CHARACTERIZATION OF THE POPULATION OF VECTOR SNAILS FROM MWEA IRRIGATION SCHEME FOR *SCHISTOSOMA MANSONI* TRANSMISSION

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN THE SCHOOL OF SCIENCE,

UNIVERSITY OF ELDORET, KENYA

JUNE, 2021

DECLARATION

DECLARATION BY THE CANDIDATE

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This work is dedicated to my wife Leah Njeri, Children Peter Ngigi and Mercy Nyambura, whose love and generous support has been a fundamental ingredient in all my undertakings, including this one.

ABSTRACT

Schistosomiasis is recognized as one of major neglected tropical diseases. Freshwater snails of the genus *Biomphalaria* serve as obligatory intermediate hosts of *Schistosoma mansoni*. The disease affects more than 249 million people worldwide with more than 90% of those requiring treatment residing in Africa. The use of synthetic molluscides to control vector snails is increasingly becoming unpopular due to its adverse effects on the environment and its associated high costs. Currently there are no vaccines proven effective. It is therefore important to target the aquatic stages of schistosome life cycle to compliment control measures. This study was set out to identify and characterize schistosome resistant snails from Mwea Irrigation Scheme. The study objectives were: To determine population proportion of resistance snails; To determine morphological characteristics of *S. mansoni* resistant and susceptible snails; To investigate evolutionary development *Biomphalaria sp*; and To assess transmission levels of resistant traits in snails under laboratory conditions. Snails infected with *S. mansoni* were determined by direct light illumination method and parasite rDNA observed by agarose gel electrophoresis. Morphological characterization of resistant and susceptible snails was determined through shell morphometric. PCR products of Snails rDNA were used for custom sequencing. Sequencing datasets were analyzed to generate the snails' phylogenetic tree. Experimental snails were infected with *S. mansoni* and infections rates determined up to F2 progeny. It was found that some *Biomphalaria pfeifferi* snails are refractory to transmission of cercaria. The mean value of infection rates for the Field, F1 and F2 snails were 36.6 ± 3.72, 1.93 ± 1.46, 0.36 ± 0.049 respectively with the infection rates decreasing from the field snail samples through to F2 snail samples. The snail morphological parameters had no significant differences in resistant and susceptible populations. The snails had mean shell height of 9.9 and 10.0 mm and shell width of 9.5 mm and 9.5 mm for resistant and susceptible snails respectively. At molecular level, PCR detection of cercaria in snails after exposure to miracidia larvae produced specific amplification of target size band of approximately 400 bp in rDNA ITS gene. Some snails had higher positive titres of cercaria DNA while others were negative. There was active transmission of resistant traits under laboratory conditions. The exhibited resistance traits *in B. pfeifferi* could not be attributed to morphological characteristics. The genetic lineage of *B. pfeifferi* was monophyletic with clusters of closely related isolates. Genus *Biomphalaria* is still undergoing population expansion through random mutation.

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LIST OF ABBREVIATIONS

BLAST	-	Basic Local Alignment Search Tool
dNTP	-	Deoxyribose Nucleotide Triphosphates
IPR	-	Institute of Primate Research
ITS	–	Internal Transcribed Spacers
MDA	-	Mass drug administration
NACOSTI	-	National Commission for Science, Technology and Innovation
NCBI	-	National Centre of Biotechnology Information
NMK	–	National Museums of Kenya
NRF	-	National Research Fund
NTD	-	Neglected Tropical Diseases
PCR	-	Polymerase Chain Reaction
WASH	-	Water, Sanitation and Hygiene
WHO	–	World Health Organization

ACKNOWLEDGEMENT

I wish to extend my heartfelt gratitude to my supervisors Prof. F.M.E. Wanjala, Dr. Stella Kirui and Dr. Kem Githui for working tirelessly in all steps from the inception of idea up to the final write up of the thesis. Their valuable guidance and mentorship are highly acknowledged.

This study was made possible through valuable efforts of several institutions and people for whom I'm greatly indebted. I'm very grateful for the funding from National Commission for Science, Technology and Innovation (NACOSTI), National Research Fund (NRF) and Institute of Primate Research (IPR) without which this work would not have been possible. I'm grateful to the Management of National Museums of Kenya (NMK) and IPR for allowing access to the laboratory facilities in their institutions.

I highly value the contribution of Dr. J. Njenga of Kibibi Sub-County Hospital in Mwea for facilitating the access of hospital data. Special thanks to the laboratory technicians and field attendants lead by Mr. Simon Kiarie who greatly assisted in both field and laboratory data collection. Their efforts made this work possible.

Finally, I wish to thanks my family for their support, encouragement and understanding which made everything so worthwhile and to whom I dedicate this thesis.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Fresh water snails of *Biomphalaria sp*are known to be the most prevalent vectors of the schistosomiasis, a vector-borne parasitic disease caused by trematodes of the genus *Schistosoma* (Souza, *et al*., 1995). This disease is endemic in the tropic and sub-tropic regions of the world but its medical and economic burdens are often neglected. The distribution of intermediate snail hosts particularly of the genus *Biomphalaria* is focal to the transmission of the parasites (Abe, *et al*., 2018).

The presence and distribution of snails intermediate hosts plays a crucial role to facilitate continued transmission in endemic areas (Toreros, *et al.,* 2015). The snails serve as obligatory hosts for the larval stage, which infects humans. This disease is one of the most prevalent parasitic infections in tropical and sub-tropical regions of the world and it has significant economic and public health consequences in these regions. It is recognized as one of the major Neglected Tropical Diseases (NTD), second to malaria in terms of its adverse socio-economic and public health impact in Africa, tropical and subtropical third-world developing countries. It is estimated that more than 249 million people worldwide are infected, with more than 90% of those requiring treatment residing in Africa (Hotez, *et al*., 2012, Colley *et al*., 2014 &WHO, 2014), and every year, more than 200,000 people lose their lives as a result of these infections worldwide(WHO, 2019).Schistosomiasis has been termed a “silent pandemic” due to the misery caused and decreased productivity of affected individuals (Morgan *et al*., 2001, King *et al*., 2005).

Schistosomiasis affects humans and other mammals including primates, cattle, birds and crocodilians (Bowles, *et al*. 1995). In human the chronic disease is debilitating particularly in early childhood, the main impact being low productivity output in terms of human resources. World Health Organization global epidemiological data shows that schistosomiasis is endemic in 78 tropical and subtropical countries with an estimated 800 million people at risk of the infection (Chitsulo, *et al*., 2000; WHO, 2014). In Kenya, it is estimated that more than six million people, accounting for approximately 23 % of the total population, are infected with urinary or intestinal schistosomiasis (Chitsulo, *et al*., 2000; Farah, *et al*., 2000).

Snails and human infections occur in Schistosomes contaminated freshwater bodies. Human populations at high risks of infections are largely those who depend on fresh water bodies inhabited by the snail hosts in their day to day economic activities(Toreros, *et al.*, 2015; Abe, *et al.*, 2018). Inadequate sanitation, human cultural habits, and the presence of the *Biomphalaria* snails contribute to the persistence of the parasite's life cycle and consequently to the disease's geographic spread. In Africa, this challenge is compounded by inadequate access to basic infrastructure by most inhabitants living in endemic areas and the limited access to chemotherapy, which constitute the main control strategy (Larding & Disso, 1998; Rocha, *et al.*, 2016; Abe, *et al.*, 2018).

It is known that Schistosomes require a Mollusca intermediate host in which they not only undergo a series of developmental stages but also asexual reproduction (Cheng &Bier, 1972). Schistosome *larvae (*miracidia) are attracted by water inhabited by snails, where they penetrate in to the body surface of the snail by a lytic enzyme secreted from the apical glands or the gut(Wajdi, 1966; Argiro, 2000). Miracidia are the infective forms for the snail

host, differentiating into sporocysts immediately after penetration. During the course of its life cycle, a Schistosome goes through distinct stages of differentiation (Maluku, *et al*., 2014). The mother sporocysts have also been found in the head-foot and tentacles of the snails (Lewis, *et al*., 2001).

One of the desired efforts in interventions of Schistosomiasis control is development of novel tools that include breaking the transmission of the schistosome parasites at the snail stage of its life cycle (King, *et al*., 2006; Kjetland, *et al*., 2006; Rollinson *et al*., 2009).There are continued efforts to provide insights into the factors that influence host-parasites compatibility on local scales (Ibikounlé, *et al*., 2012). More intervention strategies and commitment is required to ensuring the elimination of schistosomiasis from endemic regions of Africa. In efforts to eliminate schistosomiasis, snail hosts and Schistosome parasites interactions are crucial (Agola, *et al*., 2009; Bergquist, 2017)). In diversifying snails control measures, it is imperative to generate adequate information on dynamics of local snail host populations susceptibility to Schistosomes parasites (Tchuente, *et al*., 1999). As reported by Rollinson, *et al*.,(2000), global awareness need to be raised to provide adequate support mechanisms towards elimination of schistosomiasis in endemic countries. More information on susceptibility and resistant traits host snails vector is likely to provide basis for further biological interventions in snails control measures in their habitats (Hoffmann & Strand 1997; Tchuente, *et al.*, 1999; El Naga, *et al*., 2010).

Different snail-specific factors are critical in determining *Biomphalaria* susceptibility to Schistosomes (Negrao-Correa, *et al*., 2007, El Naga, *et al*., 2010). Within this context, it has been found that susceptibility of *B. glabrata* to *S. mansoni* infection is a hereditary character. Moreover, resistance of *B. glabrata* to *S. mansoni* infection was found to vary

with age and in juveniles it is controlled by at least four genes, each with several alleles, whereas in adulthood, only a single dominant gene determines this trait (Richards& Shade, 1987; Richards, *et al*., 1992; Spada, *et al*., 2002; Ittiprasert, *et al*., 2010). In *Biomphalaria tenagophila*, two dominant genes determine resistance (Rosa, *et al*.. 2005). Nevertheless, for *B. Alexandrina*, little is known about the effect of age on genetic modulation and *S. mansoni* infection compatibility. Another important factor determining the compatibility of *Biomphalaria sp* to *S. mansoni* infection is the snail's internal defense system (IDS).

With the current global interests in eliminating schistosomiasis, a considerable variety of snails control strategies have been proposed(Knight, *et al*., 2014). Nevertheless, total elimination has not yet been achieved and new cases are continuously being reported. It is clear that breaking the life cycle of this parasite will decrease its transmission and this can be achieved by targeting its intermediate host snails(Steinmann, *et al*., 2006; Knight, *et al*., 2014). Control method involving the reduction of a susceptible snails population by introducing a population that is known to be genetically resistant to the parasite has been tried but with limited success (Coelho, *et al*., 2004; Marques, *et al.*, 2014).

Recent advances in understanding of the genetics of host-parasite interactions have increased the interest in directing resistance genes into susceptible vector populations to render them resistant or even to exhibit low susceptibility when challenged by the parasite (Bonner, *et al*., 2012). A desirable better long-term solution would be to generate the field resistant vector populations or those that are less susceptible to infection, an ecologically safer way of breaking transmission cycles as reported by (Townson, *et al*., 2005; Bonner, *et al*., 2012). Indeed, studies concerning the genetic variability of *Biomphalaria sp* with different degrees of susceptibility to *S. mansoni* infection can add to the development of

control strategies for schistosomiasis (Chevreux, *et al*., 2004; Oliveira, *et al*., 2010).

1.2 Statement of the problem and Justification of the study

Globally, there are over 249 million people infected by Schistosomiasis (Colley, *et al*., 2014). The disease burden is estimated to exceed 70 million disability-adjusted life-years, and the current praziquantel-based control programs in Asia and sub-Saharan Africa are not effective or sustainable in the long term (Ismail, 2011). While a single dose of praziquantel can be used to treat the disease, humans get re-infected soon after treatment if they enter the water containing the infectious cercariae. Moreover, the drug is also found not to eliminate 100% of the worms, for the people who are heavily infected. Currently, there is a growing recognition that mass drug administration (MDA)is not likely to decrease the infection levels to where transmission will be interrupted so that there will be no additional control (King, *et al.,* 2006; Sokolow, *et al.,* 2013; King, *et al*., 2015). The use of synthetic molluscicides is also increasing becoming unpopular due to adverse effects on environment and associated high costs. The World Health assembly has recently passed the resolution of calling for the elimination of schistosomiasis, the motivation for finding schistosomiasis control means in addition to treatment must be intensified.

