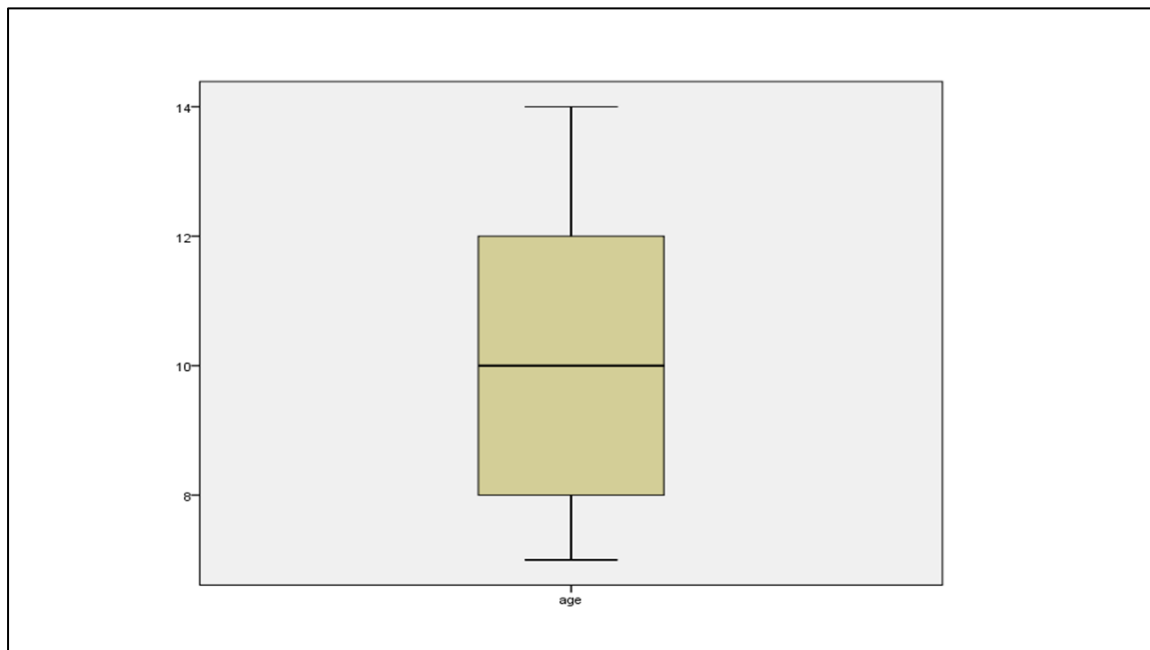


Figure 3.2. A box/whisker plot showing the age distribution of the study participants. The box represents the lower quartile (0.25), the median (0.5) and the upper quartile (0.75); the whiskers depict variability outside the lower and upper quartiles.

3.3.2 Infection prevalence and intensity

The study revealed an overall prevalence of 10.2% (95% CI: 8.9 – 11.6%) for *S. haematobium* infection, with *Schistosoma mansoni* accounting for infection in just five students giving a prevalence of 0.3% (95% CI: 0.1 – 0.6%) among the sampled primary school children. A significant difference was observed in infection rate among regions for both *S. haematobium* ($\chi^2 = 279.958$, $df = 3$, $p < 0.001$) and *S. mansoni* ($\chi^2 = 8.627$, $df = 3$, $p = 0.035$). For *S.*

haematobium, CRR was the most endemic of the 4 regions with a prevalence of 27.6% among the school children. URR, LRR and NBR had a prevalence of 12, 0.6, and 0%, respectively (Table 3.1). Four of the five students infected with *S. mansoni* were in CRR, and the fifth infected student was in URR. The rate of *S. haematobium* infection in boys (11.6%) was significantly higher than the rate of infection in girls (8.7%) across the four regions ($\chi^2 = 4.440$, $df = 1$, $p = 0.035$). For *S. mansoni*, two males and three female students were infected with the intestinal worm. Prevalence of infection with *S. haematobium* was highest (10.9%) among the 10 to 12 years age category, followed by the 13 to 14 years age group (10.2%), then the 7 to 9 years category (9.5%) of the school children, see Table 3.1. However, these differences in infection rate among age groups were not statistically significant ($\chi^2 = 0.882$, $df = 2$, $p = 0.643$). Four of the five individuals infected with *S. mansoni* were in the 10 to 12 years age group while the fifth person was in the youngest age group (7 to 9 years).

Table 3.1. Prevalence of urogenital schistosomiasis (*S. haematobium* infection) among primary school children in 4 regions of The Gambia

Variable	No. tested	No. positive	Prevalence, (95% CI)	%
Region				
NBR	501	0	0.0 (0.0 – 0.8)	
LRR	499	3	0.6 (0.2 – 1.8)	
CRR	514	142	27.6 (23.9 – 31.7)	
URR	502	60	12.0 (9.4 – 15.1)	
Gender				
Male	1010	117	11.6 (9.8 – 13.7)	
Female	1006	88	8.7 (7.2 – 10.7)	
Age category				
7 – 9	953	91	9.5 (7.8 – 11.6)	
10 – 12	750	82	10.9 (8.9 – 13.4)	

13 – 14	313	32	10.2 (7.3 – 14.1)
Total	2,016*	205	10.2 (8.9 – 11.6)

*Two of the study participants have missing data for *S. haematobium* infection and were not included in this analysis, making the total number 2,016 participants instead of 2018.

In terms of school/community prevalence, at least one student was infected with *S. haematobium* in every school sampled in CRR (Figure 3.3). Schools in CRR had *S. haematobium* prevalence ranging from 5.7% to 63.0%, and the only two schools with an infection rate more than 50% (51.0% and 63.0%) were in CRR (Figure 3.3). Half of the schools sampled in URR had *S. haematobium* infection, with infection prevalence ranging from 1.9% to 38.0%. In LRR, *S. haematobium* infection was found in just one school with a prevalence of 6.0%, while no *S. haematobium* infection was found in any of the ten schools sampled in NBR (Figure 3.3). See Supplementary Table 3.1 for *S. haematobium* prevalence of each school. Two of the four *S. mansoni*-infected students in CRR were in the same school, while the other two infected cases were in two different schools (one case each). The only school with *S. mansoni* infection in URR had just one student infected.

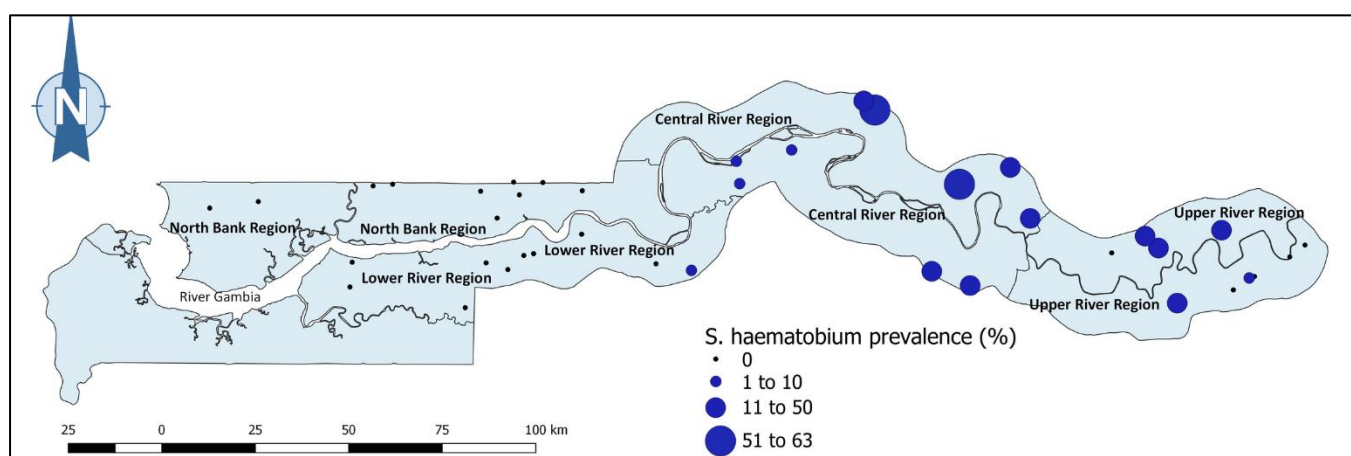


Figure 3.3. Prevalence of *S. haematobium* infection by school in 4 regions of The Gambia. Ten schools were sampled in each region. Link to map base layer used in creating this figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>.

The mean (SD) egg count for *S. haematobium* was 6.15 (40.955) eggs/10 ml of urine. Of the 205 individuals infected with *S. haematobium*, 150 (73.2%) had ‘light infection’ (1 – 49 eggs/10 ml of urine), and 55 (26.8%) manifested ‘heavy infection’ (≥ 50 eggs/10 ml of urine), see Figure 3.4. All the participants infected with *S. mansoni*, presented ‘light infection’ intensity (< 400 eggs/g of faeces). Males had significantly higher infection intensity than females for *S. haematobium* (Mann-Whitney Test = 493170.0, $p = 0.030$). There was no significant difference in *S. haematobium* egg counts among age groups (Kruskal-Wallis Test, chi-square = 1.016, $df = 2$, $p = 0.602$). A Spearman’s correlation analysis found a positive correlation between prevalence of *S. haematobium* infection and egg intensity ($r = 0.995$, $p < 0.001$).

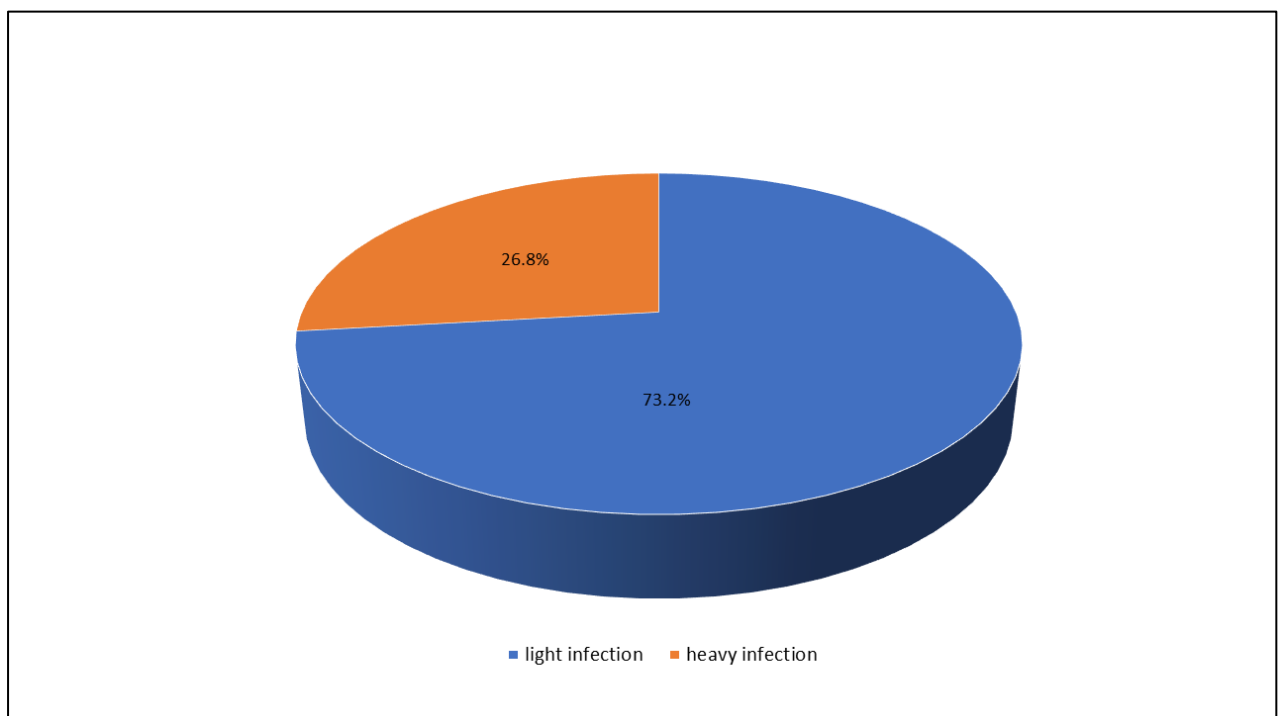


Figure 3.4. Infection intensity of *S. haematobium* (light against heavy infection) among children from 40 primary schools in 4 regions of The Gambia

3.3.3 Macro and microhaematuria

Overall, 10 (0.5%) individuals had visible blood in their urine or macrohaematuria, with 9 (90%) of them positive for *S. haematobium* eggs. Of the 2,006 participants negative for macrohaematuria, only 9.8% (196) had eggs in their urine (Table 3.2). Fisher's Exact Test showed a significant association between macrohaematuria and the presence of *S. haematobium* eggs in urine (OR=83.11, 95% CI 10.48 – 659.46, $p<0.001$). For the dipstick method (microhaematuria), 363 (18.0%) individuals were positive overall. One hundred and fifty-three (74.6%) of the 205 individuals infected with *S. haematobium* were positive for microhaematuria, and 88.8% of the total 1,811 students that were not infected with *S. haematobium* showed no presence of microhaematuria in their urine (Table 3.2). Chi-square test revealed an association between microhaematuria and *S. haematobium* infection (OR=22.43, 95% CI 15.87 – 31.70, $p<0.001$).

Table 3.2. Frequency of macro and microhaematuria among the studied school children

	<i>S. haematobium</i> infection		
	Positive (%)	Negative (%)	Total (%)
Macrohaematuria			
Yes	9 (90.0%)	1 (10.0%)	10 (100%)
No	196 (9.8%)	1810 (90.2%)	2,006 (100%)
Total	205	1811	2016
Microhaematuria			
Yes	153 (42.1%)	210 (57.9%)	363 (100%)
No	52 (3.1)	1601 (96.9)	1,653 (100%)
Total	205	1811	2,016*

*Two of the study participants have missing data for *S. haematobium* infection status, which brings the total number to 2,016 instead of 2,018 participants.

3.3.4 Risk factors

The univariate analysis showed that males were more likely to get infected with *S. haematobium* than females (OR=1.37, 95% CI 1.02 – 1.83, p=0.036), and no association was found between age category and urogenital schistosomiasis infection (Table 3.3).

Table 3.3. Demographic variables as risk factors of urogenital schistosomiasis infection among school children in 4 regions of The Gambia

Variable	<i>S. haematobium</i> infection status			OR (95% CI)	p-value
	Total (%)	Positive (%)	Negative (%)		
Gender					
Male	1,010 (100)	117 (11.6)	893 (88.4)	1.37 (1.02 – 1.83)	0.036*
Female	1,006 (100)	88 (8.7)	918 (91.3)	1	
Age category					
7 – 9	953 (100)	91 (9.5)	862 (90.5)	1	0.348
10 – 12	750 (100)	82 (10.9)	668(89.1)	1.16 (0.85 – 1.59)	
13 – 14	313 (100)	32 (10.2)	281 (89.8)	1.08 (0.71 – 1.65)	

*Statistical significance at p<0.05

OR = Odd Ratio

A generalised linear model with negative binomial log link function found a significant association between egg intensity and proximity of schools to water bodies ($b = 0.429$, 95% CI = 0.318 – 0.541, $p < 0.001$). Egg intensity was higher in schools that had a freshwater body close by 15 minutes round-trip. Logistic regression found no significant association between *S. haematobium* infection status and presence of water bodies close by the schools (OR=1.07, 95% CI 0.76 – 1.50, $p=0.719$). Water-contact behavioural risk factors such as washing clothes/dishes, fishing, fetching water, and crossing water bodies were not significantly

associated with *S. haematobium* infection (see Table 3.4). Of the 205 individuals positive for *S. haematobium*, only 25 (12.2%) responded yes for bathing in freshwater bodies, 22 (10.7%) said yes for playing in freshwater bodies, and 15 (7.3%) said yes for swimming in freshwater bodies. Binary logistic regression analyses showed an unexpected association between urogenital schistosomiasis infection and water-contact activities like bathing, playing, and swimming in water bodies. Instead of being risk for infection, bathing (OR=0.59, 95% CI 0.39 – 0.92, p=0.019), playing (OR=0.55, 95% CI 0.35 – 0.88, p=0.012) and swimming (OR=0.52, 95% CI 0.30 – 0.898, p=0.019) were found to be less risk for *S. haematobium* infection, i.e., they were found to reduce the risk of infection with *S. haematobium* (Table 3.4).

Table 3.4. Behavioural risk factors of *S. haematobium* infection in 4 regions of The Gambia

Variable	<i>S. haematobium</i> infection status			OR (95% CI)	p-value
	Total (%)	Positive (%)	Negative (%)		
Water bodies					
Yes	452 (100)	48 (10.6)	404 (89.4)	1.07 (0.76 – 1.50)	0.179
No	1564 (100)	157 (10.0)	1407 (90.0)	1	
Bathing					
Yes	368 (100)	25 (6.8)	343 (93.2)	0.59 (0.39 – 0.92)	0.019*
No	1,648 (100)	180 (10.9)	1468 (89.1)	1	
Washing clothes/dishes					
Yes	60 (100)	6 (10.0)	54 (90.0)	0.97 (0.42 – 2.31)	0.965

No	1,956 (100)	199 (10.2)	1,757 (89.8)	1		
Fishing						
Yes	69 (100)	5 (7.2)	64 (92.8)	0.68 (0.27 – 1.71)	0.417	
No	1,947 (100)	200 (10.3)	1,747 (89.7)	1		
Crossing water bodies						
Yes	33 (100)	2 (6.1)	31 (93.9)	0.57 (0.13 – 2.38)	0.437	
No	1,983 (100)	203 (10.2)	1,781 (89.8)	1		
Fetching water						
Yes	32 (100)	2 (6.3)	30 (93.8)	0.59 (0.14 – 2.47)	0.465	
No	1,984 (100)	203 (10.2)	1,781 (89.8)	1		
Playing						
Yes	345 (100)	22 (6.4)	323 (93.6)	0.55 (0.35 -0.88)	0.012*	
No	1,671 (100)	183 (11.0)	1,488 (89.0)	1		
Swimming						
Yes	253 (100)	15 (5.9)	238 (94.1)	0.52 (0.30 – 0.898)	0.019*	
No	1763 (100)	190 (10.8)	1573 (89.2)	1		

*Statistical significance at $p < 0.05$

3.4 Discussion

Human schistosomiasis was first reported in The Gambia in 1945 (Jones and Thomas, 1945), but a more detailed account was given in 1954 by Duke and McCullough (1954) on the distribution of the disease in Central River Region (CRR), then called McCarthy Island Division. Several studies on the disease followed over the next three decades until the mid-1980s (Wilkins, 1977; Goll et al., 1984; Wilkins et al., 1984; Blumenthal, 1985). After this

period, efforts geared towards the control of schistosomiasis in the country (including prevalence studies and interventions) became limited. In 2014 when The Gambia took its first steps in setting up a schistosomiasis control programme, a knowledge gap on the distribution of the disease was found (MOH, 2014). This study was conducted to provide information on the epidemiology of the disease to help fill that knowledge gap.

The study assessed the prevalence and associated risk factors of schistosomiasis among primary school children in four of the five regions of the country. As reported by previous studies (Duke and McCullough, 1954; Wilkins, 1977), urogenital schistosomiasis was the predominant form of schistosomiasis seen in this study, with intestinal schistosomiasis observed in just 0.3% of the sampled school children investigated for infection. Of the numerous past studies on schistosomiasis in The Gambia, only one study reported the occurrence of intestinal schistosomiasis in the country (Smithers, 1957). Additionally, few or no cases of intestinal schistosomiasis have presented at hospitals and health facilities in recent times (MOH, 2014). This led to the notion that *S. mansoni* does not exist in the country and that the few cases encountered in health facilities and studies might be imported cases. In this study, a follow-up on 2 of the five participants positive for *S. mansoni*, revealed they had a recent travel history. Prior to the study, the two students who are twins from the same household travelled to Guinea Conakry where *S. mansoni* is endemic (Koma and Beer, 1982; Hodges et al., 2011).

The study found the highest prevalence of schistosomiasis among the school children in CRR and URR. This corroborates previous studies that found the eastern regions (represented by CRR and URR) to be the parts of the country endemic for urogenital schistosomiasis (Duke and McCullough, 1954; Smithers, 1956; Wilkins et al., 1984; Blumenthal, 1985). In this study, CRR had a higher prevalence of *S. haematobium* than URR, with at least one student found infected in each of the ten schools sampled in the region. The River Gambia is fresh along both

regions and has many tributaries (streams), locally called ‘bolongs,’ along these regions, which serve as sources of infection in the dry season (Smithers, 1956). Further away from the River Gambia, the landscape is a sandstone plateau with rocky, laterite iron pan (Hanrion et al., 1971). When depressions formed in this laterite plateau, they hold rainwater and give rise to seasonal pools that become sources of infection in the rainy season (Goll et al., 1984; Goll and Wilkins, 1984; Senghor et al., 2015). Additionally, CRR has a huge irrigation project under the Ministry of Agriculture of The Gambia, with an irrigated area that stretches along many communities in the region engaged in rice farming. These factors might explain why the school children from these regions had the highest prevalence of urogenital schistosomiasis. Conversely, the River Gambia is brackish in NBR and LRR, and there is no laterite plateau in these regions, which might be the reason for the zero and low infection rates among the school children in these regions.

Observations for both visible blood in urine (macrohaematuria) and non-visible blood in urine (microhaematuria) were found associated with presence of *S. haematobium* eggs in urine. Previous studies also found presence of blood in urine to correlate with presence of *S. haematobium* eggs, and it has been demonstrated as a good indicator of *S. haematobium* infection (Zida et al., 2016; Hessler et al., 2017). While not all haematuria are related to *S. haematobium* infection, presence of blood in urine provides an early defining indication of infection. The presence of blood in urine is caused by granulomatous inflammation resulting from the lodging of *S. haematobium* eggs in the bladder and urogenital system (Colley et al., 2014), and is increasingly becoming an important criteria for assessing *S. haematobium* infection in studies. Self-reported blood in urine is even used to identify potential *S. haematobium* infection in people living in endemic areas (Gray et al., 2011). Prevalence of *S. haematobium* was found to increase with increase in egg intensity. Egg intensity was also higher in schools that had a water body close by 15 minutes round-trip.

Previous studies have shown schistosomiasis prevalence to be higher in males than females (Couto et al., 2014; Amuta and Houmsou, 2014; Senghor et al., 2014; Geleta et al., 2015; Hajissa et al., 2018), with males up to 16 times more likely of getting infected (Couto et al., 2014). In this study, male students had a higher infection rate and had higher risk of getting urogenital schistosomiasis than females. The context here is that 'male children' are more likely to play in freshwater bodies and/or be responsible for carrying out important designated tasks such as watering livestock and other domesticated animals. Geleta et al. (2015) found 'having a parent as a farmer' to be a predisposition for schistosomiasis while Amuta and Houmsou (2014) found 'being involved with irrigation' as a risk factor for the disease. This study found no significant difference in *S. haematobium* infection prevalence among the different age groups and no association between age groups and *S. haematobium* infection. Many previous studies that involved school aged children made similar observations (Ismail et al., 2014; Senghor et al., 2014; Geleta et al., 2015), while other studies reported the opposite (Ivoke et al., 2014; Kabuyaya et al., 2018).

Schistosomiasis is known to infect humans during water contact behaviours. Direct contact with open freshwater bodies through bathing, swimming, and wading is reported to be a risk factor of schistosomiasis (Couto et al., 2014; Amuta and Houmsou, 2014; Ismail et al., 2014; Zida et al., 2016). In this study, however, for each water contact activity, less than 13% of the children infected with *S. haematobium* indicated that they were engaged in such water contact behaviour. From experience and our broad observations, this seemed an underrepresentation of their true water contact behaviours. Regression analysis of this data further indicated that children that engaged in some water contact activities (bathing, playing, and swimming) were less likely to get *S. haematobium* infection. A possible explanation is that the responses provided by the children are not a true representation of their water contact behaviour and activity, although the field teams ensured the children were comfortable throughout the

interviews and were given gifts. While this finding did raise some questions – notably about response quality (Borges et al., 2004) around contact with water bodies, we are confident that it represented the response of the children interviewed. As most parents and teachers in these regions know about the disease, they will have advised their children against playing or bathing in these water bodies. For this reason, the children would naturally be uncomfortable disclosing contact with waterbodies for fear that they will be reprimanded by the teachers, parents or even us the researchers. The children may therefore have given inaccurate responses about their water contact behaviours.

Furthermore, if we explore the case of the children who are likely to be exposed to the water bodies by virtue of assumed responsibilities for carrying out activities such as watering livestock and other domesticated animals, an important potential risk factor is the proximity to the water source. Indeed, in a study conducted in Sudan by Hajissa and colleagues (2018), the authors identified the distance between children's place of residence and the water source as an important determinant to the risk of schistosomiasis infection in children. This factor was not assessed in the present study and is one of the limitations of the study. We however assessed the proximity of children's schools to water bodies but found no significant association with schistosomiasis disease outcome. Children spend far more time away from school than in school. Additionally, a major source of schistosomiasis infection in The Gambia is seasonal pools which were non-existent during this study as the study was conducted in the dry season. These may explain the no association between proximity of schools to water bodies and *S. haematobium* infection status. Another potential important cofounder which could have shed more light on risk factors of infection if it was assessed in this study is parents' occupation.

More innovative, collaborative Frameworks and approaches that ensure true response to questionnaires in studies involving children need to be developed and used, as children in early childhood tend to please the researcher or teacher (de Leeuw, 2011). Moreover, children are

sometimes afraid to say something wrong or undesirable, especially in school-like settings (Delfos, 2001). We suggest that, to avoid anxiety in children, questions formulated for questionnaires and interviews should not resemble a school test, and children should be reassured that there is no wrong answer and that they will not be reprimanded for giving any particular answer. The questions should take into account cultural nuances which may serve as ‘unaccounted for’ confounders in research involving children. Doing so will hopefully encourage honest feedback from children, who in some cases by virtue of associating researchers with ‘local authority’ are potentially affected by a ‘perceived power-dynamic hierarchy.’

Another point to make is that ‘The Taskforce for Global Health’ developed questionnaires we used to collect water contact data were generalised standard questionnaires and did not include country-specific activities like ‘being involved with irrigation farming’ and ‘watering livestock or domesticated animals in freshwater bodies.’ The seasonality of transmission in The Gambia was also not put into consideration. Yet, in rural Gambia, many children assist their parents in irrigation farming, watering livestock and other domesticated animals, and transmission is primarily seasonal in the country. The lesson to draw from this is that protocols/frameworks should be tailored to country-specific or local needs as much as possible.

The findings of the study revealed information that will be vital for the national schistosomiasis control efforts of The Gambia. New and continuous studies are still required to further understand the epidemiology of the disease and to provide up to date prevalence estimates. These future studies should employ more advance diagnostic methods such as PCR, which are more sensitive than the traditional parasitological techniques used in this study. The studies should also focus on all aspects of the population, as the present study has a limitation that it sampled only primary school children. More studies on *S. mansoni* and its intermediate snail hosts are also required to describe intestinal schistosomiasis status in The Gambia.

3.5 Conclusion

The study evaluated the prevalence and associated risk factors of schistosomiasis in primary school children in 4 regions of The Gambia (NBR, LRR, CRR, and URR). Urine and stool samples were collected from 2,018 students and examined for *S. haematobium* and *S. mansoni* infections. 10.2% had *S. haematobium* infection, while 0.3% had *S. mansoni* infection. CRR was the most endemic region for *S. haematobium* infection, followed by URR, then LRR, and NBR had no infection. The prevalence of *S. haematobium* infection in boys was more than the prevalence in girls. No significant difference in infection rate was observed among the different age groups involved in the study. Boys had a higher risk of getting infected than girls, and presence of blood in urine was a good indicator of urogenital schistosomiasis infection. Water contact activities such as bathing, playing and swimming were found to pose less risk for urogenital schistosomiasis infection, suggesting inaccurate responses from children about their water contact behaviours. The findings of this study provide invaluable information on the epidemiology of schistosomiasis in The Gambia which will be useful for the schistosomiasis control efforts of the country. More studies on *S. mansoni*, including investigation of its intermediate snail hosts, are required to establish the true status of intestinal schistosomiasis in the country.

4 Species diversity and distribution of schistosome intermediate snail hosts in The Gambia¹

¹This Chapter is published as “Joof E, Sanneh B, Sambou SM, Wade CM (2021) Species diversity and distribution of schistosome intermediate snail hosts in The Gambia. *PLoS Negl Trop Dis* 15(10): e0009823”.

Abstract

There is a need for recent information on intermediate snail hosts of schistosomes in The Gambia; the previous studies were conducted over three decades ago. This study assessed the incidence, species diversity, distribution and infection status of schistosome intermediate snail hosts in the country. Malacological surveys were conducted in all 5 regions of The Gambia: Central River Region (CRR), Upper River Region (URR), Western Region (WR), Lower River Region (LRR) and North Bank Region (NBR). Sampling of snails was undertaken at 114 sites that included permanent water bodies such as streams (bolongs), rice fields, irrigation canals and swamps; and temporal (seasonal) laterite pools. Ecological and physicochemical factors of sites were recorded. Snails were identified morphologically and screened for schistosome infections using molecular techniques. Freshwater snails were found at more than 50% (60/114) of sites sampled. While three species of *Bulinus* were collected, no *Biomphalaria* snails were found in any of the sites sampled. Of the total 2877 *Bulinus* snails collected, 75.9% were identified as *Bulinus senegalensis*, 20.9% as *Bulinus forskalii* and 3.2% as *Bulinus truncatus*. Seasonal pools produced the largest number of snails, and CRR was the region with the largest number of snails. *Bulinus senegalensis* was found more in seasonal pools as opposed to permanent sites, where *B. forskalii* and *B. truncatus* were observed to thrive. *Bulinus* snails were more common in seasonal sites where aquatic vegetation was present. In permanent sites, the abundance of snails increased with increase in water temperature and decrease in water pH. *Bulinus senegalensis* was found infected with both *S. haematobium* and *S. bovis*, while *B. forskalii* and *B. truncatus* had only *S. bovis* infection. While the human parasite *S.*

haematobium was restricted to just four sites, the livestock parasite *S. bovis* had a much more widespread geographical distribution across both CRR and URR. This new information on the distribution of intermediate snail hosts of *S. haematobium* group species in The Gambia will be vital for the national schistosomiasis control initiative.

4.1 Introduction

Schistosomiasis (also called bilharzia) is a parasitic disease caused by trematode worms (blood flukes) of the genus *Schistosoma*. It is the second most prevalent tropical disease after malaria and is a leading cause of morbidity in many parts of the globe, especially in Africa (Chitsulo et al., 2000). It is found in over 40 countries in Africa where nearly 192 million people are infected with the disease (Adenowo et al., 2015), making Africa the most prevalent continent in the world for schistosomiasis.

Globally, the human form of schistosomiasis is caused by six species in the genus *Schistosoma*: *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. guineensis* and *S. mekongi* (Colley et al., 2014). Most human infections result from three species, namely *S. haematobium*, *S. mansoni* and *S. japonicum*. The two most widely distributed species in Africa are *S. haematobium*, the causative agent of urogenital schistosomiasis and *S. mansoni*, which causes the intestinal form of the disease (Colley et al., 2014). In The Gambia, urogenital schistosomiasis is endemic in the eastern regions (Central River and Upper River Regions) of the country (Wilkins, 1977; Goll and Wilkins, 1984), with very few reports on the occurrence of the intestinal form of the disease (Smithers, 1957). Transmission of urogenital schistosomiasis is most prevalent in these regions during the rainy season through seasonal laterite pools formed in cuirasse depressions (Goll and Wilkins, 1984).

Schistosoma species require intermediate freshwater snail hosts to develop to the infective larval stage for human infection. Snails of the genus *Bulinus* act as the intermediate hosts of *S. haematobium* while those of the genus *Biomphalaria* acts as the intermediate hosts of *S. mansoni*. These snails occur in most parts of the African continent (Mandahl-Barth, 1965; Brown, 1994). *Bulinus globosus* (a member of the *Bulinus africanus* group), arguably the most important intermediate host of *S. haematobium* in Africa, is the most common and widely distributed species of the genus *Bulinus* in Africa (Mandahl-Barth, 1965; Brown, 1994). *Bulinus forskalii* is also widely distributed in the African continent while *B. senegalensis* is mostly found in West Africa (Mandahl-Barth, 1965). From early taxonomic literature, some *Bulinus* snails belonging to the *Bulinus africanus* group were difficult to distinguish in West Africa (Wright, 1961; Mandahl-Barth, 1965). The smaller *B. jousseaumei* was regarded as a sub-species and regional form of *B. globosus* inhabiting the Northern limit of its range based on size variation observations of snails collected from across Angola to The Gambia (Wright, 1961). A subsequent study by Mandahl-Barth (Mandahl-Barth, 1965) challenged this explanation and argued that the smaller *B. jousseaumei* is a distinct species in its own right, and he reported the occurrence of both the true *B. globosus* of typical form and normal large size as well as the smaller *B. jousseaumei* in The Gambia. *Bulinus truncatus* and *Bulinus guernei* were also reported to be separate species (McCullough and Duke, 1954; Smithers, 1956), until later studies confirmed them as the same species Brown et al., 1986; Diaw et al., 1989b).

In addition to the human parasites *Schistosoma haematobium* and *S. mansoni*, there are 3 other schistosome species belonging to the *S. haematobium* group (*S. bovis*, *S. curassoni*, *S. mattheei*) in Sub-Saharan Africa that cause animal schistosomiasis in domestic livestock (De Bont and Vercruysse; 1997; Gower et al., 2017). These schistosomes also use *Bulinus* snails as intermediate hosts and they overlap with *S. haematobium* in some endemic regions.

Schistosomiasis in livestock such as cattle, sheep and goat is thought to have potential impact on animal health and productivity (Gower et al., 2017).

Detection and identification of schistosome infection in snails is an important component of schistosomiasis transmission surveillance and plays a huge part in unravelling the epidemiology of the disease in endemic regions. The cercarial shedding method in snails remains an important schistosome infection detection approach, but more rapid and direct techniques have been developed over the years and used as a complement (Lu et al., 2016) or alternative (Amarir et al., 2014) to this long-standing traditional method. These range from simple approaches like the dot hybridisation and antibody detection methods (Hamburger et al., 1987; Hamburger et al., 1989b) to more robust techniques such as the loop-mediated isothermal amplification (LAMP) assay and polymerase chain reaction (PCR) (Notomi et al., 2000; Hamburger et al., 2004). *Bulinus* snails are known to carry different schistosome species and detection of infection in these snails is even more complicated in transmission zones where more than one schistosome species overlap. Such a need necessitated the recent development of a molecular xenomonitoring PCR-based technique that can detect and differentially identify *S. haematobium* and *S. bovis* infections in *Bulinus* snails (Pennance et al., 2020b). The method can further differentiate *S. bovis* from other *S. haematobium* group species such as *S. curassoni* (Pennance et al., 2020b).

In The Gambia, *Bulinus* species collected from streams and seasonal pools have been found to harbour *S. haematobium* and *S. bovis* (McCullough and Duke, 1954). In seasonal pools, the snails involved in the transmission of *S. haematobium* and *S. bovis* were initially identified as *Bulinus forskalii* (McCullough and Duke, 1954), but their species status was later confirmed as *Bulinus senegalensis* by Smithers (1956). Smithers (1956) also reported *Bulinus jousseaumei* and *Bulinus guernei* in permanent streams. These snails were found infected with *S. haematobium*. *Bulinus forskalii* collected in swamps was compatible to *S. bovis* in the

laboratory (Smithers, 1956). It was also found breeding in sympatry with *B. senegalensis* in alluvial pools and irrigated rice fields in The Gambia (Goll, 1981). All these past studies used morphological techniques for the identification of both snails and schistosome parasites (McCullough and Duke, 1954; Smithers, 1956; Goll, 1981). Few studies have reported on the intermediate host of *S. mansoni* in The Gambia. Smithers (Smithers, 1956) reported *Biomphalaria pfeifferi gaudi* to be a potential intermediate host of *S. mansoni* in The Gambia. Subsequently, in 1957, *Biomphalaria pfeifferi gaudi* snails collected from a stream were observed shedding *S. mansoni* cercariae and intestinal schistosomiasis infections were confirmed in inhabitants of an adjacent village, Jiboroh in the Western Region of The Gambia (Smithers, 1957). Later studies conducted in the 1980s on schistosomes and their intermediate snail hosts in The Gambia progressed from observational to interventional strategies with mollusciciding (with niclosamide) used in a study to control *B. senegalensis* populations in seasonal pools (Goll et al., 1984). *Bulinus senegalensis* populations were progressively reduced to almost 1% of the numbers in untreated pools and there was no evidence of new infection in children from the intervention area for a period of three years (Goll et al., 1984).

After the London declaration of 30th January 2012 (WHO, 2012a), which had advocated for the elimination of schistosomiasis in some countries, the move to control schistosomiasis and other neglected tropical diseases (NTDs) has intensified in many African countries. To meet the global objective of combating NTDs, The Gambia started the process of developing a national control programme for these diseases in 2014. A situation analysis was conducted from May to June 2014 to assess the status of NTDs in the country. This review based on published data and routine hospital reports revealed the endemicity of schistosomiasis in the country and established schistosomiasis (along with soil transmitted helminthiasis) as the main NTDs requiring a control program in the country. It further revealed knowledge gaps on the status of schistosomiasis infection in the country (MOH, 2014) that led to a nationwide

mapping of schistosomiasis from May to June 2015 (MOH, 2016). WHO has renewed its interest of including intermediate snail hosts in schistosomiasis surveillance and control in the new road map to the 2030 schistosomiasis elimination targets (WHO, 2020b). Since studies on snail intermediate hosts of schistosomes in The Gambia have been very limited, and the latest reported studies, covering just a few areas of the country, were conducted in the 1980s (Goll et al., 1984), there is need for a reliable, up to date knowledge on the distribution of schistosome intermediate snail hosts in a wider geographical scope, to effectively guide the national schistosomiasis control programme of the country.

This study therefore aimed to assess the current incidence, species diversity, distribution and infection status of potential intermediate snail hosts of schistosomes in The Gambia. The study sought to address the following specific research questions: 1) which intermediate snail hosts of schistosomes are most abundant and widely distributed? and 2) which habitat types and regions have the highest number of intermediate snail hosts?

4.2 Methods

4.2.1 Study area and site selection

Malacological surveys were conducted in all 5 regions of The Gambia: Central River Region (CRR), Upper River Region (URR), Western Region (WR), Lower River Region (LRR) and North Bank Region (NBR). Sampling was done at 114 sites: 50 sites in CRR, 40 sites in URR, 7 sites in WR, 9 sites in LRR and 8 sites in NBR (Figure 4.1). Communities that were found to have human schistosomiasis according to recent previous studies (MOH, 2016; Joof et al., 2021a) were selected for sampling. These communities were visited, and the inhabitants asked about the location of freshwater sites and information on human and livestock water contact at these sites. Several other communities that did not have human schistosomiasis according to

the previous studies were also selected if they had freshwater sites that were frequented by humans or livestock. The identified freshwater sites in these communities were visited and sampled. Types of habitats sampled (in 2017 and 2019) were permanent water bodies that included streams (bolongs), rice fields, irrigation canals and swamps; and temporal (seasonal) laterite pools (Figure 4.2).