In Mwea irrigation scheme in Kirinyaga County, schistosomiasis transmissions are relatively high with a prevalence of 47.4% (Mwai, *et al*., 2016). Freshwater snails, *B. pfeifferi* a major vector of *S. mansoni* has been reported to be predominant in Mwea irrigation scheme (Mutuku, *et al*., 2017). This situation calls for integrated intervention control measures. One of the popular aspiration for the control of schistosomiasis has been the development of vaccine. However, there is still no viable vaccine that has been developed. Therefore, the only experimentally available means to augment chemotherapy

treatment will be by interventions that could interrupt Schistosome parasite transmissions at aquatic life.

In has been demonstrated that destroying the snail vectors using molluscicidal agents is one way to interrupt the life cycle of parasite at snail's aquatic life and prevent human infection. However, due to costs of synthetic molluscicides, the concern about the development of resistance to them, and their toxicity to non-target organisms have led to an increasing interest in searching other measures to control the vector. Other methods employed include the introduction of biological competitors' species or natural enemies to snails and alteration of the environment but they have not succeeded to control Schistosomiasis (Steinmann, *et al*., 2006; Knight *et al*., 2014).

There are known vector snails that display Schistosome larvae (miracidia) susceptibility and resistance strains. Identifying and characterizing the resistant strains of the vector snail could provide new approaches for biological control interventions (Townson, *et al*., 2005; Bonner, *et al*., 2012). Development of *S. mansoni* in the intermediate host snails is influenced by a number of parasite and snail genes (Lewis, *et al*., 1997). Understanding these hereditary characteristics involved in host/parasite relationship may lead to a desirable approach of introducing resistant *Biomphalaria sp* into the field as a means of biological control for the parasite. This study therefore, was set out to identify and characterize resistant strains of the vector snails from a schistosomiasis endemic area.

1.3 Objectives

1.3.1 Broad objective

The study was set out to identify and characterize *Biomphalaria sp* snails resistant and susceptible to *S. mansoni* infections in Mwea irrigation scheme, Kirinyaga County with a view to expound on vector biological control interventions.

1.3.2 Specific objectives of the study

i) To determine population proportion of resistant and susceptible snails from Mwea Irrigation scheme using light illumination and molecular typing methods;

ii) To determine morphological characteristics of *S. mansoni* resistant and susceptible *Biomphalaria sp* snails samples from Mwea Irrigation scheme;

iii) To investigate evolutionary development of vector *Biomphalaria sp*;

iv) To assess transmission levels of resistant traits in *Biomphalaria sp* snails under laboratory conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution of Vector snails and parasites

Snails are invertebrate animals, belonging to the Phylum Mollusca. This group of organisms possess “shell” which is a major characteristic of the group(Abe, *et al*., 2018). Snails inhabit a wide range of habitats because they are found not only in freshwater environment but also in other ecological niches (Abe, *et al*., 2018). Many species of freshwater snail belonging to the family Planorbidae are intermediate hosts of highly infective fluke (trematode) larvae of the genus *Schistosoma* which cause schistosomiasis, also known as bilharzia in Africa, Asia and the Americas. Schistosomiasis affects humans and other vertebrate mammals including primates, cattle, birds and crocodilians (Bowles, *et al*., 1995).

Most of intermediate host snails of *Schistosoma* parasites belong to three genera, *Biomphalaria, Bulinus* and *Oncomelania*. About 350 snail species in these genera are estimated to be of possible medical or veterinary importance. The species involved can be identified by the shape of the outer shell. These vector snails can be divided into two main groups: the aquatic snails that live under water and cannot usually survive elsewhere *Biomphalaria* and *Bulinus*, and amphibious snails adapted for living in and out of water *Oncomelania* (Abe, *et al.,* 2018). Each of the common human *Schistosoma* parasite species has its own snail species as a vector, Genus *Biomphalaria* (*S. mansoni*), *Bulinus* (*S. haematobium* and *S. intercalatum*), and *Oncomelania* (*S. japonicum* and *S. mekongi*). In Africa and America, snails of the genus Biomphalaria serve as intermediate hosts of *S.*

mansoni while genus *Bulinus* serve as the intermediate hosts of *S. haematobium* and *S. intercalatum* in Africa and the Mediterranean. In Southeast Asia, *Oncomelania* serves as the intermediate host of *S. japonicum* and *S. mekongi* (Gryseels, 1989; Chen, *et al*., 2014).

Schistosomiasis infection is widespread in tropical and sub-tropical countries. Although mortality rate is relatively low, severe debilitating illness is caused in millions of people (Farah, *et al*., 2000). It is prevalent in areas where the snail intermediate hosts breed in waters contaminated by faeces or urine of infected persons. Humans acquire schistosomiasis through repeated contact with Schistosome larvae contaminated waters during fishing, farming, swimming, washing, bathing and recreational activities (Chitsulo, *et al*., 2000). Water resources development schemes in certain areas, particularly irrigation schemes, greatly contribute to the introduction and spread of schistosomiasis. The snails are considered to be the intermediate hosts because humans harbour the sexual stages of the parasites and the snails harbour the asexual stages. Humans serve as vectors by contaminating the environment. Transfer of the infection requires no direct contact between snails and humans. Freshwater snails are also intermediate hosts of food borne fluke infections affecting the liver, lungs and intestines of humans (Farah, *et al*., 2000; Chitsulo, *et al*., 2000).

In human, this chronic disease is debilitating particularly in early childhood. One of the main impacts of schistosomiasis is known to be low productivity output in terms of human resources. The World Health Organization (WHO) global epidemiological data indicates that schistosomiasis is endemic in about 78 tropical and sub-tropical countries (WHO, 2014). It is estimated that approximately 779 million people leave at risk of schistosomiasis globally with more than 230 million infected and of these 20 million suffer from

debilitating illnesses associated with schistosomiasis (Chitsulo, *et al*., 2000). In Kenya, it is estimated that there are more than 6 million people who are infected with urinary or intestinal schistosomiasis (Chitsulo, *et al*., 2000; Farah, *et al*., 2000).

In Kenya, Schistosomiasis is endemic, and the entire human population is considered to be at risk of contracting the disease (Iatroski, *et al*., 1981; Chitsulo, *et al*., 2000). Both *S. haematobium* and *S. mansonii*, the agents responsible for urinary schistosomiasis and intestinal schistosomiasis, respectively are endemic in Kenya. There are numerous intermediate host snail species acting as the environmental reservoirs of the disease and maintaining transmission, including *B. pfeifferi* and *B. sudanica* for *S. mansoni*, and *Bulinus africans* and *Bulinus glucoses* for *S. haematobium* (Iatroski, *et al*., 198; Poise, *et al*., 2011; Rollinson, *et al*., 2013; Ochanda, 2017).

Transmission of Schistosomiasis is generally associated with poverty, poor water supply and inadequate sanitation (Chitsulo, *et al*., 2000). Infection rates and intensities are high in early childhood, peak around 8 to 15 years and decrease in adulthood. The ecological conditions favoring transmission are: fresh water wetlands, tropical climate, intermediate host-specific snails and lack of proper sanitation. Therefore, Wetlands and Irrigations schemes in Kenya are sites for Schistosomiasis transmission.

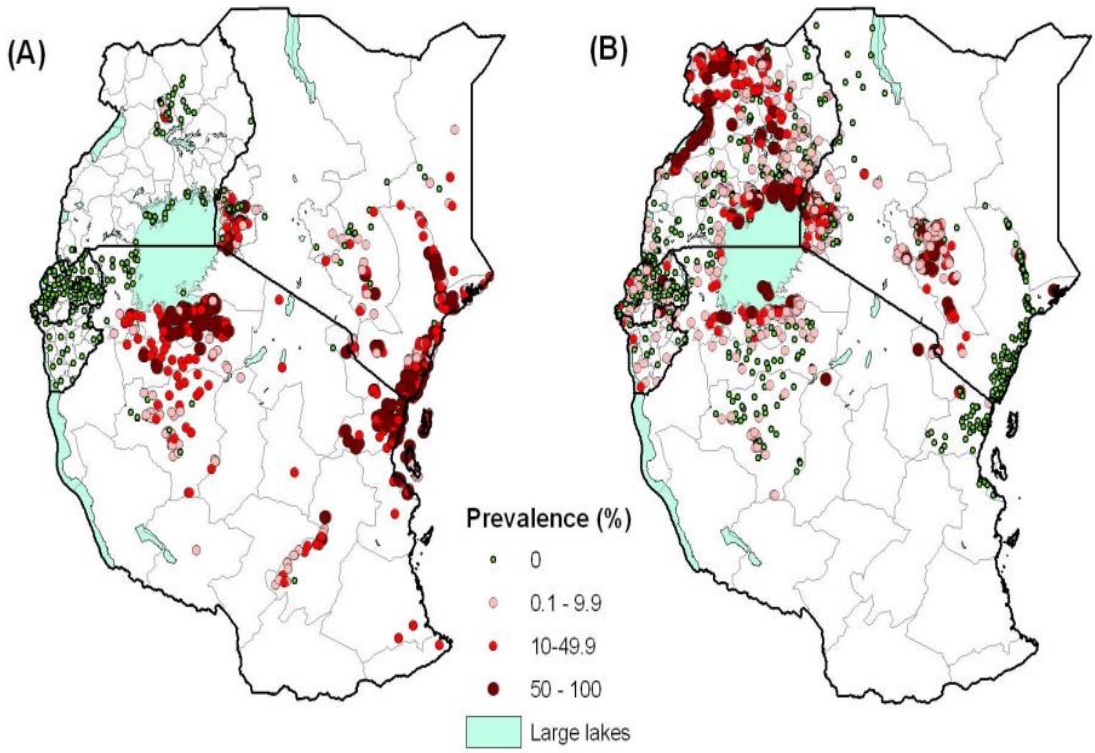

Figure 2.1Geographical distribution of schistosomiasis in East Africa

(This geographical distribution of (A) *Schistosoma haematobium* and (B) *S. mansoni* infection, based on available survey collected between 1980 and 2009, and categorized according to WHO prevalence thresholds (n = 2,405), (Brooker, *et al*., 2009).

2.2 Biology of vector snails

2.2.1 Life cycle

All species of the genus *Biomphalaria* and *Bulinus* are hermaphrodite, and are capable of self- or cross-fertilization. A single specimen can invade and populate a new habitat rapidly (WHO, 1997). Normally 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 reaching maturity in 4–7 weeks, depending on the species and environmental conditions. Temperature and food availability are among the most

important limiting factors in snails reproduction. A snail lays up to 1,000 eggs during its lifetime, which may last more than a year (Ahmed & Nabil, 2012).

The vector snail, *B. pfeifferi* is one of the air-breathing freshwater snail species, which is an aquatic culminate gastropod mollusk in the family of Planorbidae. This species is medically important vector because of transmitting schistosomiasis (Pointier, *et al.*, 2005). These snails can easily survive between 10°C to 35 °C temperature ranges. In most areas, seasonal changes in rainfall, water level and temperature cause marked fluctuations in snail population densities and transmission rates (WHO, 1997). Reservoirs that contain water for several months of the year such as in Sahelian Africa can be intensive transmission sites during a very limited period, because the vector snails can rapidly recolonize the reservoirs after the rains start.

2.2.2 Ecology of Vector Snails

Knowledge of freshwater vector snails distribution and their habitat preference is important in providing information to initiate and set-up effective snail control programmes. Snails are known to inhabit a wide range of freshwater bodies from small temporary ponds, streams to large lakes and rivers. The distribution of snails within these habitats may be patchy and detection requires examination of different sites. Moreover, snail densities vary significantly with the seasons (Pyron and Brown, 2015). In general, the aquatic snail hosts of schistosomes occur in shallow water near the shores of lakes, ponds, marshes, streams and irrigation channels. They live on water plants and mud that is rich in decaying organic matter. They can also be found on rocks, stones or concrete covered with algae or on various types of debris. They are most common in waters where water plants are abundant and in water moderately

polluted with organic matter, such as faeces and urine, as is often the case near human habitations. Plants serve as substrates for feeding and oviposition as well as providing protection from high water velocities and predators such as fish and birds. Most aquatic snail species die when stranded on dry land in the dry season. However, a proportion of some snail species are able to withstand desiccation for months while buried in the mud bottom by sealing their shell opening with a layer of mucus. Most species can survive outside water for short periods (Tchakonté, *et al.*, 2014; Pyron & Brown, 2015).