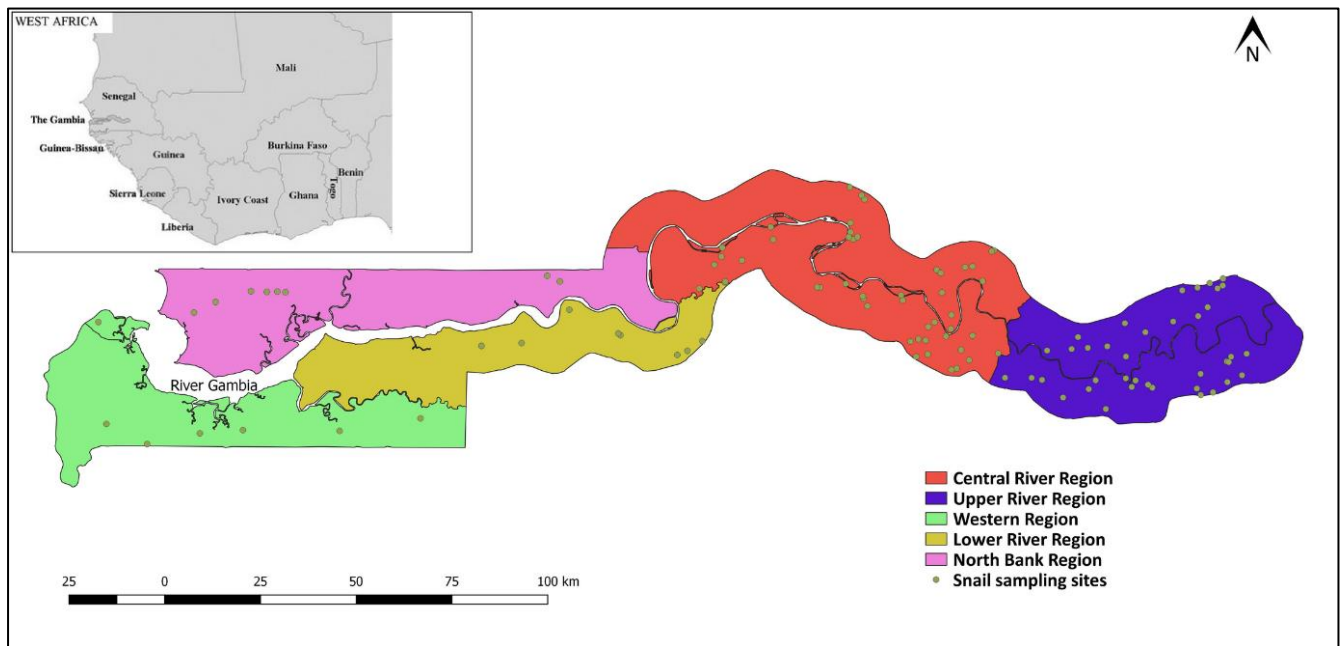


Figure 4.1. A map of The Gambia showing the snail sampling sites within the 5 regions of the country. Link to base layer of map used in the figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>.

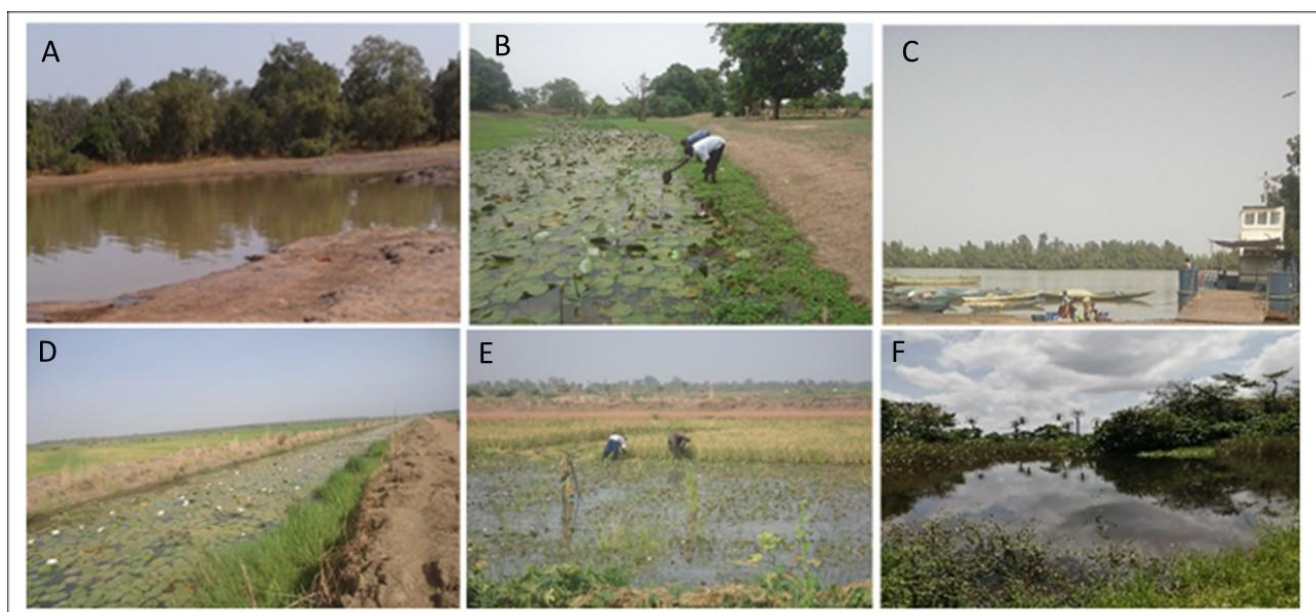


Figure 4.2. Different types of habitats from which snails were sampled; A – seasonal laterite pool, B – stream, C – river, D – irrigation canal, E – rice field, F – swamp.

4.2.2 Snail sampling and processing

Sampling of Snails was undertaken from 114 sites across The Gambia in 2017 and 2019. The 2017 collections were conducted in June in permanent sites and from October to November in seasonal sites. In 2019, the collections were undertaken in March in permanent sites and from September to October in seasonal pools. Sampling from permanent sites was carried out during the dry season because this is the time when snails are abundant at these sites. They are very scarce or non-existent during the rains due to the overflowing of water. Sampling of seasonal pools was conducted in the rainy season because this is the only time these bodies of water exist.

For each collection site, two field collectors searched for 30 minutes over a length of approximately 15m along the bank of streams, rivers and irrigation canals, and an area of approximately 5m² for seasonal pools, rice fields and swamps. Snails found were collected with a scoop, and those attached to vegetation and sticks were handpicked, as some snails attach to the underside of aquatic vegetation. The habitat type, site location, date and time of sampling

and geographical coordinates (using a GARMIN Oregon 550 GPS device) of each site were recorded. Collected snails were placed in well labelled separate containers for each site and transported to the Microbiology Laboratory of Bansang General Hospital in CRR. In the laboratory, snails were sorted, identified, and counted. The collected snails were identified based on morphological characteristics of their shells (Mandahl-Barth, 1957; Brown, 1994). *Bulinus forskalii* was differentiated from *Bulinus senegalensis* by the presence of a distinctive shoulder angle on the upper whorls (as opposed to more round and smooth upper whorls in *B. senegalensis*) (Mandahl-Barth, 1957). However, this feature is not easily identifiable in some snails. Such snails were putatively identified by morphology and then confirmed by molecular methods as described in Section 2.7.3. Furthermore, to confirm the accuracy of our morphological identifications, the identification of 10 samples per site was confirmed by molecular methods.

4.2.3 Infection screening of *Bulinus* snails

For each site, 20 *Bulinus* snails were selected at random and screened for schistosome infection using a molecular detection method. For sites with fewer than 20 *Bulinus* snails, all individuals were examined. DNA was extracted from the snails using the CTAB DNA extraction protocol as described in Section 2.7.1. A forward *Schistosoma*-specific primer, ITS2_Schisto_F (Schols et al., 2019) and a universal reverse primer, ETTS1 (Kane and Rollinson, 1994) were utilised in a PCR as described by Pennance and colleagues (2020b). The combination of these primers amplifies a 538bp fragment comprising mostly the ITS2 gene of *Schistosoma* DNA which can be sequenced to detect and differentially identify *S. haematobium* and *S. bovis* infections in *Bulinus* snails. Information on primers, PCR reactions, cycling conditions and visualisation of gel images is detailed in Section 2.7.2.

The PCR products were Sanger sequenced using the Macrogen Eco-sequencing service (Macrogen B.V, Amsterdam, Netherlands). The sequence data were managed using the

STADEN package v.1.5.3 (Staden et al., 2000) and the Genetic Data Environment (GDE) package v.2.2 (Smith et al., 1994). Sequences were then compared with *S. haematobium* and *S. bovis* reference sequences and the four single nucleotide polymorphism (SNP) sites in the ITS2 region of the two species used to confirm the species identification of each sequence. The samples that matched *S. bovis* reference sequences were further processed by amplifying an 835bp fragment comprising mostly the ITS1 region of *Schistosoma* DNA in these samples using primers ETTS2 (Kane and Rollinson, 1994) and ITS2_Schisto_R (Schols et al., 2019) to confirm if they were *S. bovis* or *S. curassoni*. *Schistosoma bovis* and *S. curassoni* have the same nucleotide bases at the four SNP sites in the ITS2 region but differ at the lone SNP site in the ITS1 region of *Schistosoma* DNA where they can be differentially identified.

4.2.4 Ecological and physicochemical parameters

For the 2017 collections, presence or absence of aquatic vegetation and algae were recorded. The water temperature and water pH of the collection sites were taken using a digital probe thermometer 31/162/0 (Brannan Thermometers, Cumbria, England) and a Mettler Toledo EL20 pH meter (Fisher Scientific, Loughborough, UK). The equipment required for the measurement and collection of physicochemical data were not available during the 2019 collections.

4.2.5 Data processing and analysis

Raw data on snails collected per site were entered on Microsoft Excel and all statistical analyses were carried out using R Version 4.1.0 (R Core Team, 2021). Differences in snail abundance among *Bulinus* species, regions and habitat types were statistically compared using Kruskal-Wallis and Wilcoxon (pairwise) Rank Sum tests, and were depicted using box and whisker plots. Maps showing the distribution of *Bulinus* snails and schistosome parasites were created using QGIS version 3.0 software (Girona Open-Source Geospatial Foundation). Negative binomial generalised linear mixed models (GLMMs) were fitted in R Version 4.1.0 using the

package ‘glmmTMB’ (Brooks et al., 2017) to test for associations between the abundance of *Bulinus* snails and environmental/physicochemical parameters. Variance inflation factor (VIF) was used to determine the relationships and collinearity between variables. A VIF value of more than 5 was indicative of collinearity (Rogerson, 2001). We used the Akaike’s Information Criterion (AIC) and negative log-likelihood values to compare models and choose the final models. The regression analyses were done separately for seasonal sites and permanent sites.

4.3 Results

4.3.1 Presence and incidence of potential schistosome snail hosts

A total of 114 sites were visited. Snails were found at 60 of these sites, while no snails were found at 54 of the sites. *Bulinus* snails were found at 59 sites. No *Biomphalaria* snails were observed at any of the 114 sites sampled. In addition to intermediate snail hosts of schistosomes, other snails (some of which are intermediate hosts of other parasites) found in these freshwater habitats included *Radix natalensis*, *Gyalus spp.* and *Lanistes spp.* One hundred and sixty-five *Radix natalensis* were recovered from three permanent sites, 120 *Gyalus spp.* were collected from 9 permanent sites, and 8 *Lanistes spp.* were found in one seasonal and two permanent sites (Supplementary Table 4.1).

4.3.2 Species diversity, distribution and abundance of *Bulinus* snails

A total of 2877 *Bulinus* snails comprising three species were collected in the study. *Bulinus senegalensis* was the most common species making up 75.9% (2184) of all *Bulinus* snails, followed by *Bulinus forskalii* with 20.9% (602), then *Bulinus truncatus* with 3.2% (91) (Figure 4.3). There was a significant difference in abundance among the three *Bulinus* species collected (Kruskal-Wallis $\chi^2 = 41.592$, $df = 2$, $p < 0.001$) with the number of *B. senegalensis* significantly higher than both *B. forskalii* ($p < 0.001$) and *B. truncatus* ($p < 0.001$) and *B.*

forskalii significantly higher than *B. truncatus* ($p < 0.001$). Abundance of *Bulinus* snails also differ among the regions (Kruskal-Wallis $\chi^2 = 19.567$, $df = 4$, $p = 0.001$) with CRR having the largest number of *Bulinus* snails, followed by URR, WR, LRR, and then NBR (Figure 4.3). CRR had significantly more *Bulinus* snails than both LRR ($p = 0.012$) and NBR ($p = 0.012$). URR also had significantly more snails than both LRR ($p = 0.024$) and NBR ($p = 0.021$).

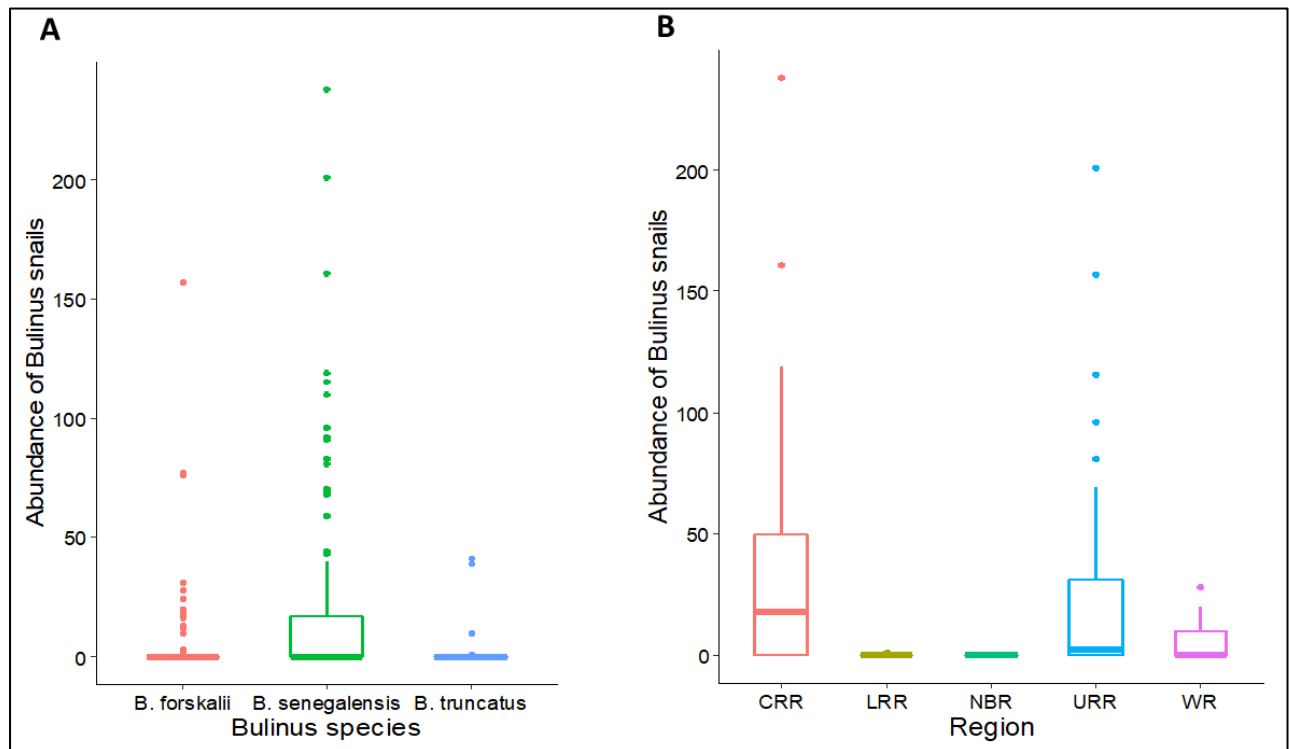


Figure 4.3. Box and Whisker plots showing the abundance of *Bulinus* snails by: (A) species and (B) region. The box represents the lower quartile (0.25), the median (0.5) and the upper quartile (0.75); the whiskers depict variability outside the lower and upper quartiles. Outliers are shown as individual points.

Bulinus senegalensis was the most abundant and widespread species found in 42 (71.2%) of the 59 sites inhabited by *Bulinus* snails. It was present on either side of the River Gambia and observed in all study regions except NBR. *Bulinus senegalensis* snails were most abundant in seasonal pools (Figure 4.4), where they were mostly observed aggregated together. Thirty-eight

of the 42 sites where *B. senegalensis* was observed were seasonal pools with 2169 *B. senegalensis* snails found in seasonal pools compared to just 2 snails in streams, 6 snails in swamps and 7 snails in rice fields. These differences in the abundance of *B. senegalensis* among habitat types were statistically significant (Kruskal-Wallis $\chi^2 = 20.015$, $df = 5$, $p = 0.001$) with the number of *B. senegalensis* significantly higher in seasonal pools than in streams ($p = 0.003$). Figure 4.5 shows the abundance and distribution of *B. senegalensis* by region. Twenty-three sites in CRR yielded no *B. senegalensis* snails, 8 sites had <10 snails, 14 sites yielded 10 to 99 snails and 5 sites produced ≥ 100 *B. senegalensis* snails (Figure 4.5). In URR, 27 sites had no *B. senegalensis* snails, a lone site yielded <10 snails, 11 sites had 10-99 snails, and just 1 site had ≥ 100 *B. senegalensis* snails. WR had two sites that yielded *B. senegalensis* and LRR had one site with *B. senegalensis* snails. NBR sites yielded no *B. senegalensis* snails (See Figure 4.5). See Supplementary Table 4.1 for the actual count of snails per species and per site and the GPS coordinates of the sites.

Bulinus forskalii was the second most common species. It was also present on both sides of the River Gambia and was collected from 35.6% (21/59) of the sites where *Bulinus* snails were found. Most of the sites (90.5%) from where this species was found were permanent sites, such as streams, swamps and rice fields (Figure 4.4). Twenty-nine *B. forskalii* were collected from seasonal pools, 382 from streams, 39 from swamps, 140 from rice fields and 12 from an irrigation canal. Abundance of *B. forskalii* was significantly different among the habitat types (Kruskal-Wallis $\chi^2 = 42.546$, $df = 5$, $p < 0.001$); seasonal pools had significantly less *B. forskalii* than streams ($p < 0.001$), swamps ($p = 0.001$), rice fields ($p < 0.001$) and irrigation canals ($p\text{-value} < 0.001$). At most permanent sites, *B. forskalii* was found in small numbers and sparsely distributed, but in a few sites (Bansang in CRR and Sotuma Sire and Misra Ba Mariama in URR) where there was abundant vegetation, they were found clustered together in large numbers. In CRR, 39 sites had no *B. forskalii* and 11 had 10-99 *B. forskalii* snails (Figure

4.5). URR which had the largest number of *B. forskalii*, had 31 sites with no *B. forskalii* snails, 2 sites with <10 snails, 6 sites with 10-99 snails and 1 site with ≥ 100 *B. forskalii* snails. WR had just one site that had *B. forskalii* and neither LRR nor NBR sites had *B. forskalii* snails (Figure 4.5). See Supplementary Table 4.1 for the actual count of snails per species and per site and the GPS coordinates of the sites.

Unlike *B. senegalensis* and *B. forskalii*, the presence and distribution of *B. truncatus* was limited. It was the least encountered species collected at only 4 (6.8%) sites. It was found only in permanent streams and one irrigation canal (Figure 4.4). Fifty *B. truncatus* were collected in streams and 41 from the irrigation canal. The difference in abundance of *B. truncatus* among habitat types was statistically significant (Kruskal-Wallis $\chi^2 = 39.042$, $df = 5$, $p < 0.001$); even though the number of *B. truncatus* collected from streams was only slightly higher than the number from the irrigation canal, the difference was still statistically significant ($p = 0.043$). The distribution of *B. truncatus* was confined to the south bank of the River Gambia, and it was found only in CRR and URR. One stream and one irrigation canal in CRR yielded 10 and 41 *B. truncatus*, respectively. One and 39 *B. truncatus* were recovered from two streams in URR (Figure 4.5). *Bulinus truncatus* was observed attached to aquatic plants such as waterlily in all these sites. See Supplementary Table 4.1 for the actual count of snails per species and per site and the GPS coordinates of the sites.

As much as these *Bulinus* species seemed to have preference for specific habitat types, they were sometimes found together in sympatry with other *Bulinus* species at the same site. *B. senegalensis* and *B. forskalii* were observed together at four permanent sites including a stream, swamp and rice field. The two species were also found together in two seasonal pools. *B. forskalii* was also observed co-existing with *B. truncatus* in a stream and in an irrigation canal. While *B. forskalii* was found in sympatry with either *B. senegalensis* or *B. truncatus*, *B. senegalensis* was never observed in the same habitat with *B. truncatus*.

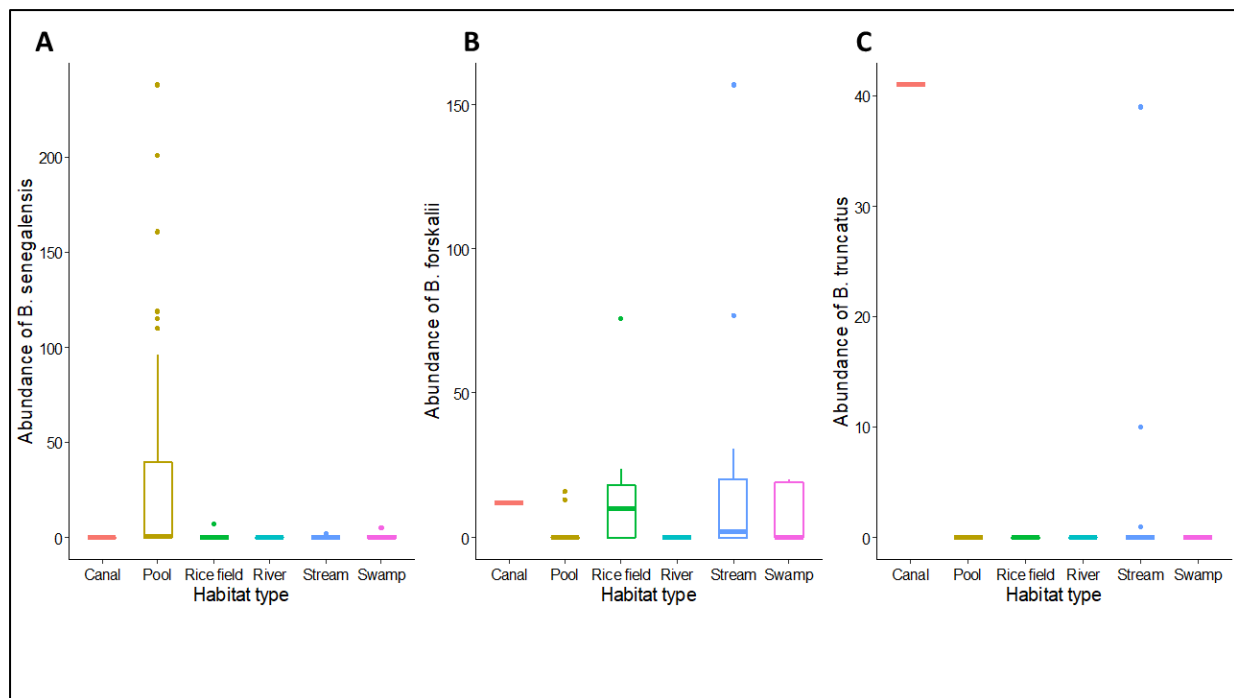


Figure 4.4. Box and Whisker plots showing the abundance by habitat type of *Bulinus* snails: (A) *B. senegalensis*, (B) *B. forskalii* and (C) *B. truncatus*. The box represents the lower quartile (0.25), the median (0.5) and the upper quartile (0.75); the whiskers depict variability outside the lower and upper quartiles. Outliers are shown as individual points.

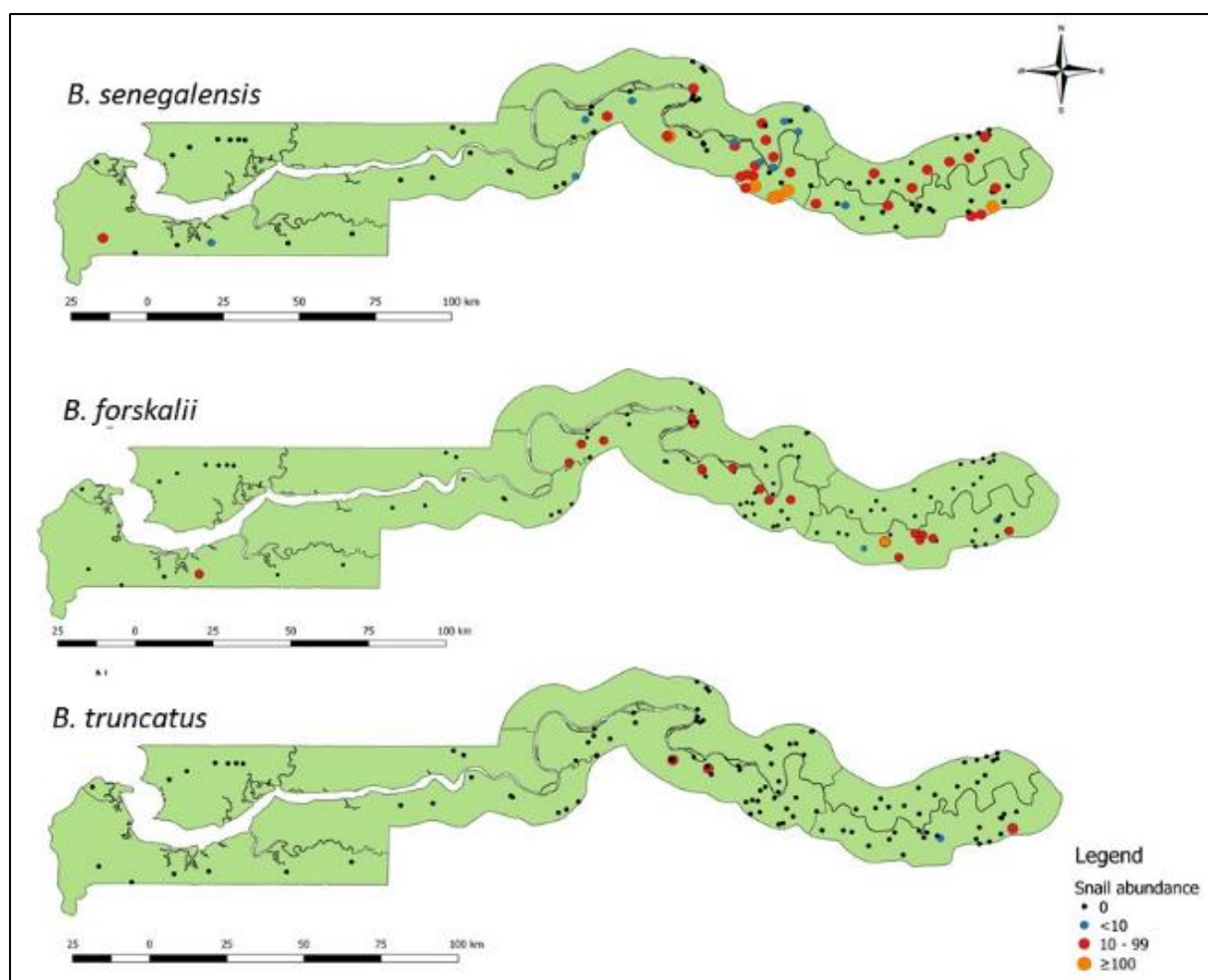


Figure 4.5. Maps of The Gambia showing the abundance and distribution of *Bulinus senegalensis*, *Bulinus forskalii*, and *Bulinus truncatus* at sampling sites across the study regions. Link to base layer of map used in the figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>.

4.3.3 Physicochemical and environmental factors

Data on ecological factors were available for only the 2017 collections. At seasonal sites, water temperature ranged from 22.1 - 38.3 °C and the median (interquartile range) water temperature was 29.3 °C (27.8 – 33.1 °C). The water pH of these seasonal sites ranged from 6.42 - 8.16 and the median (interquartile range) water pH was 6.95 (6.78 – 7.26). From the negative binomial regression in glmmTMB, only presence of aquatic vegetation had a statistically significant positive association with abundance of *Bulinus* snails in seasonal sites (p -value = 0.014). The

other variables (presence of algae, water temperature and pH) did not show any statistically significant association with abundance of *Bulinus* snails in seasonal sites (Table 4.1).

For the permanent sites, water temperature ranged from 23.1 – 33.9 °C and the median (interquartile range) water temperature was 27.6 °C (25.3 – 30.0 °C). The water pH at these permanent sites ranged from 5.52 – 7.55 and the median (interquartile range) water pH was 6.96 (6.37 – 7.17) (see Supplementary Table 4.2). Water temperature showed a significant positive relationship (p -value = 0.034) while water pH showed a significant negative relationship (p -value < 0.001) with abundance of *Bulinus* snails in permanent sites (Table 4.1). Presence of aquatic vegetation and algae did not have any significant association with abundance of *Bulinus* snails in permanent sites.

Table 4.1. Summary of negative binomial regression (GLMM) of *Bulinus* snail abundance and environmental variables. Analyses done using glmmTMB package in R 4.1.0

	Variables	Estimate	Std. Error	Confidence Interval (CI)	p -value
Seasonal sites	Intercept	2.668	6.625	-10.317 – 15.654	0.687
	Aquatic vegetation	1.565	0.637	0.317 – 2.813	0.014*
	Algae	-0.117	0.870	-1.822 – 1.587	0.893
	Temperature	-0.056	0.081	-0.215 – 0.102	0.484
	pH	0.268	0.670	-1.046 – 1.581	0.690
Permanent sites	Intercept	3.605	8.958	-16.829 – 17.039	0.967
	Aquatic vegetation	10.240	8.760	-15.807 – 18.287	0.906
	Algae	0.085	0.629	-1.148 – 1.317	0.893
	Temperature	0.250	0.118	0.018 – 0.482	0.034*
	pH	-2.729	0.777	-4.253 – -1.206	0.0004*

*Statistical significance at $p < 0.05$

Negative binomial glmm analyses performed separately for seasonal sites and permanent sites.

4.3.4 Infection of *Bulinus* snails with schistosome parasites

Of the 883 *Bulinus* snails screened for pre-patent schistosome infection, 77 (8.7%) were infected with schistosomes (either *S. haematobium* or *S. bovis*). These comprised 52 (5.9%) *B. senegalensis*, 19 (2.2%) *B. forskalii* and 6 (0.7%) *B. truncatus*. Both *S. haematobium* and *S. bovis* infections were observed in *B. senegalensis* snails with 7 (1.2%) *B. senegalensis* infected with *S. haematobium* and 45 (7.7%) infected with *S. bovis* (Table 4.2). No co-infection of *S. haematobium* and *S. bovis* was observed in *B. senegalensis* snails. *Bulinus forskalii* had only *S. bovis* infections, with 19 (7.7%) of this species found harbouring this livestock parasite. *Bulinus truncatus* also had only *S. bovis* infection, with 6 (12.0%) snails infected (Table 4.2).

Table 4.2. Infection of *Bulinus* snails with *Schistosoma haematobium* and *Schistosoma bovis*

	<i>B. senegalensis</i>	<i>B. forskalii</i>	<i>B. truncatus</i>
Number of snails screened	586	247	50
No. Infected with <i>S. haematobium</i> (%)	7 (1.2%)	0 (0%)	0 (0%)
No. Infected with <i>S. bovis</i> (%)	45 (7.7%)	19 (7.7%)	6 (12.0%)
Total (%)	52 (8.9%)	19 (7.7%)	6 (12.0%)

Infected *Bulinus* snails were found at 27 sites, which included seasonal pools, streams, a swamp, ricefield and irrigation canal. Nineteen sites had infected *B. senegalensis*, 5 sites had infected *B. forskalii*, 1 site had infected *B. truncatus* and 2 sites had both infected *B. forskalii* and *B. truncatus*. All these sites were in CRR and URR (Figure 4.6).

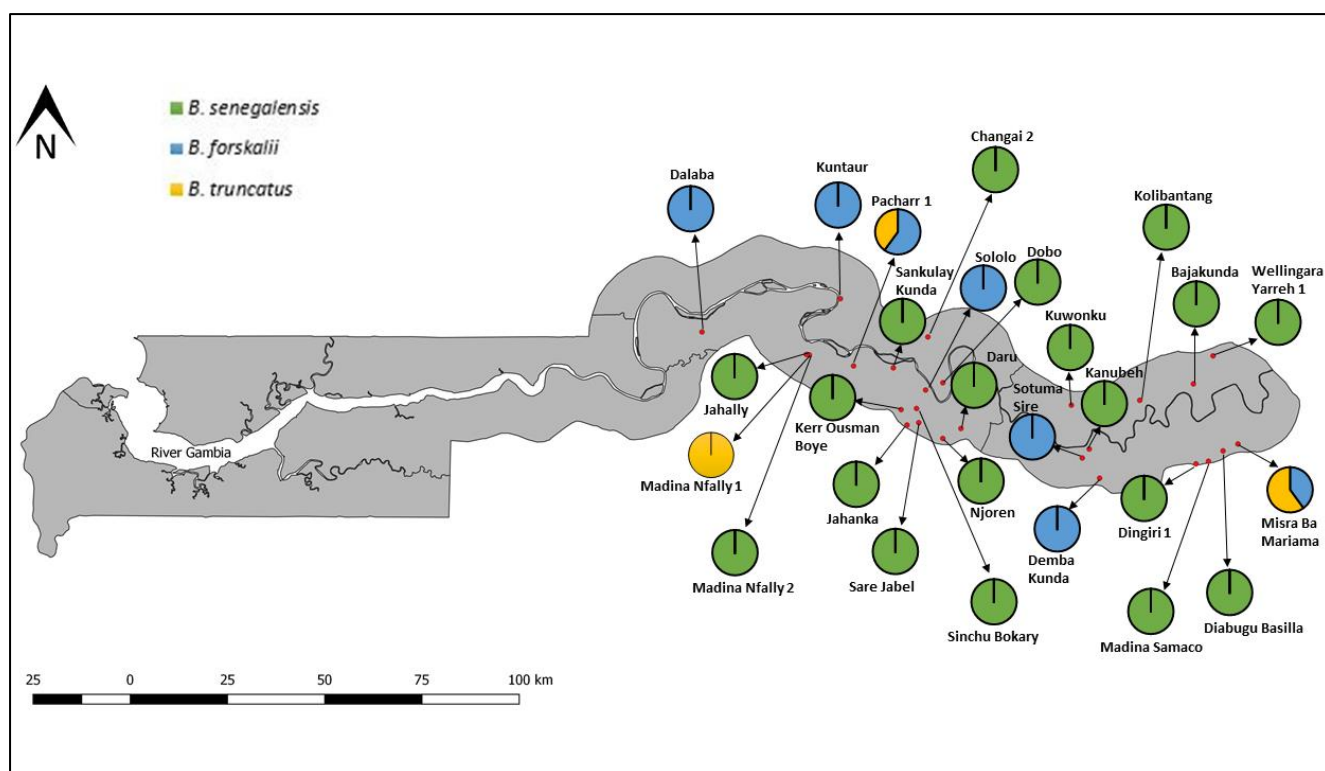


Figure 4.6. Distribution of infected *Bulinus* species in the study. Link to base layer of map used in the figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>.

Schistosoma haematobium was discovered in 4 study sites which were all seasonal pools and had only *B. senegalensis* snails, with three of the sites (Jahanka, Njoren and Sare Jabel) in CRR and 1 site (Dingiri 1) in URR (Figure 4.7). Jahanka and Njoren had 3 (15%) and 1 (5%) infected snails, respectively; all infections were *S. haematobium*. Sare Jabel had 1 snail (5%) infected with *S. haematobium* and 5 snails (25%) infected with *S. bovis*. Dingiri 1 was the only site with *S. haematobium* infected snails in URR, with 2 (10%) snails infected with *S. haematobium* and another 2 (10%) infected with *S. bovis*. Only *S. bovis*, which is the predominant schistosome species observed in this study, occurred in the remaining 23 sites where infected snails were found, with 13 of the sites in CRR and 10 in URR (Figure 4.7). *Schistosoma bovis* infection rate at these sites ranged from 5% to 40% for all three *Bulinus* species (See Supplementary Table 4.3). *Schistosoma bovis* infection rates for CRR sites ranged from 5% to 40% and

infection rates for URR sites ranged from 5% to 20%. These sites included all the habitat types in which snails were found in this study.

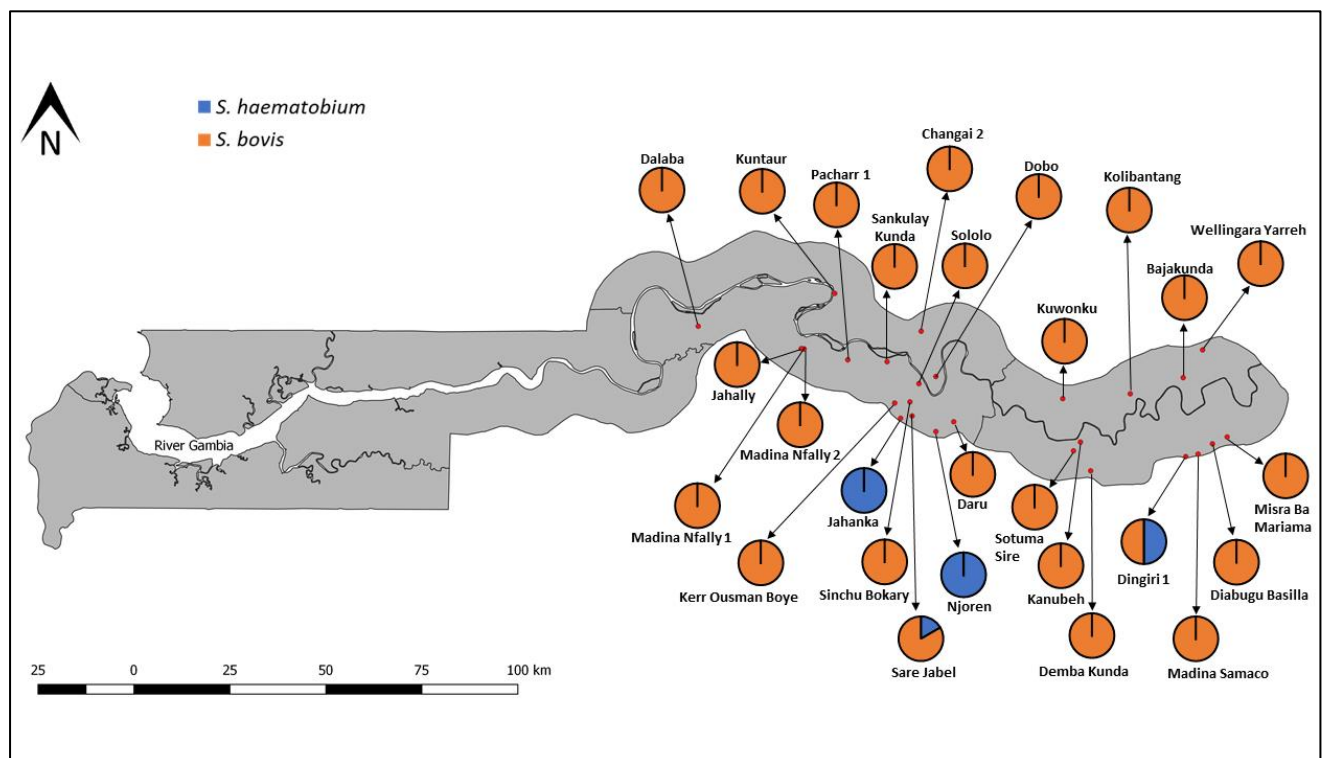


Figure 4.7. Distribution of *Schistosoma haematobium* and *Schistosoma bovis* in the study. Link to base layer of map used in the figure: <https://data.humdata.org/dataset/gambia-administrative-boundaries>.