The ecological conditions facilitating active transmission of schistosomiasis are known to be fresh water wetlands, tropical climate, intermediate host-specific snail and lack of proper sanitation. While wetlands are valuable focal points for provision of domestic water for consumption, irrigation agriculture, fishing, they have one major drawback in these ecosystems in that they represent sites for active disease transmission (Farah, *et al.*, 2,000).

2.3 Transmission of Schistosomes

Schistosomes have an indirect life cycle, involving asexual reproduction in a snail intermediate host and sexual reproduction in a mammalian or avian definitive host (Seubert, *et al*., 1977). Aquatic snails of the genus *Biomphalaria* act as intermediate hosts in the Schistosome parasite life cycle. *S. mansoni* remains an important parasitic disease of man which is endemic in large parts of sub-Saharan Africa, Middle East, South America and the Caribbean. The distribution of parasites among hosts is the result of interaction between numerous factors including genetic, biological, behavioral, and ecological processes (Adema, *et al*., 1994). During the

life cycle of trematodes, parasites need to penetrate into the host, develop, multiply asexually and finally leave the host to continue their life cycle (Almeida, 1980; Davies & Mckerrow, 2003).

One of key the factors in transmission of schistosomiasis in endemic areas is the Snail infection and contact patterns of human with water infested with cercaria. The life cycle of Schistosomes involves an invertebrate (snails) and a vertebrate host (e.g. man). Between the two hosts occur transient free-living stages in water. The eggs released into the water by the vertebrate host hatch to miracidia (larva) that infect snails. After several weeks of asexual multiplication in the snail tissue, the next larvae (cercaria) are released into the water where it infects the vertebrate host (Neva &Brown, 1994; Hamburger, *et al*., 1998).

Schistosomes eggs excreted by an infected person hatch to release a tiny parasite (miracidium) that swims actively through the water by means of fine hairs (cilia) covering its body. The miracidium survives for about 8–12 hours, during which time it must find and penetrates the soft body of a suitable freshwater snail in order to develop further (WHO, 1997). Depending on the species of snail and parasite, and on environmental conditions, this phase of development may take 3 weeks in hot areas, and 4–7 weeks or longer elsewhere. The fork-tailed cercariae shed by infected snails can live for up to 48 hours outside the snail's body. Within that time they must penetrate the skin of a human being in order to continue their life cycle (Bundy, 1981; WHO, 1997).

There are five species of schistosomes that are considered important parasites of human. This include *S. mansoni* (from Africa and later introduced to South America),

S. haematobium (from Africa and adjacent regions- Middle East) and *S. japonicum* (from S.E. Asia). The other two; *S. intercalatum* and *S. mekongi* have more localized geographic location (Desires *et al.,* 1993). Elsewhere, *S. bovis* has been reported as an important parasite for angulates (Barber, *et al*., 2006). Fresh water snails of the genus *Oncomelania* are the important vectors for *S. japonicum* while the culminate snails of the genera *Bulinus* and *Biomphalaria* transmit *S. haematobium* and *S. mansonii*, respectively (Sturrock, 2001).

The difference between Schistosomiasis and other water borne diseases is that other waterborne diseases occur through drinking contaminated water or through raw foods or fruits which have been washed with the contaminated water while the Schistosomiasis is through the skin getting in contact with fresh water that contain the parasite cercaria stage (Montressor, *et al*., 2012). It has been found that the strategies of treating of drinking water to avoid waterborne diseases like using chlorine or boiling does not prevent the transmission of Schistosome infection since the way of fetching water, bathing on untreated water and other way expose individuals to infection. All over the world there are about 200 million people infected by one of the three species of Schistosomes and there are about 700 million people who are at risk to be infected in developing countries in Africa, Asia, and South America (Montressor, *et al*., 2012; Steinmann *et al*., 2006).

With the right vertebrate host the cercariae changes into a larval which is known as Schistosomula. For a duration of about 4–6 weeks, the young worms move through the various organs of the human body and finally mature to adults which then reside in blood vessels near the intestines. Both sexes of the worms female and male live

together in copula and the female lay about 300 and 3000 eggs per day (WHO, 1997). The eggs, which get out of the host must reach the fresh water for them to hatch into miracidia which are infectious to intermediate snail hosts. In snails, sporocysts produce the infectious cercariae in a period of about 4 to 6 weeks after infection with miracidia (Bundy, 1981; WHO, 1997).

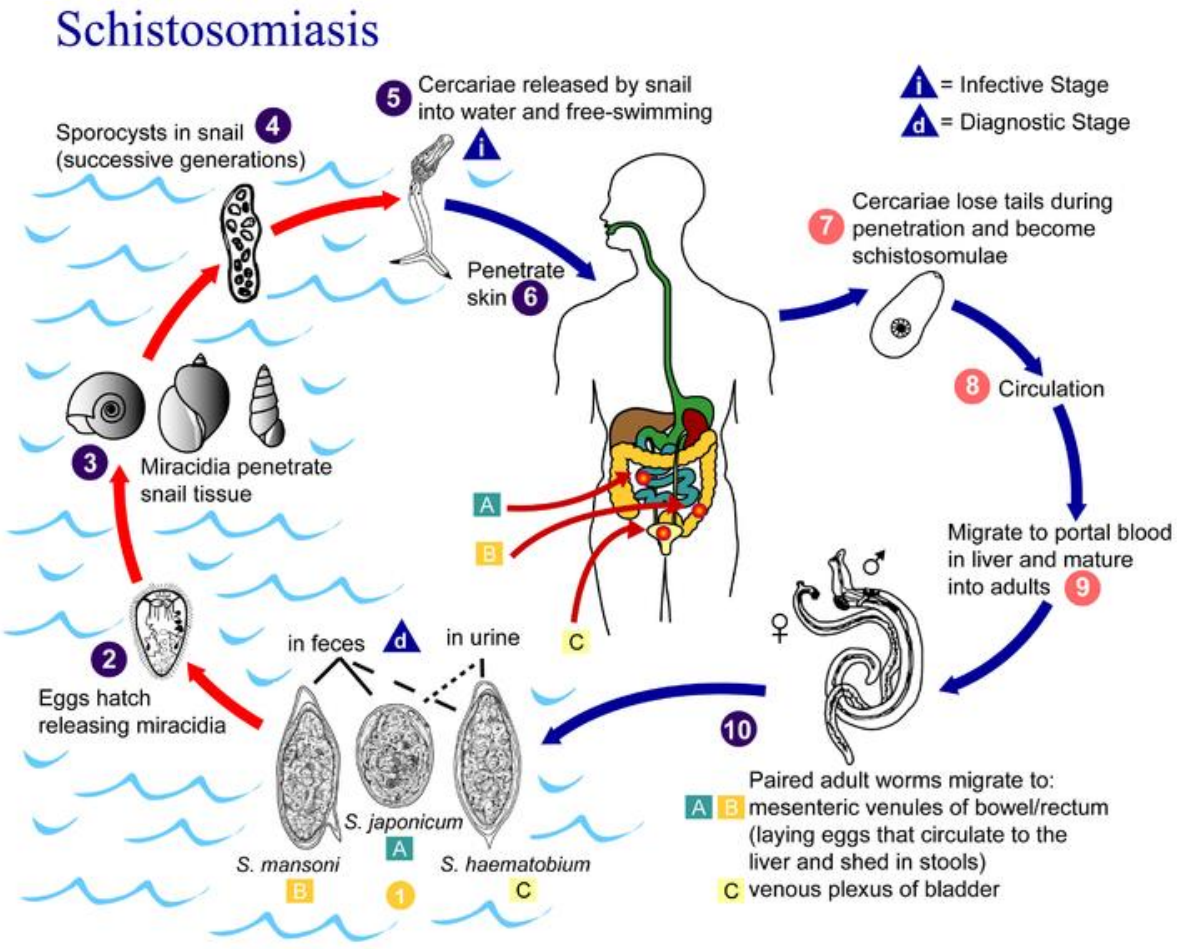

Figure 2.3.1 Demonstration of Schistosomes life cycle involving snails as intermediate host and human host (*Source: Rose, et al., 2014)*.

2.4 Vector snails control strategies

The use of praziquantel has been widely employed in treatment of human schistosomiasis for many years (Seubert, *et al*., 1977). While major attention has been focusing on control measures through human chemotherapy for morbidity control and prevalence reduction, increasingly over time, there is evidence to suggest that praziquantel-based control programs are not likely to be sufficient to achieve sustainable transmission control alone (Cook, *et al*., 1977; Gray, *et al*., 2010; Lamberton, *et al*., 2014; Lelo, *et al*., 2014; King, *et al*., 2015). Drugs alone, although they have public health benefits in reducing the pathological consequences of infection, have not eliminated transmission of schistosomiasis. Retreatment is essential to maintain initial reductions of prevalence and intensity (Chen & Feng, 1999).

Commercially-produced molluscicides have been used to control snails. However, molluscicides are not widely used due to their high cost and growing environmental concerns (Webbe, 1987). Other alternative means of transmission control have been reported to include snails' biological control of snails, provision of safe water supplies and sanitation with varied levels of achievement (WHO, 1998). After the discovery of effective treatment, other efforts to develop strategies to reduce Schistosomiais transmission were considered less critical (Chambray, 2012).

Currently, there is no single method of controlling schistosomiasis regardless of the location that has been shown to work effectively due large number of environmental variables involved in its transmission (Kings, 2009). However, four approaches to controlling infection have proven effective at the community level. These include control of snail population, community-based chemotherapy, public health education and

sanitation. It is becoming evident that interruption of Schistosome transmission in high-risk areas will require more integrated control strategies such as combination drug treatment, efficient water management, snail population control, and effective treatment of sewage and public health education (Amado, *et. al*., 2006; Kings, 2009). Other reported interventions includes removal of cattle from snail infested grassland, provision of farmers with mechanized farm equipments and transmission control via interventions targeting animal reservoirs particularly buffaloes (Goo, *et. al*., 2006; Long-De *et. al*., 2009). Integrated approach to minimize transmission has been shown to work. In a study that involved health education aimed to change human behavior regarding water contact, environmental engineering schemes to create alternative water sources and latrines, and mass chemotherapy with praziquantel, proved to greatly reduce the rate and intensity of schistosomiasis (Katsivo, *et al*., 1993).