4.4 Discussion

This study is the first malacological survey of intermediate snail hosts of schistosomes conducted in The Gambia in more than 30 years and is the first study to include all the regions of the country. *Bulinus* snails were found in 98% of the aquatic sites where snails were observed.

Bulinus senegalensis was found to be the most common and widely distributed species in The Gambia in this study. It was found on both sides of the River Gambia and in all but one region of the country. This widespread distribution of *B. senegalensis* is similar to what was observed

in neighbouring Senegal as the snail species was found in many of its regions (Diaw et al., 1989b; Diaw et al., 1992; Senghor, 2015). *Bulinus senegalensis* was most common in CRR and URR which share the same ‘tall-grass savanna shrub’ ecological zone with the Senegalese regions in which the snail was mostly reported (Diaw et al., 1989b; Diaw et al., 1992; Senghor, 2015). They were mostly found in seasonal pools often in large numbers in these two regions. Away from the river in these regions lies a sandstone plateau which has a rocky, laterite iron pan (cuiasse) termed a laterite plateau (Hanrion et al., 1971). Depressions formed in this plateau hold rainwater and form seasonal pools during the rainy season which become breeding grounds for *B. senegalensis* (Goll and Wilkins, 1984). Despite the short (4-5 months) span of these pools *B. senegalensis* can continue breeding year after year in these sites due to its short reproductive cycle and its behaviour of aestivation (Diaw et al., 1989a; Senghor, 2015). African Bulinid snails are known for their ability to aestivate and survive in dried habitats during long dry periods (Goll and Wilkins, 1984; Brown, 1994). In this study *B. senegalensis* were observed in seasonal pools in CRR and URR, even those at the verge of drying and holding little water. The other 3 regions (LRR, NBR and WR) where the laterite plateau is not found have few seasonal pools and thus few *B. senegalensis*. Just as has been reported in other parts of Africa (Greer et al., 1990), this study shows that seasonal rain-fed pools are important habitats of *S. haematobium* intermediate snail hosts. *Bulinus senegalensis* was also found in small numbers in streams and rice fields. This is consistent with the findings of previous studies undertaken in The Gambia and elsewhere (Goll, 1981; Betterton et al., 1983).

Bulinus forskalii was the second most encountered species. It was also observed on both sides of the River Gambia, and found in CRR, URR and WR. The geographical range of *B. forskalii* has been reported to extend across many parts of Africa including Madagascar and São Tomé Island (Brown, 1994). Although *B. forskalii* was found in two seasonal pools, it was mostly found in streams and rice fields in this study. Previous studies in Senegambia have reported the

existence of *B. forskalii* in such habitat types (Diaw et al., 1989b; Goll, 1981). The River Gambia is fresh along CRR and URR and its tributaries (streams) along these regions (locally called ‘bolongs’) are also freshwater. The river also provides water supply to a large irrigation scheme in CRR which is used for rice growing. These freshwater streams and irrigated rice fields provide breeding grounds for *B. forskalii* which proliferate more in permanent habitats (De Kock and Wolmarans, 2005). *Bulinus forskalii* was more common in URR than CRR because URR has a lot more streams than CRR. WR has a single site (swamp) where *B. forskalii* was collected. No *B. forskalii* were found in sites in either LRR or NBR. Since the River Gambia is brackish along these 3 regions, the existence of freshwater streams and swamps in these regions is rare.

Bulinus truncatus was the least common and least distributed species in this study. It was observed in streams and in an irrigation canal in only CRR and URR. An earlier study in the 1950s reported snails from some localities in The Gambia as *Bulinus truncatus* (McCullough and Duke, 1954), but a later study identified similar snails from these same localities as *Bulinus guernei* (Smithers, 1956). In later years, studies on various *Bulinus truncatus* and *Bulinus guernei* samples from different parts of Africa that utilised shell morphology, biochemical and cytological analyses found no taxonomic subdivision between the species and suggested they are the same (Brown et al., 1986; Diaw et al., 1989b). *Bulinus guernei* reported in Gambia by Smithers (1956) were collected from streams in URR, the same region and type of habitat in which *B. truncatus* was found in this study. *B. truncatus* was also found in CRR in this study.

Bulinus senegalensis was mostly found alone in seasonal pools but was occasionally observed together with *B. forskalii* in seasonal pools and in permanent sites (stream, swamps and rice fields). *Bulinus forskalii* also co-inhabited a single stream and a single irrigation canal with *B. truncatus*. Goll (1981) also found *B. senegalensis* and *B. forskalii* breeding together in alluvial pools and irrigated rice fields in The Gambia.

Despite this huge incidence and presence of *Bulinus* snails, *Biomphalaria* snails were not found in any of the sites sampled. The occurrence of *Biomphalaria pfeifferi gaudi* in The Gambia was reported in the 1950s in just few studies (Mandahl-Barth, 1957; Smithers, 1957) but numerous studies conducted on freshwater snails in the country between the 1950s and 1980s have failed to identify *Biomphalaria* in The Gambia (McCullough and Duke, 1954; Goll, 1981; Goll and Wilkins, 1984; Goll et al., 1984). This corresponded with very few reports on the existence of intestinal schistosomiasis among the human population (Smithers, 1957). In more recent times, there were few or no reports of intestinal schistosomiasis cases in health facilities across the country as opposed to the urogenital form of the disease (MOH, 2014), which is consistent with findings of recent prevalence studies of the disease (MOH, 2016; Sanneh et al., 2017).

Bulinus snails were observed to be more common at seasonal sites where aquatic vegetation such as water lilies and ‘Mandinka rice’ were present. Some previous studies have found snails to be more abundant at sites where aquatic vegetation is found (Mutuku et al., 2019). At permanent sites, we found both water temperature and pH to have influence on *Bulinus* snail abundance. Snail density was observed to increase with rise in water temperature in permanent habitats. The reverse was observed with water pH with snail numbers observed to decrease with increase in water pH at permanent sites. Most previous studies found water temperature to be a more important predictor of snail numbers (Stensgaard et al., 2006; Opisa et al., 2011), but one study also found that snail abundance increased with a decrease in water pH (Levitz, 2013).

Molecular techniques were used to detect and differentially identify *Schistosoma haematobium* and *Schistosoma bovis* parasites in *Bulinus* snails in this study. This technique was chosen because *S. haematobium* and *S. bovis* are both known to occur in The Gambia where they utilise the same intermediate host snails to accomplish their life processes (Smithers, 1956). Schistosomiasis transmission surveillance is usually complicated in areas where more than one

schistosome species co-exist, and thus well robust species-specific detection techniques are required in such areas (Pennance et al., 2020b).

The study revealed that all three *Bulinus* snails collected in the study are involved in the transmission of at least one schistosome species. *Bulinus senegalensis*, the most widely distributed of all the *Bulinus* species in this study, was the only snail found infected with the human parasite, *Schistosoma haematobium*, albeit at just four study sites (3 in CRR and 1 in URR). These sites are in communities (Jahanka, Njoren, Sare Jabel and Dingiri) that are known hotspots of urogenital schistosomiasis (MOH, 2016; Joof et al., 2021a). The rate of *S. haematobium* infection in *B. senegalensis* observed in this study corresponds to the prevalence of urogenital schistosomiasis infection observed in our human study. In our human study involving school children, urogenital schistosomiasis was more common in CRR than URR. Previous studies conducted in The Gambia and other parts of West Africa also reported *B. senegalensis* as an intermediate host of *S. haematobium* (Smithers, 1956; Vera et al., 1992; Senghor et al., 2015). In contrast, the bovine schistosome (*Schistosoma bovis*) had a much more widespread distribution occurring in 25 sites across CRR and URR, with all three *Bulinus* species found in this study involved in its transmission. It overlapped with *S. haematobium* at two of these sites. A lot of these sites are used by humans as well as livestock but are more frequented by livestock as was observed during the sample collection period. This finding is similar to what was recently observed in Ivory Coast (Tian-Bi et al., 2019) and Niger (Pennance et al., 2020a), where the livestock schistosome was found to be more common and occur in a wider geographical range compared to its human schistosome relative. Previous studies have also confirmed *B. senegalensis* as an intermediate host of *S. bovis* (Smithers, 1956). As *B. senegalensis* was found infected with both *S. haematobium* and *S. bovis* in this study, it might have the potential to harbour hybrids of these two species. *Bulinus forskalii* is also a confirmed intermediate host of the bovine schistosome in many parts of Africa (Diaw et al., 1989b;

Pennance et al., 2020a), even though its role in the transmission of the human schistosome (*S. haematobium*) is not clear. One study implicated *B. forskalii* in the transmission of *S. haematobium* in Niger (Labbo et al., 2007) but other studies undertaken in the same area disagreed with this finding, suggesting misidentification of *B. senegalensis* as *B. forskalii* and the notion that *B. forskalii* was not compatible with *S. haematobium* in the region (Vera et al., 1992; Pennance et al., 2020a). Despite *B. truncatus* being the least common *Bulinus* species encountered in this study, it has the highest schistosome infection rate (12%) among the three *Bulinus* species and this shows how competent it is as an intermediate host of schistosomes. *Bulinus truncatus* is a proven intermediate host of *S. haematobium* and *S. bovis* in several areas endemic for schistosomiasis (Sene et al., 2004; Tian-Bi et al., 2019; Pennance et al., 2020b; Léger et al., 2020). It is also reported to harbour the hybrids of *S. haematobium* and *S. bovis* in many parts of West Africa Huyse et al., 2009; Tian-Bi et al., 2019; Pennance et al., 2020b). Additionally, a study conducted in the 1950s in The Gambia found *B. truncatus* (then referred to as *Bulinus guernei*) infected with *S. haematobium* (Smithers, 1956). The absence of *S. haematobium* infection in *B. truncatus* in this study is likely attributed to the low numbers of this snail in this study, with it found at just a few sites.

The existence of *Bulinus* snails infected with *S. haematobium* only in seasonal pools, confirms the important role of temporal rain-fed pools in the transmission of *S. haematobium* in Africa (Greer et al., 1990; Vera et al., 1992; Senghor et al., 2015; Léger et al., 2020). Conversely, *Bulinus* snails infected with *S. bovis* were recovered from all habitat types from which snails were found in this study and previous studies found snails infected with *S. bovis* in several habitat types including seasonal pools, streams, swamp, ponds, ricefields, canals and dams (Smithers, 1956; Pennance et al., 2020b, Léger et al., 2020).

The Gambia has initiated its national control programme for schistosomiasis and has implemented several activities. These activities were focused on humans. Subsequent

interventions will include intermediate host snail control, sanitation and hygiene approaches which require knowledge on the intermediate host snails. The findings of this study provide new insight on the distribution and infection status of intermediate snail hosts of schistosomes in The Gambia which will be vital for the national schistosomiasis control initiative. The finding that *S. haematobium* transmission was observed at only four sites provides an opportunity for targeted interventions towards these communities. It also demonstrates the need for setting up a transmission surveillance system within the already established national schistosomiasis control programme, as snail sampling was undertaken at just a few time points in this study. Since transmission of schistosomiasis may vary from one time point to another, sustained sampling of snails will be required to better understand the transmission dynamics of *S. haematobium* in endemic areas of the country. The high incidence and wide geographical distribution of *S. bovis* in the study area serve as a reminder that this neglected ruminant schistosome needs to be given more attention in the study area, as the calls for a ‘one health approach’ to the control of schistosomiasis grow louder (Gower et al., 2017; Schols et al., 2019; Léger et al., 2020; Stothard et al., 2020). Additionally, intestinal schistosomiasis in livestock caused by *S. bovis* can result to potential economic loss through enteritis, emaciation and even death of livestock (De Bont and Vercruysse, 1997).

Although *Bulinus* snail sampling was conducted over a large geographical range across The Gambia, the sites were sampled during only four time points across two different years (2017 and 2019). More sustained sampling should be considered in future studies to better understand the snail-schistosome interactions at transmission sites in the country. Further studies are also needed to investigate hybrids of *S. haematobium* group species as there is potential for *B. senegalensis* and *B. truncatus* to harbour hybrids in The Gambia (Stothard et al., 2020). For hybrids identification, the *cox1* gene need to be amplified in the parasite which could not be achieved in this study due to the lack of well robust markers that can amplify parasite DNA

from within snail DNA extract. Another limitation of the study was, only a sub-set of snails were able to be molecularly identified. As the infection detection method used in this study detects only pre-patent infections in snails, it is worthwhile to highlight that not all pre-patent infections lead to shedding snails. The epidemiological implication of that is, not all snails found pre-patently infected with schistosome parasites necessarily contribute to the transmission of schistosomiasis.

5 Genetic variation of *Bulinus senegalensis* in relation to *Schistosoma haematobium* and *Schistosoma bovis* infections in The Gambia

Abstract

Understanding the genetic diversity of intermediate host snails is vital for a better understanding of the role they play in the transmission of schistosomiasis. We conducted a population genetic study on *Bulinus senegalensis* from The Gambia to characterise its genetic diversity in relation to schistosome infection. *Bulinus senegalensis* was widely sampled across the country and the mitochondrial cytochrome oxidase subunit 1 (cox1) and the nuclear internal transcribed spacer 2 (ITS-2) genes were analysed in samples collected from 30 sites. The cox1 gene was more variable than the ITS-2 gene, with an overall haplotype diversity of 0.886 for cox1 compared to 0.662 for ITS-2 and nucleotide diversity of 0.006 for cox1 compared to 0.002 for ITS-2. The cox1 loci had 27 haplotypes (9 shared and 18 private), which revealed a clear structure in their distribution. Only 1 haplotype was found in the Western Region (WR), some shared haplotypes were restricted to either only the Central River Region (CRR) or the Upper River Region (URR), and some were found in both CRR and URR. The nuclear genetic marker (ITS-2) had 7 haplotypes (4 shared and 3 private) which showed less structure in their distribution compared to the mitochondrial marker (cox1). Only one haplotype was found in WR, and all 4 shared haplotypes were present in both CRR and URR. The fixation index (Fst) values suggested moderate to restricted gene flow between *B. senegalensis* populations of CRR and URR, and highly restricted gene flow between these two populations and that of the WR. Of the 27 cox1 haplotypes, one was associated with *S. haematobium* infection, 6 were associated with *S. bovis* infection and 3 were associated with both *S. haematobium* and *S. bovis* infections. One of these haplotypes was significantly associated with *S. bovis* infection. Two of the 7 ITS-2 haplotypes were associated with *S. bovis* infection and 2 were associated with

both *S. haematobium* and *S. bovis* infections. One of these haplotypes was significantly associated with *S. bovis* infection. This study provides first-time insight into the genetic structure and its link to schistosome infection in *Bulinus senegalensis*, the intermediate host of *Schistosoma haematobium* and *Schistosoma bovis* in The Gambia. The findings of this study in combination with further understanding of parasite genetic diversity may help us better understand the role of host-parasite interactions in the transmission dynamics of schistosomiasis.

5.1 Introduction

Schistosomiasis is a chronic parasitic disease that has both medical and veterinary significance. It is most common in Sub-Saharan Africa but also occurs in other parts of the world. In Sub-Saharan Africa, urogenital schistosomiasis caused by *Schistosoma haematobium* is the most common form of schistosomiasis in humans. Urogenital schistosomiasis affects approximately 112 million people, with a further 436 million individuals estimated to be at risk of infection (Chala and Torben, 2018).

Several freshwater *Bulinus* species act as intermediate hosts of *Schistosoma haematobium* group species. *Bulinus senegalensis* is a sinistral-shaped bulinid snail belonging to the *Bulinus forskalii* group and is the type species of the genus *Bulinus*. This freshwater snail species was originally known from only The Gambia and Senegal where it mostly inhabited temporal/seasonal pools (Mandahl-Barth, 1957). Over time, it has been reported in other parts of Africa including Nigeria, Niger and Cameroon (Betterton et al., 1983; Vera et al., 1992; Zein-Eddine et al., 2014). *Bulinus senegalensis* is one of the most important intermediate snail hosts of *S. haematobium* group species in Senegal (Senghore et al., 2015). In the Gambia, it is the main intermediate host of *Schistosoma haematobium* and *Schistosoma bovis* and is the snail

species involved in the seasonal transmission of urogenital schistosomiasis in the country (Joof et al., 2021b).

The co-adaptation and interactions between intermediate host snails and schistosome parasites are believed to be influenced by genetic diversity of local snail populations (Minchella and LoVerde, 1993; Webster et al., 2007). For instance, high genetic variation of snail populations was found to reduce schistosome parasite transmission potential and a decrease in snail genetic diversity corresponded to an increase in parasite transmission (Hoffman *et al.*, 1998; Sandland *et al.*, 2007). Schistosome infection was observed to result in reduced fitness in *Biomphalaria* (Webster and Woolhouse, 1999), but refractoriness to infection itself does not come without a cost, as it is reported to cause reduced reproductive capacity in snails (Webster and Woolhouse, 1999). Since relationships and co-adaptations between intermediate snail hosts and schistosome parasites is influenced by the genetic diversity of local snail populations, understanding the genetic variation and structure of *B. senegalensis* populations from The Gambia will shed more light on the local schistosomiasis transmission dynamics. It can further provide information that will be useful for the design of new approaches for schistosomiasis control.

Several genetic studies have been conducted on *Bulinus* (Kane et al., 2008; Jorgensen et al., 2011; Zein-Eddine *et al.*, 2014; Abe et al., 2018; Mutsaka-Makuvaza et al., 2020). Most of these studies have focused on examining the phylogenetic relationships among species (Kane et al., 2008; Jorgensen et al., 2011; Zein-Eddine *et al.*, 2014), with just a handful focusing on analyses at the population level (Zein-Eddine *et al.*, 2014; Abe et al., 2018; Mutsaka-Makuvaza et al., 2020). A genetic study on several *Bulinus* species including *B. senegalensis* revealed high levels of genetic diversity within species and clear segregation between populations of the same species (Zein-Eddine *et al.*, 2014). In contrast, *Bulinus truncatus* collected from different locations in Egypt were found to have low haplotype diversity and low nucleotide diversity

(Abe et al., 2018). *Bulinus globosus* and *B. truncatus* sampled from a schistosomiasis endemic area in Zimbabwe were characterised as having high overall haplotype diversity and low nucleotide diversity (Mutsaka-Makuvaza et al., 2020). More extensive genetic studies have been undertaken on *Biomphalaria* (the intermediate snail host of *Schistosoma mansoni*), examining both evolutionary relationships among species (Estrada et al., 2006; Jørgensen et al., 2007; Attwood et al. 2015) and genetic variation in populations (Wethington et al., 2007; Tian-Bi et al., 2013; Standley et al., 2014). *Biomphalaria pfeifferi* collected from several sites in Ivory Coast was found to have low genetic variation within populations and no isolation by distance between populations (Tian-Bi et al., 2013). *Biomphalaria choanomphala* specimens collected from three countries along Lake Victoria exhibited high intrapopulation diversity and elevated levels of population structure (Standley et al., 2014).

In the present study, we conducted population genetic analyses on *Bulinus senegalensis* populations from sites across The Gambia utilising the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene. We assessed the genetic diversity and structure of the *B. senegalensis* populations and assessed the level of gene flow between the populations. We further investigated relationships between snail genotype and schistosome infection.

5.2 Methods

5.2.1 Snail samples

Sampling of *Bulinus* snails was undertaken from 114 sites across The Gambia in 2017 and 2019. The 2017 collections were conducted in June in permanent sites and from October to November in seasonal sites. In 2019, the collections were undertaken in March in permanent sites and from September to October in seasonal pools. Sampling from permanent sites was

carried out during the dry season because this is the time when snails are abundant at these sites. Sampling of seasonal pools was conducted in the rainy season because this is the only time these bodies of water exist. *Bulinus senegalensis* was mostly found in temporal/seasonal sites (Joof et al., 2021b) and in total, *B. senegalensis* samples were collected from 30 sites: 1 site in Western Region (WR), 18 sites in the Central River Region (CRR) and 11 sites in the Upper River Region (URR) (Figure 5.1). CRR and URR are schistosomiasis endemic regions. WR is a non-schistosomiasis endemic region. For each collection site where *B. senegalensis* was found, 10 *B. senegalensis* snails were randomly selected for genetic analysis.

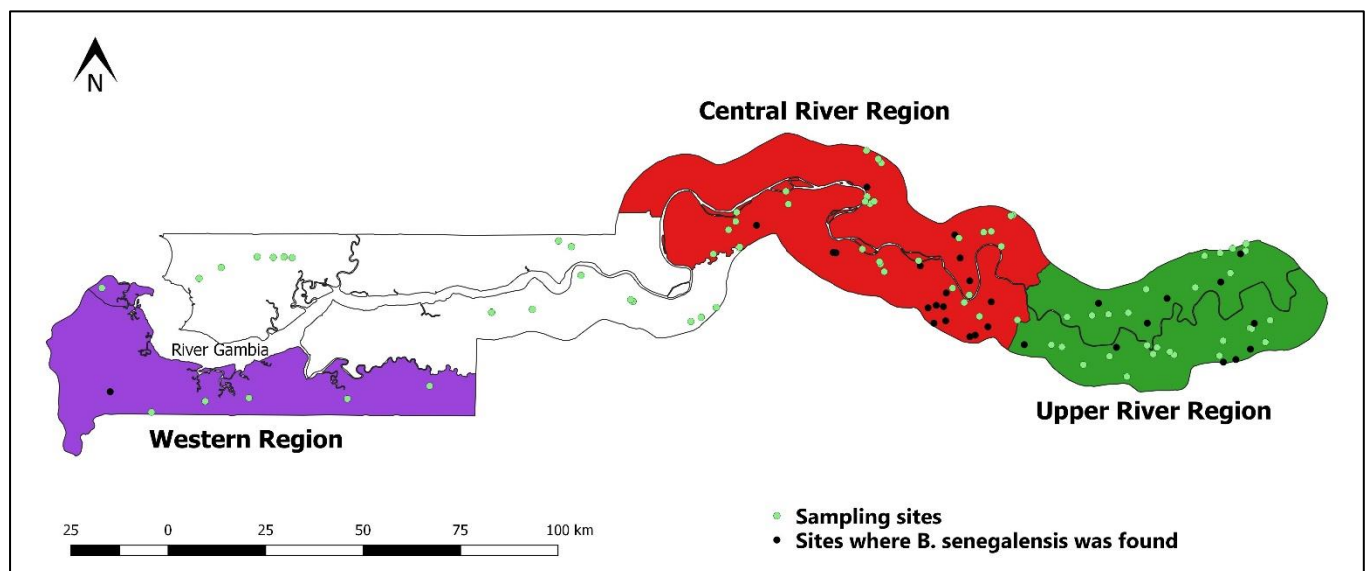


Figure 5.1. Map of The Gambia showing snail sampling sites across the country and the sites where *B. senegalensis* was found.

5.2.2 DNA extraction, PCR amplification and sequencing

DNA was extracted from snails using the CTAB (hexadecyltrimethylammonium bromide) method (Goodacre and Wade, 2001; see Section 2.7.1 for details). For each snail specimen we amplified both the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene. For cox1, we used the Folmer et al (1994) primers

LCO and HCO. For the ITS2 gene, we utilised the primers LSU1iii and LSU3iii (Fontanilla et al., 2017). See Section 2.7.3 for details on primers, PCR reactions, cycling conditions, gel image visualisation and sequencing.

5.2.3 Sequence processing and population genetic analyses

The DNA sequences were processed using the STADEN package v1.5.3 (Staden et al., 2000) and aligned using the Genetic Data Environment (GDE) package v2.2 (Smith et al., 1994). Maximum likelihood (ML) phylogenetic trees were constructed in PhyML version 3.0 (Guindon et al., 2010) using the general time reversible model of evolution incorporating gamma correction (GTR+ Γ) to correct for multiple substitutions. Additionally, median-joining haplotype networks were generated in PopART (Leigh and Bryant, 2015) using the algorithm described by Bandelt et al (1999). Genetic variation of *B. senegalensis* populations was assessed by estimating haplotype diversity (Hd) and nucleotide diversity (π) in DnaSP v6.12.03 (Rozas et al., 2017). Tajima's test of neutrality (Tajima, 1989) was carried out in DnaSP to detect violations of mutation-drift equilibrium caused by selection or changes in population size. Genetic distance (average evolutionary divergence over sequence pairs) within and between *B. senegalensis* populations was estimated in MEGA v11 (Tamura et al., 2021) using the Maximum Composite Likelihood method. Pairwise fixation index (Fst) was estimated in Arlequin v3.5 (Excoffier and Lischer, 2010) as a measure of genetic differentiation and gene flow between populations. The analysis was conducted with 10,000 permutations and at significance level of $p < 0.05$. P-values were corrected with the Bonferroni method using the "p.adjust" function of the "stats" package in R v4.1.0 (R Core Team, 2021). Gene flow was determined from the Fst values as follows: < 0.05 = high gene flow, $0.05 - 0.15$ = moderate gene flow, $0.15 - 0.25$ = restricted gene flow and > 0.25 = highly restricted gene flow (Hartl and Clark, 1997). Additionally, the novel *B. senegalensis* sequences generated in this study were included in a phylogeographic analysis with published *B. senegalensis* cox1 sequences

from Senegal and Cameroon available on GenBank (5 sequences from 3 locations in Senegal: Poudaye, Diohine and Toukar; and 6 sequences from 4 locations in Cameroon: Kousseri, Kaélé, Mora and Maroua; GenBank accession numbers KJ157477-87 (Zein-Eddine *et al.*, 2014)).

5.2.4 Association between snail genotype and schistosome infection

B. senegalensis snails were screened for schistosome parasite infections. We related genetic diversity (number of haplotypes) of snail populations to schistosome infections through logistic regression analysis. We further tested for associations between haplotypes and schistosome infections using the Fisher's exact test at significance level of $p < 0.05$ in GraphPad Prism v8.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

5.3 Results

Analysis of the mitochondrial cytochrome oxidase 1 gene for the 300 *B. senegalensis* samples from 30 sites across The Gambia revealed an overall high haplotype diversity (0.886) and low nucleotide diversity (0.006) (Table 5.1). The analysis showed a total of 27 haplotypes, of which 9 were shared haplotypes (found at more than one site) and 18 were private haplotypes (found at just a single collection site) (Figure 5.2). CRR sites alone had 19 haplotypes with a haplotype diversity of 0.878 and a nucleotide diversity of 0.006. URR sites had 14 haplotypes with haplotype diversity of 0.693 and nucleotide diversity of 0.005. In contrast to these two regions, WR had just a single haplotype and thus low haplotype and nucleotide diversity (Table 5.1). The number and frequency of haplotypes per site are detailed in Supplementary Table 5.1. There was structure in the distribution of the haplotypes. The most common or shared haplotypes were H9 (found in 10 sites), H24 (found in 11 sites) and H25 (found in 11 sites) (Figure 5.2). Only H9 was found in WR. H9 was also found throughout CRR, with just one

site in URR having this haplotype. H24 never overlapped with H9 and was mostly found in URR, with just two sites in CRR having this haplotype. H25 was present in both CRR and URR and overlapped with both H9 and H24 in some sites (Figure 5.2). Other shared haplotypes such as H12, H26 and H27 also showed some structure in their distribution. H12 and H27 were present in only CRR with H12 confined to sites on the south bank of the River Gambia. H26 was found in only URR restricted to sites on the north bank of the river in this region (Figure 5.2). Sites Daru and Sanka Bari had only private haplotypes (H4, H5 and H8) despite being quite close to sites like Njoren which had high presence of common shared haplotypes (Figure 5.2). There was a low genetic diversity as exemplified by short branch lengths on the tree and with most sequences separated by a single nucleotide change in the network (Figure 5.3). Bootstrap support for variation between haplotypes was low, further confirming species delimitation of the samples as *B. senegalensis*. Mean pairwise genetic distances within and between regions were generally low and are shown in Table 5.2. Mean pairwise genetic distances within and between *B. senegalensis* collection sites ranged from 0.00000 to 0.00834 and 0.00000 to 0.01435 (Supplementary Table 5.2). Tajima's D statistic showed non-significant values ($p > 0.10$) for all *B. senegalensis* populations for the *cox1* gene (Table 5.1). Pairwise fixation index (F_{st}) values between the regional populations were significant (Table 5.3). F_{st} between CRR and URR was 0.16824, suggesting restricted gene flow between the two regions. F_{st} values between each of these two regions and WR were greater than 0.25 (Table 5.3), indicating highly restricted gene flow between each of these regions and WR.

Table 5.1. Genetic diversity based on the mitochondrial *cox1* and nuclear ITS-2 gene of *Bulinus senegalensis* from The Gambia

Gene	Region	No. of samples	Number of haplotypes (h)	Haplotype diversity (Hd) (SD)	Nucleotide diversity (π)	Tajima's D (p-value)	Fu's Fs

Cox1	All	300	27	0.886 (0.010)	0.006	-1.094 (p>0.10)	-5.264
	CRR	180	19	0.878 (0.014)	0.006	-0.736 (p>0.10)	-1.333
	URR	110	14	0.693 (0.044)	0.005	-0.891 (p>0.10)	-1.440
	WR	10	1	0.000 (0.000)	0.000	-	-
ITS-2	All	300	7	0.662 (0.018)	0.002	-0.074 (p>0.10)	-0.558
	CRR	180	6	0.711 (0.016)	0.002	-0.084 (P>0.10)	0.103
	URR	110	5	0.546 (0.040)	0.002	0.408 (P>0.10)	-0.465
	WR	10	1	0.000 (0.000)	0.000	-	-

Table 5.2. Estimates of genetic distances $\pm$ SE over sequence pairs between and within regional populations of *B. senegalensis*

		CRR	URR	WR
Cox1	CRR	0.00632 $\pm$ 0.00160		
	URR	0.00617 $\pm$ 0.00153	0.00458 $\pm$ 0.00123	
	WR	0.00393 $\pm$ 0.00108	0.00298 $\pm$ 0.00099	0.00000 $\pm$ 0.00000
ITS-2	CRR	0.00227 $\pm$ 0.00127		
	URR	0.00215 $\pm$ 0.00127	0.00159 $\pm$ 0.00113	
	WR	0.00208 $\pm$ 0.00143	0.00114 $\pm$ 0.00083	0.00000 $\pm$ 0.00000

Table 5.3. Fixation index (Fst) estimates for pairwise comparisons of *B. senegalensis* populations using the cox1 and ITS-2 genes

		CRR	URR	WR
Cox1	CRR	-		
	URR	0.16824*	-	
	WR	0.31571*	0.48175*	-
ITS-2	CRR	-		
	URR	0.07126*	-	
	WR	0.26086*	0.14251	-

*Fst value significant at $p < 0.05$

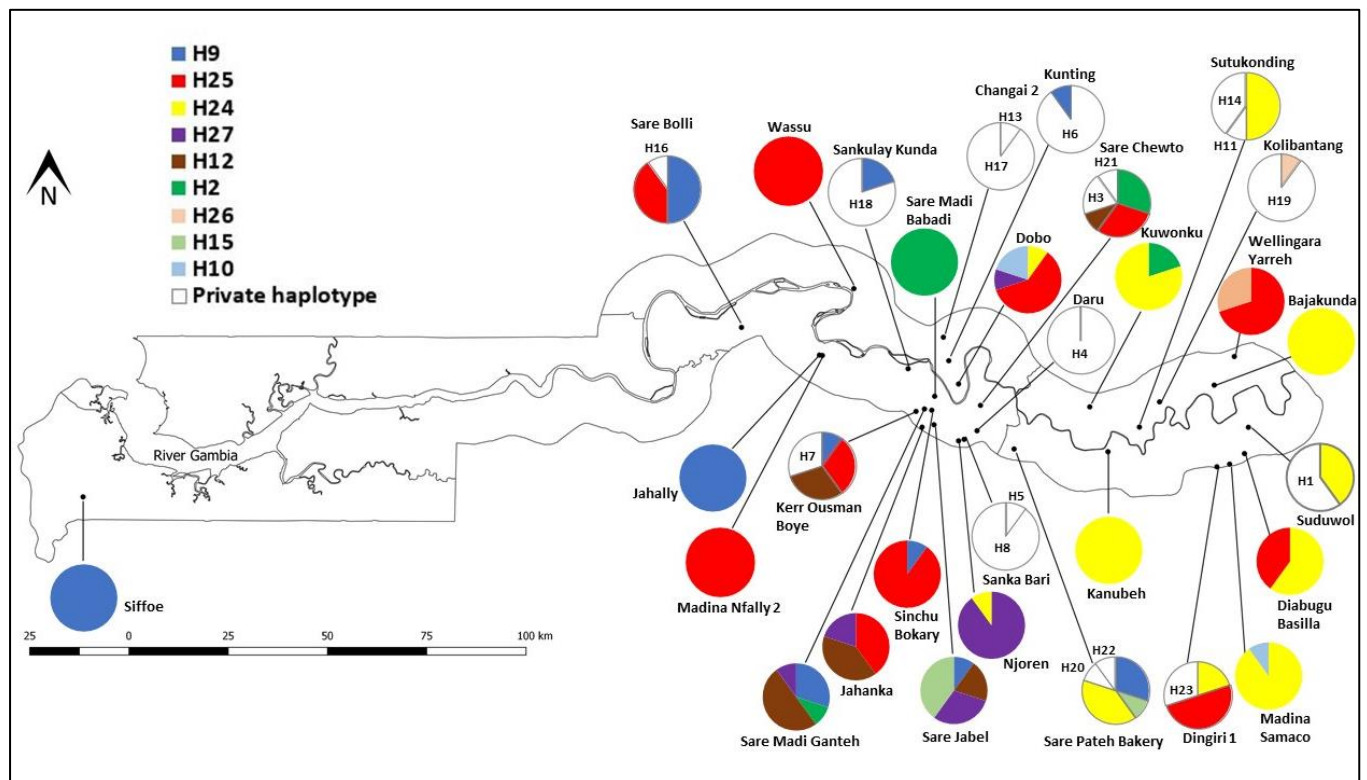
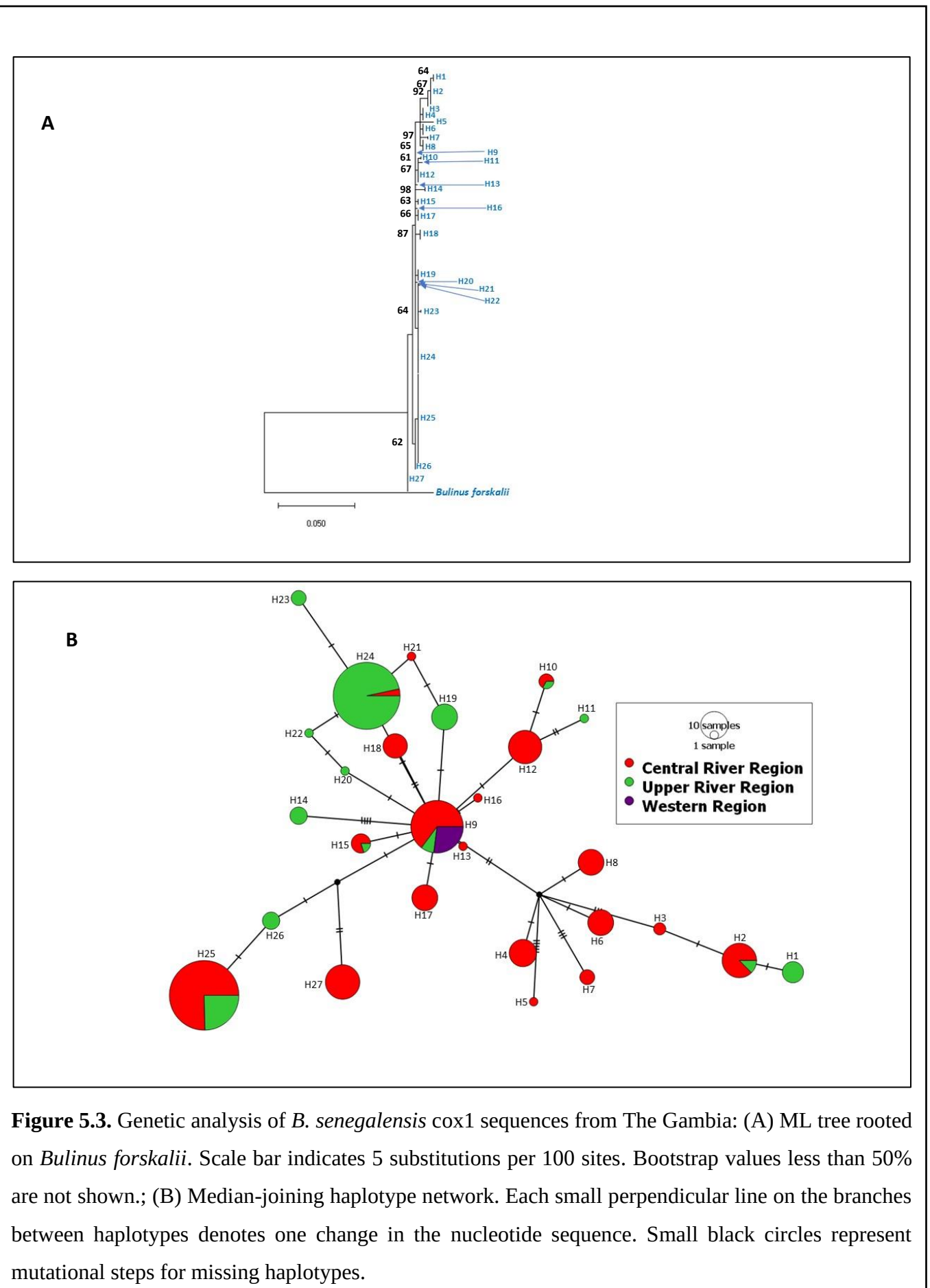


Figure 5.2. Distribution of *B. senegalensis* cox1 haplotypes in the 30 collection sites where *B. senegalensis* was found across The Gambia. Shared haplotypes are coloured according to the key. Private haplotypes are shown in white and are labelled on individual pie charts.



The nuclear ITS-2 gene revealed less variation compared to the mitochondrial Cox1 gene. The ITS-2 marker had 7 haplotypes, of which 4 were shared haplotypes and 3 were private haplotypes (Figure 5.4), with an overall haplotype diversity of 0.662 and nucleotide diversity of 0.002 (Table 5.1). The regional haplotype numbers and diversities, and nucleotide diversities are detailed in Table 5.1. The ITS-2 gene showed less structure in the distribution of haplotypes compared to the *cox1* gene, as the four shared haplotypes overlapped at many sites. The four shared haplotypes are H1 (found in 20 sites), H7 (found in 15 sites), H5 (found in 9 sites) and H3 (found in 6 sites) (Figure 5. 4). Only H1 was found in WR. This haplotype is also present in both CRR (11 sites) and URR (8 sites). H7, H5 and H3 were also present in both CRR and URR (Figure 5.4). Daru had only a private haplotype (H6) despite this site being located very closed to sites with high presence of common shared haplotypes (Figure 5.4). The low genetic diversity of the ITS-2 gene was revealed by the comparatively lower haplotype diversity and nucleotide diversity values (Table 5.1). This low variation was emphasised by the short branch lengths on the tree and the single nucleotide change separation between all unique haplotypes (except haplotype 1 and 2) in the network (Figure 5.5). Support values on the tree were low. The evolutionary distance within and between regional populations are shown in Table 5.2. The evolutionary distance within and between the collection sites ranged from 0.00000 to 0.00253 and 0.00000 to 0.00547 (Supplementary Table 5.2). Tajima's D statistic was not significant for the ITS-2 gene ($p > 0.10$). The pairwise F_{st} was significant for all comparisons except the one between WR and URR (Table 5.3). F_{st} between CRR and URR suggested moderate gene flow between the two regions and F_{st} between CRR and WR is indicative of a high restriction in gene flow between the two regions.

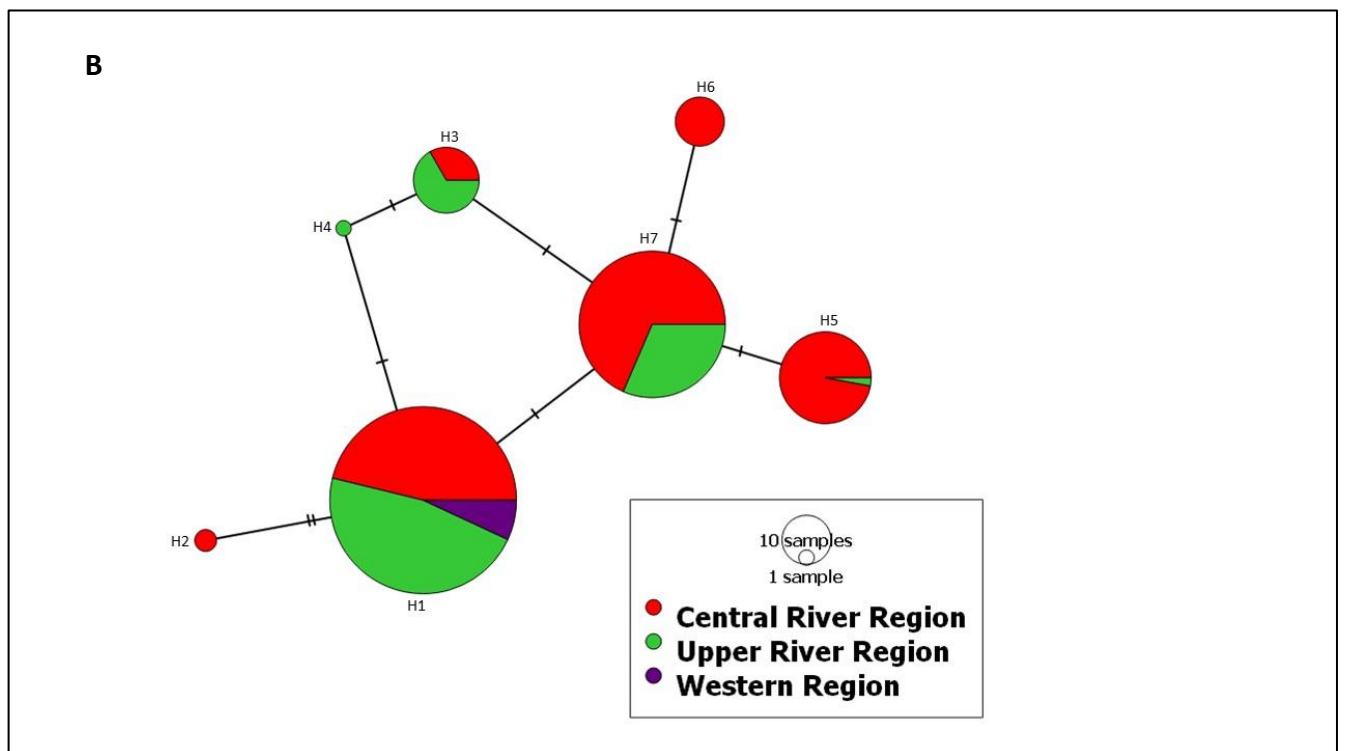
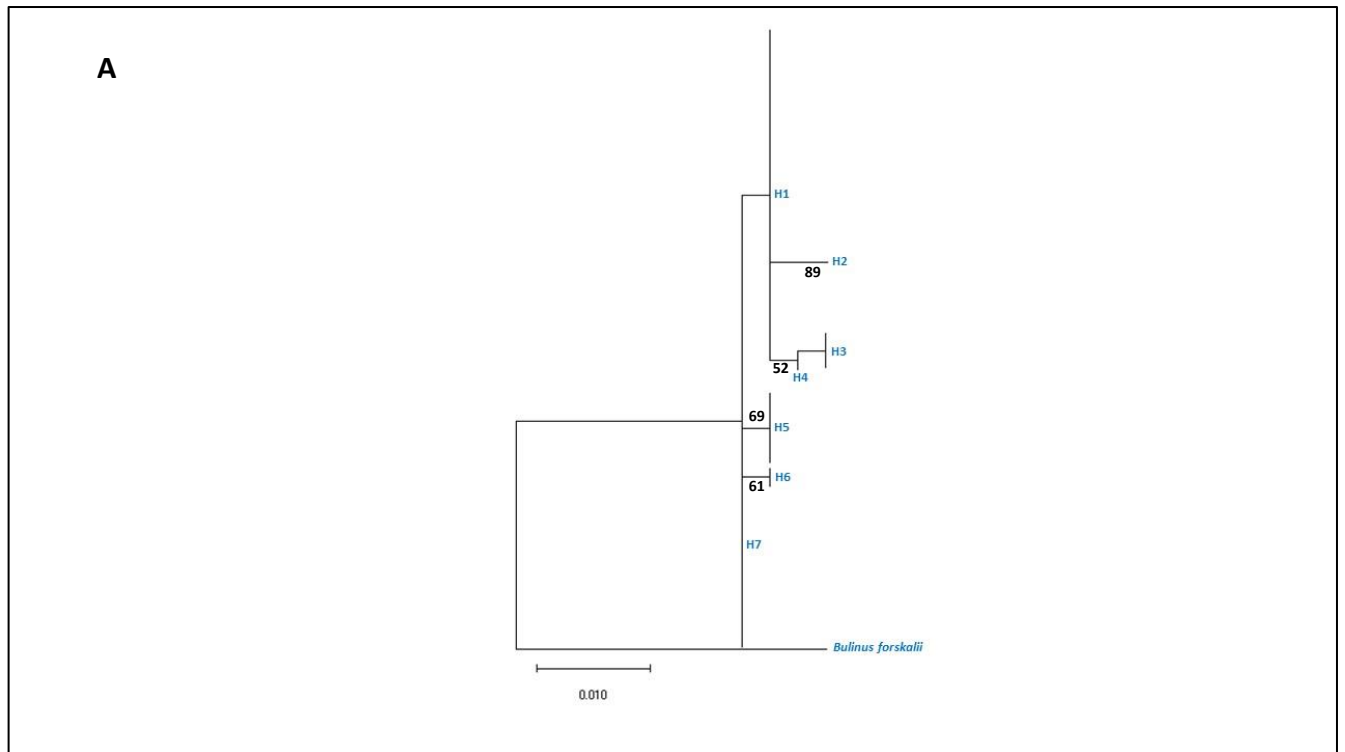


Figure 5.5. Genetic analysis of *B. senegalensis* ITS-2 sequences from The Gambia: (A) ML tree rooted on *Bulinus forskalii*. Scale bar indicates 1 substitution per 100 sites. Bootstrap values less than 50% are not shown; (B) Median-joining haplotype network. Each small perpendicular line on the branches between haplotypes denotes one change in the nucleotide sequence. Small black circles represent mutational steps for missing haplotypes.

The 300 *cox1* sequences were further analysed together with 5 published *B. senegalensis* *cox1* sequences from neighbouring Senegal and 6 sequences from Cameroon that were available on GenBank. The samples were resolved according to the geographic partitioning of their origins. The Senegalese samples and the Cameroonian samples formed two distinct clades within the ML tree, with low bootstrap support (Figure 5.6). In terms of genetic distance depicted by branch lengths, the Gambian population was closer to the Senegalese samples than the Cameroonian ones (Figure 5.6).

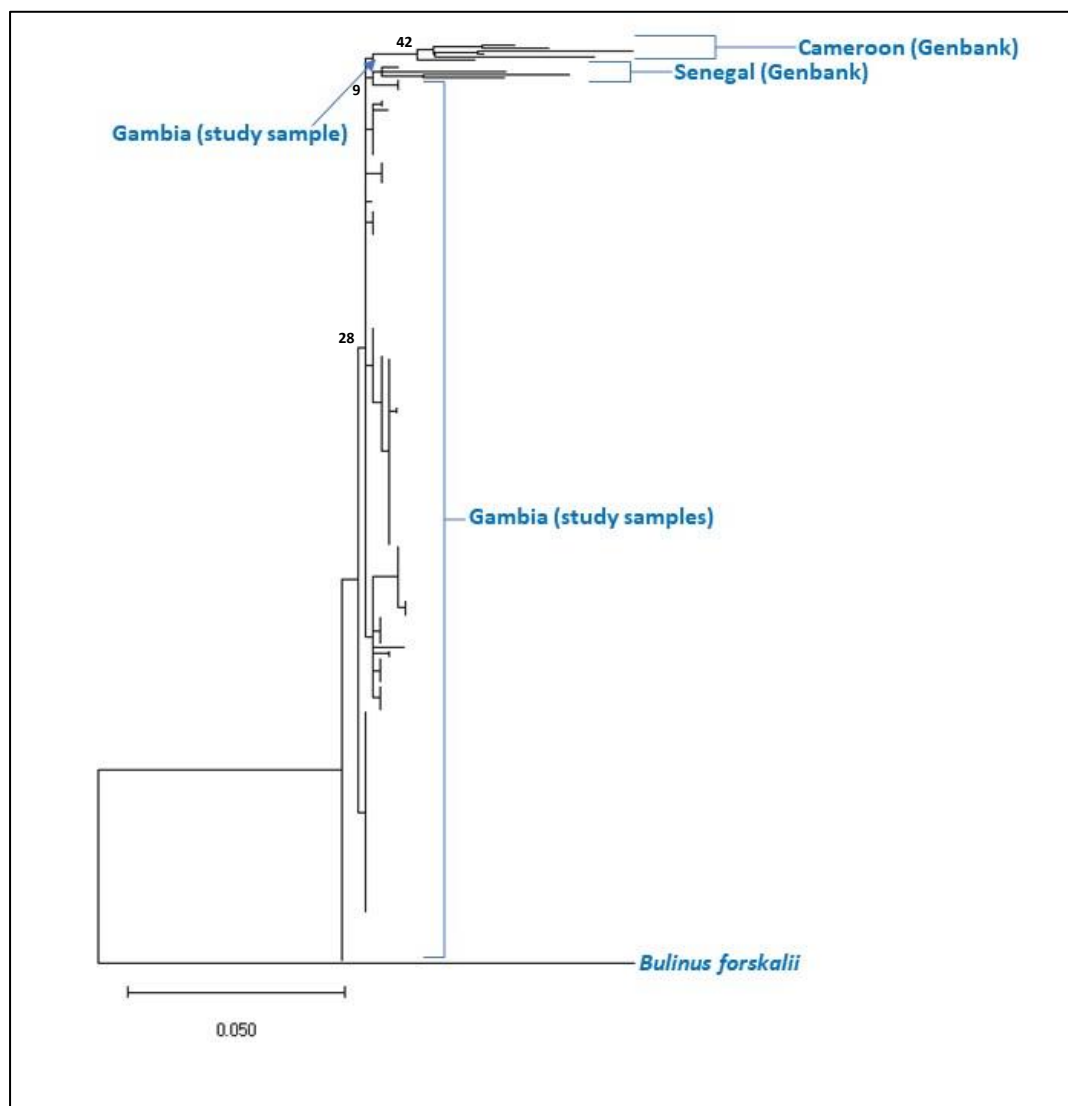


Figure 5.6. Maximum likelihood tree of *B. senegalensis* *cox1* sequences from The Gambia together with GenBank *B. senegalensis* *cox1* sequences from Senegal and Cameroon. The tree is rooted on *Bulinus forskalii*. Scale bar indicates 5 substitutions per 100 sites. Bootstrap values are shown for only the main groups.

No clear pattern was observed between schistosome infection and diversity (number of haplotypes) in *B. senegalensis* populations, as infection (both *S. haematobium* and *S. bovis*) was observed both in sites with high number of haplotypes and those with low number of haplotypes (Supplementary Table 5.1). Moreover, logistic regression analyses found no association between infection and diversity (number of haplotypes) in *B. senegalensis* populations ($p > 0.05$). Overall, 10 of the 27 *cox1* haplotypes were linked to schistosome infection (Table 5.4). One was linked to *S. haematobium* infection, 6 were linked to *S. bovis* infection and 3 were linked to both *S. haematobium* and *S. bovis* infections (Table 5.4). Of all these haplotypes linked with schistosome infection, only haplotype 24 (found in 20% of snails) was statistically associated with infection. Fisher's exact test found H24 to be significantly associated with *S. bovis* infection ($p = 0.0394$), with a relative infection risk of 2.353 (95% CI: 1.138 to 4.733). No statistically significant association was found between any of the haplotypes and *S. haematobium* infection ($p > 0.05$).

Table 5.4. Distribution of schistosome infection across the 27 *cox1* haplotypes identified in *B. senegalensis* from The Gambia

Haplotype name/designation	Frequency of haplotype (%)	No. infected with <i>S. haematobium</i> (%)	No. infected with <i>S. bovis</i> (%)	Overall schistosome infection (%)
H1	6 (2.0%)	0	0	0
H2	16 (5.3%)	0	0	0
H3	2 (0.7%)	0	0	0
H4	10 (3.3%)	0	0	0
H5	1 (0.3%)	0	0	0
H6	9 (3.0%)	0	0	0
H7	3 (1.0%)	0	1 (0.3%)	1 (0.3%)
H8	9 (3.0%)	0	0	0
H9	37 (12.3%)	0	2 (0.7%)	2 (0.7%)

H10	3 (1.0%)	0	0	0
H11	1 (0.3%)	0	0	0
H12	15 (5.0%)	1 (0.3%)	1 (0.3%)	2 (0.7%)
H13	1 (0.3%)	0	0	0
H14	4 (1.3%)	0	0	0
H15	5 (1.7%)	0	2 (0.7%)	2 (0.7%)
H16	1 (0.3%)	0	0	0
H17	9 (3.0%)	0	1 (0.3%)	1 (0.3%)
H18	8 (2.7%)	0	0	0
H19	9 (3.0%)	0	1 (0.3%)	1 (0.3%)
H20	1 (0.3%)	0	0	0
H21	1 (0.3%)	0	0	0
H22	1 (0.3%)	0	0	0
H23	3 (1.0%)	2 (0.7%)	0	2 (0.7%)
H24	60 (20.0%)	1 (0.3%)	10 (3.3%)*	11 (3.7%)
H25	65 (21.7%)	3 (1.0%)	8 (2.7%)	11 (3.7%)
H26	4 (1.3%)	0	0	0
H27	16 (5.3%)	0	1 (0.3%)	1 (0.3%)
Total	300 (100%)	7 (2.3%)	27 (9.0%)	34 (11.3%)

*Association significant at $p < 0.05$.

Out of the 7 ITS-2 haplotypes, 4 were linked to schistosome infection (Table 5.5). Two were linked to *S. bovis* infection and another two were linked to both *S. haematobium* and *S. bovis* infections (Table 5.5). Of these 4 haplotypes found linked with infection, only H7 (found in 29.7% of snails) was statistically associated with infection according to the Fisher's exact test. H7 was significantly associated with *S. bovis* infection ($p = 0.0035$, risk = 2.963, 95% CI: 1.462 – 5.970). No significant association was found between any of the haplotypes and *S. haematobium* infection ($p > 0.05$).

Table 5.5. Distribution of schistosome infection across the seven ITS-2 haplotypes identified in *B. senegalensis* from The Gambia

Haplotype name/designation	Frequency of haplotype (%)	No. infected with <i>S. haematobium</i> (%)	No. infected with <i>S. bovis</i> (%)	Overall schistosome infection (%)
H1	145 (48.3%)	6 (2.0%)	7 (2.3%)	13 (4.3%)
H2	2 (0.7%)	0	0	0
H3	18 (6.0%)	0	1 (0.3%)	1 (0.3%)
H4	1 (0.3%)	0	0	0
H5	35 (11.7%)	1 (0.3%)	4 (1.3%)	5 (1.7%)
H6	10 (3.3%)	0	0	0
H7	89 (29.7%)	0	15 (5.0%)*	15 (5.0%)
Total	300 (100%)	7 (2.3%)	27 (9.0%)	34 (11.3%)

*Association significant at $p < 0.05$.

5.4 Discussion

The genetic diversity of intermediate snail hosts has an influence on the dynamics of schistosome parasitism in local snail populations (Minchella and LoVerde, 1993; Hoffman *et al.*, 1998; Webster *et al.*, 2007; Sandland *et al.*, 2009; King and Lively, 2012). Therefore, understanding the level of genetic variation and structure of local snail populations can help in understanding and unravelling local schistosome-snail host relationships and transmission dynamics.

We performed population genetic analyses on *Bulinus senegalensis* populations from The Gambia using a mitochondrial and a nuclear marker to understand their population genetic diversity and segregation. Maternally inherited mitochondrial genes have a fast rate of evolution which makes them desirable choice of markers for population genetic analysis (Rollinson *et al.*, 1998). The mitochondrial cytochrome oxidase subunit I (cox1) gene we utilised in this study is a popular marker for population genetics due to its high level of variation

that allows evolutionary inference at intraspecific/population level (Rollinson et al., 1998; Zein-Eddine et al., 2014). Bi-parentally inherited nuclear markers are generally less variable than mitochondrial markers, but some such as the internal transcribed spacer (ITS) region used in this study have some variation, and allow for investigation of more detailed demographical factors, such as the history of past bottlenecks (Zhang and Hewitt, 2003).

The mitochondrial cytochrome oxidase subunit 1 (cox1) gene revealed more structure and pattern in the distribution of haplotypes across the country than the nuclear (ITS-2) gene. In addition to the high presence of private haplotypes (haplotypes found at a single collection site), some commonly shared cox1 haplotypes were restricted to only certain parts of the country and did not overlap with other shared haplotypes in their distribution. Siffoe, the only site from the non-schistosomiasis endemic WR where *B. senegalensis* was found, had a single *B. senegalensis* haplotype. The cox1 gene showed relatively high haplotype diversity but low nucleotide diversity while the ITS-2 gene had relatively lower haplotype and nucleotide diversity. The level of genetic diversity found in this study was lower than that found by Zein-Eddine et al (2014) in *B. senegalensis* from Senegal where they found 30/34 unique haplotypes with a haplotype diversity of 0.985 and nucleotide diversity of 0.053 for the same cox1 fragment. This is most likely explained by the difference in geographic size of the two countries. Senegal (size: 196,722 km²) is over 18 times bigger than The Gambia (size: 10,698 square km²) where sampling was conducted over a smaller geographic area. As expected, the cox1 gene was more variable than the ITS-2 gene in this study. Previous studies that utilised both mitochondrial cox1 gene and nuclear genes like the ITS region in *Bulinus* genetic studies found more diversity in the mitochondrial gene than the nuclear genes (Kane et al., 2008; Zein-Eddine et al., 2014; Abe et al., 2018). Some sites such as Daru and Sanka Bari had only private haplotypes and did not have some commonly distributed shared haplotypes which had high presence in nearby sites. This could be associated with the fact that the sites under study here

are isolated seasonal pools cut off from other sites, even those nearby. Ecological factors such as geographic isolation are believed to have great influence on snail genetic diversity (Jarne et al., 1991; Zein-Eddine *et al.*, 2014). As snails inhabit isolated habitats, geographic isolation gives rise to reproductive isolation and limits gene flow leading to independent evolution and divergent individuals (Zein-Eddine et al., 2014).

The non-significant Tajima's D statistic values showed that the *B. senegalensis* populations under study here are evolving according to the expected mutation-drift equilibrium. In other words, there is no recent demographic changes among the *B. senegalensis* populations from 30 sites across The Gambia. The significant Fst values between CRR and URR suggested moderate to restricted gene flow between *B. senegalensis* populations of the two regions. The significant high Fst values between each of these two regions and WR suggested extremely low gene flow between *B. senegalensis* populations from WR and CRR or URR. CRR and URR are geographically closer to each other than with WR and shared more similar ecological features with each other than with WR. A study by Abe et al. (2018) also observed a correspondence between genetic differentiation and geographic distance of *B. truncatus* populations from selected African countries. A similar observation was made in a study on *Biomphalaria* snails collected on a small spatial scale in Brazil (Wethington et al., 2007). Geographic and ecological features are believed to affect genetic differentiation and gene flow of snail populations (Jarne et al., 1991; Zein-Eddine *et al.*, 2014). In addition to geographic isolation, other factors (e.g., historical differentiation, reproductive isolation or fluctuations in effective population sizes) can influence genetic segregation among local snail populations (Wethington et al., 2007).

The *Bulinus senegalensis* samples from this study were analysed together with Genbank reference *B. senegalensis* cox1 sequences from Senegal and Cameroon to assess the phylogeographic differentiation of these samples. The samples were generally resolved

according to the geographic partitioning of the origin of the three sets of samples. Zein-Eddine *et al.* (2014) also found a clear geographic segregation between Senegalese and Cameroonian *B. senegalensis* *cox1* sequences. However, one might still expect some similarities between the Senegalese samples and The Gambian samples, if the Senegalese samples were collected just across the border with The Gambia. The contrary is the case in this study. The Senegalese samples were from the regions of Diourbel (Poudaye) and Fatick (Diohine and Toukar) which lie far away from The Gambia. This might help explain the genetic segregation of the Senegalese samples from The Gambian samples.

Co-adaptation between intermediate snail host populations and schistosome parasites is a driver for genetic diversity in local snail populations (Minchella and LoVerde, 1993; Webster *et al.*, 2007). A reduction in genetic variation of *Biomphalaria* populations was found to correlate with high *S. mansoni* infection rate and an increase in snail genetic diversity corresponded with low parasitism in local snail populations (Hoffman *et al.*, 1998; Sandland *et al.*, 2009). In this study, no pattern was observed between schistosome infection and genetic diversity (number of haplotypes) in populations of *B. senegalensis*. However, we found a statistically significant association between one *cox1* haplotype and *S. bovis* infection. Additionally, one ITS-2 haplotype was associated with *S. bovis* infection in snails. This means the two haplotypes have an influence on *S. bovis* infection outcome in *B. senegalensis*. The lack of association between snail genotype and *S. haematobium* infection may be attributed to the low number of *S. haematobium* infected snails found in the study. Some of the limitations of the study are low sample size for population genetic analysis, microsatellite markers which could provide further genetic information were not utilised, and lack of appropriate methods to correlate snail genotypes (haplotypes) with snail infections.

5.5 Conclusion

Understanding the genetic variation and structure of local freshwater snail populations can help in the elucidation of local schistosomiasis transmission dynamics and can further provide relevant information for the design of novel control approaches. The *B. senegalensis* populations analysed in this study are characterised as having high haplotype diversity and low nucleotide diversity with some sites having only private haplotypes and missing widely distributed shared haplotypes present in nearby sites. The *cox1* gene revealed more clear structure than the ITS-2 gene in the distribution of haplotypes, with some shared haplotypes restricted to only certain parts and regions of the country. The statistically significant estimates of genetic differentiation (F_{st}) suggested moderate to restricted gene flow between *B. senegalensis* populations of CRR and URR, and highly restricted gene flow between these two populations and that of WR. One *cox1* haplotype and one ITS-2 haplotype were found to have an influence in determining schistosome infection outcome in snails. The current study provides first-time insight into the genetic structure of a *Bulinus* species that acts as the intermediate host of both the human parasite (*S. haematobium*) and the livestock parasite (*S. bovis*) in a country that is striving to eliminate schistosomiasis by the year 2030. It also revealed a link between snail genotype and schistosome infection status. The findings of this study in combination with further understanding of parasite genetic diversity in the country may help us better understand the role of host-parasite interactions in the disease transmission dynamics which is ultimately useful for the designing of novel control strategies.

6 Genetic variation of the *Schistosoma* snail hosts *Bulinus forskalii* and *Bulinus truncatus* in relation to *Schistosoma bovis* infection in The Gambia

Abstract

Exploration of snail genetic structure is necessary for a better understanding of the host-parasite interactions in local schistosomiasis transmission zones. We conducted a study on *Bulinus forskalii* and *Bulinus truncatus* from The Gambia to characterise their genetic structure in relation to schistosome infection. The two *Bulinus* species were widely sampled across the country and analysed using the mitochondrial cytochrome oxidase subunit 1 (cox1) and the nuclear internal transcribed spacer 2 (ITS-2) genes. High genetic diversity was observed within *B. forskalii*. The cox1 gene had 18 haplotypes, with an overall haplotype diversity of 0.899 and nucleotide diversity of 0.029. The ITS-2 gene had 4 haplotypes, with an overall haplotype diversity 0.621 and nucleotide diversity of 0.006. There was a clear structure in the distribution of haplotypes for both genes with more haplotype sharing and homogeneity observed in the Upper River Region (URR) where the collection sites were streams than in the Central River Region (CRR) where the collection sites were mostly temporal sites. Western Region (WR) had just a single haplotype. Genetic differentiation was strong among *B. forskalii* populations from the three regions, and Tajima's D statistic revealed evidence of selection or recent population contraction. For *B. truncatus*, diversity was generally low. The cox1 gene had 6 haplotypes, with an overall haplotype diversity of 0.777 and nucleotide diversity of 0.002. The ITS-2 gene had 2 haplotypes, with an overall haplotype diversity of 0.497 and nucleotide diversity of 0.00109. There was strong genetic differentiation among *B. truncatus* populations, with less haplotype sharing. Tajima's D statistic revealed no evidence of selection or recent demographic changes occurring in *B. truncatus*. While we found no association between snail

population diversity or snail genotypes and *S. bovis* infection for either *B. forskalii* or *B. truncatus*, we observed a higher infection rate of *S. bovis* in *B. truncatus* (16.7%) compared to *B. forskalii* (11.4%). This study reported for the first time, the population genetic structure of *B. forskalii* and *B. truncatus* in relation to schistosome infection in The Gambia. The findings will foster our understanding of the role played by *Bulinus* snail genetics in the epidemiology of schistosomiasis.

6.1 Introduction

The genus *Bulinus* is a group of pulmonate snails distributed mainly in Africa, the Middle East and the Mediterranean region (Brown, 1994). The genus has 37 species some of which are involved in the transmission of schistosome parasites (Brown, 1994). *Bulinus forskalii* and *B. truncatus* are among the most widely distributed *Bulinus* species in Africa (Mandahl-Barth, 1957; Brown, 1994). *B. forskalii* is distributed in Egypt, Sudan, West, Central, East and Southern Africa, Madagascar and Sao Tome Island (Brown, 1994; Gow et al., 2004; Zein-Eddine et al., 2014). *B. truncatus* is found in Egypt, Sudan, Cameroon, West and East Africa, southern parts of Africa and the Mediterranean (Mandahl-Barth, 1957; Brown, 1994; Zein-Eddine et al., 2014, Abe et al., 2018; Chibwana et al., 2020; Mutsaka-Makuvaza et al., 2020). *B. forskalii* and *B. truncatus* act as intermediate hosts for several *Schistosoma* parasites. However, the compatibility and role of each bulinid species in the transmission of these parasites varies from region to region. For instance, *B. forskalii* is the main intermediate host of the medically important parasite *Schistosoma guineensis* in the Lower Guinea areas, mainly in Cameroon (Jourdane et al., 2001; Pages et al., 2003), while in West Africa it is involved in the transmission of the livestock parasite *S. bovis* (Diaw et al., 1989b, Pennance et al., 2020). *Bulinus truncatus* is the main intermediate host of the medically important *S. haematobium* parasite in Egypt and Cameroon (Zein-Eddine et al., 2014). In West African countries including

Ivory Coast, Niger and Senegal, *B. truncatus* is involved in the transmission of *S. haematobium*, *S. bovis* and *S. haematobium* x *S. bovis* hybrids (Sene et al., 2004; Huyse et al., 2009; Tian-Bi et al., 2019; Pennance et al., 2020; Leger et al., 2020). In The Gambia both *B. forskalii* and *B. truncatus* are intermediate hosts of the livestock parasite *S. bovis* (Joof et al., 2021b).

Bulinus snails are hermaphrodites and reproduce through either self-fertilisation or cross-fertilisation (Jarne and Charlsworth, 1993). Both *B. forskalii* and *B. truncatus* prefer self-fertilization over cross-fertilisation (Gow et al., 2004; Zein-Eddine et al., 2017). High selfing behaviour and population bottlenecks increase genetic variation between snail populations and decrease variation within local snail populations (Gow et al., 2004; Zein-Eddine et al., 2017). Inbreeding in a population decreases polymorphism and genetic variation within the population (Gow et al., 2004). Genetic diversity of snails, in turn influences host snail and schistosome parasite compatibility (Minchella and LoVerde, 1993; Sandland et al., 2007). This makes the exploration of snail genetic structure necessary for a good understanding of host-parasite interactions in local schistosomiasis transmission zones (Zein-Eddine et al., 2014, Abe et al., 2018; Chibwana et al., 2020; Mutsaka-Makuvaza et al., 2020). The knowledge gained can provide useful insight on disease epidemiology that empowers the formation of effective control approaches.

Several molecular studies have been undertaken in African countries to determine the genetic diversity and structure of *Bulinus forskalii* and *B. truncatus* (Gow et al., 2004; Nalugwa et al., 2010; Zein-Eddine et al., 2014; Zein-Eddine et al., 2017; Abe et al., 2018; Tumwebaze et al., 2019; Mutsaka-Makuvaza et al., 2020; Chibwana et al., 2020). High levels of genetic variation were observed in *B. forskalii* populations from Cameroon (Gow et al., 2004; Zein-Eddine et al., 2014). In contrast, Abe et al (2018) found low haplotype diversity in *B. truncatus* collected from Giza and Sharkia governorates in Egypt while Tumwebaze et al. (2019) observed a single haplotype in *B. truncatus* from the Crater Lakes of Western Uganda. Additionally, a study by

Mutsaka-Makuvaza et al (2020) found an overall low level of genetic variation within *B. truncatus* populations collected from a urogenital schistosomiasis endemic area in Zimbabwe. On the contrary, a study found high haplotype and nucleotide diversity with limited haplotype sharing within *B. truncatus* populations collected from Lake Victoria and neighbouring aquatic systems (Chibwana et al., 2020). No information is available on the genetic structure of *B. forskalii* and *B. truncatus* from The Gambia.

This study assessed the genetic diversity and structure of *B. forskalii* and *B. truncatus* from The Gambia using the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene. It further tested for relationships between snail genetics and schistosome infection.

6.2 Methods

6.2.1 Snail samples

Sampling of *Bulinus* snails was undertaken from 114 sites across The Gambia in 2017 and 2019. The 2017 collections were conducted in June in permanent sites and from October to November in seasonal sites. In 2019, the collections were undertaken in March in permanent sites and from September to October in seasonal pools. Sampling from permanent sites was carried out during the dry season because this is the time when snails are abundant at these sites. Sampling of seasonal pools was conducted in the rainy season because this is the only time these bodies of water exist. *Bulinus forskalii* samples were collected from 14 sites: 1 site in the Western Region (WR), 9 sites in the Central River Region (CRR) and 4 sites in the Upper River Region (URR) (Figure 6.1). The lone site in WR where *B. forskalii* was found was a swamp. CRR sites included swamps, rice fields, an irrigation canal, a seasonal pool and a stream. URR sites were all streams. *Bulinus truncatus* samples were collected from 3 sites:

Pacharr 1 and Madina Nfally 1 in CRR, and Misra Ba Mariama in URR. Pacharr 1 was an irrigation canal while Madina Nfally 1 and Misra Ba Mariama were streams. CRR and URR are schistosomiasis endemic regions. WR is a non-schistosomiasis endemic region. For each collection site where *B. forskalii* and *B. truncatus* were found, 10 specimens of each species were randomly selected for genetic analysis.

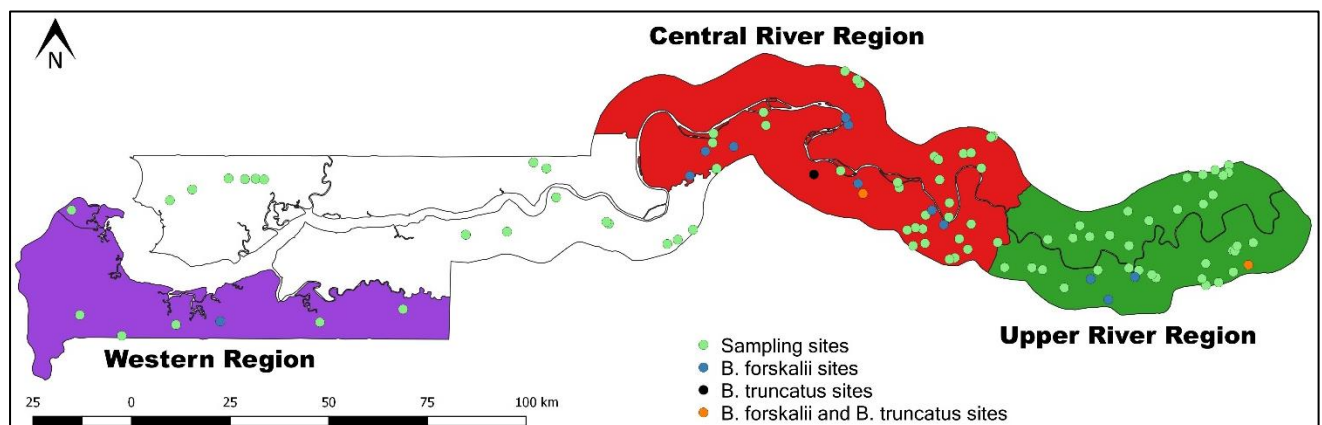


Figure 6.1. Map of The Gambia showing snail sampling sites across the country and the sites where *B. forskalii* and *B. truncatus* were found. Note that there were two sites that had both *B. forskalii* and *B. truncatus*.

6.2.2 DNA extraction, PCR amplification and sequencing

DNA was extracted from snails using the CTAB (hexadecyltrimethylammonium bromide) method (Goodacre and Wade, 2001; see Section 2.7.1 for details). For each snail specimen we amplified both the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene. For cox1, we used the Folmer et al (1994) primers LCO and HCO. For the ITS2 gene, we utilised the primers LSU1iii and LSU3iii (Fontanilla et al., 2017). See Section 2.7.3 for details on primers, PCR reactions, cycling conditions, gel image visualisation and sequencing.

6.2.3 Sequence processing and population genetic analyses

The DNA sequences were processed using the STADEN package v1.5.3 (Staden et al., 2000) and aligned using the Genetic Data Environment (GDE) package v2.2 (Smith et al., 1994). Maximum likelihood (ML) phylogenetic trees were constructed in PhyML version 3.0 (Guindon et al., 2010) using the general time reversible model of evolution incorporating gamma correction (GTR+ Γ) to correct for multiple substitutions. Additionally, median-joining haplotype networks were generated in PopART (Leigh and Bryant, 2015) using the algorithm described by Bandelt et al. (1999). Genetic variation of *B. forskalii* and *B. truncatus* populations was assessed by estimating haplotype diversity (H_d) and nucleotide diversity (π) in DnaSP v6.12.03 (Rozas et al., 2017). Tajima's test of neutrality (Tajima, 1989) was carried out in DnaSP to detect violations of mutation-drift equilibrium caused by selection or changes in population size. Mean genetic distance (average evolutionary divergence over sequence pairs) within and between populations of each species was estimated in MEGA v11 (Tamura et al., 2021) using the Maximum Composite Likelihood method. Pairwise fixation index (F_{st}) was estimated in Arlequin v3.5 (Excoffier and Lischer, 2010) as a measure of genetic differentiation between populations. The analysis was conducted with 10,000 permutations and at significance level of $p < 0.05$. P-values were corrected with the Bonferroni method using the "p.adjust" function of the "stats" package in R v4.1.0 (R Core Team, 2021). Genetic differentiation was determined from the F_{st} values as follows: < 0.05 = low genetic differentiation, $0.05 - 0.15$ = moderate genetic differentiation, $0.15 - 0.25$ = high genetic differentiation and > 0.25 = very high genetic differentiation (Hartl and Clark, 1997). Additionally, the novel Gambian *B. forskalii* and *B. truncatus* sequences generated in this study were included in a phylogeographic analysis with published cox1 GenBank sequences from 6 African countries for *B. forskalii* and 9 African countries for *B. truncatus* (Kane et al., 2008; Nalugwa et al., 2010; Akinwale et al., 2011; Zein-Eddine et al., 2014; Tumwebaze et al., 2019;

Chibwana et al., 2020; Pennance et al., 2020). Details of the GenBank sequences are shown in Supplementary Table 6.1. An amino acid change analysis was conducted on the novel *B. forskalii* sequences to estimate the number of synonymous and non-synonymous substitutions/mutations using the single-likelihood ancestor counting (SLAC) method on Datamonkey (<http://www.datamonkey.org/>) and DnaSP v6.12.03 (Rozas et al., 2017). A phylogenetic analysis was also conducted on the novel *B. forskalii* sequences with reference sequences of closely related *Bulinus* species from GenBank (Kane et al., 2008). A maximum likelihood tree was constructed using the GTR+ Γ model and 1000 bootstrap replicates. The tree was rooted on *Biomphalaria pfeifferi* (DQ084835; Jørgensen et al., 2007).