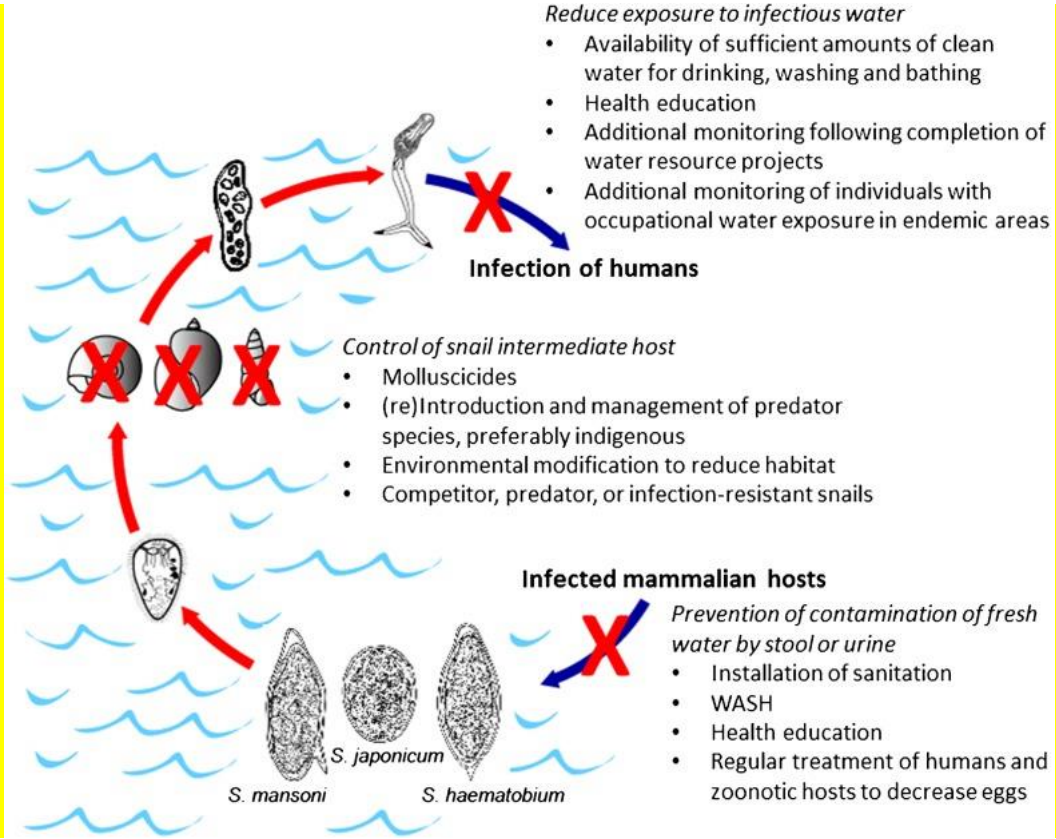

Figure 2.4.1. Schematic representation of schistosomiasis control measures targeting intermediate hosts *(Source: Secor, 2014)*

2.4.1 Biological control of Vector Snails

In biological control of Schistosomiasis vector snails, other species that are not known to be hosts of Schistosomes have been used to compete with or predate on intermediate host snails with reported success. Recent studies on snail control using predators have focused on fish and some crustacean species (Sokolow, *et al*., 2015). In carinnean region, an endemic area there has been use of ampullarid *Marisa cornuarietis* and *Melanoidees tuberculata* snails species. It was found that the

species successfully replaced populations of vector snail, *Biomphalaria sp* snails, recording reduction or interruption of transmission of *S. mansoni* (Pointier, & Journdane, 2000). In Northern Tanzania, it was reported that *M. cornuarietis* rapidly replaced population of *Biomphalaria* and other pulmonate snails following its introduction into a man-made water bodies(Nguma& Tenga, 2000).The interaction with competitor snails function by consuming and depleting food sources and egg clusters of Schistosome-transmitting snails while they have higher rates of reproduction (Negron-Aponte& Jobin, 1979; Pointier& McCullough, 1989; Chingwena *et al*., 2002 Gashaw, *et al.*, 2008). As reported by Jobin, *et al*., (1977) it was cost effective while using competitor snails in comparison to the use of molluscicides that does require frequent re-application

The use of snails predators is another approach that can be used to reduce snail populations and impacting transmission of schistosomiasis. One of the reported predatory species is the African river prawn *Macrobrachium vollenhovenii*. In Senegal, it was reported by Sokolow *et al*., (2014), that interruption of predator prawn life cycle may have contributed to the raise of schistosomiasis epidemic that occurred following construction of the Diama Dam. The crayfish *Procambarus clarkii* is another known predatory species. When it was introduced in water bodies in Kenya for aquaculture purpose, researchers made an observation that in those water bodies, it was either crayfish or snails, but not both co-existing together (Hofkin, *et al*., 1991). Under laboratory and some field enclosure studies, it was established that *P. clarkii* readily consumes *Bulinus* and *Biomphalaria* snails, as well as their egg masses. It has been reported that introduction of Louisiana red swamp

crayfish into man-made ponds has recorded significant decreases in snail populations and subsequent reductions in Schistosome infections rates among children in high transmission areas (Mkoji*et al.,* 1999). Unlike in the case of prawns in Senegal, *P. clarkii* is not a native species in Africa. This therefore may pose a potential impact of an introduced exotic species on the overall ecosystem and as such it requires consideration that could limit it's introduction in schistosomiasis endemic areas.

There are certain species of fish that are also known to be predators of snails that transmit schistosomiasis. The case of Cichlids which are molluscivores in Lake Malawi they preferentially feed on *Bulinus sp* compared to other snails with thicker shells (Evers, *et al*., 2006). The observed increase in transmission of *S. haematobium* in Lake Malawi in around mid-1980s was reported to have coincided with decreases in populations of cichlid attributed to overfishing (Stauffer, *et al.,* 1997). As the larger fish populations dwindled, fishermen resulted into use of nets with smaller openings to obtain food. This over fishing eventually led to rapid *Bulinus* populations increase, which resulted in recorded increased transmissions of schistosomiasis that had an inverse correlation between transmission and density of molluscivorous fish (Stauffer, *et al*., 2006; Madsen& Stauffer, 2011). Others research works have demonstrated that, fishes indigenous to Brazil, Zimbabwe, and Ethiopia that are molluscivorous in nature could be stocked into local waters with an aim of controlling vector snails populations. (Weinzettle & Jurberg, 1990; Chambray, 1997; Madsen & Stauffer, 2011).

There has been much focus on the use of snail competitors, but generally these are introduced species and their use may therefore not be acceptable ecologically given the risk that some species may become invasive. Also, potential predators should be from within the major catchment area of the sites where used (Nguma, *et al*., 1982; Sokolow, *et al*., 2015).

One other focus in biological control of snails is based on the premise that snails resistant to parasitic infection could be used as biological competitors to replace existing susceptible snails in endemic areas (Coelho, *et al*., 2004). To determine genetic basis of resistance (Larson *et al*., 1996; Allan, *et al.*, 2013) conducted inbreeding experiments to produce snail population with both susceptible and resistant individual lines of *B. glabrata*. The inbred lines were found to have line-specific differences in numbers of spreading haemocytes; these were enumerated in both juvenile and adult snails. Lines with high cell numbers were invariably resistant to *S. mansonii*, whereas lines with lower cell numbers could be resistant or susceptible(Pointier& Journdane, 2002). Since snail displays strains highly susceptible or completely resistant to the parasite infection, the knowledge of that would be a useful tool to propagate clonally breed of snail resistance to transmission of schistosomiasis as a biological control method. Biological control using invading fresh water snails in the reduction of schistosomiasis transmission has achieved appreciable successes in the Caribbean Islands and in Brasil (Beck, *et al*., 1993; Pointier& Giboda, 1999; Pointier & Journdane, 2002). A study carried out in West Indies on biological control of *B. glabrata* and *B. stamina*, using a competitive snail, a third, *Melanoides tuberculata* showed that the invading snail rapidly colonized the vector snail. Population of *B. glabrata* and *B. stamina* declined and completely disappeared within a period of two years (Pointier, *et al.*, 1989).

In similar studies in Brasil, several species of snails were identified as probable competitors of culminate species(Guimaraes, *et al*., 2001).

In other works, it had been reported that certain species of trematode parasites are known not to be infectious to humans while they infect intermediate host snails consequently rendering them non-fertile or prevent infection by schistosome miracidia (Tang, 2009). However, the use of trematodes in control of snails, just like the use of predator species interventions require great consideration as recorded success in one ecological area may not necessarily be reproducible in a different endemic area. More importantly, over time it has been observed that introducing non-indigenous species in an area is replete with unintended consequences and such is discouraged (El-Nassery, 2013). Introduced competitor species of snails or parasites poses a risk of greater harm to agriculture or other animals in an ecosystem. Alternatively, it is desirable to identify certain strains of Schistosome intermediate host snails that are refractory to miracidial infections and their associated defense mechanisms suggest a more promising strategy (Hanington, *et al*., 2012; El-Nassery, 2013). Efforts to replace infection-susceptible snails population with infection-resistant strains of the same species may be possible to boost the gains of competitor snail approaches while minimizing harmful consequences in the ecosystem.

Various other studies have noted that not all strains within the snail species transmit schistosomiasis. It has been noted that not all species within the genus *Bulinus africans* act as intermediate host for Schistosomes. *S. mansoni* miracidia has been found to infects only certain species of *Biomphalaria* and only some strains of *B. glabrata* are compatible with this parasite. Similarly, *S. mansoni* miracidia infects only certain species of *Biomphalaria*

and only some strains of *B. glabrata* are compatible with this parasite and *B. ugandae* appears refractory to infection (Borges, *et al*., 1998; Stothard, *et al*., 2002). In other studies, (El-Nassery, *et al*., 2013) demonstrated the genetic variations between susceptible and resistant strains to *Schistosoma* infection within *B. alexandrina* snails using random amplified polymorphic DNA marker showed that 39.8% of the examined field snails were resistant, while 60.2% of these snails showed high infection rates.

Studies in Brazil have shown that Taim strain of *B. tenagophila* shows absolute resistance against the trematode parasite. In experiments where Taim strain was challenged to infection with miracidia, systematic resistance of this strain to *S. mansoni* was recorded (Paraense &Cornea, 1978; Bezerra, *et al*., 2003, Martins-Souza, *et al*., 2003, Rosa *et al*., 2004). This resistance to parasite infection is a dominant characteristic during crossbreeding with susceptible *B. tenagophila* strains. The transmission of the resistance phenotype of *B. tenagophila* (Taim) resistance was successfully transmitted to individuals in the F1, F2 and F3 generations of the susceptible population, regardless of the presence of parasite pressure (Rosa, *et al*., 2006; Marques, *et al*., 2014). These experiments also identified a 350 bp molecular marker that is exclusive to the Taim strain (Rosa, *et al*., 2004). Samples of offspring captured 4, 11 and 14 months after the introduction of the Taim strain were examined, and the susceptibility of the snails to *S. mansoni* infection dropped from high of 38.6 to 2.1% during the 14 months after the introduction of the Taim snail strain.

Investigation on variability of *B. glabrata* strains found to be resistant or susceptible to *S. mansoni* infection using RAPD-PCR primers previously been useful to detect polymorphism among *B. glabrata* and *B. tenagophila* showed polymorphic bands only in

the susceptible strain (Spada, *et al*., 2002). In other studies the genetic variations between susceptible and resistant strains to Schistosoma infection within *B. Alexandrina* snails using random amplified polymorphic DNA marker showed that 39.8% of the examined field snails were resistant, while 60% of these snails showed high infection rates (DeJong, *et al*., 2001; Lockyer, *et al*., 2004; El-Nassery, *et al*., 2013).

2.4.2 Use of Molluscicides

Molluscicides are chemicals or plant extracts that are used to control snails and which may be of synthetic or natural origin (Mott, 1987). The control of freshwater snails requires molluscicides to be added to freshwater bodies or on dried mud surfaces that are frequently used by people and their livestock for everyday activities or for agriculture. Recent reviews of the application of molluscicides around the world have reported their importance in schistosomiasis control (King & Bertsch, 2015; King, *et al*., 2015). Snails control can be achieved by focal use of molluscicides, especially in combination with other methods like chemotherapy, sanitation and health education, offers considerable promise for reduction of disease transmission.

Snail control has relied primarily on a single compound, namely niclosamides which was selected as molluscicides in the 1950s after screening of well over 20,000 compounds for toxicity against the schistosome-transmitting snail *B. glabrata* (Andrews, *et al*., 1983). Currently, Bayluscide, containing niclosamides or its ethanolamine salt, is still being applied in many endemic areas, mostly in Africa and Asia (Yang, *et al*., 2012, Kariuki, *et al*., 2013, Knopp, *et al*., 2013; Dai, *et al*., 2014).

In a number of early studies based on physiological and biochemical assays suggested that niclosamides affects snail oxygen consumption and carbohydrate metabolism. High

concentrations of niclosamides (above 0.15 mg/L) reduce oxygen uptake whereas low concentrations increase oxygen uptake (Gonnert, *et al*., 1959). Niclosamide may also interfere with glucose metabolism (El-Gindy & Mohamend, 1978). Nevertheless, the underlying mechanism of niclosamides potent activity in killing snails remains unclear even though its molluscicidal properties were revealed many years ago (Ishak, *et al*., 1972; Andrews, *et al*., 1983).