6.2.4 Association between snail genotype and schistosome infection

B. forskalii and *B. truncatus* snails were screened for schistosome parasite infections. We related genetic diversity (number of haplotypes) of snail populations to schistosome infection through logistic regression analysis. We further tested for associations between snail haplotypes and schistosome infection using the Fisher's exact test at significance level of $p < 0.05$ in GraphPad Prism v8.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

6.3 Results

6.3.1 Genetic variation of *B. forskalii*

Analysis of the *B. forskalii* mitochondrial cytochrome oxidase 1 gene revealed high genetic diversity with an overall haplotype diversity of 0.899 and nucleotide diversity of 0.029 (Table 6.1). There was a total of 18 *cox1* haplotypes within the 140 *B. forskalii* samples analysed, of which 5 were shared (found at more than one site) and 13 were private (found at just a single collection site) (Figure 6.2). CRR had 14 haplotypes with a haplotype diversity of 0.882 and a

nucleotide diversity of 0.027. URR had 6 haplotypes with haplotype diversity of 0.723 and nucleotide diversity of 0.015. WR had a single haplotype and thus no diversity (Table 6.1). The occurrence and frequency of each haplotype differed among sites and regions (Supplementary Table 6.2). The lone haplotype (H9) found in WR was restricted to only this region. CRR had less haplotype sharing than URR, with 11 of its 14 haplotypes each locally restricted to just a single site in the CRR region and only 3 haplotypes found at more than one site in the region (Figure 6.2). Two of the 6 haplotypes in URR were locally restricted to just a single site in the region with 4 haplotypes observed at more than one site in the region (Figure 6.2). The five shared haplotypes (H1, H2, H4, H6 and H17) had a clear pattern in their distribution within CRR and URR. H17 was observed in only CRR while H2 was restricted to only URR. H1, H4 and H6 were found in both CRR and URR (Figure 6.2). Sequence diversity was high, exemplified by long branch lengths on the tree and with many sequences separated by numerous nucleotide changes in the network. (Figure 6.3). The tree and the network resolved the sequences into 3 distinct groups, with the first group (Group 1) comprised of 2 haplotypes from CRR and URR samples, the second group (Group 2) containing 11 haplotypes from CRR and WR samples and the third group (Group 3) having 5 haplotypes from CRR and URR samples (Figure 6.3). The support values of the phylogenetic tree were not generally significant, but there was a very high support for the branch leading to Group 2. To assess if the divergence of Group 2 from the other two groups was just intraspecies variation or involvement of another *Bulinus* species, amino acid change (synonymous and non-synonymous mutations) and phylogenetic analyses were conducted. The amino acid change analysis revealed that all nucleotide substitutions in the data were synonymous substitutions (Supplementary Table 6.4). From the phylogenetic analysis, all *B. forskalii* haplotypes found in this study clustered with reference GenBank *B. forskalii* sequences with strong bootstrap support (92%), further confirming their species status as *B. forskalii* (see the phylogenetic tree

in Supplementary Figure 6.1). Mean pairwise genetic distance within regions was 0.00000 for WR, 0.01710 for CRR and 0.00863 for URR. Mean pairwise distance between regions was 0.01710 for WR and CRR, 0.03295 for WR and URR and 0.02039 for CRR and URR. Mean pairwise genetic distances within and between *B. forskalii* collection sites ranged from 0.00000 to 0.01603 and 0.00106 to 0.03530 respectively (Supplementary Table 6.3). Tajima's D statistic showed significant positive values for *B. forskalii* populations for all, CRR and URR (Table 6.1), indicating a violation of mutation-drift equilibrium caused by selection maintaining variation or a recent population contraction. Pairwise fixation index (Fst) values between the regional populations were significant (Table 6.2). Fst between CRR and URR was 0.13140, suggesting moderate genetic differentiation between the two regions. Fst values between each of these two regions and WR were greater than 0.25 (Table 6.2), indicating very high genetic differentiation between each of these regions and WR.

Table 6.1. Genetic diversity based on the mitochondrial cox1 and nuclear ITS-2 genes of *B. forskalii* from The Gambia

Gene	Region	No. of samples	Number of haplotypes (h)	Haplotype diversity (Hd) (SD)	Nucleotide diversity (π)	Tajima's D (p-value)	Fu's Fs
Cox1	All	140	18	0.899 (0.011)	0.029	2.863 (p < 0.01)	15.653
	CRR	90	14	0.882 (0.015)	0.027	2.226 (p < 0.05)	14.303
	URR	40	6	0.723 (0.051)	0.015	2.812 (p < 0.01)	10.848
	WR	10	1	0.000 (0.000)	0.000	-	-
ITS-2	All	140	4	0.621 (0.025)	0.006	2.584 (p < 0.05)	7.259

	CRR	90	3	0.584 (0.032)	0.005	2.888 (p < 0.01)	6.837
	URR	40	1	0.000 (0.000)	0.000	-	-
	WR	10	1	0.000 (0.000)	0.000	-	-

Table 6.2. Fixation index (Fst) estimates for pairwise comparisons of *B. forskalii* populations using the cox1 and ITS-2 genes

		CRR	URR	WR
Cox1	CRR	-		
	URR	0.13140*	-	
	WR	0.39316*	0.50971*	-
ITS-2	CRR	-		
	URR	0.47714*	-	
	WR	0.56244*	1.00000*	-

*Fst value significant at p < 0.05

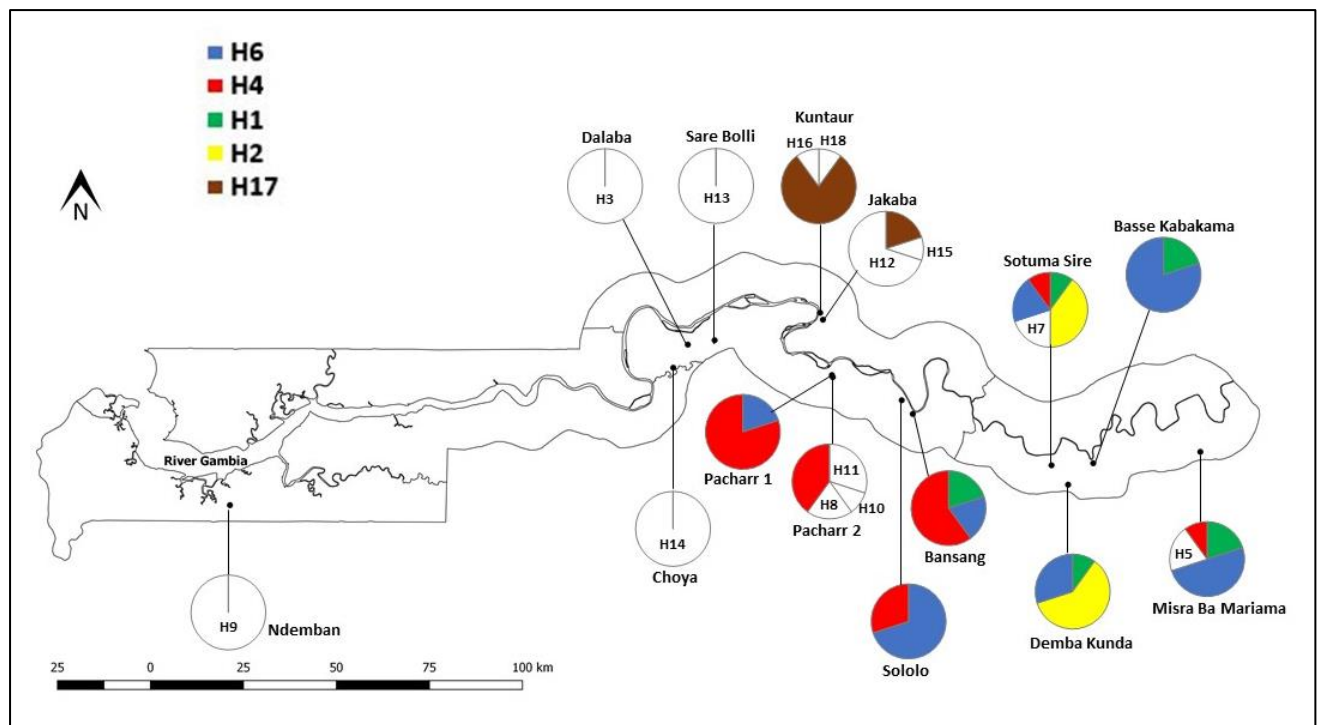
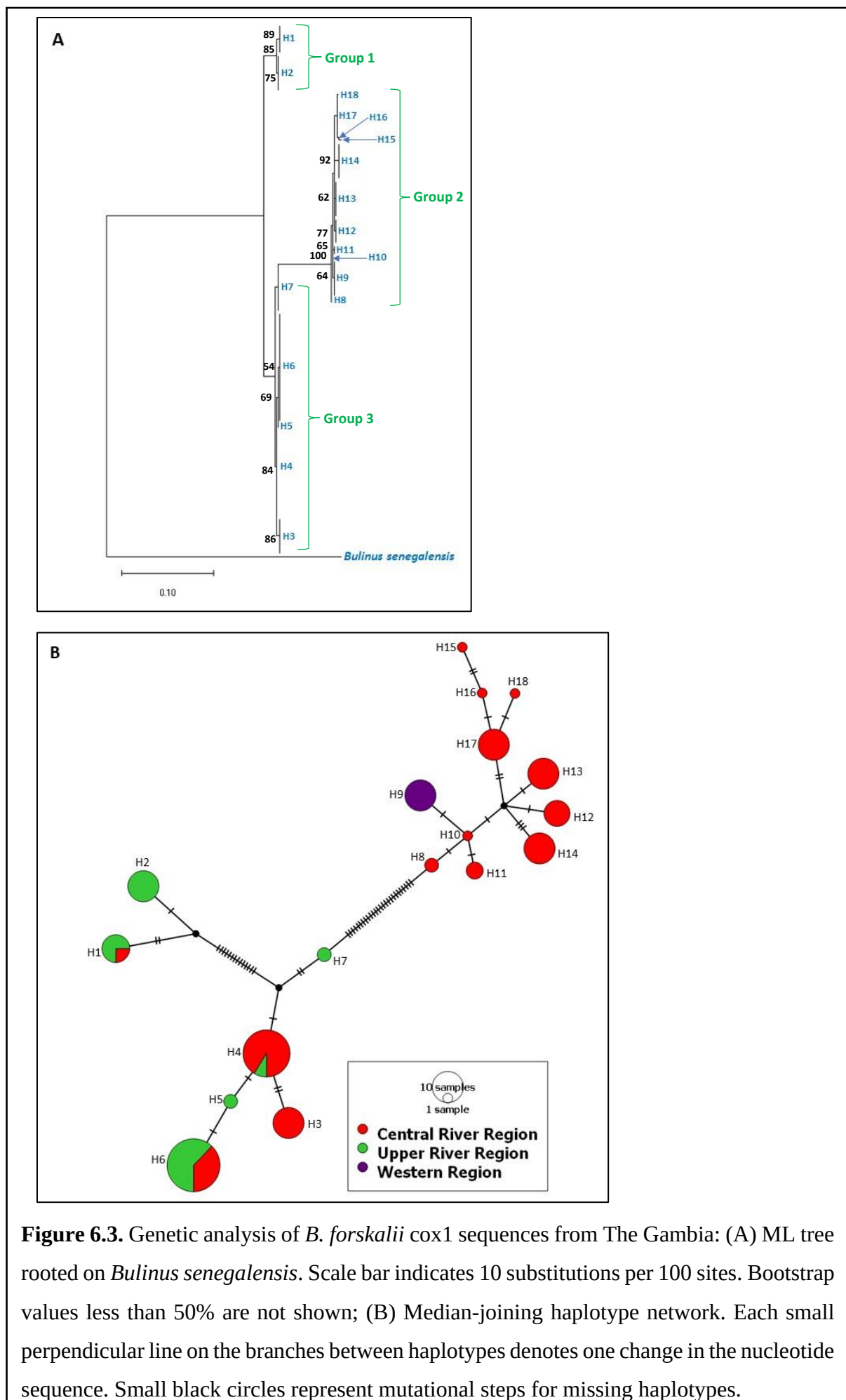


Figure 6.2. Distribution of *B. forskalii* cox1 haplotypes in the 14 collection sites where *B. forskalii* was found in The Gambia. Shared haplotypes are coloured according to the key. Private haplotypes are shown in white, and the haplotype number is labelled on individual pie charts.



The nuclear ITS-2 marker had 4 haplotypes, with an overall haplotype diversity of 0.621 and nucleotide diversity of 0.006 (Table 6.1). The regional haplotype numbers and diversities, and nucleotide diversities are outlined in Table 6.1. The number of haplotypes within sites was low, with all sites having one haplotype, except Pacharr 2 which had two haplotypes (Figure 6.4). One of the 4 haplotypes (H3) was a private haplotype found only in WR. The other 3 haplotypes (H1, H2 and H4) were shared haplotypes and showed a pattern in their distribution in CRR and URR (Figure 6.4). All three haplotypes were found in CRR, with H1 and H4 confined to the south bank of the River Gambia and H2 found in both the south and north banks of the River Gambia in the region (Figure 6.4). URR had only H4 shared by all the sites in the region (Figure 6.4). There was diversity in the ITS-2 gene as emphasised by the long branch lengths on the tree and the many nucleotide changes between unique haplotypes in the network (Figure 6.5). There was no overall significant support for intraspecific variation on the tree. The tree splits the sequences into two groups, with one comprised of 3 haplotypes (H1, H2 and H3) and the other having one haplotype (H4) (Figure 6.5). The network had a central haplotype (H1) from which the other 3 haplotypes branch off by 1 nucleotide change (H2), 2 nucleotide change (H3) and 4 nucleotide change (H4) (Figure 6.5). Mean pairwise genetic distance within regions was 0.00000 for WR, 0.00513 for CRR and 0.00000 for URR. Mean pairwise distance between regions was 0.00866 for WR and CRR, 0.01352 for WR and URR and 0.00718 for CRR and URR. The mean pairwise distance within and between the collection sites ranged from 0.00000 to 0.00044 and 0.00000 to 0.01351 respectively (Supplementary Table 6.3). Tajima's D statistic values were significant and positive for all and CRR (Table 6.1), indicative of selection or recent population contraction occurring. The pairwise F_{st} was high and significant for all pairwise comparisons (Table 6.2), suggesting very high genetic differentiation amongst the regional populations of *B. forskalii*.

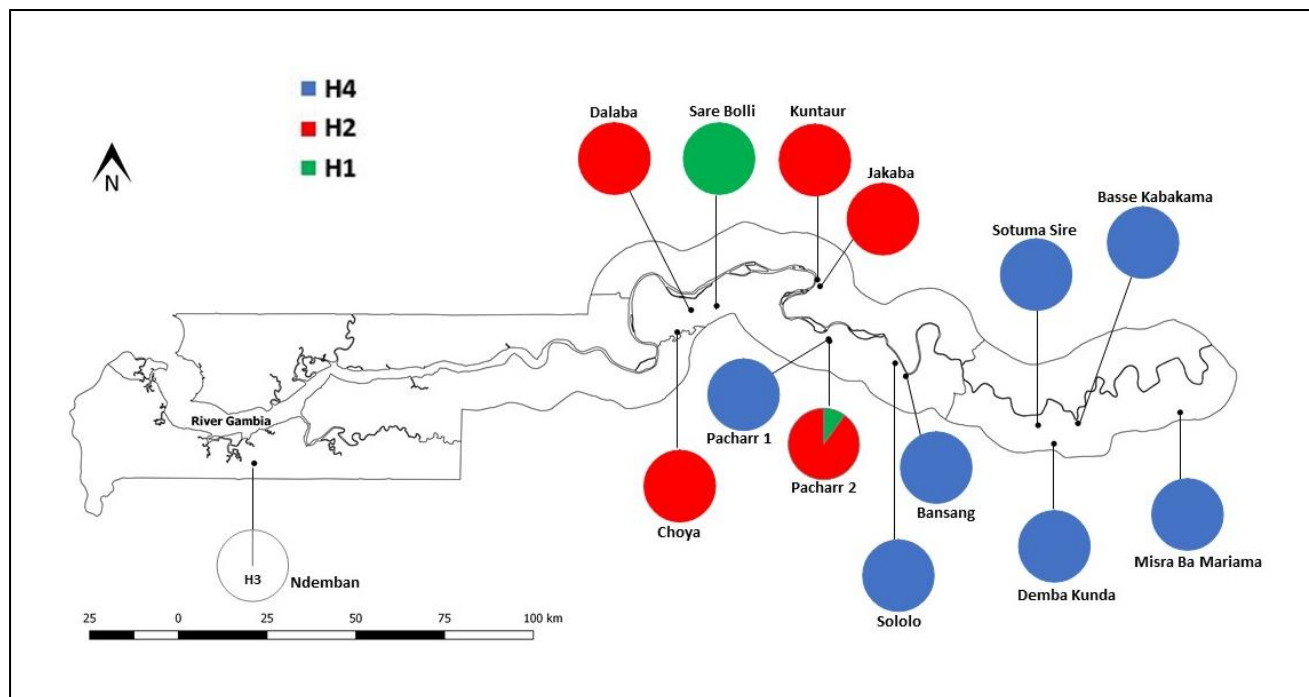
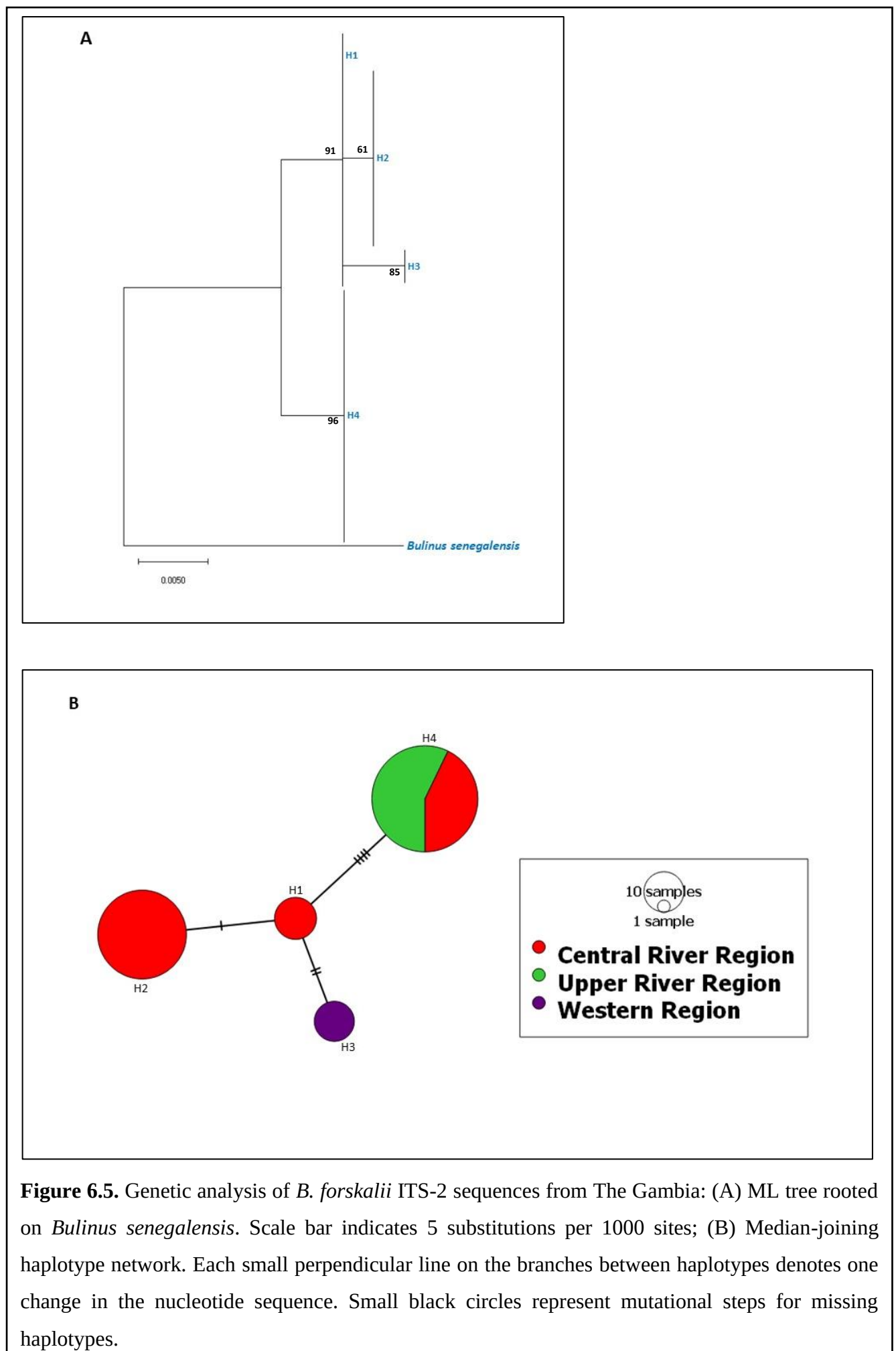


Figure 6.4. Distribution of *B. forskalii* ITS-2 haplotypes in the 14 collection sites where *B. forskalii* was found in The Gambia. Shared haplotypes are coloured according to the key. Private haplotypes are shown in white, and the haplotype number is labelled on individual pie charts.



6.3.2 Genetic variation of *B. truncatus*

There were 6 unique *cox1* haplotypes from the 30 *B. truncatus* samples analysed, with an overall haplotype diversity of 0.777 and nucleotide diversity of 0.002 (Table 6.3). All 6 haplotypes were private haplotypes (Figure 6.6). The number of each haplotype per site are detailed in Supplementary Table 6.2. Pacharr 1 had the highest number of haplotypes (three), with a haplotype diversity of 0.644 and nucleotide diversity of 0.00285 (Table 6.3). Madina Nfally 1 had two haplotypes, with a haplotype diversity of 0.200 and nucleotide diversity of 0.00031. Misra Ba Mariama had one haplotype, and thus no diversity (Table 6.3). *Cox1* genetic diversity was low, exemplified by the short branch lengths on the tree and the single nucleotide change separation of all unique *cox1* haplotypes in the network (Figure 6.7). There was no bootstrap support for intraspecific variation between haplotypes. Mean pairwise genetic distance within sites was 0.00242 for Pacharr 1, 0.00027 for Madina Nfally 1 and 0.00000 for Misra Ba Mariama. Mean pairwise genetic distance between sites was 0.00226 for Pacharr 1 and Madina Nfally 1, 0.00319 for Pacharr 1 and Misra Ba Mariama, and 0.00279 for Madina Nfally 1 and Misra Ba Mariama. Tajima's D statistic showed non-significant values (Table 6.3), indicating no selection or recent population size changes within the *B. truncatus* populations. All the *Fst* values were significant and high (Table 6.4), implying very high genetic differentiation between each of the three *B. truncatus* populations. *Fst* was lowest between Pacharr 1 and Madina Nfally 1 (0.57778) and highest between Madina Nfally 1 and Misra Ba Mariama (0.90000) (Table 6.4).

Table 6.3. Genetic diversity based on the mitochondrial cox1 and nuclear ITS-2 genes of *B. truncatus* from The Gambia

Gene	Site	No. of samples	Number of haplotypes (h)	Haplotype diversity (Hd) (SD)	Nucleotide diversity (π)	Tajima's D (p-value)	Fu's Fs
Cox1	All	30	6	0.777 (0.040)	0.002	0.226 (p > 0.10)	-0.206
	Madina Nfally 1	10	2	0.200 (0.154)	0.00031	-1.112 (p > 0.10)	-0.339
	Pacharr 1	10	3	0.644 (0.101)	0.00285	1.109 (p > 0.10)	1.755
	Misra Ba Mariama	10	1	0.000 (0.000)	0.000	-	-
ITS-2	All	30	2	0.497 (0.043)	0.00109	1.507 (p > 0.10)	1.626
	Madina Nfally 1	10	1	0.000 (0.000)	0.000	-	-
	Pacharr 1	10	2	0.356 (0.159)	0.00078	0.015 (p > 0.10)	0.417
	Misra Ba Mariama	10	1	0.000 (0.000)	0.000	-	-

Table 6.4. Fixation index (Fst) estimates for pairwise comparisons of *B. truncatus* populations using the cox1 and ITS-2 genes

		Madina Nfally 1	Pacharr 1	Misra Ba Mariama
Cox1	Madina Nfally 1	-		
	Pacharr 1	0.57778*	-	
	Misra Ba Mariama	0.90000*	0.67778*	-
ITS-2	Madina Nfally 1	-		
	Pacharr 1	0.77778*	-	
	Misra Ba Mariama	1.00000*	0.11111	-

*Fst value significant at $p < 0.05$

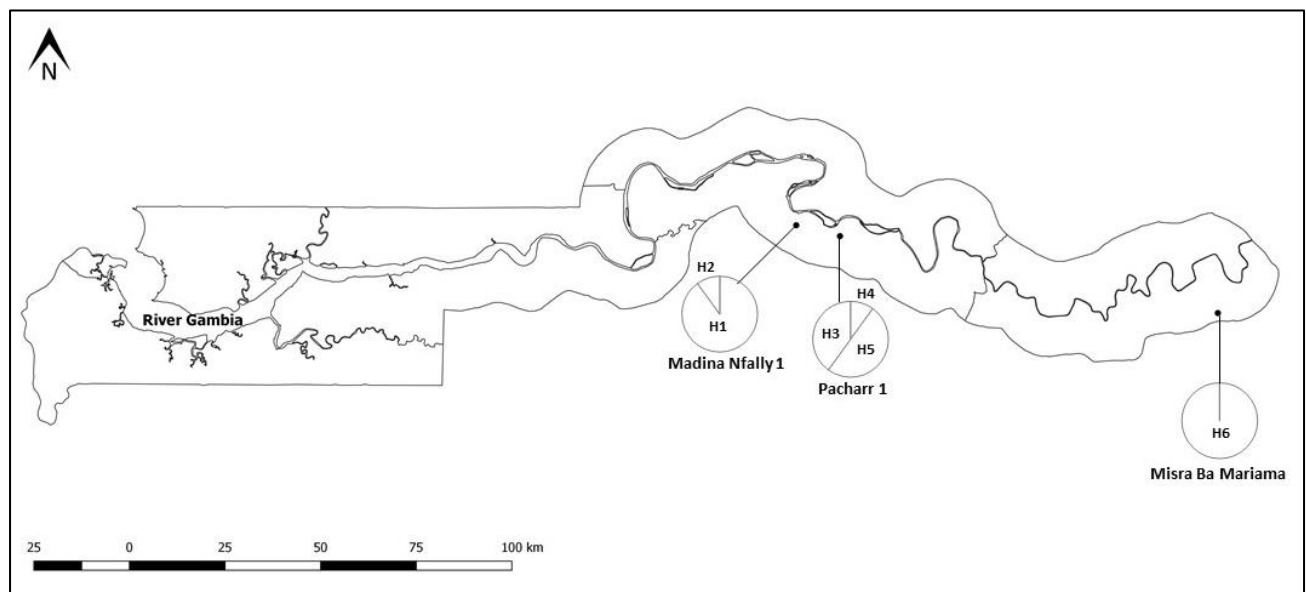
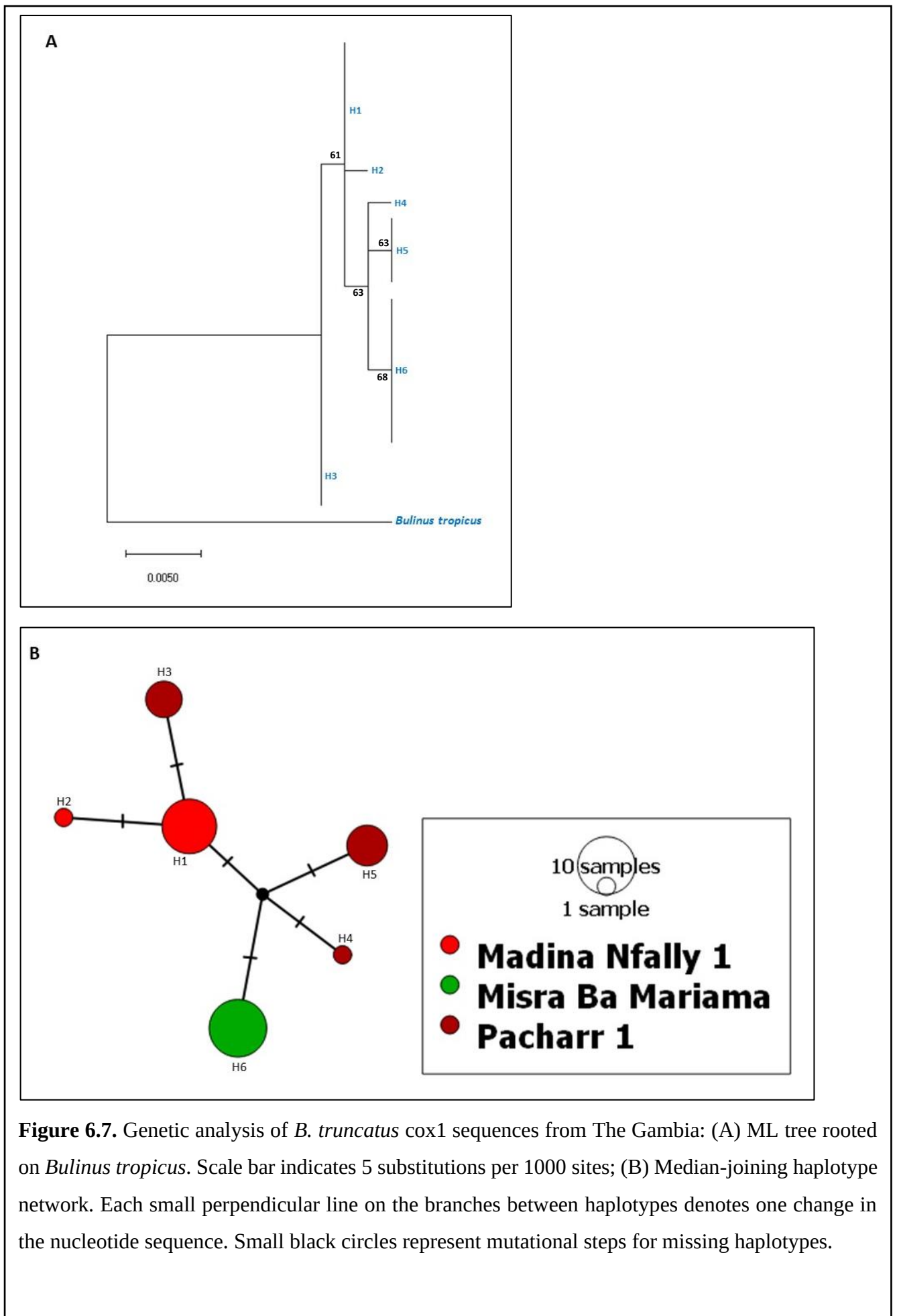


Figure 6.6. Distribution of *B. truncatus* cox1 haplotypes in the 3 collection sites where *B. truncatus* was found in The Gambia. All haplotypes are private haplotypes and are labelled on individual pie charts.



The analysis of the ITS-2 gene revealed two unique haplotypes within the three *B. truncatus* populations, with an overall haplotype diversity of 0.497 and nucleotide diversity of 0.00109 (Table 6.3). Pacharr 1 had two haplotypes, with a haplotype diversity of 0.356 and nucleotide diversity of 0.00078 (Table 6.3). The other two sites (Madina Nfally 1 and Misra Ba Mariama) each had a single haplotype and no diversity (Table 6.3). H1 was found in both Pacharr 1 and Madina Nfally 1, and H2 was shared between Pacharr 1 and Misra Ba Mariama (Figure 6.8). Genetic diversity was quite low within the ITS-2 gene and the two unique haplotypes differed by a single nucleotide change (Figure 6.9). There was no support for variation between the haplotypes on the tree. Mean pairwise genetic distance within sites was 0.00078 for Pacharr 1, 0.00000 for Madina Nfally 1 and 0.00000 for Misra Ba Mariama. Mean pairwise genetic distance between sites was 0.00177 for Pacharr 1 and Madina Nfally 1, 0.00044 for Pacharr 1 and Misra Ba Mariama, and 0.00221 for Madina Nfally 1 and Misra Ba Mariama. Tajima's D test showed no selection or recent population size changes within the *B. truncatus* populations for the ITS-2 gene (Table 6.3). Fst was significant for the pairwise comparisons between Pacharr 1 and Madina Nfally 1, and between Madina Nfally 1 and Misra Ba Mariama (Table 6.4). Genetic differentiation was very high between Pacharr 1 and Madina Nfally 1 (0.77778), and between Madina Nfally 1 and Misra Ba Mariama (1.00000) (Table 6.4).

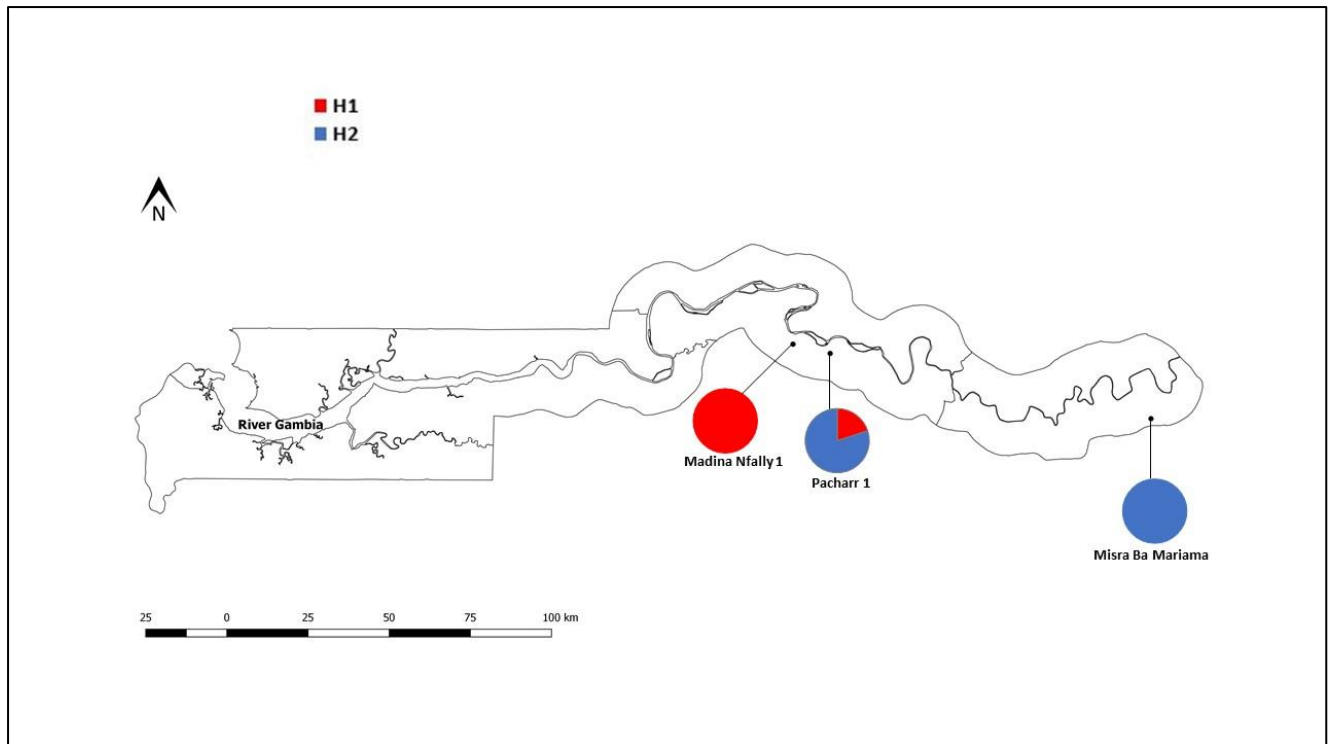


Figure 6.8. Distribution of *B. truncatus* ITS-2 haplotypes in the 3 collection sites where *B. truncatus* was found in The Gambia. Haplotypes are coloured according to the key.

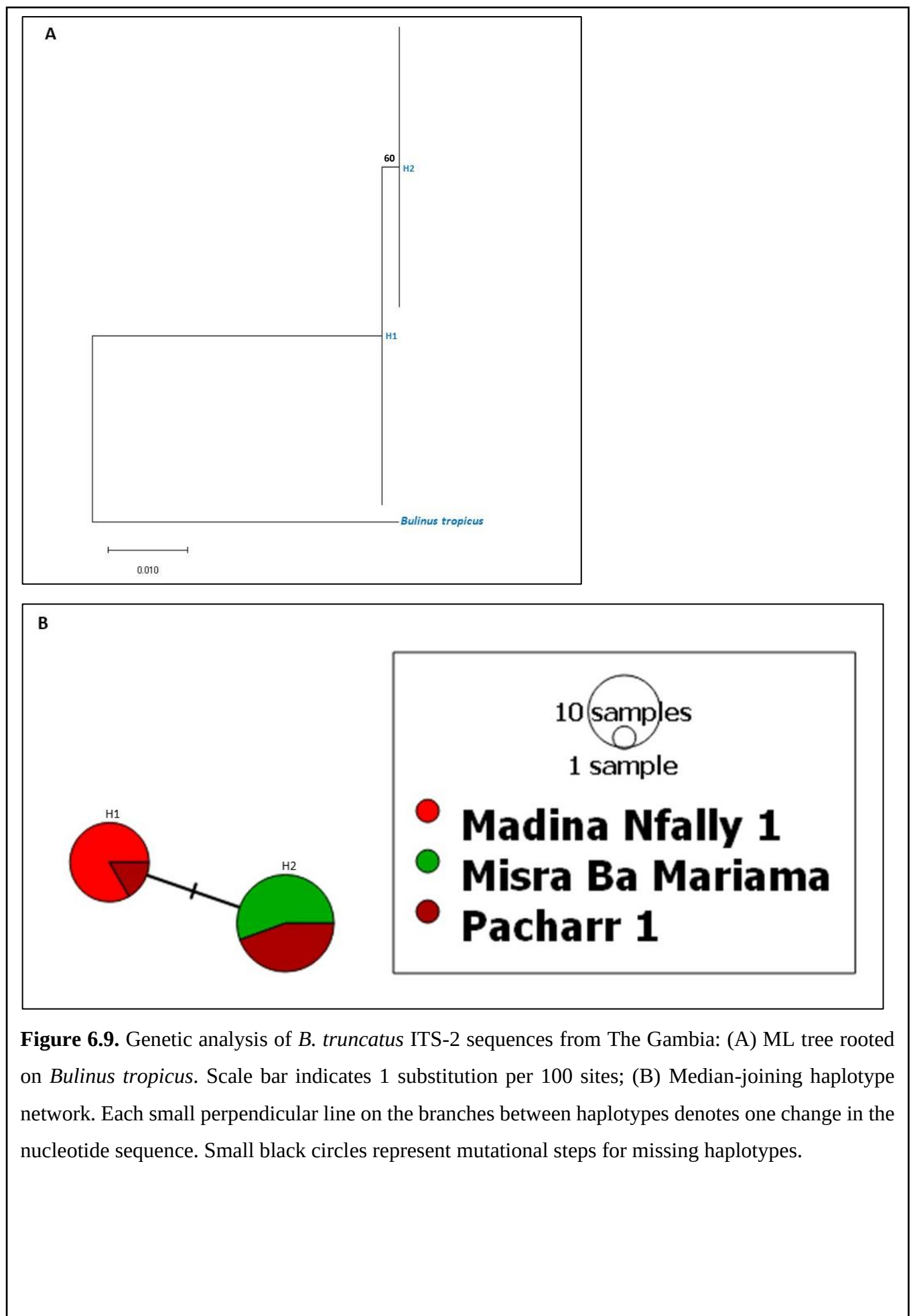


Figure 6.9. Genetic analysis of *B. truncatus* ITS-2 sequences from The Gambia: (A) ML tree rooted on *Bulinus tropicus*. Scale bar indicates 1 substitution per 100 sites; (B) Median-joining haplotype network. Each small perpendicular line on the branches between haplotypes denotes one change in the nucleotide sequence. Small black circles represent mutational steps for missing haplotypes.