Interest in molluscicides began to wane when new drugs finally appeared and their decline was hastened by the sharp rise in the price of pesticides after the oil crisis in the 1970s, and growing fear of adverse environmental effects that led to complete abandonment in some places (Hong, *et al.*, 2013; Ronaldo-de & Clelia, 2018). However, the real turning point in the use of molluscicides was the arrival in the early 1980s of praziquantel, effective and single-dose drug active for all Schistosomes and many other human and veterinary helminthes (WHO, 1985). It promised to boost community chemotherapy against schistosomiasis, although it was initially too expensive for widespread use and its price has at last dropped greatly and it is now the major weapon for community control of schistosomiasis. New drugs revived hopes of eradicating Schistosomes directly by treating people where single-dose treatment programmes certainly had dramatic results, but transmission persisted and re-infection invariably occurred. Experience has shown that repeated treatments were essential to maintain initial gains. The most successful control programmes have been those that included some method of curbing transmission, including molluscicide, even at a reduced level (Webbe& El Hak, 1990).

Given the concerns about the sustainability of chemotherapy-based control, potential emergence of resistance to praziquantel, and lack of an anti-schistosome vaccine in the

near future, development of additional methods of snail control, including a new generation of highly specific and environmentally friendly molluscicides, is a consideration in light of WHO's call for elimination of schistosomiasis where possible by 2025 (WHO, 2012).As shown from a number of studies of the effects of pesticides on insect disease vectors (David, *et al*., 2013; Mougabure-Cueto & Picollo, 2015; Nakao &Banba, 2015), understanding the toxicology of niclosamides in snails would be helpful in developing effective new molluscicides, ultimately benefiting schistosomiasis control. In addition to its potent molluscicidal activity, niclosamides has also been used as an anthelmintic drug for treatment of adult tapeworm infection for decades (Al-Hadaka, 2005).

There are certain negative consequences in the use of molluscicides. The chemicals in use, that kill snails could be non-specific and toxic to other aquatic animals such as fish, which provide rich source of protein for many communities living in schistosomiasis endemic areas. It is reported that fish toxicity and yellowing of treated water by niclosamides led to decreased use and acceptability of molluscicides in many communities (Takougang, *et al*., 2007; Secor, 2014). Beside the harmful ecological impact, synthetic chemicals for snail control are expensive. Sprayed chemicals as well could be rapidly washed down streams following rains or diluted to non-toxic concentrations in larger water bodies leading to frequent re-applications. Additionally, the personnel handling molluscicides require proper skills since the efficacy of molluscicides is influenced by many other environmental factors such as temperature and water hardness. However, despite these limitations, combination use of molluscicides and praziquantel treatment has been reported to be more effective for reducing prevalence of schistosomiasis in humans than is praziquantel treatment alone (Kariuki, *et al.,* 2013). However, despite the uses of molluscicides and chemotherapy

schistosomiasis prevalence in many endemic areas has continued to soar (Dida, *et al*., 2014).

2.4.3 Environment alteration

Alteration of snails environmental habitat is possible approach to control snails as reported in the works by Boelee and Hammou (2004) and Ohmae, *et al*., (2003).Altering snail habitats through removal of vegetations that provide feeding ground, lining water canals with cement and draining water bodies significantly lower population. However, the removal of vegetation may have a negative impact and could pose an increasing risk of infection to workers engaged without proper tools or protective clothing. Similarly, it has been reported that efforts to directly remove snails from inhabited waters, with financial incentives for numbers of snails collected has been employed in parts of China (Sleigh, *et al*., 1998). However, efficacy of such intervention of physical removal of snails remain questionable (Fan, 2012).

In areas reporting high schistosomiasis transmission rates, it has been observed that the percentages of infected snails are generally low. This point to the fact that it takes a few positively infected snails to sustain the parasite life cycles. This therefore, suggests that the prospects of finding and removing a sufficient numbers of intermediate host snails to disrupt the transmission significantly is unlikely. As control measure, environmental alterations methods are more effective but the costs associated with it may not be practical particularly to resource constrained endemic areas in the tropical countries (Ohmae, *et al.*, 2003).

2.4.4 Reduction of Human Exposure to Contaminated Water

The most effective solution to control human infection with schistosomiasis is provision of clean water supplies, which is applicable even to most of other waterborne diseases. However, to have such water systems it is costly in both installation and maintenance in the midst of other competing demands for resource-constrained countries (Susanne, *et al*., 2016). Schistosomes control is usually complicated than other water-borne diseases that can be managed through water filtration or chemical treatment of water for drinking or cooking due to the volumes of water needed domestic consumption. Consequently, it has been shown that in Countries that have succeeded in eliminating schistosomiasis have managed to do so as a result of their economic growth (Secor, 2014, Susanne, *et al*., 2016).The reported successful management of schistosomiasis is linked to countries economic development resulting to increased clean water access and reduction of transmission sites (Secor, 2014). It has been shown that concrete is the most effective control measure for schistosomiasis as the economic conditions improve, children who are more at risk also find other entertainment options other than playing in infectious waters (Secor, 2014; Susanne, *et al*., 2016).

Similarly, economic development can as well have consequences that point to increased risks as opposed to decreased exposure to infected waters. Agricultural practices such as Irrigation have been associated with human Schistosome infections for over 6000 years (Hibbs, *et al.,* 2011; Anastasiou *et al*., 2014). Several water resource development projects set up for provision of hydroelectric power, improved irrigation, and piped water systems have been shown to contribute to increased snail habitats and human water contact. Particularly, it has been report that two major water projects, Aswan High Dam on the

River Nile and the Diama Dam in Senegal River, are linked to unprecedented with outbreaks of schistosomiasis, where local prevalence rates rose rapidly from less than 5% to over 70% in a period of few years(Steinmann, *et al*., 2006).

The effect of the Diama Dam was found to be compounded by interruption the life cycles of the snail predator freshwater prawn *Macrobrachium vollenhovenii* that naturally serve to check the population of snails(Sokolow, 2014). It was demonstrated that the reduction in prawns population, coupled with an increased snail habitat along the irrigation schemes, led to rapid growth Schistosomiasis cases. Elsewhere, the potential adverse impact water resource projects on increased local Schistosomiasis resulted in great concern in advancement of the construction of the Three Gorges Dam on the Yangtze River and its effect on China's progress toward reducing the prevalence of *S. japonicum* infections (Zhen, *et al*., 2002; Zhu *et al*., 2008). Fortunately, to date, it has not been shown to result in increased schistosomiasis transmission rates (Gray, *et al*., 2012).This could have been attributed to consistent use of chemotherapy efforts to the neighboring population.

Public health campaigns, particularly through schools, and enactment of alternative position for washing or swimming are important interventions to reduce exposure to contaminated water (Kosinski, *et al*., 2012; Mwanga& Lwambo, 2013; Zhou *et al.*, 2013 and Musuva, *et al*., 2014).Well thought out considerations of sociological factors are important aspects in the design of any intervention as effecting changes in behaviors is much more challenging than simply performing Mass Drug Administration (MDA) or installing new facilities. It was shown that the Rockefeller Foundation-supported schistosomiasis control studies in Saint Lucia, researchers found that despite provision of

adequate household water supplies, women in that are still preferred to do their laundry at the river points as it provided for an opportunity for social interactions (Jordan, *et al*., 1978). The subsequent development of communal washing stations that provided clean water gained more acceptances. Despite such failures of these initiatives, water supplied at individual households was readily accepted for domestic purposes and bathing (Jordan, *et al*., 1982). It also had the collateral benefits of clean water for prevention of other waterborne diseases. Unfortunately, it has been shown that for many areas that are endemic for schistosomiasis, getting clean drinking water still remain a challenge, much less having alternatives to contaminated water for washing, bathing, and swimming. In addition, certain occupations such as car washing, sand harvesting, canal cleaning, and fishing expose workers to a higher risk of infections (Tameim *et al*., 1985; Karanja, *et al*., 2002; Peng, *et al*., 2010).

2.4.5 Prevention of Fresh Water Contamination

Prevention of fecal and urine contamination of water bodies is a key intervention in reducing risks of infection. If stool in the case of (*S. mansoni* and *S. japonicum*) infections and urine (*S. haematobium*) from infected individuals is prevented from getting into in to fresh water bodies, Schistosome eggs eventually will not hatch and as result there will be no miracidia larvae to infect snails. However, just as the case in snails control, unless these efforts are highly effective, the life cycle can be maintained by the considerable amplification that takes place in the snail host. The situation is further complicated for *S. japonicum* infections that are zoonotic, with infections of bovines making a substantial contribution to the continuation of transmission in China (Freeman, *et al*., 2013).

Traditional sanitation systems are expensive as they require an extensive infrastructure installation and maintenance costs that may be beyond the means of most areas where schistosomiasis is endemic. In the absence of sanitation infrastructure, Water, Sanitation, and Hygiene (WASH) has been promoted as a means to reduce excreta from reaching fresh water as an alternative. This component usually consists of point of use treatment, sanitation involves construction of latrines and hygiene involves public health education and advocacy to behavioral modification approaches such as community-led total sanitation that discourages open defecation through social pressure (Freeman, *et al*., 2013). There has been very little operational research that have successfully identified what sanitation practices are effective for lowering the transmission rates of schistosomiasis in many areas. In a similar randomized control trials in Kenya that was designed to evaluate the effect of WASH on soil-transmitted helminth infections, it was reported that there was no effect of the WASH intervention on re-infection with *S. mansoni* after treatment (Freeman, *et al*., 2013). Elsewhere in Zanzibar, it has been shown that WASH interventions failed to lower the risk of *S. haematobium* infection (Knopp, *et al*., 2013). Simply the provision of latrines may not be sufficient, even if they are consistently used, it has been recorded in areas where toilet paper is scarce as hygienic bathing after defecation may be the main source of the vast majority of miracidia larvae released into transmission sites rather than stool being washed into the water body (Sow, *et al*., 2008). For urogenital schistosomiasis, preventing urination into the water is challenging as it can occur unobserved and the sense of urgency to urinate may be increased by the combination of bladder irritation from infection and being in the water (Knopp, *et al*., 2013). The relationship between schistosomiasis and sanitation on one side may look obvious but there

need to generate more additional investigation in this area, particularly in connection with to human behavior.

2.4.6 Use of vaccines

Vaccine tests for Schsitosomiasis have failed to achieve effective protection (Berquist & Colley, 1998; Alsallaq, *et al*., 2017). In the four decade, there have been several scientific researches and review articles done but the results lack the required efficacy because. This is because vaccine-induced protective immunity generated in animal models may not translate to humans; there is uncertainty about the type of human response most appropriate for protective immunity; and that the antigens may not be expressed on the Schistosome apical surface, and will not therefore be exposed to the human host immune system (Ismail, 2011). While there is an effective drug (Praziquantel) for schistosomiasis, the costs are still prohibitive, especially when considering the income bracket of the subsistence-based communities in Africa. Moreover, re-infection after drug treatment is common. Human population growth and erratic weather patterns have prompted the continued expansion of irrigated agricultural schemes in Africa hence transmission of schistosomiasis in this region is expected to increase (Wilson & Coulson, 2006; 2009). It is therefore, important that various public health control measures for schistosomiasis are effected concurrently (Alsallaq *et al*., 2017). This include disrupting transmission involving awareness for good sanitation, provision of praziquantel for Schistosomes through community health workers and the management of wetland habitats to favor population of snails that that are refractory to miracia infection (Famakinde, 2017; Campbell, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling of Field Snails

The experimental snails in this study were collected from Mwea Irrigation Scheme which is located in Kirinyaga County. The sampling sites were selected within Mwea East and West sub-counties, which have a population size of approximately 237,382. This area occupies the lower altitude zone of Kirinyaga County with an expansive low-lying wet-savannah ecosystem with an average temperatures range from 16 – 27°C. The Mwea rice irrigation scheme covers approximately 13,640 hectares where, more than 50% of the scheme area was used for rice cultivation while the remaining area was under subsistence farming, grazing and community activities.

Snails sampling sites were randomly selected along water canals within irrigated farmlands. The sampling points were selected 100 metre apart along the irrigation water canals. Snails were systematically searched at sampling points and collected using a scoop (30 by 30 cm) covered with a 2 × 2 mm size mesh (Plate 1). Sampled live snails were picked with forceps and placed on wet cotton wool in perforated containers. Sampled snails were transported to aquaculture facility at Institute of Primate Research (IPR) within with 24 hrs.