6.3.3 Phylogeographic structuring

Phylogeographic analysis was conducted for the 140 novel *B. forskalii* sequences generated in this study together with GenBank *B. forskalii* sequences from 6 African countries. The sequences were split into two clusters (Groups 1 and 2) with low bootstrap support. The first cluster (Group 1) comprised sequences from Burkina Faso, Niger and Cameroon along with our Gambian haplotypes H1 – H7 (Figure 6.10A). The second cluster (Group 2) was further divided into two well supported sub-groups: one comprised of Senegalese and our Gambia haplotypes H8 – H18 and the other was made up of samples from East Africa (Tanzania and Uganda) (Figure 6.10A).

The novel *B. truncatus* sequences generated by this study were also analysed with GenBank Sequences from 9 African countries. There was a strong geographic structuring, as the samples

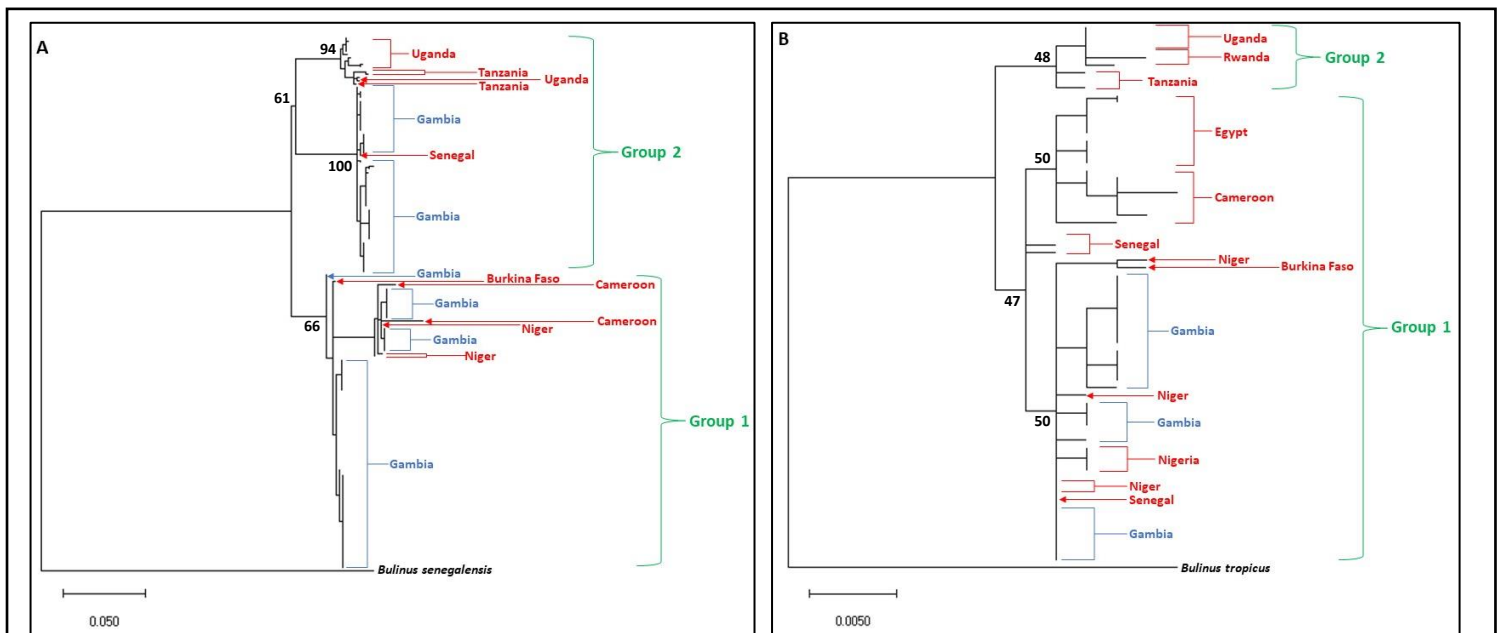


Figure 6.10. Maximum likelihood tree of: (A) our novel *B. forskalii* *cox1* sequences with GenBank *B. forskalii* *cox1* sequences from 6 African countries. Tree rooted on *B. senegalensis*. Scale bar indicates 5 substitutions per 100 sites; (B) our novel *B. truncatus* *cox1* sequences with GenBank *B. truncatus* *cox1* sequences from 9 African countries. Tree rooted on *B. tropicus*. Scale bar indicates 5 substitutions per 1000 sites. The novel Gambian sequences are shown in blue and the GenBank sequences in red. Bootstrap values are shown for only the main groups.

were mostly clustered according to their respective subregional origins (Figure 6.10B). The samples were split into two less supported groups, with one group (Group 2) exclusively comprised of samples from East Africa (Tanzania, Uganda and Rwanda) (Figure 6.10B). The other group (Group 1) is further divided into a West African lineage (Senegal, Burkina Faso, Niger and Nigeria) and a Central/North African lineage (Cameroon and Egypt) (Figure 6.10B). Our Gambian sequences all fell within the West African sub-group (Figure 6.10B). A couple of Senegalese sequences lie between these two sub-groups but were closest to the West African sub-group (Figure 6.10B).

6.3.4 Association of snail genetics and infection

Only *Schistosoma bovis* infection was detected in *B. forskalii* with an infection rate of 11.4% (Table 6.5). No pattern was observed between infection and diversity (number of haplotypes) in *B. forskalii* populations, as *S. bovis* infection was observed both in populations with high number of haplotypes and those with low number of haplotypes (Supplementary Table 6.2). Moreover, logistic regression analysis found no association between infection and diversity (number of haplotypes) in *B. forskalii* populations ($p > 0.05$). *S. bovis* infection was identified in 9 of the 18 *cox1* haplotypes and in 2 of the 4 ITS-2 haplotypes (Table 6.5). Fisher's exact test found no statistically significant association between any of these haplotypes and *S. bovis* infection ($p > 0.05$).

B. truncatus was also found infected with only *S. bovis* with an infection rate of 16.7% (Table 6.5). Just like for *B. forskalii*, no pattern was observed between infection and diversity for *B. truncatus*, as *S. bovis* infection was identified in all *B. truncatus* populations (Supplementary Table 6.2). Furthermore, logistic regression analysis found no relationship between infection and diversity (number of haplotypes) in *B. truncatus* populations ($p > 0.05$). *S. bovis* infection

was identified in 4 of the 6 unique *cox1* haplotypes, and in both two ITS-2 haplotypes (Table 6.5). No statistically significant association was found between these haplotypes and *S. bovis* infection ($p > 0.05$).

Table 6.5. Distribution of *S. bovis* infection across the *cox1* and ITS-2 haplotypes identified in *B. forskalii* and *B. truncatus* from The Gambia

Species	Gene	Haplotype name/designation	Frequency of haplotype (%)	No. infected with <i>S. bovis</i> (%)
<i>B. forskalii</i>	Cox1	H1	8 (5.7%)	1 (0.7%)
		H2	10 (7.1%)	1 (0.7%)
		H3	10 (7.1%)	1 (0.7%)
		H4	23 (16.4%)	5 (3.6%)
		H5	2 (1.4%)	0
		H6	29 (20.7%)	3 (2.1%)
		H7	2 (1.4%)	1 (0.7%)
		H8	2 (1.4%)	0
		H9	10 (7.1%)	0
		H10	1 (0.7%)	0
		H11	3 (2.1%)	0
		H12	7 (5.0%)	0
		H13	10 (7.1%)	0
		H14	10 (7.1%)	0
		H15	1 (0.7%)	0
		H16	1 (0.7%)	1 (0.7%)
		H17	10 (7.1%)	2 (1.4%)
		H18	1 (0.7%)	1 (0.7%)
		Total	140 (100%)	16 (11.4%)
	ITS-2	H1	11 (7.8%)	0
		H2	49 (35.0%)	5 (3.6%)

		H3	10 (7.1%)	0
		H4	70 (50.0%)	11 (7.8%)
		Total	140 (100%)	16 (11.4%)
<i>B. truncatus</i>	Cox1	H1	9 (30.0%)	0
		H2	1 (3.3%)	1 (3.3%)
		H3	4 (13.3%)	1 (3.3%)
		H4	1 (3.3%)	1 (3.3%)
		H5	5 (16.7%)	0
		H6	10 (33.3%)	2 (6.7%)
		Total	30 (100%)	5 (16.7%)
	ITS-2	H1	12 (40.0%)	1 (3.3%)
		H2	18 (60.0%)	4 (13.3%)
		Total	30 (100%)	5 (16.7%)

6.4 Discussion

Bulinus forskalii and *Bulinus truncatus* are freshwater snail species of medical and veterinary importance in different parts of Africa (Jourdan et al., 2001; Pages et al., 2003; Tian-Bi et al., 2019; Pennance et al., 2020a; Léger et al., 2020). The two bullinid species are confirmed intermediate hosts of *Schistosoma bovis* in The Gambia (Joof et al., 2021b). In this study, we analysed the mitochondrial cytochrome oxidase subunit 1 (cox1) gene and the nuclear internal transcribed spacer 2 (ITS-2) gene in populations of these two important *Bulinus* species from The Gambia to understand their genetic variation and structure in relation to schistosome infection within the country.

For *B. forskalii*, the mitochondrial gene (cox1) showed relatively high variation with a high overall haplotype and nucleotide diversity. Previous studies that utilised the same cox1 fragment in *B. forskalii* from Cameroon and the Albertine Rift in East Africa also found high

genetic diversity in this bulinid snail (Nalugwa et al., 2010; Zein-Eddine., 2014). However, haplotypes denoted Group 2 are quite divergent from the other two groups (Group 1 and 3) but phylogenetic analysis revealed all three haplogroups clustering with reference GenBank *B. forskalii* sequences with strong support away from other closely related *Bulinus* species. A GenBank *B. camerunensis* sequence included in the analysis clustered with *B. forskalii*, further questioning the status of *B. camerunensis* as a separate species from *B. forskalii* (Kane et al., 2008). As expected, variation was lower in the ITS-2, but it mirrored the pattern observed in the *cox1* gene, with haplotype H4 highly divergent from haplotypes H1, H2 and H3. Despite the low number of haplotypes observed for the nuclear gene (ITS-2), some variation exists with 4 unique haplotypes. For both the *cox1* and the ITS-2 genes, there was a clear haplotype structuring, with more haplotype sharing and homogeneity within *B. forskalii* from URR compared to *B. forskalii* from CRR. This is probably linked to the habitat types from which the samples were collected. URR samples were all collected from streams that are open and can easily flow into other water systems. On the contrary, CRR samples were collected largely from confined habitats such as swamps, rice fields, irrigation canals and seasonal pools that are mostly cut off from other habitats. Ecological isolation of snail populations can lead to independent evolution that give rise to divergent individuals in these populations (Zein-Eddine., 2014). Some studies on *B. forskalii* and other *Bulinus* species found more genetic variation in populations inhabiting temporal and less stable habitats (Viard et al., 1996; Viard et al., 1997; Gow et al., 2004). In contrast, other studies on freshwater snails such as *Bulinus globosus* and *Biomphalaria grabrata* observed more genetic diversity in populations from more stable and connected habitats (Mukaratirwa et al. 1996; Mavárez et al. 2002). The significant positive Tajima's D statistics for *B. forskalii* implied selection or a recent population shrinkage occurring. This is backed by the fact that most of the *B. forskalii* collection sites in this study are less stable habitat types like swamps, seasonal pools, rice fields and irrigation

canals that are susceptible to bottleneck events, e.g., seasonal floods, droughts and even man-made habitat modifications for agricultural use. Even for the less common, more stable sites (streams) in this study, there are chances of population size changes occurring through flood from seasonal heavy rains or severe water level recedence due to the long dry season (7-8 months) in The Gambia. The significant high F_{st} estimates implied high genetic differentiation between the regional populations. A high degree of genetic differentiation has been observed in highly selfing freshwater snail species such as *B. forskalii* (Gow et al., 2004), *B. truncatus* (Viard et al. 1996; Viard et al., 1997; Zein-Eddine., 2017) and *Biomphalaria pfeifferi* (Charbonnel et al. 2002). This high genetic segregation is attributed to self-fertilisation and population bottlenecks increasing the influence of genetic drift in shaping genetic structure (Gow et al., 2004). The phylogeographic analysis of these *B. forskalii* samples with GenBank sequences of *B. forskalii* from other African countries showed some of our Gambian samples clustering with the GenBank Sequences from West Africa and Cameroon. Some Senegalese and Gambian samples also clustered with East African Samples. Kane et al. (2008) also found a clustering of West African *B. forskalii* with *B. forskalii* group isolates from East Africa.

While the overall number of *cox1* haplotypes for *B. truncatus* was high, nucleotide diversity was low with no haplotype sharing between the sites. Low genetic variation and strong population differentiation is a characteristic of self-fertilising snails like *B. truncatus* (Viard et al., 1996; Viard et al., 1997; Zein-Eddine et al., 2017). Diversity within the ITS-2 gene was low with just two haplotypes separated by a single nucleotide difference. This low diversity observed for *B. truncatus* in this study is not surprising, as several previous studies that utilised the same genes found low diversity in *B. truncatus* (Zein-Eddine., 2017; Abe et al., 2018; Tumwebaze et al., 2019). For instance, *B. truncatus* from Upper Egypt and the Nile Delta were observed to have little genetic diversity (Zein-Eddine et al., 2017). Additionally, a study found low haplotype and nucleotide diversity in *B. truncatus* collected from Giza and Sharkia

governorates in Egypt (Abe et al., 2018). Tumwebaze et al. (2019) also observed a single haplotype in *B. truncatus* from Crater Lakes of Western Uganda. In contrast to these findings, a couple of studies found high genetic diversity in *B. truncatus* (Zein-Eddine et al., 2014; Chibwana et al., 2020). Chibwana et al. (2020) found high haplotype and nucleotide diversity with limited haplotype sharing within *B. truncatus* populations collected from Lake Victoria and surrounding aquatic habitats. Tajima's D statistic showed no selection among the *B. truncatus* populations involved in the study. The significant and high Fst values means high genetic differentiation between the populations. Strong genetic differentiation among populations is typical of self-fertilising freshwater snails such as *B. truncatus* (Viard et al. 1996; Viard et al., 1997; Charbonnel et al. 2002; Gow et al., 2004; Zein-Eddine et al., 2017). The phylogeographic analysis of *B. truncatus* showed a strong sub-regional partitioning, with West African sequences clearly separated from samples from Cameroon, Egypt and East Africa. Abe et al. (2018) observed a clear separation of West African *B. truncatus* from those of Egypt, Sudan and Zimbabwe. Our novel Gambian *B. truncatus* samples cluster as expected with GenBank *B. truncatus* sequences from W. Africa.

Our study found no association between snail population diversity or snail genotype and *S. bovis* infection for either *B. forskalii* or *B. truncatus*. However, we observed higher infection rate of *S. bovis* in *B. truncatus* (16.7%) compared to *B.forskalii* (11.4%). Loss of genetic variation can increase host population susceptibility to parasitic infection (Schmid-hempel and Stauffer, 1998; Coltman et al., 1999). Both *B. forskalii* and *B. truncatus* are implicated in the transmission of only *S. bovis* in The Gambia according to a recent study (Joof et al., 2021b). However, an earlier study (Smithers, 1956) observed natural specimens of *B. truncatus* (then referred to as *B. guernei*) shedding cercariae of *S. haematobium* in The Gambia. This difference in observations could be due to various reasons. Firstly, it could be due to the small number of *B. truncatus* found in this study. Secondly, it might be attributed to the drastic decline in the

prevalence of urogenital schistosomiasis in the country over the period separating the two studies through availability of praziquantel and pipe-borne water, improved sanitation, and less use of freshwater sites by people. Lastly, the reason could be genetic as host-parasite co-adaptation and genetic interplay have an implication for the level parasitism in local host snail populations (Minchella and LoVerde, 1993). The limitations of the study are low sample size for population genetic analysis, microsatellite markers which could provide further genetic information were not utilised, and lack of appropriate methods to correlate snail genotypes (haplotypes) with snail infections.

6.5 Conclusion

Molecular studies provide information on the genetic diversity and structure of intermediate snail hosts, which in turn fosters our understanding of host-parasite interactions. Snail population genetics is increasingly becoming important in the epidemiology and integrated control approaches of schistosomiasis. Our study reported for the first time, the population genetic structure of *B. forskalii* and *B. truncatus* in relation to *S. bovis* infection in The Gambia. The study found high genetic variation with lower infection rate in *B. forskalii* and less genetic variation with higher infection rate in *B. truncatus*. Future studies involving extensive field sampling and parasite genetics are required to further understand the demography and host-parasite interactions of these two bulinid species in The Gambia. These studies should also include laboratory experiments to test compatibility of *B. truncatus* and *S. haematobium* in the country.

7 General discussion

This study was intended to provide an all-inclusive understanding of the transmission dynamics of schistosomiasis in The Gambia through disease prevalence assessment in humans and malacological studies including intermediate snail host distribution, infection and genetics. The goal was to provide the essential information required to lay the foundation for an integrated control approach for schistosomiasis in the country. The two broad areas that underpinned the study are discussed.

7.1 Disease epidemiology in humans

As expected, our study found urogenital schistosomiasis as the dominant form of schistosomiasis in The Gambia, with very rare cases of intestinal schistosomiasis. In line with historical documentation of the disease in The Gambia, the eastern part of the country (Central River Region – CRR and Upper River Region – URR) was found to carry almost the entire burden (>98%) of urogenital schistosomiasis. The prevalence levels observed among school children from these two regions in our study represent a decreasing trend in the burden of urogenital schistosomiasis when compared to the prevalence levels observed about 40 years ago (Goll et al., 1984). Availability of Praziquantel, safe water supply, improved sanitation, and less use of freshwater sites by people are credited for this decline. However, infection was observed to be high and focal in some communities such as Jahanka (48%), Njoren (46%), Changai (63%) and Pallol (51%) in CRR, and Kiskis (38%) and Kolibantang (34%) in URR. In the absence of comprehensive control strategies, ultimate control and eradication will remain an illusion. Mass drug administration (MDA) will be useful in the control of schistosomiasis in The Gambia. Following MDA, further prevalence assessment studies will be required to assess its impact in the country. It is a recommendation that more sensitive techniques such as point of care-circulating cathodic antigen (POC-CCA) test or polymerase chain reaction (PCR) are used as a complement to traditional parasitological methods in surveys following MDA.

Given the findings of numerous studies that found POC-CCA unsuitable for mapping in *S. haematobium* endemic areas like The Gambia (Sanneh et al., 2017), PCR becomes one of the most viable complementary options for MDA impact assessment studies in The Gambia. Circulating anodic antigen (CAA) assay is another promising method that can be utilised in these studies as it works well for all *Schistosoma* species (Corstjens et al., 2020). We found detection of blood in urine (haematuria) as a useful supplementary parameter for urogenital schistosomiasis diagnosis. This technique is increasingly becoming important as a supplementary technique for field diagnosis of urogenital schistosomiasis, and self-reported blood in urine has been used to diagnose infection in adults living in endemic areas (Gray et al., 2011).

As often seen in many studies that assessed risk factors of schistosomiasis, our study found more infection rate and risk for boys than girls. This is highly attributed to the lifestyle and high-risk behaviour of boys around infested water bodies. In addition to these inherent male gender behaviours, certain socio-cultural factors predispose boys to schistosomiasis infection in The Gambia. These socio-cultural factors include boys being tasked to work on farms near freshwater sites and rearing of family livestock that utilised freshwater sites for drinking (Figure 7.1A). An unexpectedly low number of infected children responded that they visit freshwater sites. Given the aetiology and epidemiology of schistosomiasis and our broad observations, we think the children may have given inaccurate responses about their water contact behaviours. Anxiety and fear of being reprimanded might be the reason for the children giving false responses. To ensure accurate responses to questionnaires in studies involving children, more innovative, collaborative frameworks and approaches should be utilised. Survey questionnaires and interviews should not resemble a school test, and children should be reassured that there is no wrong answer and that they will not be reprimanded for giving any particular answer. Cultural nuances which may have ‘unaccounted for’ confounder effects

should also be considered when designing research questionnaires for children. Future epidemiological studies on schistosomiasis in The Gambia should assess the knowledge, attitude and practices of children and their parents/guardians. Health education on schistosomiasis transmission and prevention should be promoted by the Ministry of Health in schistosomiasis endemic regions like CRR and URR. Efforts should also be made to increase access to clean and safe water supply in these regions where a good proportion of the community members still rely on freshwater pools and streams as source of water for some chores like washing clothes (Figure 7.1B).



Figure 7.1. Use of freshwater sites by people during our snail sampling period: A – young boys watering livestock such as cattle at a seasonal pool in Jahanka, CRR; B – women washing clothes at a stream in Misra Ba Mariama, URR.

7.2 Intermediate host snail dynamics

Following the first report on the existence of human schistosomiasis in The Gambia in 1945 (Jones and Thomas, 1945), several studies were conducted on the intermediate host snails over the next 40 years (McCullough and Duke, 1954; Smithers et al., 1956; Goll et al., 1984; Blumenthal, 1985). After this period, studies on intermediate host snails involved in the transmission of schistosomiasis in the country has stalled. Our study provides the first report on intermediate snail host of schistosomes in The Gambia in over three decades. *Bulinus* snails

are one of the most predominant freshwater snail species in The Gambia; we found them in 98% of collection sites where freshwater snails exist. *Bulinus senegalensis* was the most predominant *Bulinus* species and exists on both the south bank and north bank of the River Gambia. It was also observed in all regions of the country except the North Bank Region (NBR), though only one specimen of this species was found in the Lower River Region (LRR). This pattern of distribution of *B. senegalensis* in our study is similar to its distribution in neighbouring Senegal, especially in the central regions where it maintains seasonal transmission of urogenital schistosomiasis (Senghor et al., 2015). The distribution of urogenital schistosomiasis in our study is similar to the distribution pattern of *B. senegalensis* as the species was predominantly found in CRR and URR. This is attributed to the occurrence of a sandstone plateau that supports the existence of seasonal pools, the preferred habitat type of *B. senegalensis*, in these two regions (Goll et al., 1984). Our study revealed that *B. senegalensis* is the only intermediate host of the human parasite *S. haematobium* in The Gambia. It is also involved in the transmission of the livestock parasite *S. bovis* in the country. *B. senegalensis* was particularly found infected with *S. haematobium* in four communities: Jahanka, Njoren and Sare Jabel in CRR and Dingiri in URR. Our human study found high prevalence of urogenital schistosomiasis among school children from Jahanka and Njoren. Sare Jabel and Dingiri were not sampled in our human study due to the random sampling approach, but human surveys conducted in these communities prior to our malacological sampling found high prevalence of urogenital schistosomiasis in these communities (Serigne J. Ceesay - Medical Research Council, The Gambia, Personal Communication). Our study demonstrates the importance of *B. senegalensis* in maintaining transmission of urogenital schistosomiasis at seasonal rain-fed pools in West Africa as previously reported in other studies (Vera et al., 1992; Senghor, 2015).

It is a common view that genetic diversity of intermediate snail host populations has an influence on the dynamics of schistosome parasitism in the populations (Minchella and LoVerde, 1993; Campbell et al., 2010; King and Lively, 2012). Populations with low diversity are more prone to infection than more genetically diverse populations (Campbell et al., 2010; King and Lively, 2012). To further our understanding of schistosome-host snail interactions, we characterised the genetic diversity and structure of *B. senegalensis* populations from The Gambia. Although we found several haplotypes (genotypes), diversity was relatively low in *B. senegalensis* from The Gambia compared to other studies (Zein-Eddine et al., 2014). There was a pattern in the distribution of haplotypes with some shared haplotypes restricted to only certain parts and regions of the country while some were found broadly across multiple regions of the country. We observed no association between genetic diversity and schistosome infection in *B. senegalensis*. We however found a statistically significant link between *B. senegalensis* genotype and *S. bovis* infection. One *cox1* haplotype (H24) and one ITS2 haplotype (H7) were found associated with *S. bovis* infection. This *cox1* haplotype (H24) was found in 20% of *B. senegalensis* analysed and was distributed in 11 sites, mostly in URR. The ITS2 haplotype (H7) was found in about 30% of *B. senegalensis* and was distributed in 15 sites across CRR and URR. The implication of this is that the two haplotypes may have an influence on *S. bovis* infection outcome in *B. senegalensis*, and the transmission of the parasite might be high in areas where these snail genotypes are found. No statistically significant relationship was observed between *B. senegalensis* genotypes and *S. haematobium* infection, but quite possibly this is due to the low number of *S. haematobium* infected snails in this study.

Bulinus forskalii was the second most common species in this study and was mostly found in CRR and URR often inhabiting permanent streams and rice fields as reported by previous studies in Senegambia (Goll et al., 1981; Diaw et al., 1989b). It was also observed in a swamp in the Western Region (WR). *Bulinus truncatus*, the rarest species of all *Bulinus* species found

in this study, exists in CRR and URR inhabiting streams and irrigation canals. The freshwater tributaries (streams) of the River Gambia in CRR and URR support the occurrence of *B. forskalii* and *B. truncatus* in these regions. Additionally, activities of a rice growing irrigation scheme in CRR by The Ministry of Agriculture create a suitable ecology for these two *Bulinus* species. While *B. forskalii* and *B. truncatus* were not found infected with the human parasite *S. haematobium*, they were both found harbouring infections of the livestock parasite *S. bovis* at high rates across CRR and URR. *B. forskalii* may not be a natural host of *S. haematobium* in West Africa but is an important intermediate host of *S. bovis* in many parts of the sub-region (Diaw et al., 1989b, Pennance et al., 2020a). In contrast, *Bulinus truncatus* is a confirmed intermediate host of *S. haematobium* and *S. bovis* in several West African countries (Sene et al., 2004; Tian-Bi et al., 2019; Pennance et al., 2020a; Léger et al., 2020). It is also reported to harbour the hybrids of *S. haematobium* and *S. bovis* in many parts of West Africa (Huyse et al., 2009; Tian-Bi et al., 2019; Pennance et al., 2020a). Indeed, an earlier study observed natural specimens of *B. truncatus* (then referred to as *B. guernei*) shedding cercariae of *S. haematobium* in The Gambia (Smithers, 1956). The difference in observations between this earlier study and our current study might be attributed to the small number of *B. truncatus* found in this study, the drastic decline in the prevalence of urogenital schistosomiasis in the country or a combination of the two factors. The high incidence of *S. bovis* observed in CRR and URR should give attention to this neglected livestock schistosome and a ‘one health approach’ to the control of schistosomiasis in The Gambia should be considered (Léger et al., 2020).

Our population genetic analysis found high genetic variation within *B. forskalii* with a clear haplotype structuring. More haplotype sharing and homogeneity was observed in *B. forskalii* from URR compared to *B. forskalii* from CRR. *B. truncatus* had a relatively low genetic diversity with less haplotype sharing between populations. No statistically significant

association was found between snail population diversity or snail genotype and *S. bovis* infection for either *B. forskalii* or *B. truncatus*.

Despite the high incidence and presence of *Bulinus* snails in this study, no *Biomphalaria* snails were found at any of the sites sampled. This observation, coupled with no report of *Biomphalaria* in several previous studies and the corresponding low detection of *S. mansoni* infection in humans, adds weight to the general view that intestinal schistosomiasis might not exist in The Gambia. The rare cases of the disease occasionally detected in people in the country might be imported cases from neighbouring countries such as Senegal and Guinea Conakry which are endemic for intestinal schistosomiasis (Hodges et al., 2011; Catalano et al., 2020). People in The Gambia has close family ties to Senegal and Guinea Conakry, and there is constant movement of people between The Gambia and these two countries. Future studies on schistosomiasis in The Gambia should collect information on travel history of participants to neighbouring countries that are endemic for intestinal schistosomiasis.

The findings of our study will be essential for the initiation and set-up of an integrated control approach for schistosomiasis in The Gambia. This integrated control strategy should include disease surveillance, prevention and control in humans through chemotherapy and health education as well as snail surveillance and control. Further operational studies will be required to effectively inform a continuous and successful implementation of this control approach in The Gambia. These studies should include both human epidemiological and malacological components. The studies should investigate the potential occurrence of *S. haematobium* group hybrids in humans and snail species such as *B. senegalensis* and *B. truncatus*. These studies should also investigate the compatibility of *B. truncatus* and *S. haematobium* in The Gambia. The elimination of schistosomiasis from The Gambia is within reach due to the focal nature and seasonality of transmission in the country. Future works in the country should employ

integrated control approaches of chemotherapy, snail control, health educational/behavioural interventions, and improved sanitation and safe water supply.

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Supplementary material

Supplementary Table 3.1. Prevalence of urogenital schistosomiasis by school

School Name	Region	<i>S. haematobium</i> Prevalence (%)	Latitude	Longitude
Chagai wollof lower basic school	CRR	63	13.58341	-14.6659
Jahanka Lower Basic School	CRR	48	13.37859	-14.7312
Raneru Lower Basic School	CRR	17.6	13.62318	-14.5462
Fory Lower Basic School	CRR	11.8	13.50314	-14.4993
Njoren Basic Cycle School	CRR	46	13.34511	-14.6408
Jamagan Lower Basic School	CRR	5.9	13.58508	-15.1838
Kudang Lower Basic School	CRR	7.8	13.66437	-15.0614
Jarreng Badala Lower Basic School	CRR	5.7	13.63774	-15.1918
Pallol Lower Basic School	CRR	51	13.7584	-14.8652
Jockul Ndowen Lower Basic School	CRR	19.2	13.77997	-14.8916
Fatoto Lower Basic School	URR	0	13.41254	-13.8877
Sotuma Lower Basic School	URR	0	13.44038	-13.8518
Murreh Kunda Lower Basic School	URR	24	13.47533	-14.0485
Suduwol Basic Cycle School	URR	0	13.38153	-13.9588
Kiskis Lower Basic School	URR	38	13.30364	-14.1529
Njayel Lower Basic School	URR	0	13.33473	-14.0206
Koli Bantang Lower Basic School	URR	34	13.43335	-14.1971
Touba Woppa Lower Basic School	URR	22	13.46138	-14.2286
Sare Alpha Lower Basic School	URR	1.9	13.38153	-13.9588
Kuwonku Lower Basic School	URR	0	13.42195	-14.3072
Pakalinding Lower Basic School	LRR	0	13.46594	-15.5562
Badumeh Kuta Lower Basic School	LRR	0	13.39619	-15.381
Dongoroba Lower Basic School	LRR	6	13.38078	-15.2975
Nema kuta Basic Cycle School	LRR	0	13.41579	-15.6924
Kolior Lower Basic School	LRR	0	13.42018	-15.6694
Sibito Lower Basic School	LRR	0	13.38273	-15.7301
Bumari Lower Basic School	LRR	0	13.39839	-15.7814
Kuyang Lower Basic School	LRR	0	13.34174	-16.1021
Jouli Lower Basic School	LRR	0	13.40005	-16.0969
Nioro Jattaba Lower Basic School	LRR	0	13.29287	-15.8305
Challa Lower Basic School	NBR	0	13.56832	-15.5547
Ngerr Lower Basic School	NBR	0	13.58771	-15.6475
Yallal Tankonjala Lower Basic School	NBR	0	13.55862	-15.703
Katchang Lower Basic School	NBR	0	13.50389	-15.7553
Konteh kunda Lower Basic School	NBR	0	13.56722	-15.7942
Kerr Ndongo Lower Basic School	NBR	0	13.58864	-15.7165
Kerr Ardo Lower Basic School	NBR	0	13.57961	-16.0476
Mbamori Kunda Lower Basic School	NBR	0	13.5836	-16.0015
Ndungu Kebbeh Basic Cycle School	NBR	0	13.5427	-16.3181
Ndofan Lower Basic School	NBR	0	13.52746	-16.4325

Supplementary Table 4.1. Actual snail counts and GPS location coordinates of sampling sites

No	Site	GPS coordinates	Region	Habitat type	Snail abundance				
					<i>B. senegalensis</i>	<i>B. forskalii</i>	<i>B. truncatus</i>	Total <i>Bulinus</i>	Other snails
1	Sare Bolli	13.609017 -15.14675	CRR	Seasonal pool	12	13	0	25	<i>Lanistes spp.</i> : 5
2	Madina Nfally 1	13.543967 -14.959617	CRR	Stream	0	0	10	10	
3	Madina Nfally 2	13.54395 -14.959117	CRR	Seasonal pool	110	0	0	110	
4	Kudang Bridge	13.658867 -15.071667	CRR	Seasonal pool	1	0	0	1	
5	Kundang Tenda	13.6891 -15.077283	CRR	River	0	0	0	0	
6	Jarreng Bridge	13.617917 -15.196067	CRR	Stream	0	0	0	0	
7	Jarreng Badala	13.6396 -15.19395	CRR	River	0	0	0	0	
8	Sare Anis 1 (sofaniama Bolong)	13.556167 -15.188367	CRR	Stream	0	0	0	0	
9	Sare Anis 2	13.558167 -15.1867	CRR	Stream	0	0	0	0	
10	Daru	13.36944 -14.60019	CRR	Seasonal pool	161	0	0	161	
11	Sanka Bari	13.34949 -14.63018	CRR	Seasonal pool	115	0	0	115	
12	Njoren	13.34598 -14.64303	CRR	Seasonal pool	119	0	0	119	
13	Sinchu Bokar	13.41695 -14.70525	CRR	Seasonal pool	83	0	0	83	

14	Sare Madi Ganteh	13.41991 -14.72219	CRR	Seasonal pool	91	0	0	91	
15	Kerr Ousman Boye	13.41423 -14.74174	CRR	Seasonal pool	70	0	0	70	
16	Sare Jabel	13.38333 -14.70000	CRR	Seasonal pool	238	0	0	238	
17	Jahanka	13.37776 -14.72773	CRR	Seasonal pool	68	0	0	68	
18	Sare Madi Babadi	13.44930 -14.69858	CRR	Seasonal pool	17	0	0	17	
19	Sare Chewto	13.42820 -14.59223	CRR	Seasonal pool	44	16	0	60	
20	Bansang	13.42670 -14.65623	CRR	Rice field	0	76	0	76	
21	Sololo	13.46059 -14.68370	CRR	Swamp	5	20	0	25	
22	Dobo	13.47782 -14.64302	CRR	Seasonal pool	39	0	0	39	
23	Dembakally	13.59259 -14.61033	CRR	Seasonal pool	2	0	0	2	
24	Changai 2	13.58624 -14.67834	CRR	Seasonal pool	92	0	0	92	
25	Changai 1	13.57868 -14.66852	CRR	Seasonal pool	0	0	0	0	
26	Raneru 2	13.63299 -14.54088	CRR	Seasonal pool	1	0	0	1	
27	Raneru 1	13.63092 -14.54611	CRR	Seasonal pool	0	0	0	0	
28	Kuntaur Fulakunda/ Jakaba	13.65983 -14.87867	CRR	Rice field	0	24	0	24	
29	Jokul	13.78485	CRR	Seasonal pool	0	0	0	0	

	Ndowen	-14.88732							
30	Pallol 2	13.75574 -14.85203	CRR	Seasonal pool	0	0	0	0	
31	Pallol 1	13.76528 -14.85914	CRR	Seasonal pool	0	0	0	0	
32	Sapu	13.552733 -14.89715	CRR	Rice field	0	0	0	0	
33	Pacharr 1	13.517733 -14.8543	CRR	Irrigation canal	0	12	41	53	<i>Radix natalensis</i> : 57; <i>Gyralus spp</i> : 4
34	Pacharr 2	13.522917 -14.856383	CRR	Rice field	0	10	0	10	<i>Gyralus spp</i> : 32
35	Dalaba	13.598217 -15.21305	CRR	Stream	2	18	0	20	<i>Gyralus spp</i> : 27
36	Kuntaur Wharf Town	13.676867 -14.885517	CRR	Rice field	0	18	0	18	<i>Radix natalensis</i> : 1; <i>Gyralus spp</i> : 7; <i>Lanistes spp</i> : 2
37	Pallang	13.66535 -14.86895	CRR	Stream	0	0	0	0	<i>Radix natalensis</i> : 107
38	Sankulay Kunda	13.51318 -14.76011	CRR	Seasonal pool	26	0	0	26	
39	Janjanbureh	13.52557 -14.7639	CRR	Rice field	7	12	0	19	
40	Kuntaur 2	13.66493 -14.89007	CRR	Rice field	0	0	0	0	
41	Wassu	13.69895	CRR	Seasonal pool	36	0	0	36	