The data for recorded incidences of by *S. mansoni* and other Helminth infections in the study area was collected from Kimbimbi Sub-County hospital for the period of 2015-2019.

Plate 3.1: Collection of experimental snails from water canals in irrigated farmland in Mwea East

(Source : Author, 2018)

3.2 Determining the Proportion of Resistance Snails from Field Collected Samples

3.2.1 Culturing and Infection of Snails with *S. mansoni* Miracidia

Snails collected from Mwea Irrigation Scheme were transferred to IPR, where they were housed for two weeks in glass aquaria containing snail-conditioned water to acclimatize to laboratory conditions. After two weeks the live snails were picked for the trials.

The selected experimental snails were fed on lettuce leaves, tetramine fish food and calcium carbonate, under suitable environmental laboratory conditions, using the method described by Eveland and Hayseed (2011). Schistosome eggs were obtained from feaces of infected Baboons in on-going schistosomiasis experiments at Institute of Primate Research (IPR). The eggs were exposed to light for 2-3 hours to stimulate hatching into miracidia. In 10 ml beakers, the snails were exposed individually to 8-10 freshly hatched miracidia under direct sunlight for 3-4 hours.

After exposure to miracidia, the experimental snails were transferred into troughs each containing 48 snails and this was replicated 10 times in a complete randomized design experiment. Miracidia exposed snails were kept under laboratory condition at room temperature.

3.2.2 Checking infected snails using light illumination procedure

After four (4) weeks period of incubation, *S. mansoni* cercaria shedding from individual snail was determined using the direct light illumination procedure. Individual snail was put in 10 ml water in beaker under direct light and larval cercaria shedding determined by

observation on X 10 magnification stage microscope. The observation was repeated once per week for four (4) week period to ascertain if the snails were infected or not. The percentage of snails that were shedding and those not shedding cercaria was determined. Snails that failed to shed any cercaria were kept separately and propagated to increase that population of potential resistant strains.

The snail Infection Rate (IR) was determined by dividing the number of cercaria shedding snails by the number of exposed snails. The percentage of susceptible and resistant snails was determined as described by Yousif et al. (1998) as:-

$$\text{IR \%} = \frac{\text{Number of shedding snails in each subgroup}}{\text{Number of exposed snails in each subgroup}} \times 100$$

The number of dead snails was counted and recorded starting from the day of exposure to the parasite to determine mortality rates(MR). This was determined as:

$$\text{MR \%} = \frac{\text{Number of dead snails in each subgroup}}{\text{Number of exposed snails in each subgroup}} \times 100$$

3.2.3 Molecular detection of Cercaria in infected snails

Tissues biopsy from snails exposed to miracidia were obtained and subjected to DNA extraction using Qiagen kit (Qiagen Inc. MD, USA) to maximize yield. DNA samples were extracted from snails that were shedding cercaria and those that were not shedding.

The procedure used 20µl Proteinase K and 200µl of digestion/lysis buffer added to snail tissues biopsy samples and incubated at 56ºC water-bath for 1 hr. Lysine was pelleted to remove debris by centrifugation (X1000 g) and the supernatant aspirated into silica matrix spin column to bind DNA. After initial centrifugation at 10,000 g for 2 min the DNA in the column matrix was cleaned in wash buffer centrifugation then eluted in 200µl nuclease free distilled de-ionized water (ddH_20), as described in Qiagen kit manual. DNA was visually analyzed on 1% agarose gel with Ethdium bromide under utra-violet illumination and quantity determined by 260/280 nm ratio. The DNA samples were labelled and stored at -20ºC for subsequent analysis.

Using Polymerase Chain Reaction (PCR) technique cercaria infection was determined at molecular level. The PCR was carried out using specific primers dependent on target gene of the extracted DNA, *Schistosoma sp* cercaria specific primers targeting conserved region of 5S rDNA (TGCATACTGCTTTGAACATTC; CCTGACTAGGCTGGT) and *Biomphalaria sp* ribosomal target 16S rDNA(TCGAAGCGCACGAACGCG; GGAAGGATCATTAAAGGCTT). The PCR amplification was done using approximately 70 ng/ul of the template DNA, 0.5 µM of each primer, 200 µM each dNTP, 10 X buffer with 2.0 mM $MgCl_2$ and 0.5 U Takara Taq polymerase, made up to 25 µl with ddH_2O. The cycling conditions were: Taq polymerase activation 92ºC, 3 min; then 30 cycles profile: denaturation, 92ºC, 1 min; annealing 52ºC-57 ºC, 1 min; polymerase nucleotide elongation 70ºC, 1 min; final polymerase extension step 70ºC, 3 min and final storage in the PCR machine at 8ºC.

PCR was performed using primers franking rDNA 5S and partial 16S intergenic regions with the target product size of approximately 350bps in all samples. The PCR fragments were excised from the gel and dissolved in Sodium Iodide at 56^{o}C for 15 min in gene clean procedures (Jenna Kit). Cleaned PCR fragments were used for custom Sanger fluorescent sequencing method in Applied Biosystems squencer.

3.3 Determination of Morphological characteristics of *S. mansoni* susceptible and resistant snails

Morphometry of the shell of snails is a useful tool in taxonomic identification and ecological studies of mollusks(Schnabel, *et al*., 2013; Wullschleger & Jokela, 2002). This tool was used to identify and characterize the sampled snails. To determine morphological variations of *S. mansoni* susceptible and resistant snails. A sample of 20 adult snails from each set (shedding cercaria and non-shedding) was randomly selected for characterization at NMK.

The snail shell morphometric parameters (Fig 3.3) were used to characterize the samples. These included shell height (SH), shell width (SW), aperture height (AH), aperture width (AW) and spiral length (SL). These parameters were also taken for F1 and F2 progeny of laboratory bred snails.

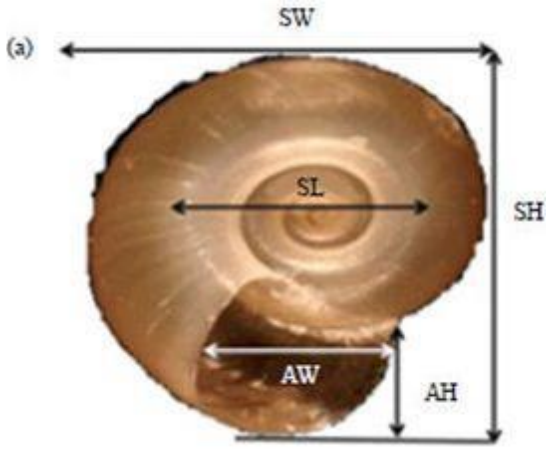

Figure 3.3.1. The shell morphometric parameters of *B. pfeifferi* that were used as described by Falade and Otarigho (2015)

3.4 Evolutionary development of vector *Biomphalaria sp*

3.4.1 *Biomphalaria sp* phylogenetic tree

To determine snail varieties in the sampled population, PCR products of the rDNA fragment were gene cleaned using commercial protocols. Gene clean procedures on the PCR products were carried out according to innuPREP procedures (Life science Jena, Germany). The 450 bps PCR products obtained using primers for *Biomphalaria* snails rDNA 5.8s, ITS1 were used for custom sequencing. The raw sequences were analyzed and edited in BioEdit Suite (BioEdit v7.0.5, Tom Hall Ibis Therapeutics) to get consensus sequences and trim 3' and 5' ends. Verification was done via Basic Local Alignment Search Tool (BLAST)analysis to determine highly related sequences in National Centre of Biotechnology Information (NCBI) nucleotide database. The *Biomphalaria* rDNA ITS1 sequences were deposited via BankIt procedures in NCBI to GenBank (Accession: 579768.1-579823.1).

The *Biomphalaria sp* rDNA Sequence data obtained was used to construct the *snails'* evolutionally history. Using Phylip software in MEGA 6, *Biomphalaria sp* the snails phylogenetic tree was constructed. To identify the population structure of sample snails using *Biomphalaria sp* rDNA gene, species and sub-species analysis was performed in Phylip software as described in (Felsenstein, 1985; Saitou & Nei, 1987; Tamura, *et al.*, 2013).

3.4.2 Tajima's neutrality test for *Biomphalaria sp*

Tajima's neutrality test for *Biomphalaria sp* was performed using the rDNA gene sequencing data to assess the snail population drift from equilibrium. The Tajima-D test is important to distinguish between DNA sequences in a sample population that is evolving randomly (naturally) and those evolving under balancing selection. A given population is normally at constant size and natural mutation with no effects on its fitness and survival when it reaches equilibrium of gene frequencies (Tamura, *et al*., 2013.

A population at gene frequencies equilibrium, the number of DNA segregating sites (DNA sites that are polymorphic) denoted as $\{S\}$and nucleotides diversity as $\{\pi\}$ (average number of mutations between paired samples) is expected to be the same, Tajima-D test value =0. To assess population neutrality of *Biomphalaria sp* population, a total 56 and 83 nucleotide sequences of *Biomphalaria sp* from the study area were compared with other Africa region composite samples respectively. The Tajima-D test analysis carried out using MEGA Version 6 software program as described by (Tajima, 1989; Tamura, *et al*., 2013).

3.5 Assessing transmission levels of resistant traits under laboratory conditions

3.5.1 Laboratory breeding for snails and infection with *S. mansoni* larvae

Populations of *B. pfeifferi* snails were collected from Mwea irrigation scheme for laboratory rearing. The snails were cultured under laboratory conditions as described in section 3.2.1 above. The field sample snails, were individually exposed to *S. mansoni* miracidia larvae and selection of susceptible and resistant snail isolates carried out by biological shedding of cercaria under light illumination. The selected resistant isolates, snails that remained uninfected after exposure to miracidia infection after 4-5 weeks were isolated and reared separately. Their progeny (F1) were selected as resistant experimental group and reared separately for multiplication. Their progeny (F2) were also selected as the experimental resistant group similar to earlier study by (Zanotti-Magalhães, *et al.,* 1997) and subjected to miracidia infection.

3.5.2 Assessing transmission of resistant traits in(F1) and (F2) progenies

Schistosomes eggs collected from Baboons feaces were exposed to light for 2-3 hours to stimulate hatching into miracidia. In 10ml beakers, 480 resistant isolates snails were exposed individually to 8-10 freshly hatched miracidia under direct sunlight for 3-4 hours. The exposed snails were maintained in aquarium each with 48 snails replicated 10 times. The snails were kept under laboratory condition at room temperature (El Naga, *et al*., 2010, Eveland & Hayseed, 2011; Mostafa &El-Defray 2011).

At four weeks post-exposure to the parasite, the snails were individually checked for cercarial shedding twice weekly, repeatedly for four weeks. The cercaria shedding or non-shedding from individual snail was determined using a direct light illumination procedure

where individual snails were put in 10 ml water in beaker and cercaria shedding determined by observation on 10 X magnification stage microscope. During the shedding period of three weeks, the snails were kept in darkness throughout as described by (Yousif, *et al.*, 1998). The snail infection and mortality rates were determined. This observation experiment was carried out to both F1 & F2 snails populations.

CHAPTER FOUR
RESULTS

4.1 Schistosomiasis incidences in study area

The data on incidences of waterborne infections kept at Kimbibi Sub-County Hospital, indicated that *S. mansoni* infections were reported throughout the observation period as from 2015-2019. *S. mansoni* infections recorded higher incidences than other observed helminth worms infections i.e. (*Taenia sp*, Hookworms, Round worms) with highest number reported of 23 patients reported in October-December, 2018 as shown in (Fig 4.1.1). This was diagnosed cases in patients who visited the hospital with gastrointestinal infections.