		-14.88602							
42	Pacharr 3	13.49936 -14.84492	CRR	Seasonal pool	0	0	0	0	
43	Jahally	13.54481 -14.9659	CRR	Seasonal pool	27	0	0	27	
44	Choya	13.54108 -15.24912	CRR	Stream	0	31	0	31	
45	Cha Kunda	13.38555 -14.53051	CRR	Seasonal pool	0	0	0	0	
46	Sare Modou	13.39387 -14.61993	CRR	Seasonal pool	0	0	0	0	
47	Dobo 2	13.44479 -14.64437	CRR	Seasonal pool	6	0	0	6	
48	Kunting	13.53171 -14.66579	CRR	Seasonal pool	40	0	0	40	
49	Sami Karantaba	13.55906 -14.56813	CRR	Seasonal pool	1	0	0	1	
50	Dembakally Njagga	13.59446 -14.59227	CRR	Seasonal pool	0	0	0	0	
51	Dingiri 1 (Dingiri Kore)	13.28580 -14.04369	URR	Seasonal pool	81	0	0	81	
52	Dingiri 2	13.30101 -14.05310	URR	Seasonal pool	0	0	0	0	
53	Kuwonku (Koilu Dalo)	13.42475 -14.33871	URR	Seasonal pool	96	0	0	96	
54	Touba Woppa	13.45798 -14.226	URR	Seasonal pool	0	0	0	0	
55	Madina Samaco (Toro)	13.29197 -14.01417	URR	Seasonal pool	68	0	0	68	
56	Bajakunda	13.47509 -14.04987	URR	Seasonal pool	30	0	0	30	

57	Koli Bantang (bamba dala)	13.43636 -14.17679	URR	Seasonal pool	33	0	0	33	
58	Murreh Kunda	13.53689 -14.0881	URR	Seasonal pool	0	0	0	0	
59	Diabugu Basilla (misigi)	13.316533 -13.979717	URR	Seasonal pool	201	0	0	201	
60	Kisskiss	13.30309 -14.16010	URR	Stream	0	0	1	1	
61	Basse Kobakunda	13.32553 -14.22385	URR	Stream	0	10	0	10	
62	Demba Kunda	13.25185 -14.27178	URR	Stream	0	20	0	20	
63	Dampha Kunda	13.32033 -14.20051	URR	Stream	0	28	0	28	<i>Gyralus spp:</i> 2
64	Chamoi	15.55500 -14.17032	URR	Stream	0	16	0	16	<i>Gyralus spp:</i> 5
65	Sare Alpha	13.36609 -13.98098	URR	Stream	0	3	0	3	<i>Gyralus spp:</i> 1
66	Sare Alpha2	13.364717 -13.976367	URR	Stream	0	0	0	0	
67	Missira Ba Mariama	13.33295 -13.94445	URR	Stream	0	77	39	116	<i>Gyralus spp:</i> 33
68	Sotuma Sire/Samba	13.29975 -14.312933	URR	Stream	0	157	0	157	<i>Gyralus spp:</i> 9; <i>Lanistes spp:</i> 1
69	Sutukonding	13.37796 -14.22345	URR	Seasonal pool	69	0	0	69	
70	Kanubeh	13.3208 -14.2964	URR	Seasonal pool	59	0	0	59	
71	Hellakunda	13.27951 -14.37461	URR	Stream	0	2	0	2	

72	Madina Samba Jawo	13.32174 -14.42531	URR	Seasonal pool	1	0	0	1	
73	Mankamang Kunda	13.3267 -14.45029	URR	Seasonal pool	0	0	0	0	
74	Sare Pateh Bakery	13.32705 -14.51422	URR	Seasonal pool	16	0	0	16	
75	Darsilameh	13.40236 -14.26843	URR	Seasonal pool	0	0	0	0	
76	Sumakunda	13.39877 -14.3152	URR	Seasonal pool	0	0	0	0	
77	Sare Fodike	13.39596 -14.35416	URR	Seasonal pool	0	0	0	0	
78	Diabug Batapa	13.39236 -14.41309	URR	Seasonal pool	0	0	0	0	
79	Kuwonku 2	13.39877 -14.3152	URR	Seasonal pool	0	0	0	0	
80	Chamoi 2	13.46208 -14.10999	URR	Seasonal pool	17	0	0	17	
81	Sutukoba	13.49646 -14.02709	URR	Seasonal pool	0	0	0	0	
82	Makka Masireh	13.56516 -13.99013	URR	Seasonal pool	0	0	0	0	
83	Wellingara Yarreh	13.54152 -14.00332	URR	Seasonal pool	43	0	0	43	
84	Wellingara Yarreh 2	13.54845 -13.99094	URR	Seasonal pool	0	0	0	0	
85	Gunjur	13.55409 -14.02202	URR	Seasonal pool	0	0	0	0	
86	Murreh Kunda 2	13.54345 -14.05137	URR	Seasonal pool	0	0	0	0	
87	Sare Juldeh	13.33624 -14.04408	URR	Seasonal pool	0	0	0	0	

88	Suduwol	13.37747 -13.97064	URR	Seasonal pool	27	0	0	27	
89	Kusan/ Temato	13.38461 -13.93328	URR	Seasonal pool	0	0	0	0	
90	Basse Kabakamma	13.30485 -14.20964	URR	Stream	0	20	0	20	
91	Kanjapat	13.22957 -15.9194	WR	Seasonal pool	0	0	0	0	
92	Ndemban	13.20117 -16.34642	WR	Swamp	1	19	0	20	
93	Kampanti	13.19921 -16.11379	WR	Seasonal pool	0	0	0	0	
94	Kafuta	13.19351 -16.44991	WR	Seasonal pool	0	0	0	0	
95	Jiboro	13.1678 -16.57662	WR	Swamp	0	0	0	0	
96	Sifoe/Kitty	13.21601 -16.67436	WR	Seasonal pool	28	0	0	28	
97	Kotu	13.460706 -16.69386	WR	Swamp	0	0	0	0	
98	Wellengara Ba 1	13.414983 -15.242017	LRR	Seasonal pool	1	0	0	1	
99	Wellengara Ba 2	13.391717 -15.277667	LRR	Stream	0	0	0	0	
100	Dongoro Ba	13.381733 -15.302017	LRR	Stream	0	0	0	0	
101	Nioro Jataba	13.42922 -15.43882	LRR	Seasonal pool	0	0	0	0	
102	Kiang Bambako 1	13.40347 -15.77289	LRR	Seasonal pool	0	0	0	0	
103	Kiang Bambako 2	13.40349 -15.77303	LRR	Rice field	0	0	0	0	

104	Kolior	13.4104 -15.67651	LRR	Seasonal pool	0	0	0	0	
105	Jarra Buiba	13.43324 -15.44416	LRR	Seasonal pool	0	0	0	0	
106	Jenoi	13.49045 -15.56171	LRR	Swamp	0	0	0	0	
107	Farafenni 1	13.55827 -15.58444	NBR	Rice field	0	0	0	0	
108	Farafenni 2	13.57205 -15.61475	NBR	Seasonal pool	0	0	0	0	
109	Bangally	13.50906 -16.41188	NBR	Seasonal pool	0	0	0	0	
110	Ndungu Kebbeh	13.53477 -16.32685	NBR	Seasonal pool	0	0	0	0	
111	Kerr Selleh	13.53314 -16.28884	NBR	Seasonal pool	0	0	0	0	
112	Njama Sinyan	13.53407 -16.26364	NBR	Seasonal pool	0	0	0	0	
113	Jamagen	13.53238 -16.24413	NBR	Seasonal pool	0	0	0	0	
114	Berending	13.4835 -16.46419	NBR	Stream	0	0	0	0	
Total					2184	602	91	2877	

Supplementary Table 4.2. Physicochemical and environmental parameters

Habitat type	Site name	Aquatic vegetation present	Algae present	Water temp.		Water pH	
Seasonal	Sare Bolli/Bamba Kolong	Yes	No	23.2	Range: 22.1°C – 38.3°C	7.1	Range: 6.42 – 8.16
	Daru	Yes	No	27.8		6.42	
	Njoren/Sanka Bari Pool 1	No	No	29.3		6.62	
	Njoren/Sanka Bari Pool 2	Yes	Yes	30.1	Median: 29.3°C	7.26	Median: 6.95
	Sinchu Bokar	Yes	No	33.3		6.78	
	Sare Madi Ganteh	Yes	No	35.9	Interquartile range: 27.8°C – 33.1°C	6.79	Interquartile range: 6.78 – 7.26
	Kerr Ousman Boye	yes	Yes	33.1		6.95	
	Sare Jabel	yes	No	28.9		6.91	
	Jahanka	yes	No	31.4		6.67	
	Sare Madi Babadi	No	No	36.4		6.67	
	Sare Chewto	Yes	No	29.1		7.19	
	Dobo	No	No	34.9		6.99	
	Dembakally	No	No	31.5		7.24	
	Changai Pool 2	Yes	No	26.5		7.64	
	Raneru Pool 2	Yes	No	34.6		6.87	
	Jokul Ndowen	No	No	32.3		7.53	
	Pallol Pool 2	No	No	27.2		7.05	
	Dingiri pool 1 (Dingiri Kore)	yes	No	28.3		6.91	
	Dingiri pool 2	No	No	29.5		6.95	
	Kuwonku (Koilu Dalo)	Yes	No	22.1		6.91	
	Madina Samaco (Toro)	Yes	No	28.3		6.67	
	Bajakunda	yes	yes	24.4		6.7	
	Koli Bantang (bamba dala)	No	No	38.3		7.54	
	Diabugu Basilla (misigi)	yes	No	28.3		7.44	
	Wellengara Ba 1	No	No	30.5		7.84	
	Kudang Bridge	yes	No	28.3		7.05	
Permanent	Madina Nfally pool 1	Yes	No	25.6	Range: 23.1°C – 33.9°C	6.63	Range: 5.52 – 7.55
	Madina Nfally pool 2	No	No	24.5		8.16	
	Kundang Tenda	No	No	23.2		7.55	
	Jarreng Bridge/Bolong	yes	No	27.3	Median: 27.6°C	7.18	Median: 6.96
	Jarreng Badala	No	No	27.9		7.49	
	Sare Anis (sofaniama Bolong)	Yes	No	28.3	Interquartile range: 25.3°C – 30.0°C	7.16	Interquartile range: 6.37 – 7.17
	Sare Anis Pool	Yes	Yes	23.1		7.13	
	Bansang Rice field	yes	No	30.5		6.73	
	Sololo	yes	No	33.9		5.52	
	Kuntaur Fulakunda/Jakaba	yes	No	26.6		6.94	
	Kisskiss	yes	No	25.1		6.38	

	Basse Kobakunda	yes	No	25.5		6.17	
	Demba Kunda	Yes	No	23.3		6.34	
	Dampha Kunda	Yes	No	25.3		6.33	
	Chamoi	Yes	Yes	30		7.09	
	Sare Alpha	Yes	No	24.8		6.97	
	Suduwoi Sare Alpha Bridge/Bolong	Yes	No	24.3		6.99	
	Sapu	yes	No	26.9		7.37	
	Pacharr 1	yes	No	28.1		6.69	
	Pacharr 2	yes	yes	28.2		7.13	
	Dalaba	Yes	Yes	30.1		6.5	
	Kuntaur Wharf Town	yes	No	33		6.98	
	Pallang	Yes	Yes	31		6.8	
	Missira Ba Mariama	Yes	Yes	31.1		5.97	
	Sotuma Sire/Samba	Yes	Yes	27.9		5.99	
	Wellengara Ba 2	Yes	No	27.7		7.52	
	Dongoro Ba	No	No	27.4		7.35	

Supplementary Table 4.3. Rate of Infected *Bulinus* species per site

No.	Site	Region	<i>Bulinus</i> species	No. Tested	No. Infected (rate)	
					<i>S.</i> <i>haematobium</i>	<i>S. bovis</i>
1	Sare Bolli	CRR	<i>B.</i> <i>senegalensis</i>	10	0	0
			<i>B. forskalii</i>	11	0	0
2	Madina Nfally 1	CRR	<i>B. truncatus</i>	10	0	1 (10%)
3	Madina Nfally 2	CRR	<i>B.</i> <i>senegalensis</i>	20	0	6 (30%)
4	Kudang Bridge	CRR	<i>B.</i> <i>senegalensis</i>	1	0	0
5	Njoren	CRR	<i>B.</i> <i>senegalensis</i>	20	1 (5%)	0
6	Daru	CRR	<i>B.</i> <i>senegalensis</i>	20	0	1 (5%)
7	Sanka Bari	CRR	<i>B.</i> <i>senegalensis</i>	20	0	0
8	Sinchu Bokary	CRR	<i>B.</i> <i>senegalensis</i>	20	0	2 (10%)
9	Sare Madi Ganteh	CRR	<i>B.</i> <i>senegalensis</i>	20	0	0
10	Kerr Ousman Boye	CRR	<i>B.</i> <i>senegalensis</i>	20	0	8 (40%)
11	Sare Jabel	CRR	<i>B.</i> <i>senegalensis</i>	20	1 (5%)	5 (25%)
12	Jahanka	CRR	<i>B.</i> <i>senegalensis</i>	20	3 (15%)	0
13	Sare Madi Babadi	CRR	<i>B.</i> <i>senegalensis</i>	17	0	0
14	Sare Chewto	CRR	<i>B.</i> <i>senegalensis</i>	20	0	0

			<i>B. forskalii</i>	10	0	0
15	Bansang	CRR	<i>B. forskalii</i>	20	0	0
16	Sololo	CRR	<i>B. senegalensis</i>	5	0	0
			<i>B. forskalii</i>	20	0	2 (10%)
17	Dobo	CRR	<i>B. senegalensis</i>	20	0	1 (5%)
18	Dembakally	CRR	<i>B. senegalensis</i>	2	0	0
19	Changai 2	CRR	<i>B. senegalensis</i>	20	0	1 (5%)
20	Kuntaur Fulakunda/Jakaba	CRR	<i>B. forskalii</i>	20	0	0
21	Pacharr 1	CRR	<i>B. forskalii</i>	12	0	3 (25%)
			<i>B. truncatus</i>	20	0	2 (10%)
22	Pacharr 2	CRR	<i>B. forskalii</i>	10	0	0
23	Dalaba	CRR	<i>B. forskalii</i>	17	0	1 (5.9%)
24	Kuntaur Wharf Town	CRR	<i>B. forskalii</i>	15	0	6 (40%)
25	Sankulay Kunda	CRR	<i>B. senegalensis</i>	19	0	1(5.3%)
26	Janjanbureh	CRR	<i>B. senegalensis</i>	6	0	0
			<i>B. forskalii</i>	10	0	0
27	Wassu	CRR	<i>B. senegalensis</i>	20	0	0
28	Jahally	CRR	<i>B. senegalensis</i>	20	0	2(10%)
29	Choya	CRR	<i>B. forskalii</i>	20	0	0
30	kunting	CRR	<i>B. senegalensis</i>	20	0	0
31	Dingiri 1	URR	<i>B. senegalensis</i>	20	2 (10)	2 (10%)

32	Kuwonku	URR	<i>B. senegalensis</i>	20	0	3 (15%)
33	Madina Samaco	URR	<i>B. senegalensis</i>	20	0	1 (5%)
34	Bajakunda	URR	<i>B. senegalensis</i>	20	0	4 (20%)
35	kolibantang	URR	<i>B. senegalensis</i>	20	0	1 (5%)
36	Diabugu Basilla	URR	<i>B. senegalensis</i>	20	0	4 (20%)
37	Demba Kunda	URR	<i>B. forskalii</i>	11	0	1 (9.1%)
38	Misra Ba Mariama	URR	<i>B. forskalii</i>	20	0	2 (10%)
			<i>B. truncatus</i>	20	0	3 (15%)
39	Sotuma Sire/Samba	URR	<i>B. forskalii</i>	20	0	4 (20%)
40	Sutukonding	URR	<i>B. senegalensis</i>	20	0	0
41	Kanubeh	URR	<i>B. senegalensis</i>	20	0	2 (10%)
42	Madina Samba Jawo	URR	<i>B. senegalensis</i>	1	0	0
43	Sare Pateh Bakery	URR	<i>B. senegalensis</i>	12	0	0
44	Wellingara Yarreh	URR	<i>B. senegalensis</i>	20	0	1 (5%)
45	Suduwol	URR	<i>B. senegalensis</i>	20	0	0
46	Basse Kabakama	URR	<i>B. forskalii</i>	20	0	0
47	Ndemban	WR	<i>B. senegalensis</i>	1	0	0
			<i>B. forskalii</i>	11	0	0
48	Siffoe/Kitty	WR	<i>B. senegalensis</i>	12	0	0
Total				883	7 (0.8)	70 (7.9)

Supplementary Table 5.1. *Bulinus senegalensis* haplotype distribution by site of collection for cox1 and ITS-2 genes. Red text denotes private haplotype.

Region	Site	GPS coordinates	Cox1		ITS-2		<i>S. haematobium</i> infection rate	<i>S. bovis</i> infection rate	Overall Schistosome Infection rate
			Sample size (no. of haplotypes)	Haplotype name (no. of individuals)	Sample size (no. of haplotypes)	Haplotype name (no. of individuals)			
Central River Region (CRR)	Sare Madi Ganteh	13.41991 -14.72219	10 (4)	H2 (1), H9 (3), H12 (5), H27 (1)	10 (3)	H3 (2), H5 (4), H7 (4)	0%	0%	0%
	Changai 2	13.58624 -14.67834	10 (2)	H13 (1), H17 (9)	10 (1)	H1 (10)	0%	10%	10%
	Sare Bolli	13.609017 -15.14675	10 (3)	H9 (5), H16 (1) H25 (4)	10 (2)	H1 (6), H7 (4)	0%	0%	0%
	Sare Jabel	13.38333 -14.70000	10 (4)	H9 (1), H12 (2), H15 (4), H27 (3)	10 (3)	H1 (6), H5 (2), H7 (2)	10%	30%	40%
	Kerr Ousman Boye	13.41423 -14.74174	10 (4)	H7 (3), H9 (1), H25 (3), H12 (3)	10 (3)	H3 (2), H5 (3), H7 (5)	0%	40%	40%
	Madina Nfally 2	13.54395 -14.959117	10 (1)	H25 (10)	10 (1)	H7 (10)	0%	40%	40%
	Daru	13.36944 -14.60019	10 (1)	H4 (10)	10 (1)	H6 (10)	0%	0%	0%
	Sinchu Bokary	13.41695 -14.70525	10 (2)	H9 (1), H25 (9)	10 (3)	H1 (6), H5 (2), H7 (2)	0%	10%	10%
	Dobo	13.47782 -14.64302	10 (4)	H10 (2), H24 (1), H25 (6), H27 (1)	10 (3)	H1 (7), H2 (2), H3 (1),	0	0%	0%
	Sare Madi Babadi	13.44930 -14.69858	10 (1)	H2 (10)	10 (1)	H1 (10)	0%	0%	0%
	Njoren	13.34598 -14.64303	10 (2)	H24 (1), H27 (9)	10 (2)	H1 (1), H5 (9)	10%	0%	10%
	Jahanka	13.37776 -14.72773	10 (3)	H12 (4), H25 (4), H27 (2)	10 (2)	H1 (7), H5 (3)	30%	0%	30%
	Sanka Bari	13.34949 -14.63018	10 (2)	H5 (1), H8 (9)	10 (1)	H7 (10)	0%	0%	0%
	Jahally	13.54481 -14.9659	10 (1)	H9 (10)	10 (1)	H1 (10)	0%	10%	10%
	Kunting	13.53171 -14.66579	10 (2)	H6 (9), H9 (1)	10 (2)	H1 (1), H7 (9)	0%	0%	0%
	Sankulay Kunda	13.53171 -14.66579	10 (2)	H18 (8), H9 (2)	10 (2)	H3 (1), H7 (9)	0%	10%	10%
	Wassu	13.69895 -14.88602	10 (1)	H25 (10)	10 (1)	H5 (10)	0%	0%	0%
	Sare Chewto	13.42820 -14.59223	10 (5)	H2 (3), H3 (2), H21 (1), H25 (3), H12 (1)	10 (3)	H1 (3), H5 (1), H7 (6)	0%	0%	0%
Upper River Region (URR)	Kuwonku	13.42475 -14.33871	10 (2)	H24 (8), H2 (2)	10 (3)	H1 (4), 5 (1), 7 (5)	0%	30%	30%
	Diabugu Basilla	13.316533 -13.979717	10 (2)	H24 (6), H25 (4)	10 (1)	H1 (10)	0%	10%	10%
	Bajakunda	13.47509 -14.04987	10 (1)	H24 (10)	10 (1)	H7 (10)	0%	30%	30%
	Dingiri 1	13.28580 -14.04369	10 (3)	H23 (3), H24 (2), H25 (5)	10 (1)	H1 (10)	20%	0%	20%
	Madina Samaco	13.29197 -14.01417	10 (2)	H24 (9), H10 (1)	10 (1)	H7 (10)	0%	10%	10%

	Sutukonding	13.37796 -14.22345	10 (3)	H11 (1), H14 (4), 24 (5)	10 (1)	H1 (10)	0%	0%	0%
	Wellingara Yarreh	13.54152 -14.00332	10 (2)	H26 (3), 25 (7)	10 (2)	H1 (8), H7 (2)	0%	10%	10%
	Kolibantang	13.43636 -14.17679	10 (2)	H19 (9), H26 (1)	10 (1)	H3 (10)	0%	10%	10%
	Suduwol	13.37747 -13.97064	10 (2)	H1 (6), H24 (4)	10 (3)	H1 (7), H3 (2), H4 (1)	0%	0%	0%
	Kanubeh	13.3208 -14.2964	10 (1)	H24 (10)	10 (1)	H1 (10)	0%	20%	20%
	Sare Pateh Bakery	13.32705 -14.51422	10 (5)	H9 (3), H15 (1), H20 (1), H22 (1), H24 (4)	10 (2)	H1 (9), H7 (1)	0%	0%	0%
Western Region (WR)	Siffoe/Kitty	13.21601 -16.67436	10 (1)	H9 (10)	10 (1)	H1 (10)	0%	0%	0%

Supplementary Table 5.2. Estimates of average evolutionary divergence over sequence pairs within sites

Cox1		ITS-2	
Site d		Site d	
KW	0.00395	KW	0.00167
SMG	0.00366	SJ	0.00202
CHA	0.00063	SC	0.00151
SB	0.00284	SPB	0.00045
SJ	0.00361	WAS	0.00000
KOB	0.00666	KNB	0.00000
MNF	0.00000	SMG	0.00202
DIA	0.00337	CHA	0.00000
DAR	0.00000	SB	0.00121
BJK	0.00000	KUN	0.00045
DIN	0.00425	SAN	0.00045
MSC	0.00094	SIF	0.00000
SBO	0.00094	SUD	0.00187
DOB	0.00449	JHY	0.00000
SMB	0.00000	SUT	0.00000
NJ	0.00126	WY	0.00081
JHK	0.00449	KOL	0.00000
SBA	0.00190	SBA	0.00000
JHY	0.00000	JHK	0.00212
SUT	0.00520	NJ	0.00091
WY	0.00073	SMB	0.00000
KOL	0.00094	MNF	0.00000
KUN	0.00094	DIA	0.00000
SAN	0.00112	DAR	0.00000
SIF	0.00000	BJK	0.00000
SUD	0.00679	DIN	0.00000
KNB	0.00000	MSC	0.00000
WAS	0.00000	SBO	0.00202
SC	0.00834	DOB	0.00253
SPB	0.00174	KOB	0.00187

Supplementary Table 6.1. Sample accession number, country and collection locality information of Genbank sequences included in the phylogeographic analysis

Species	Country	Sample Accession No	Locality of collection	Reference
<i>B. forskalii</i>	Senegal	AM286307	Dakar	Kane et al., 2008
	Burkina Faso	AM286310	Mogtedo barrage	Kane et al., 2008
	Niger	AM286308	Satoni	Kane et al., 2008
		MT272322 MT272323	Niger	Pennance et al., 2020
	Cameroon	KJ157488 KJ157489	Gounougou	Zein-Eddine et al., 2014
	Uganda	MT707433	Lake Nakivale	Chibwana et al., 2020
		MN551573	Crater lake Kibungo	Tumwebaze et al., 2019
		MN551574 MN551559	Crater lake Mirambi	Tumwebaze et al., 2019
		HQ121580 HQ121581 HQ121582	Lake Albert	Nalugwa et al., 2010
		HQ121583 HQ121584	Lake Edward	Nalugwa et al., 2010
		HQ121585 HQ121586	Lake George	Nalugwa et al., 2010
		HQ121587 HQ121588	Katosho Swamp	Nalugwa et al., 2010
	Tanzania	HQ121589 HQ121590	Lake Tanganyika	Nalugwa et al., 2010
<i>B. truncatus</i>	Senegal	KJ135306	Diana	Zein-Eddine et al., 2014
		KJ135307	Guede	Zein-Eddine et al., 2014
		KJ135308	Ndombo	Zein-Eddine et al., 2014
		AM921806	Mbane	Kane et al., 2008
		AM921807	Bouton Batt	Kane et al., 2008
	Burkina Faso	AM286315	Mogtedo barrage	Kane et al., 2008
	Niger	MT272327 MT272328 MT272332	Niger	Pennance et al., 2020
		AM286316 AM286317	Satoni	Kane et al., 2008
	Nigeria	FN546809 FN546810	Musawa	Akinwale et al., 2011
		FN546812	Yuna	Akinwale et al., 2011
		FN546813	Tada	Akinwale et al., 2011

	Cameroon	KJ135288 KJ135289 KJ135290	Kekem	Zein-Eddine et al., 2014
		KJ135291	Mokolo	Zein-Eddine et al., 2014
		KJ135292	Barombi-kotto	Zein-Eddine et al., 2014
		KJ135293	Dschang	Zein-Eddine et al., 2014
		KJ135294	Ngaoundere	Zein-Eddine et al., 2014
		KJ135295	Loum	Zein-Eddine et al., 2014
	Egypt	KJ135296 KJ135297	Behera	Zein-Eddine et al., 2014
		KJ135298 KJ135299	Tanta	Zein-Eddine et al., 2014
		KJ135300 KJ135301	Giza	Zein-Eddine et al., 2014
		KJ135302 KJ135303	Mansouria	Zein-Eddine et al., 2014
		KJ135304 KJ135305	Quena	Zein-Eddine et al., 2014
	Tanzania	AM286313	Nyanguge	Kane et al., 2008
		HQ121562 HQ121563	Katosho Swamp	Nalugwa et al., 2010
	Rwanda	MN551580 MN551581	Lake Muhazi	Tumwebaze et al., 2019
		MN551578	Rice scheme dam lake	Tumwebaze et al., 2019
	Uganda	MN551575 MN551576	Crater lake Kyasanduka,	Tumwebaze et al., 2019
		MT707427	Lake Mburo	Chibwana et al., 2020

Supplementary Table 6.2. *B. forskalii* and *B. truncatus* haplotype distribution by site of collection for cox1 and ITS-2 genes. Red text denotes private haplotype.

Species	Region	Site	GPS coordinates	Cox1		ITS-2		<i>S. bovis</i> infection rate
				Sample size (no. of haplotypes)	Haplotype name (no. of individuals)	Sample size (no. of haplotypes)	Haplotype name (no. of individuals)	
<i>B. forskalii</i>	Central River Region (CRR)	Choya	13.54108 -15.24912	10 (1)	H14 (10)	10 (1)	H2 (10)	0
		Dalaba	13.598217 -15.21305	10 (1)	H3 (10)	10 (1)	H2 (10)	1 (10%)
		Sare Bolli	13.609017 -15.14675	10 (1)	H13 (10)	10 (1)	H1 (10)	0
		Pacharr 1	13.517733 -14.8543	10 (2)	H4 (8), 6 (2)	10 (1)	H4 (10)	3 (30%)
		Pacharr 2	13.522917 -14.856383	10 (4)	H4 (4), H8 (2), H10 (1), H11 (3)	10 (2)	H1 (1), H2 (9)	0
		Kuntaur	13.676867 -14.885517	10 (3)	H16 (1), H17 (8), H18 (1)	10 (1)	H2 (10)	4 (40%)
		Jakaba	13.65983 -14.87867	10 (3)	H12 (7), H15 (1), H17 (2)	10 (1)	H2 (10)	0
		Sololo	13.46059 -14.68370	10 (2)	H4 (3), H6 (7)	10 (1)	H4 (10)	2 (20%)
		Bansang	13.42670 -14.65623	10 (3)	H1 (2), H4 (6), H6 (2)	10 (1)	H4 (10)	0
	Upper River Region (URR)	Sotuma Sire	13.29975 -14.312933	10 (5)	H1 (1), H2 (4), H4 (1), H6 (2), H7 (2)	10 (1)	H4 (10)	4 (40%)
		Demba Kunda	13.25185 -14.27178	10 (3)	H1 (1), H2 (6), H6 (3)	10 (1)	H4 (10)	0
		Basse Kabakama	13.30485 -14.20964	10 (2)	H1 (2), H6 (8)	10 (1)	H4 (10)	0
		Misra Ba Mariama	13.33295 -13.94445	10 (4)	H1 (2), H4 (1), H5 (2), H6 (5)	10 (1)	H4 (10)	2 (20%)
	Western Region (WR)	Ndemban (Foni)	13.20117 -16.34642	10 (1)	H9 (10)	10 (1)	H3 (10)	0
<i>B. truncatus</i>	Central River Region (CRR)	Madina Nfally 1	13.543967 -14.959617	10 (2)	H1 (9), H2 (1)	10 (1)	H1 (10)	1 (10%)
		Pacharr 1	13.517733 -14.8543	10 (3)	H3 (4), H4 (1), H5 (5)	10 (2)	H1 (2), H2 (8)	2 (20%)
	Upper River Region (URR)	Misra Ba Mariama	13.33295 -13.94445	10 (1)	H6 (10)	10 (1)	H2 (10)	2 (20%)

Supplementary Table 6.3. Mean genetic distance within and between *B. forskalii* populations for the cox1 and ITS-2 genes

<i>Bulinus forskalii</i>													
cox1 Intrapopulation mean distance													
Site	d												
SS	0.00958												
DAL	0												
BSG	0.00611												
SOL	0.0008												
PCH1	0.00061												
PCH2	0.01603												
DEM	0.00804												
JAK	0.00146												
MBM	0.00641												
KTR	0.00034												
NDE	0												
BKM	0.00617												
SB	0												
CHO	0												
cox1 Interpopulation mean distance													
	SS	DAL	BSG	SOL	PCH1	PCH2	DEM	JAK	MBM	KTR	NDE	BKM	SB
SS													
DAL	0.01002												
BSG	0.00863	0.00519											
SOL	0.00897	0.00293	0.00417										
PCH1	0.00840	0.00206	0.00364	0.00106									

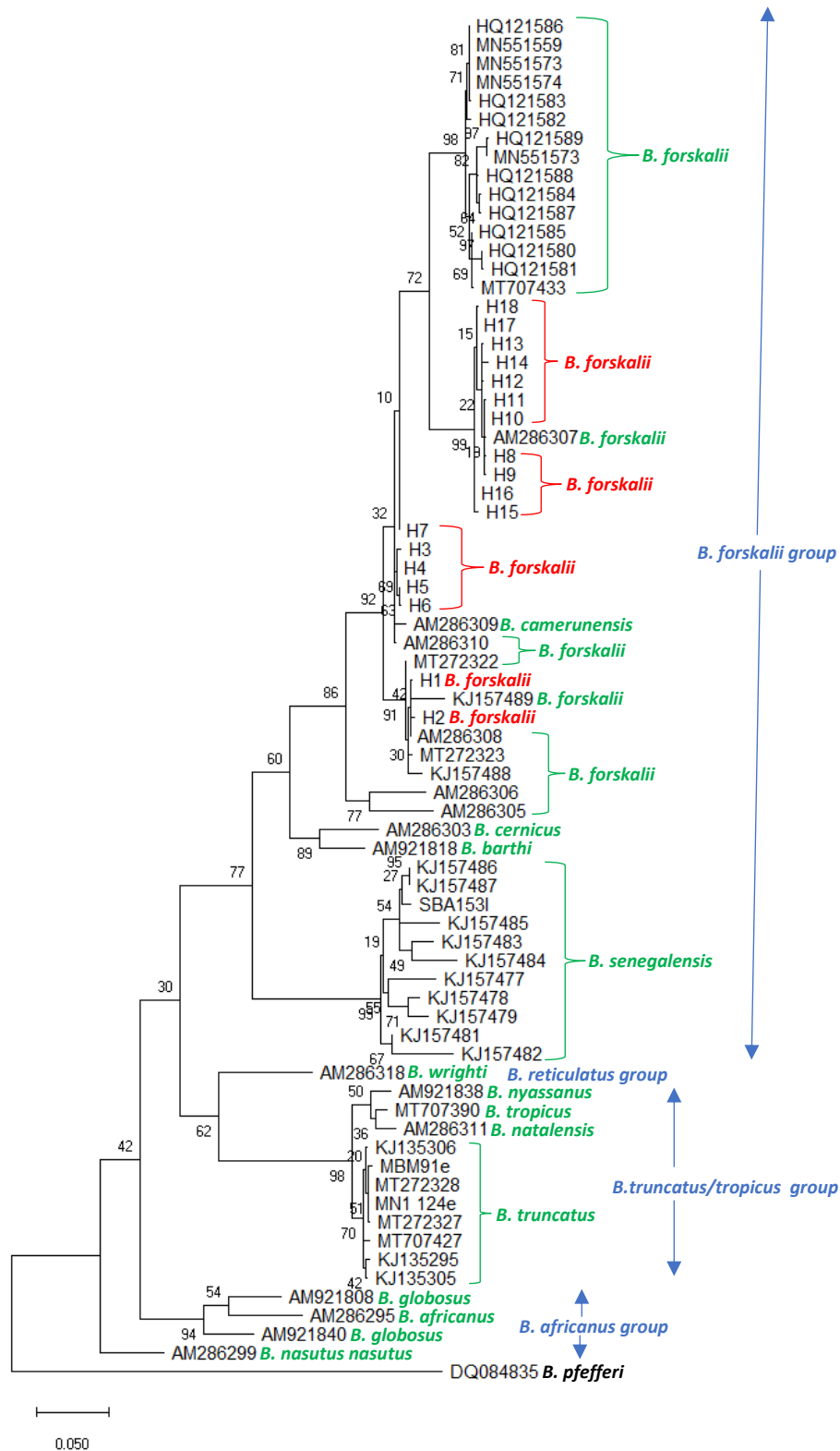
PCH2	0.02214	0.01973	0.01996	0.01846	0.01789								
DEM	0.00856	0.01260	0.01009	0.01129	0.01088	0.02416							
JAK	0.03366	0.03410	0.03318	0.03178	0.03178	0.01439	0.03530						
MBM	0.00909	0.00589	0.00592	0.00390	0.00405	0.02049	0.01042	0.03341					
KTR	0.03265	0.03309	0.03218	0.03079	0.03079	0.01441	0.03427	0.00222	0.03240				
NDE	0.03273	0.03317	0.03225	0.03086	0.03086	0.01329	0.03435	0.00293	0.03248	0.00362			
BKM	0.00931	0.00623	0.00614	0.00376	0.00426	0.02063	0.01058	0.03318	0.00575	0.03218	0.03225		
SB	0.03276	0.03204	0.03160	0.02976	0.02976	0.01336	0.03483	0.00206	0.03183	0.00275	0.00257	0.03160	
CHO	0.03239	0.03190	0.03100	0.02963	0.02963	0.01437	0.03307	0.00381	0.03122	0.00452	0.00433	0.03100	0.00345
ITS2 Intrapopulation mean distance													
	Site	d											
KTR	0												
PCH2	0.00044												
PCH1	0												
DEM	0												
JAK	0												
SS	0												
MBM	0												
DAL	0												
BSG	0												
SOL	0												
NDE	0												
BKM	0												
CHO	0												
SB	0												
ITS2 Interpopulation mean distance													
	KTR	PCH2	PCH1	DEM	JAK	SS	MBM	DAL	BSG	SOL	NDE	BKM	CHO
KTR													

PCH2	0.00022												
PCH1	0.01118	0.01095											
DEM	0.01118	0.01095	0.00000										
JAK	0.00000	0.00022	0.01118	0.01118									
SS	0.01118	0.01095	0.00000	0.00000	0.01118								
MBM	0.01118	0.01095	0.00000	0.00000	0.01118	0.00000							
DAL	0.00000	0.00022	0.01118	0.01118	0.00000	0.01118	0.01118						
BSG	0.01118	0.01095	0.00000	0.00000	0.01118	0.00000	0.00000	0.01118					
SOL	0.01118	0.01095	0.00000	0.00000	0.01118	0.00000	0.00000	0.01118	0.00000				
NDE	0.00664	0.00642	0.01351	0.01351	0.00664	0.01351	0.01351	0.00664	0.01351	0.01351			
BKM	0.01118	0.01095	0.00000	0.00000	0.01118	0.00000	0.00000	0.01118	0.00000	0.00000	0.01351		
CHO	0.00000	0.00022	0.01118	0.01118	0.00000	0.01118	0.01118	0.00000	0.01118	0.01118	0.00664	0.01118	
SB	0.00219	0.00197	0.00892	0.00892	0.00219	0.00892	0.00892	0.00219	0.00892	0.00892	0.00442	0.00892	0.00219

Supplementary Table 6.4. Amino acid change analysis (estimation of number of synonymous and non-synonymous mutations) result for *Bulinus forskalii* cox1 data.

Polymorphic Sites

Input Data File: C:\...\co1.bf.snp.phy
Number of sequences: 18 Number of sequences used: 18
Selected region: 1-639 Number of sites: 639
Total number of sites (excluding sites with gaps / missing data): 639
Sites with alignment gaps or missing data: 0
Invariable (monomorphic) sites: 588
Variable (polymorphic) sites: 51 (Total number of mutations: 53)
Singleton variable sites: 8
Parsimony informative sites: 43
===== Protein Coding Region =====
Genetic Code: mtDNA Drosophila
Protein Coding, and Non-Coding Regions:
Number of protein coding regions (exons): 1
Number of noncoding regions (intronic and flanking regions): 0
Protein coding region, from site: 1 to 639
Total number of sites in coding regions: 639
Total number of sites excluding complex codons or codons with gaps: 639
===== Synonymous/Replacement Changes =====
Segregating sites: 51 Total number of mutations: 53
Total number of Synonymous changes: 53
54 58 165 174 192 201 204 207 216 222 240 243 244
246 267 271 276 279 282 321 324 327 333 336 336 339 345
348 351 381 382 384 387 444 462 465 471 492 495 517 519
523 528 531 534 543 558 567 567 609 612 624 627
Total number of Replacement changes: 0



Supplementary Figure 6.1. Maximum likelihood tree for the 18 novel *B. forskalii* *cox1* haplotypes found in this study with reference GenBank sequences representing all four *Bulinus* species groups. 1000 bootstrap replicates have been used. Tree rooted on *Biomphalaria pfeifferi*. The novel *B. forskalii* haplotypes are marked in red and the reference GenBank sequences are marked in green.

Appendices

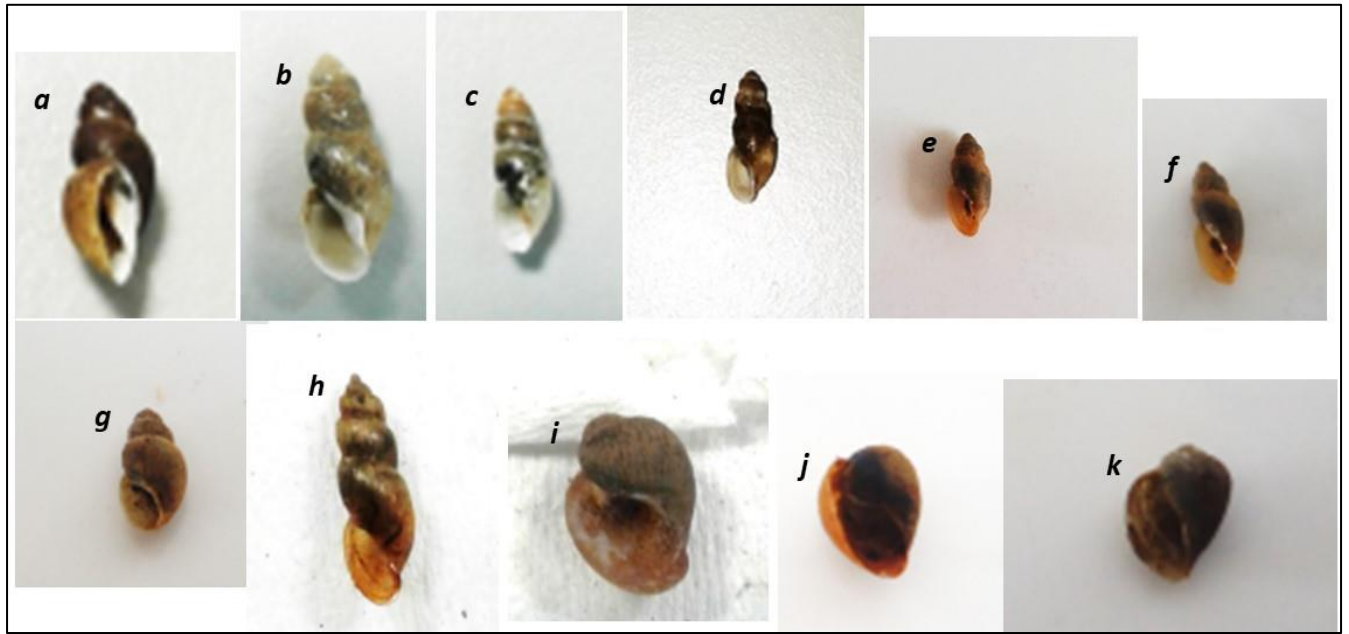
		7	Schisto and/or STH						
Q3_Community_code1	Enter community/village code	User entered integer							
Q4_Community_code2	Enter community/village code again	User entered integer							
Q5_School_code1	Enter school code	User entered integer							
Q6_School_code2	Enter school code again	User entered integer							
Q7_Consent	Select enrollment type	<table border="1"> <tr><td>1</td><td>Consent</td></tr> <tr><td>2</td><td>Absent</td></tr> <tr><td>9</td><td>Refused</td></tr> </table>		1	Consent	2	Absent	9	Refused
1	Consent								
2	Absent								
9	Refused								
Consent	Hidden from user								
Q7a_Name	Enter name	User entered text							
Q8_Sex	Select sex	<table border="1"> <tr><td>1</td><td>Male</td></tr> <tr><td>2</td><td>Female</td></tr> </table>		1	Male	2	Female		
1	Male								
2	Female								
Q9_Age	Enter age	User entered integer							
Q9a_Age_STH	Enter age	User entered integer							
Q10a_Barcode	Barcode scan or manual entry?	<table border="1"> <tr><td>1</td><td>Scan</td></tr> <tr><td>2</td><td>Manual</td></tr> </table>		1	Scan	2	Manual		
1	Scan								
2	Manual								
Q10b_Scan	Scan barcode	User captured barcode							

Q28_School_urinate	When you are at school, where do you usually go to urinate?	<table border="1"> <tr><td>1</td><td>In the school toilet</td></tr> <tr><td>2</td><td>Around school compound</td></tr> </table>		1	In the school toilet	2	Around school compound								
1	In the school toilet														
2	Around school compound														
		<table border="1"> <tr><td>3</td><td>Outside of school compound</td></tr> <tr><td>4</td><td>In river/body of water</td></tr> <tr><td>5</td><td>I wait/hold it</td></tr> <tr><td>6</td><td>Other</td></tr> </table>		3	Outside of school compound	4	In river/body of water	5	I wait/hold it	6	Other				
3	Outside of school compound														
4	In river/body of water														
5	I wait/hold it														
6	Other														
Q28a_Urinate_other	If other, specify	User entered text													
Q29_School_defecate	When you are at school, where do you usually go to defecate?	<table border="1"> <tr><td>1</td><td>In the school toilet</td></tr> <tr><td>2</td><td>Around school compound</td></tr> <tr><td>3</td><td>Outside of school compound</td></tr> <tr><td>4</td><td>In river/body of water</td></tr> <tr><td>5</td><td>I wait/hold it</td></tr> <tr><td>6</td><td>Other</td></tr> </table>		1	In the school toilet	2	Around school compound	3	Outside of school compound	4	In river/body of water	5	I wait/hold it	6	Other
1	In the school toilet														
2	Around school compound														
3	Outside of school compound														
4	In river/body of water														
5	I wait/hold it														
6	Other														
Q29a_Defecate_other	If other, specify	User entered text													

Water	Hidden from user						
Q30b_Bathing	Bathing	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30c_Washing	Washing clothes/dishes	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30d_Fishing	Fishing	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30e_Crossing	Crossing water	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30f_Fetching	Fetching water	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30g_Playing	Playing	<table border="1"> <tr><td>1</td><td>Yes</td></tr> </table>		1	Yes		
1	Yes						

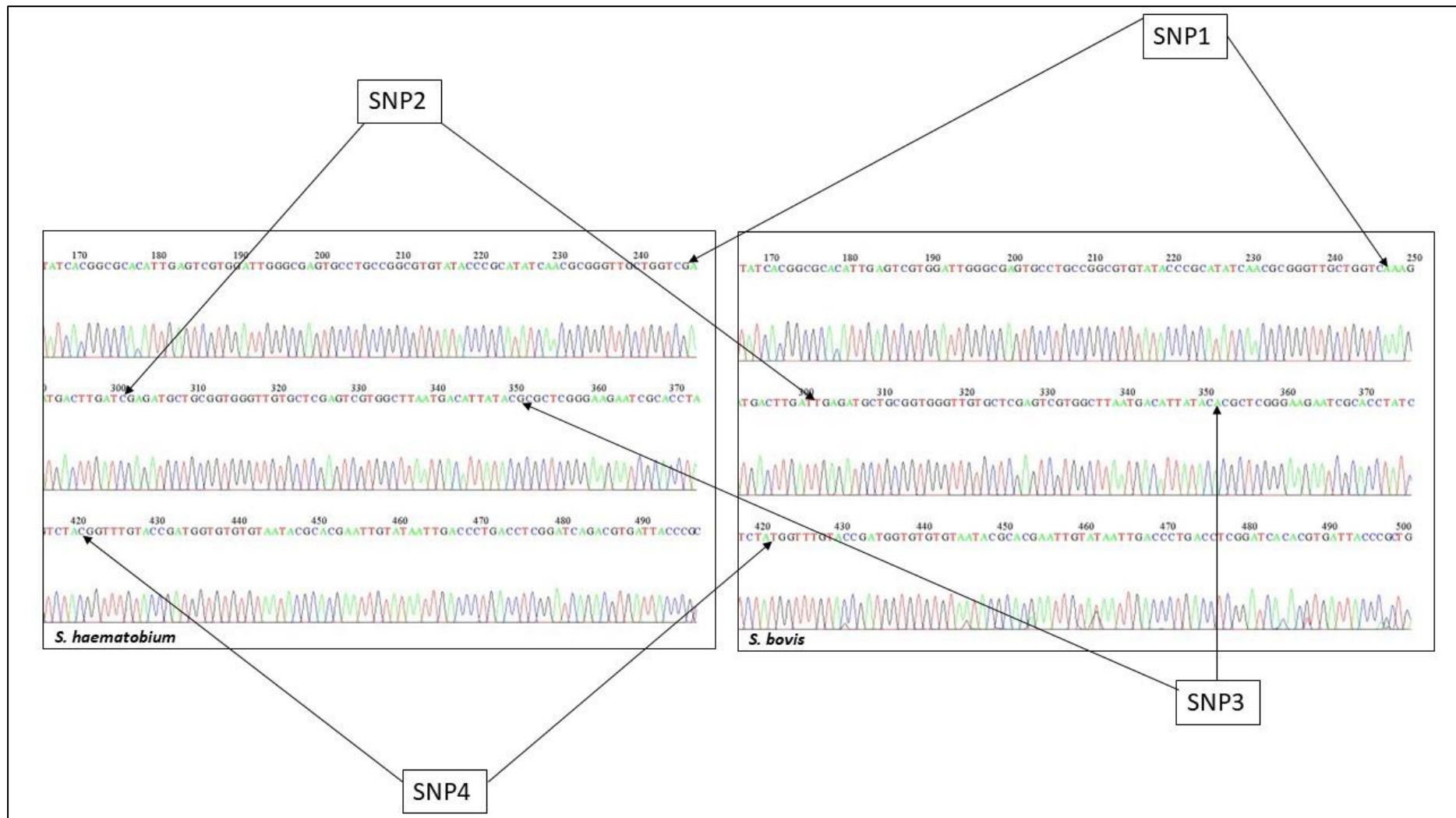
Q30h_Swimming	Swimming	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30i_No_activity	No activity	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30a_None	No nearby water bodies	<table border="1"> <tr><td>1</td><td>Yes</td></tr> <tr><td>2</td><td>No</td></tr> </table>		1	Yes	2	No
1	Yes						
2	No						
Q30k_Other	Other (specify)	User entered text					
Q31_Notes	Additional notes	User entered text					
Start_time	Hidden from user	Timestamp of form open					
End_time	Hidden from user	Timestamp of form save					

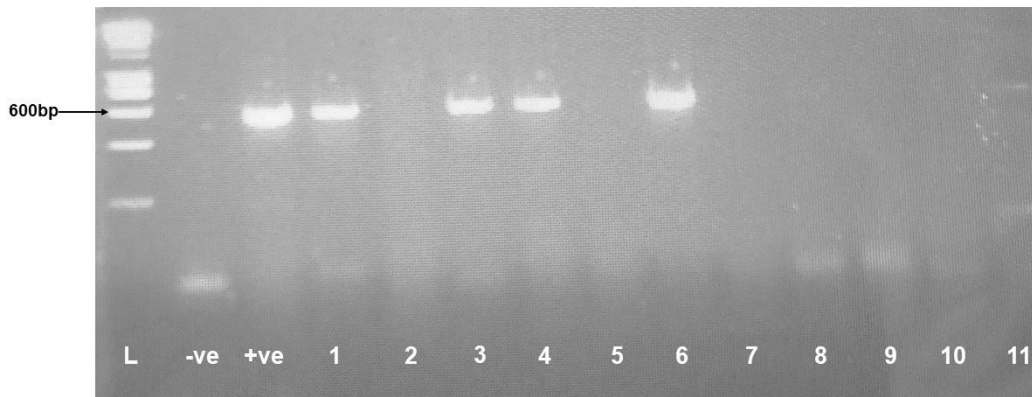
Appendix 2.1. Questionnaire used for the collection of demographic and water contact data of study participants in the human epidemiological study.



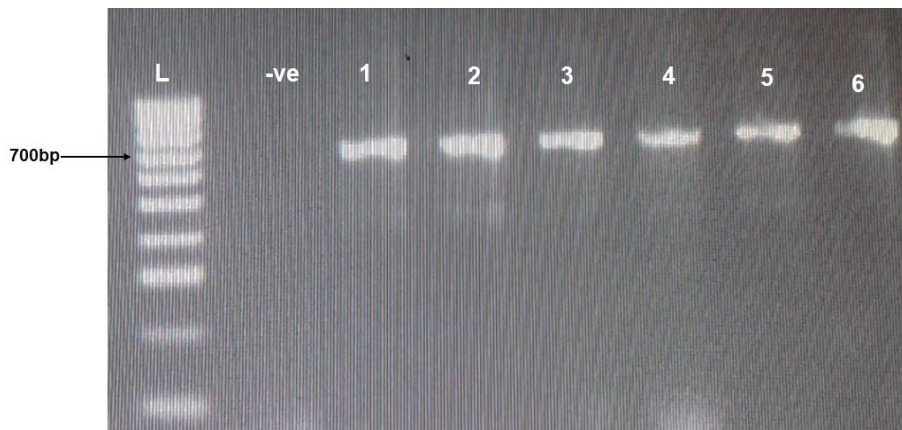
Appendix 2.2. *Bulinus senegalensis*, *Bulinus forskalii* and *Bulinus truncatus* from The Gambia. (a) *B. senegalensis* from Madina Samaco, URR; (b) *B. senegalensis* from Kuwonku, URR; (c) *B. senegalensis* from Dobo, CRR; (d) *B. senegalensis* from Sare Madi Babadi, CRR; (e, f) *B. forskalii* from Dalaba, CRR; (g) *B. forskalii* from Misra Ba Mariama, URR; (h) *B. forskalii* Sotuma Sire, URR; (i, j) *B. truncatus* from Misra Ba Mariama, URR; (k) *B. truncatus* form Pacharr 1, CRR.

Appendix 2.3. Sequence traces showing the 4 SNP sites in the ITS-2 gene at which *S. haematobium* and *S. bovis* were differentiated

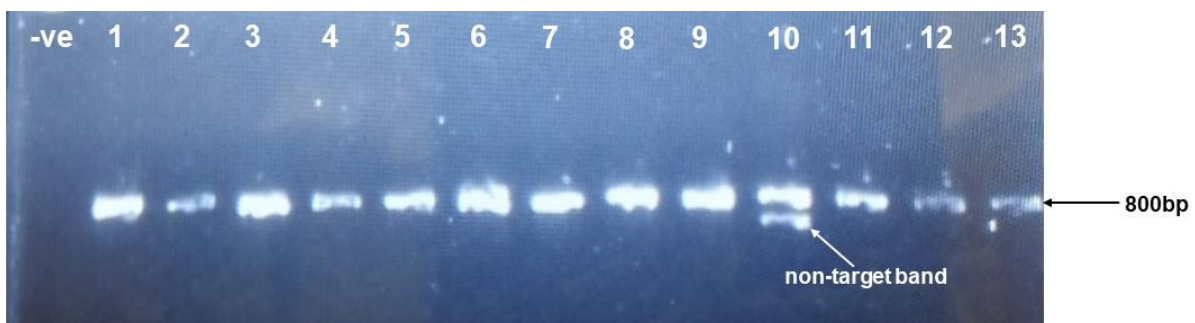




Appendix 2.4. Gel electrophoresis image of infection detection secondary PCR (with primers ITS2_Schisto_F and ETTS1) for field collected *Bulinus senegalensis* showing the 538bp *Schistosoma* amplicon in infected samples. L: HyperLadder I (Bioline, UK); -ve: negative control (no template DNA); +ve: positive control (infected snail DNA); Lanes 1, 3, 4 and 6: infected field snails; Lanes 2, 5, 7 – 11: uninfected field snails.



Appendix 2.5. Gel electrophoresis image of the ~700bp cox1 amplicons (amplified by primers LCO and HCO) from *Bulinus* DNA for population genetic analysis. L: HyperLadder IV (Bioline, UK); -ve: negative control (no template DNA); Lanes 1 – 6: *Bulinus* snail DNA.



Appendix 2.6. Gel electrophoresis image of the ~800 rRNA gene fragment (amplified by primers LSU1iii and LSU3iii) from *Bulinus* DNA for population genetic analysis. -ve: negative control (no template DNA); Lanes 1 – 13: *Bulinus* snail DNA. Lane 10 has a smaller non-target band in addition to the ~800 target snail band.

Appendix 2.7. Gel extraction protocol using the QIAquick gel extraction kit (Qiagen)

according to the manufacturer's instructions

1. PCR products were excised from the agarose gel with a clean, sharp scalpel and placed in a clean 1.5 ml microfuge tube.
2. The gel slice was weighed and three volumes of the buffer QG were added to each volume of the excised gel.
3. The gel slices were incubated at 50 °C for 10 min (or until the gel slices completely dissolved). To help dissolve the gel, tubes were inverted every 2-3 min during the incubation.
4. Once gel slices were completely dissolved, one gel volume of isopropanol was added and mixed.
5. A spin column with a membrane filter was placed in a 2 ml collection tube. Then, the sample was applied to the spin column and centrifuged at 13,000 rpm for 1 min to bind the PCR products. The flow-through was discarded and the column was placed back in the same collection tube. The maximum volume of the column reservoir is 800 µl. Therefore, the samples with volume more than 800 µl were loaded again in the same spin column until all the sample was used up.
6. To wash the samples, 750 µl of the washing buffer (Buffer PE) was added and centrifuged at 13,000 rpm for 1 min. The flow-through was discarded and the samples were centrifuged for an additional 1 min at 13,000 rpm to remove the residual ethanol from Buffer PE completely.
7. The spin column was placed into a clean 2 ml collection tube. 30 µl of Elution Buffer EB was added to the centre of the membrane filter, and the collection tube was centrifuged at 13,000 rpm for 1 min to elute the PCR products. The spin column was discarded, and the DNA was stored at -20 °C until further use.

Appendix 2.8. Outputs of chi square tests for *S. haematobium* infection among demographic variables (regions, gender and age group) in SPSS.

S. haematobium infection * region

Crosstab

			region			
			NBR	LRR	CRR	URR
S. haematobium infection	-ve	Count	501	496	372	442
		% within region	100.0%	99.4%	72.4%	88.0%
	+ve	Count	0	3	142	60
		% within region	0.0%	0.6%	27.6%	12.0%
Total		Count	501	499	514	502
		% within region	100.0%	100.0%	100.0%	100.0%

Crosstab

			Total
S. haematobium infection	-ve	Count	1811
		% within region	89.8%
	+ve	Count	205
		% within region	10.2%
Total		Count	2016
		% within region	100.0%

Chi-Square Tests:

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	279.958 ^a	3	<.001
Likelihood Ratio	315.618	3	<.001
N of Valid Cases	2016		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 50.74.

S. haematobium infection * gender

Crosstab

			gender		
			Male	Female	Total
S. haematobium infection	-ve	Count	893	918	1811
		% within gender	88.4%	91.3%	89.8%
	+ve	Count	117	88	205
		% within gender	11.6%	8.7%	10.2%
Total		Count	1010	1006	2016

% within gender	100.0%	100.0%	100.0%
-----------------	--------	--------	--------

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.440 ^a	1	.035		
Continuity Correction ^b	4.135	1	.042		
Likelihood Ratio	4.453	1	.035		
Fisher's Exact Test				.039	.021
N of Valid Cases	2016				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 102.30.

b. Computed only for a 2x2 table

S. haematobium infection * age_group

Crosstab

		age_group			
		1	2	3	Total
-ve	Count	862	668	281	1811

S. haematobium infection		% within age_group	90.5%	89.1%	89.8%	89.8%
	+ve	Count	91	82	32	205
		% within age_group	9.5%	10.9%	10.2%	10.2%
Total		Count	953	750	313	2016
		% within age_group	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.882 ^a	2	.643
Likelihood Ratio	.879	2	.644
N of Valid Cases	2016		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 31.83.

Appendix 2.9. Generalised linear mixed models (GLMMs) outputs for relationship test between *Bulinus* snail abundance and environmental/physicochemical parameters

Seasonal sites

Family: nbinom2 (log)

Formula: abundance ~ veg + algae + temp + ph

Data: ssnaill

AIC	BIC	logLik	deviance	df.resid
269.5	277.2	-128.7	257.5	21

Overdispersion parameter for nbinom2 family (): 0.566

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.66843	6.62519	0.403	0.687
vegy	1.56491	0.63668	2.458	0.014 *
algaey	-0.11723	0.86957	-0.135	0.893
temp	-0.05649	0.08077	-0.699	0.484
ph	0.26750	0.66999	0.399	0.690

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> confint(m1)

	2.5 %	97.5 %	Estimate
(Intercept)	-10.3167097	15.6535738	2.66843206
vegy	0.3170369	2.8127920	1.56491442
algaey	-1.8215576	1.5870903	-0.11723366
temp	-0.2147957	0.1018163	-0.05648969
ph	-1.0456550	1.5806621	0.26750356

> m1 <- glmmTMB(abundance ~ veg + algae + temp + ph + (1|site), data = ssnaill,

+ family = nbinom2)

Permanent Sites

Family: nbinom2 (log)

Formula: abundance ~ veg + algae + temp + ph

Data: psnail

AIC	BIC	logLik	deviance	df.resid
173.0	180.6	-80.5	161.0	20

Overdispersion parameter for nbinom2 family (): 0.61

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	3.60495	8.95763	0.041	0.966932
veg	10.23972	8.76027	0.118	0.906049
algaey	0.08463	0.62901	0.135	0.892978
temp	0.24998	0.11814	2.116	0.034355 *
ph	-2.72945	0.77714	-3.512	0.000444 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> confint(m1)

	2.5 %	97.5 %	Estimate
(Intercept)	-16.82887663	17.0387797	3.60495154
veg	-15.80728385	18.2867258	10.23972097
algaey	-1.14822109	1.3174718	0.08462535
temp	0.01842034	0.4815323	0.24997632
ph	-4.25262119	-1.2062743	-2.72944773

> m1 <- glmmTMB(abundance ~ veg + algae + temp + ph + (1|site), data = psnail,
+ family = nbinom2)

Appendix 2.10. Snail genotype and *S. bovis* infection association output in GraphPad Prism for cox1 haplotype H24 and ITS-2 haplotype H7 of *B. senegalensis*

Cox1 Haplotype H24

Table Analyzed Data 1

P value and statistical significance

Test Fisher's exact test

P value 0.0394

P value summary *

One- or two-sided Two-sided

Statistically significant (P < 0.05)? Yes

Effect size Value 95% CI

Relative Risk 2.353 1.138 to 4.733

Reciprocal of relative risk 0.4250 0.2113 to 0.8791

Methods used to compute CIs

Relative Risk Koopman asymptotic score

Data analyzed	S. bovis	No Infection	Total
Hap_24 10	50	60	
NHap_0	17	223	240
Total 27	273	300	

Percentage of row total S. bovis No Infection

Hap_24 16.67% 83.33%

NHap_0 7.08% 92.92%

Percentage of column total S. bovis No Infection

Hap_24 37.04% 18.32%

NHap_0 62.96% 81.68%

Percentage of grand total Schistosome Infection No Infection

Hap_243.33%	16.67%	
NHap_0	5.67%	74.33%

ITS-2 Haplotype H7

Table Analyzed Data 1

P value and statistical significance

Test Fisher's exact test

P value 0.0035

P value summary **

One- or two-sided Two-sided

Statistically significant (P < 0.05)? Yes

Effect size	Value	95% CI
Relative Risk	2.963	1.462 to 5.970
Reciprocal of relative risk	0.3374	0.1675 to 0.6842

Methods used to compute CIs

Relative Risk Koopman asymptotic score

Data analyzed	S. bovis	No Infection	Total
Hap_7 15	74	89	
Row 2 12	199	211	
Total 27	273	300	

Percentage of row total S. bovis No Infection

Hap_7 16.85% 83.15%

Row 2 5.69% 94.31%

Percentage of column total S. bovis No Infection

Hap_7 55.56% 27.11%

Row 2 44.44% 72.89%

Percentage of grand total S. bovis No Infection

Hap_7 5.00% 24.67%

Row 2 4.00% 66.33%

Appendix 6.1. Complete snail infection and molecular dataset including sample ID, collection site type, region, morphological and molecular ID, schistosome infection status and cox1 and ITS2 haplotypes

Snail sample ID	Site name	Site type	Region	Morphological ID	Molecular ID/cox1 barcoding	S. haematobium infection	S. bovis infection	cox1 haplotype	ITS2 haplotype
KW7	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
KW8	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
KW9	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
KW10	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H2	H5
KW11	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H2	H1
KW12	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
KW25	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	yes	H24	H7
KW26	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
KW27	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	yes	H24	H1
KW28	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
KW29	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
KW30	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
KW318	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW319	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW320	Kuwonku	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	yes	H24	H7
KW321	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW322	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW323	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW324	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
KW325	Kuwonku	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SMG13	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H7
SMG14	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H5
SMG15	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H3
SMG16	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H3
SMG17	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SMG18	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H5
SMG19	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SMG20	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H5
SMG21	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H7
SMG22	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
SMG23	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H7
SMG24	Sare Madi Ganteh	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H7
CHA31	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H17	H1
CHA32	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA33	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA34	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA35	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA36	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA37	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA38	Changai 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
CHA39	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA40	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
CHA41	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H13	H1
CHA42	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H17	H1
CHA23C	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
CHA24C	Changai 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
CHA574	Changai 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
CHA575	Changai 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ49	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SJ50	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	N/A	N/A

SJ51	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H1
SJ52	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H1
SJ53	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H5
SJ54	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H15	H1
SJ55	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H15	H7
SJ56	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H1
SJ57	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H15	H1
SJ58	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H15	H5
SJ59	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	yes	no	H12	H1
SJ60	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SJ61	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ63	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ64	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	yes	N/A	N/A
SJ65	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ69	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ70	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SJ71	Sare Jabel	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H27	H7
SJ72	Sare Jabel	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB73	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H7	H7
KOB74	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H5
KOB75	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H7
KOB76	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	N/A	N/A
KOB77	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H3
KOB78	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H7	H7
KOB79	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H3
KOB80	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H12	H7
KOB81	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H7	H5
KOB82	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	N/A	N/A
KOB83	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H7
KOB84	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H24	H5
KOB85	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB86	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB87	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	yes	N/A	N/A
KOB88	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB89	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB90	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB91	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KOB95	Kerr Ousman Boye	Seasonal pool	CRR	B. senegalensis	N/A	no	yes	N/A	N/A
MNF97	Madina Nfally 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H7
MNF98	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H7
MNF99	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H7
MNF100	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF101	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF102	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF103	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF104	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF105	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF106	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF107	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF108	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF109	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
MNF110	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H7
MNF111	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF112	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
MNF113	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	N/A	N/A
MNF118	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	yes	N/A	N/A
MNF119	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
MNF120	Madina Nfall 2	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DIA121	Diabugu Basilla	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1

BJK184	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
BJK185	Bajakunda	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
BJK186	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
BJK187	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
BJK188	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	yes	H24	H7
BJK189	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
BJK190	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
BJK191	Bajakunda	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
BJK192	Bajakunda	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
BJK193	Bajakunda	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN202	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
DIN203	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1
DIN205	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
DIN209	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1
DIN210	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	yes	no	H23	H1
DIN211	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	yes	N/A	N/A
DIN213	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
DIN214	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1
DIN216	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H23	H1
DIN218	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1
DIN219	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H25	H1
DIN220	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
DIN394	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN395	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN396	Dingiri 1	Seasonal pool	URR	B. senegalensis	B. senegalensis	yes	no	H23	H1
DIN397	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN398	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN399	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN400	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
DIN401	Dingiri 1	Seasonal pool	URR	B. senegalensis	N/A	no	yes	N/A	N/A
MSC222	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	yes	H24	H7
MSC223	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H10	H7
MSC226	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC227	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC228	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC230	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC231	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC232	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC233	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC234	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC235	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H7
MSC236	Madina Samaco	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
MSC237	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC238	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC239	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC240	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC241	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC242	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC243	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
MSC244	Madina Samaco	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
JHK253	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H1
JHK554	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H1
JHK255	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H1
JHK264	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
JHK371	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	yes	no	H25	H5
JHK381	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	yes	no	H25	H1
JHK391	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	yes	no	H25	H1
JHK401	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H1
JHK411	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H1

JHK421	Jahanka	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H5
JHK448	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK451	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK251	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK258	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK260	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK262	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK263	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHLK265	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK266	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
JHK267	Jahanka	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB279	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB281	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB289	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB476	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H10	H2
DOB478	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB483	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H10	H2
DOB484	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB485	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
DOB486	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H1
DOB487	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H24	H3
DOB488	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
DOB490	Dobo	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	N/A	N/A
DOB272	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB276	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB288	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB289	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB290	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB291	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB292	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
DOB293	Dobo	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO317	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
SBO407	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SBO411	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	yes	H25	H1
SBO414	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO415	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	yes	N/A	N/A
SBO416	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SBO417	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO418	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO419	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO420	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO421	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO422	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SBO423	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SBO424	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H5
SBO425	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SBO426	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H5
SBO427	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SBO558	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SBO559	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SBO561	Sinchu Bokary	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H7
NJ452	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ453	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ454	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	yes	no	H24	H1
NJ455	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ456	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ457	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ458	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ459	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A

NJ460	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ461	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ462	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ463	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ464	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
NJ465	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ466	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
NJ467	Njoren	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H27	H5
NJ468	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ469	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ470	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
NJ471	Njoren	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SMB378	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB379	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB380	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SMB381	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SMB382	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB383	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB384	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB385	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB386	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB387	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB388	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SMB389	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SMB390	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SMB391	Sare Madi Babadi	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H1
SC568	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H12	H7
SC570	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H3	H7
SC494	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SC497	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC492	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC493	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H7
SC495	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC498	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC502	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H7
SC569	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC496	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC499	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC271I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H2	H7
SC272I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SC273I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H21	H5
SC274I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SC275I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H25	H1
SC276I	Sare Chewto	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H3	H7
SUT1I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H14	H1
SUT2I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H14	H1
SUT3I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SUT4I	Sutukonding	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SUT5I	Sutukonding	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SUT6I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H14	H1
SUT7I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H11	H1
SUT8I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SUT9I	Sutukonding	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1

[illegible]

CHO81I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO82I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO83I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO84I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO85I	Choya	Stream	CRR	B. forskalii	N/A	no	no	N/A	N/A
CHO86I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO87I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO89I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
CHO90I	Choya	Stream	CRR	B. forskalii	B. forskalii	no	no	H14	H2
KUN91I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN92I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN93I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN94I	Kunting	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KUN95I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN96I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN97I	Kunting	Seasonal pool	CRR	B. senegalensis	N/A	no	no	N/A	N/A
KUN98I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN99I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN100I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
KUN101I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H9	H1
KUN102I	Kunting	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H6	H7
BKM103I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM104I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM105I	Basse Kabakama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
BKM106I	Basse Kabakama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
BKM107I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H1	H4
BKM108I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM109I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H1	H4
BKM110I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM111I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM112I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM113I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
BKM114I	Basse Kabakama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
SUD127I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD128I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SUD129I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD130I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H4
SUD131I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD132I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD133I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD134I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H3
SUD135I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H1	H1
SUD136I	Suduwol	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H3
SUD137I	Suduwol	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SUD138I	Suduwol	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
NDE139I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE140I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE141I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE142I	Ndemban	Swamp	WR	B. forskalii	N/A	no	no	N/A	N/A
NDE143I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE144I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE145I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE146I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE147I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE148I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE149I	Ndemban	Swamp	WR	B. forskalii	B. forskalii	no	no	H9	H3
NDE150I	Ndemban	Swamp	WR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SBA151I	Sanka Bari	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H8	H7
SBA152I	Sanka Bari	Seasonal pool	CRR	B. senegalensis	B. senegalensis	no	no	H8	H7

SIF204I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SIF205I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SIF206I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SIF207I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	N/A	no	no	N/A	N/A
SIF208I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SIF209I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SIF210I	Sifoe/Kitty	Seasonal pool	WR	B. senegalensis	B. senegalensis	no	no	H9	H1
SPB247I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H9	H7
SPB248I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SPB249I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H22	H1
SPB250I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H20	H1
SPB251I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H9	H1
SPB252I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H15	H1
SPB253I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SPB254I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SPB255I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SPB256I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H9	H1
SPB257I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	N/A	no	no	N/A	N/A
SPB258I	Sare Pateh Bakery	Seasonal pool	URR	B. senegalensis	B. senegalensis	no	no	H24	H1
SOL519	Sololo	Swamp	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SOL625	Sololo	Swamp	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
SOL265I	Sololo	Swamp	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SOL266I	Sololo	Swamp	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SOL267I	Sololo	Swamp	CRR	B. senegalensis	N/A	no	no	N/A	N/A
SOL515	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL516	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H4	H4
SOL517	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL522	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL525	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL624	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL627	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL628	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	yes	H4	H4
SOL629	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	yes	H4	H4
SOL653	Sololo	Swamp	CRR	B. forskalii	B. forskalii	no	no	H6	H4
SOL518	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL520	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL521	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL523	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL524	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL526	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL626	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL651	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL652	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SOL654	Sololo	Swamp	CRR	B. forskalii	N/A	no	no	N/A	N/A
SC491	Sare Chewto	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SC500	Sare Chewto	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SC501	Sare Chewto	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SC571	Sare Chewto	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SC573	Sare Chewto	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SB169	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB171	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB173	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB543	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB544	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB545	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB547	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	N/A	N/A
SB548	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB549	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
SB550	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1

SB225l	Sare Bolli	Seasonal pool	CRR	B. forskalii	B. forskalii	no	no	H13	H1
BSG562	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H6	H4
BSG563	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG564	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H6	H4
BSG565	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG566	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG567	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG503	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG504	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H1	H4
BSG507	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H4
BSG512	Bansang	Rice field	CRR	B. forskalii	B. forskalii	no	no	H1	H4
BSG505	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG506	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG508	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG509	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG510	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG511	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG513	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG514	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG645	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
BSG646	Bansang	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
SS30e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	yes	H7	H4
SS41e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H4	H4
SS42e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H1	H4
SS46e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H7	H4
SS53e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
SS54e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	yes	H6	H4
SS55e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	yes	H2	H4
SS56e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	yes	H6	H4
SS61e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
SS65e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
SS37e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	N/A	N/A
SS44e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	N/A	N/A
SS60e	Sotuma Sire	Stream	URR	B. forskalii	B. forskalii	no	no	N/A	N/A
SS45e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS47e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS50e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS51e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS52e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS57e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
SS62e	Sotuma Sire	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM67e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM68e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM69e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM70e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM71e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM72e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM74e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM75e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM79e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	yes	H1	H4
MBM80e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
MBM81e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	yes	H6	H4
MBM82e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H5	H4
MBM83e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
MBM84e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H1	H4
MBM85e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H4	H4
MBM86e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
MBM87e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
MBM88e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4

MBM89e	Misra Ba Mariama	Stream	URR	B. forskalii	B. forskalii	no	no	H5	H4
MBM90e	Misra Ba Mariama	Stream	URR	B. forskalii	N/A	no	no	N/A	N/A
DAL141e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL142e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL143e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL144e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	yes	H3	H2
DAL145e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL146e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL147e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL150e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL151e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL152e	Dalaba	Stream	CRR	B. forskalii	B. forskalii	no	no	H3	H2
DAL148e	Dalaba	Stream	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
DAL149e	Dalaba	Stream	CRR	B. senegalensis	B. senegalensis	no	no	N/A	N/A
KTR153e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	H17	H2
KTR154e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	H18	H2
KTR155e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR156e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	H17	H2
KTR157e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR158e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR159e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	H16	H2
KTR160e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR161e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR162e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
KTR163e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	N/A	N/A
KTR164e	Kuntaur Wharf Town	Rice field	CRR	B. forskalii	B. forskalii	no	yes	N/A	N/A
PCH2_165e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H10	H2
PCH2_166e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H8	H2
PCH2_167e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H2
PCH2_168e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H8	H2
PCH2_169e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H11	H2
PCH2_170e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H11	H2
PCH2_171e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H11	H2
PCH2_172e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H2
PCH2_173e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H2
PCH2_174e	Pacharr 2	Rice field	CRR	B. forskalii	B. forskalii	no	no	H4	H1
PCH1_175e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H4	H4
PCH1_176e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H4	H4
PCH1_177e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	yes	H4	H4
PCH1_178e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H4	H4
PCH1_179e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H4	H4
PCH1_180e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H4	H4
PCH1_181e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	yes	H4	H4
PCH1_182e	Pacharr 1	Irrigation canal	CRR	B. forskalii	N/A	no	no	N/A	N/A
PCH1_183e	Pacharr 1	Irrigation canal	CRR	B. forskalii	N/A	no	no	N/A	N/A
PCH1_184e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H6	H4
PCH1_185e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	no	H6	H4
PCH1_186e	Pacharr 1	Irrigation canal	CRR	B. forskalii	B. forskalii	no	yes	H4	H4
JAK576	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
JAK577	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK578	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
JAK579	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H15	H2
JAK580	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK581	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK582	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK583	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK584	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2
JAK585	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H17	H2
JAK586	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	B. forskalii	no	no	H12	H2

JAK587	Jakaba/Kuntaur Fulakunda	Rice field	CRR	B. forskalii	N/A	no	no	N/A	N/A
DEM600	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
DEM601	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
DEM602	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM603	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H1	H4
DEM604	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM605	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM606	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM607	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM608	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H2	H4
DEM609	Demba Kunda	Stream	URR	B. forskalii	B. forskalii	no	no	H6	H4
DEM610	Demba Kunda	Stream	URR	B. forskalii	N/A	no	yes	N/A	N/A
MBM2e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	no	N/A	N/A
MBM10e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	no	N/A	N/A
MBM11e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM12e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM13e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM21e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	no	N/A	N/A
MBM22e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	N/A	N/A
MBM91e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM92e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM93e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM94e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM95e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	H6	H2
MBM96e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	N/A	N/A
MBM97e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	yes	H6	H2
MBM98e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	yes	H6	H2
MBM99e	Misra Ba Mariama	Stream	URR	B. truncatus	B. truncatus	no	no	N/A	N/A
MBM100e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	yes	N/A	N/A
MBM101e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	no	N/A	N/A
MBM102e	Misra Ba Mariama	Stream	URR	B. truncatus	N/A	no	no	N/A	N/A
MN1_115e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_116e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	yes	H2	H1
MN1_117e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_118e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_119e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_120e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_121e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_122e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_123e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
MN1_124e	Madina Nfally 1	Stream	CRR	B. truncatus	B. truncatus	no	no	H1	H1
PCH1_109e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A
PCH1_110e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A
PCH1_111e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A
PCH1_112e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H5	H2
PCH1_113e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H5	H3
PCH1_114e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H5	H4
PCH1_127e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H5	H5
PCH1_128e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	yes	H3	H6
PCH1_129e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	N/A	N/A
PCH1_130e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H3	H1
PCH1_131e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H3	H2
PCH1_132e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	N/A	N/A
PCH1_133e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	yes	H4	H2
PCH1_134e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H3	H1
PCH1_135e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	N/A	N/A
PCH1_136e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	N/A	N/A
PCH1_137e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A
PCH1_138e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A

PCH1_139e	Pacharr 1	Irrigation canal	CRR	B. truncatus	B. truncatus	no	no	H5	H2
PCH1_140e	Pacharr 1	Irrigation canal	CRR	B. truncatus	N/A	no	no	N/A	N/A