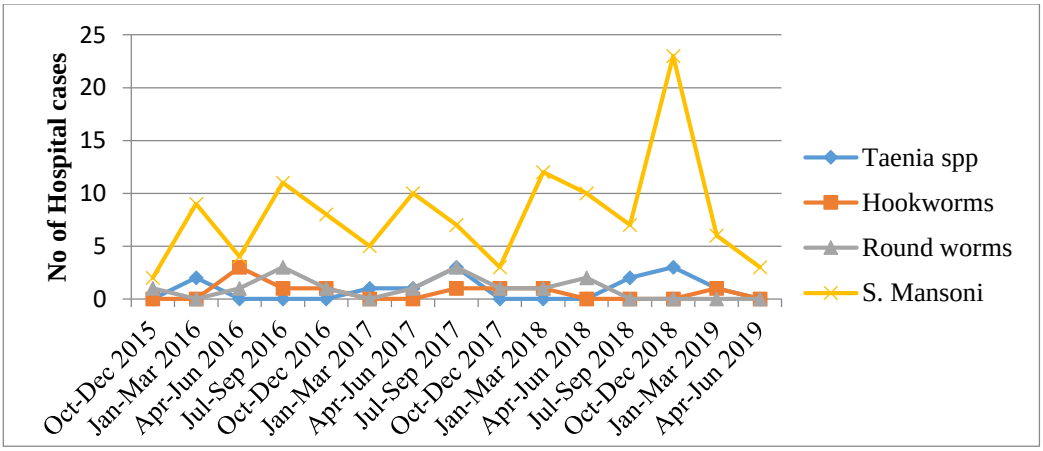

Figure 4.1.1. The observed *S. mansoni* infection incidences in study as compared to other Helminth worms based on local hospital data

4.2 Determining population proportions of *B. pfeifferi* resistant and susceptible snails

4.2.1 Detection of cercaria infection in snails using direct light method

Significantly, high populations of *B. pfeifferi* snails from the study area were found to be susceptible to *S. mansoni*. However, importantly it was found that there existed a population proportion of snails that are resistant to *S. mansoni*. The field sampled population had a mean of 36.6 ± 3.7 for infected and 5.4 ±1.2 for non-infected snails. Comparing the infected and non-infected snails, F = 1,334 and P = 0.0007 at 0.05 confidence level, the two population were found to be significantly different (Table 4.2.1). As shown in (Figure 4.2.1), a proportion of 76% of sampled snails were susceptible while, 24% exhibited resistance to *S. mansoni* infection.

Table 4.2.1. The mean infection rates of field collected *B. pfeifferi snails*

Groups	N	SS	Mean	Variance
Infected Snails	30	14,633	36.6± 3.7	13.8
Non-Infected Snails	30	536	5.4±1.2	3.1
	F = 1,334			
	P value = 0.0007			
	F critical = 4.01			

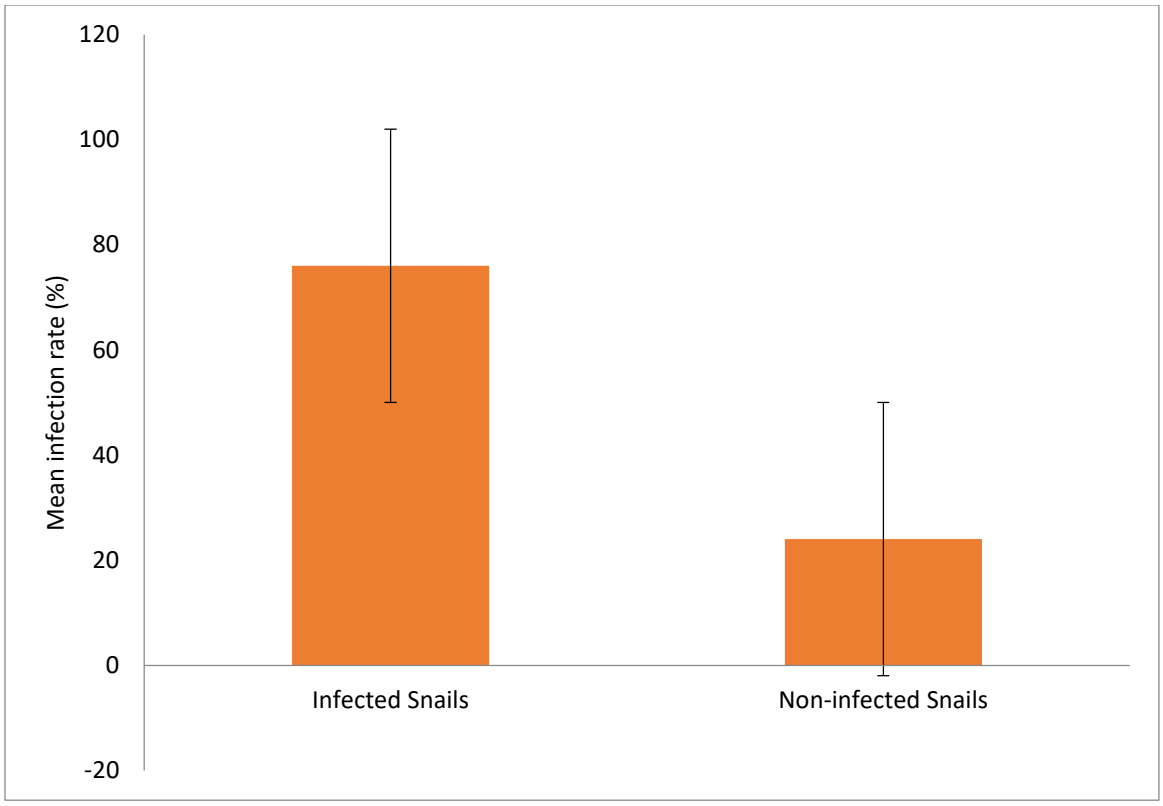

Figure 4.2.1. Proportions ofsusceptible and resistant field collected*B. pfeifferi* against *S. mansoni*miracidia larvae infection

4.2.2 Molecular detection of cercaria infection in snails

The PCR tests done on cercaria DNA using pair of primers franking the ITS 1 schistosome of rDNA yielded a distinct band approximate 350bps (Figure 4.2.2.1). Detection tested by limiting dilution was sensitive at micromole/ femtoMole concentrations (1×10^{-12}-1×10^{-15}g) of DNA.

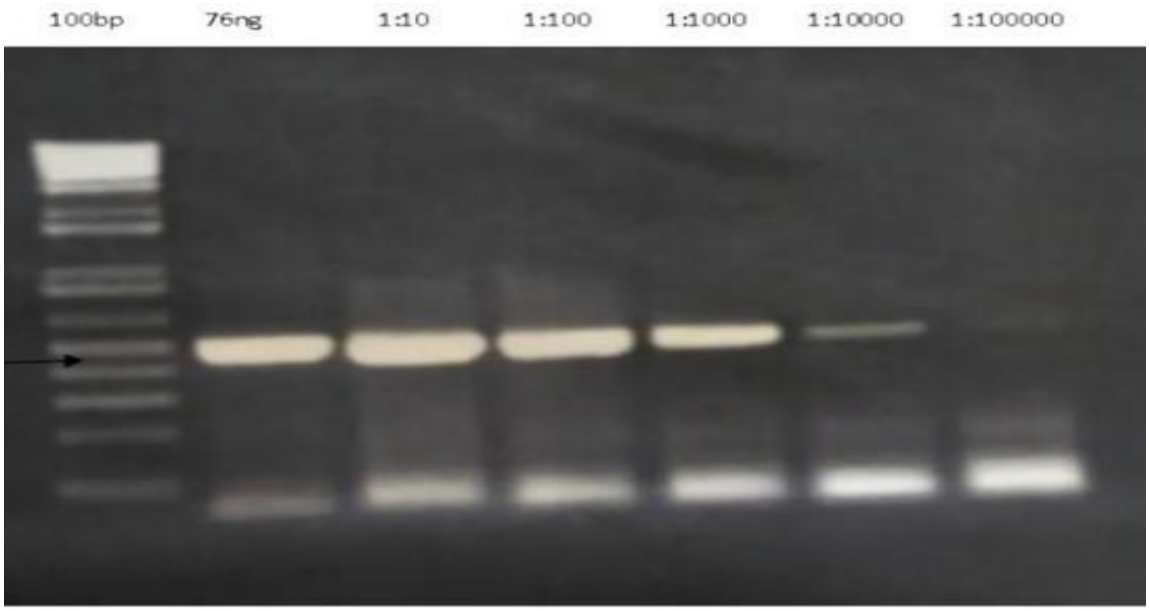

Figure 4.2.2.1 Limiting dilution test of sensitivity of PCR probes for cercaria detection (Lane 1, 100bp marker; lane 2-7, limiting dilution PCR tests)

The PCR on DNA extracted from snails after exposure to infection by miracidia produced specific amplification of target size band of approximately 400 bps in rDNA ITS gene (Figure 4.2.2.2) but there were amorphous amplification, possibly degraded target. In the target amplification, the results were not uniform, with some snails demonstrating higher positive titre of sporocyst/cercaria DNA while others had limiting titre while others were negative. The PCR detection of cercaria in snail samples was either positive amplification, partial amplification or no amplification.

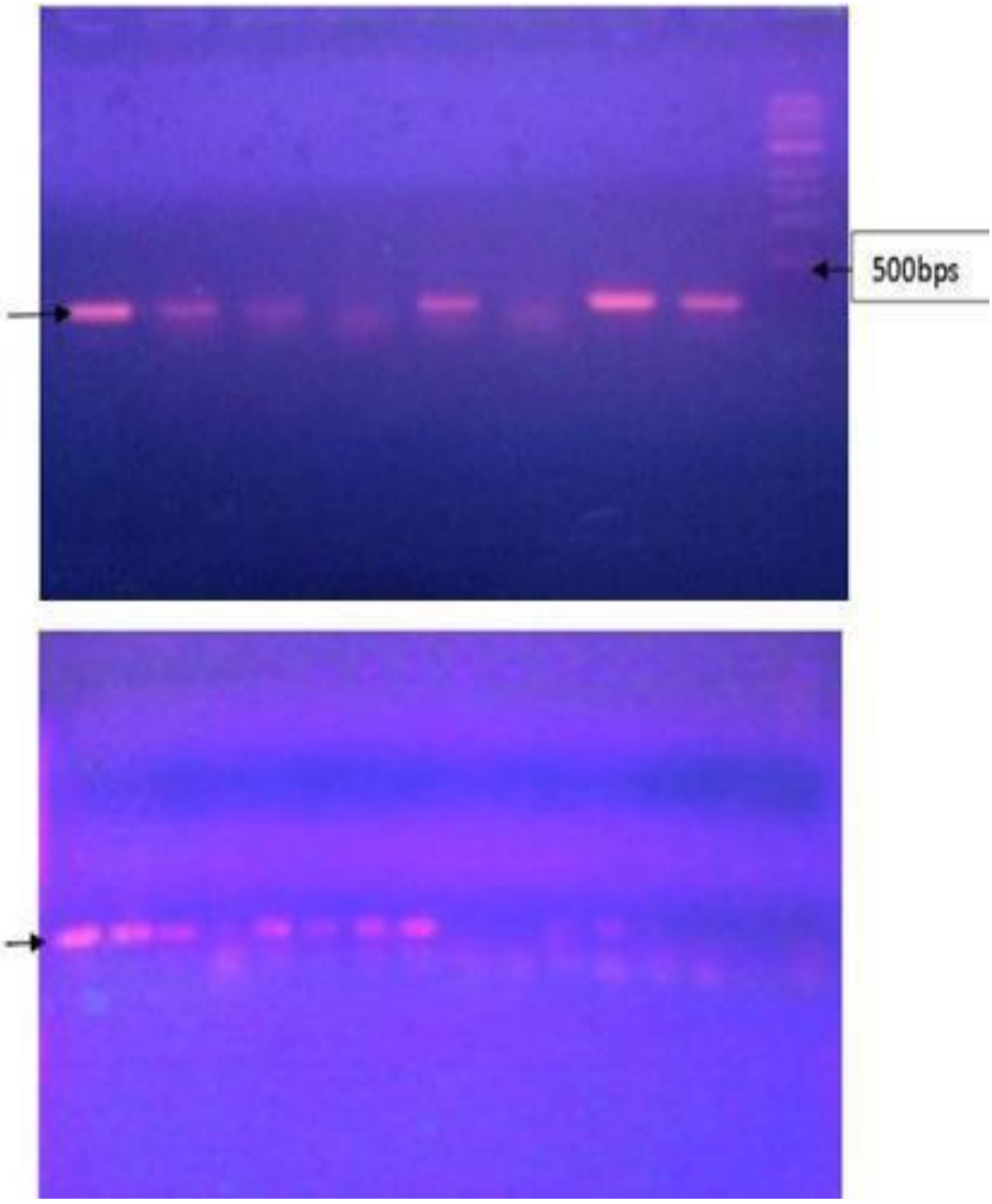

Figure 4.2.2.2 PCR detection of sporocyst/cercaria DNA in snails samples at target size band of approximately 400 bps (Arrow indicating 500 bp maker)

4.3 Morphological characteristics of *S. mansoni* susceptible and resistant snails samples from Mwea Irrigation scheme

Using morphometric identification, snails dominating the Mwea irrigation scheme in Kenyan Highland were determined *as B. pfeifferi* although there also occurs other vector snails for example the *Bulinus sp* vector for *S. intercalatum*.

The morphological parameters as reported in (Table 4.3.1) indicated that snails had a mean shell height of 9.9 and 10.0 mm for resistant and susceptible snails respectively. Comparing the two populations, F= 2.49, P=0.13 at 0.05 confidence level, there were no significant differences. The shell width had a mean of 9.5 and 9.5 mm for resistant and susceptible respectively, where at F= 0.23, P = 0.23 at 0.05 confidence level there were no significant differences.

Similarly for the other parameters: Aperture height, Aperture width and Spiral length at 0.05 confidence level there were no significant differences observed. The results showed that there were no differences in physical characteristics of both the resistant and susceptible populations of *B. pfeifferi* snails from the study region.

Table 4.3.1. Mean Morphological parameters of *B. pfeifferi* Snails from Mwea Irrigation Scheme

Group	Shell Height (SH)	Shell width (SW)	Aperture height (AH)	Aperture width (AW)	Spiral length (SL)
Resistant Snails	9.9 ± 1.3	9.5±1.2	4.9±0.6	3.9±0.3	5.0±1.1
Susceptible Snails	10 ± 1.4	9.5±1.2	4.8± 0.5	4.0± 0.1	5.0±1.2
	P = 0.13	*P = 0.23*	*P = 1.82*	*P = 0.62*	*P = 0.59*
	F = 2.49	*F = 0.23*	*F = 0.21*	*F = 1.82*	*F = 0.28*
	F crit. = 4.5	*F crit.= 4.6*	*F crit. = 5.32*	*F crit. = 4.40*	*F crit. = 4.50*

4.4 Evolutionary development of vector *Biomphalaria sp*

4.4.1 Phylogenetic structure of *Biomphalaria sp* Snails

The phylogenetic structure showed that certain lineages of *Biomphalaria sp* are monophyletic with long distance time separation lineages (I, II and IIIA). Some of the sampled snails populations clusteres were phylogenetically indistinguishable (IB, IIIB and other were clonal off shoots within cluster II and III (Figure 4.4.1.1). Larger proportion of sampled snails were found to belong to *B. pfeifferi* but there is ample representation in the other clusters that included *B. sudanica*, *B. choanomphala*, *B. stanleyi*, *B. alexandria* and *B. glabrata.*

Snails rDNA sequences obtained in this study together were compared with similar sequences datasets from across Africa obtained in GenBank. The comparison showed that most snails were clusters of closely related populations, isolates or are derived from clonally propagation (Figure 4.4.1.2, curly brackets) with few monophyletic lineages. While this nomenclature of *Biomphalaria sp* may need revision, four clusters were evident

in phylogenetic tree structure of Kenyan and African composite samples (brackets I, II, III, and IV).

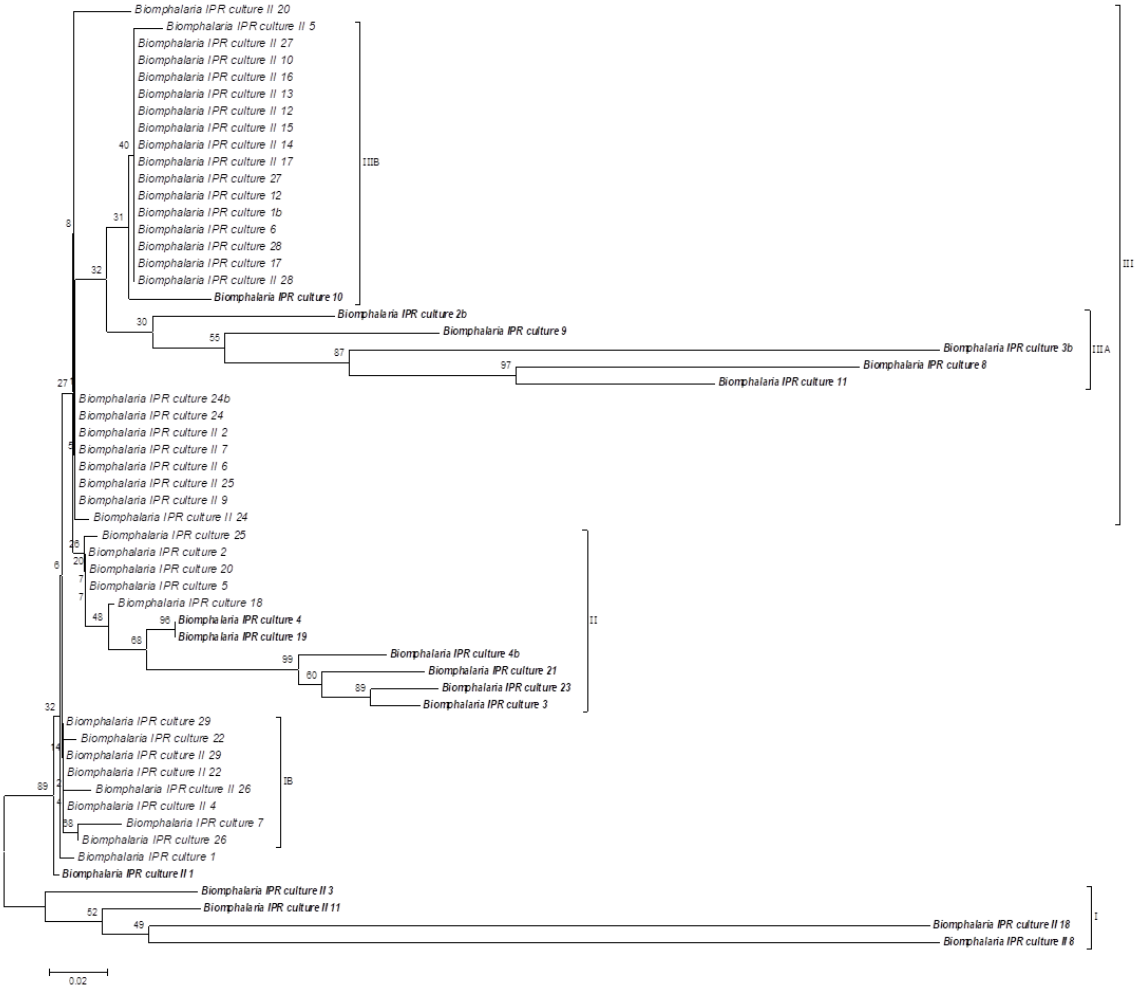

Figure 4.4.1.1 Evolutionary history of *Biomphalaria* snails population within Kenya the Neighbor-Joining method

(The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 56 nucleotide sequences. There were a total of 207 positions in the final dataset. Evolutionary analyses were conducted in MEGA6**)**

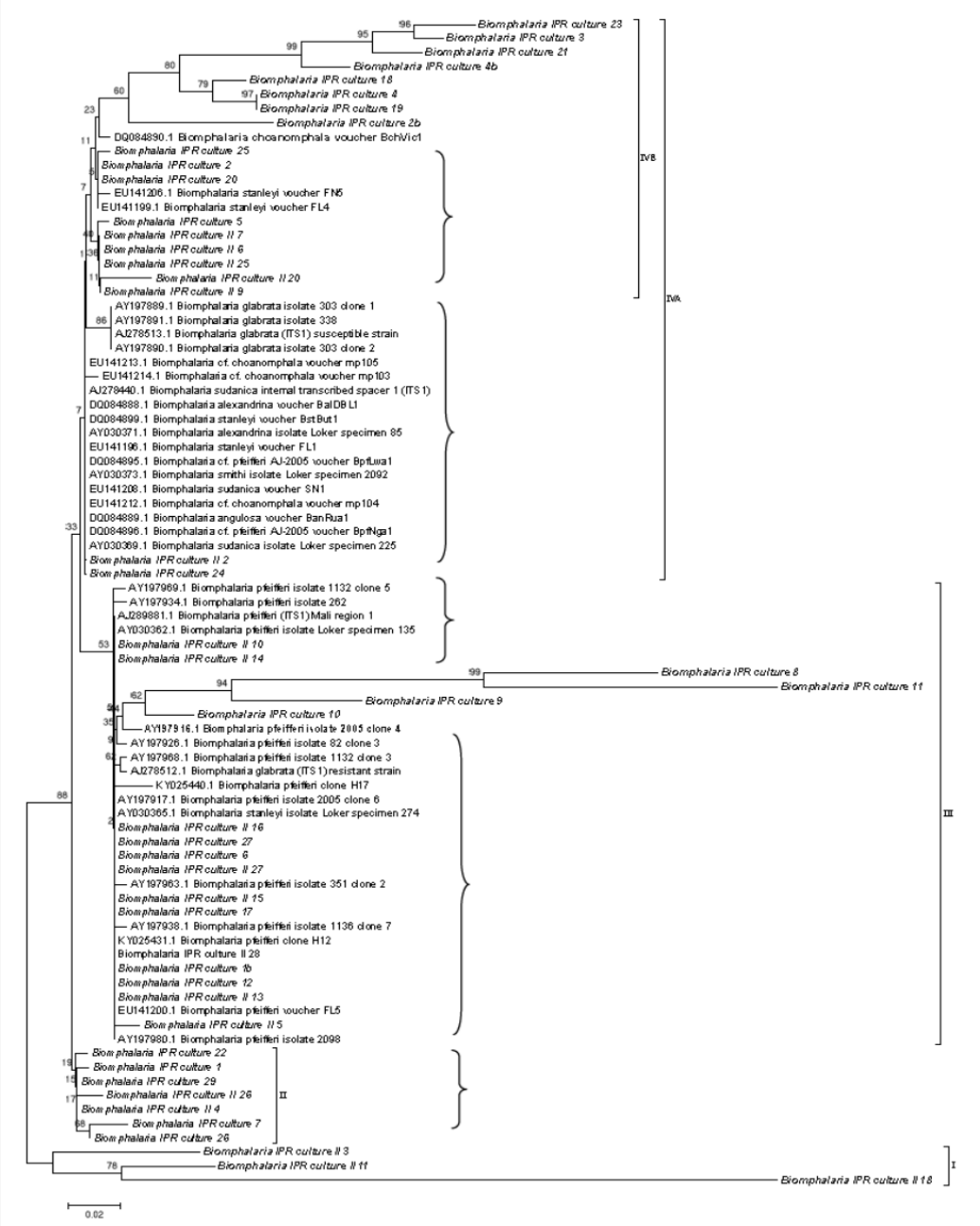

Figure 4.4.1.2 Evolutionary history relationships of *biomphalaria* snails within Africa using the Neighbor-Joining method

(The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches .The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 83 nucleotide sequences with were a total of 207 positions in the final dataset. Evolutionary analyses were conducted in MEGA6)

4.4.2 Population drift of *Biomphalaria* snails

Test of drift from neutrality was performed using Tajima's D test. When population is at equilibrium neutrality, the nucleotide diversity (π) and the number of nucleotide segregating sites are indistinguishable. In this study, a Tajima D test values of -1.681654 and -2.003619 for Kenyan and Africa composite samples were recorded (Table 4.4.2). In the tested *Biomphalaria* populations {S}is significantly greater than (π) resulting in pronounced negative Tajima's D-test value.

Table 4.4.2. Tajima's Neutrality Test for *Biomphalaria* snails populations

Population	No. of sequences (M)	No. of segregating sites {S}	ps	Θ	Nucleotide diversity (π)	Tajima's test Statistic (D)
Kenya regional *Biomphalaria*	56	146	0.705314	0.153542	0.080617	-1.681654
Africa composite *Biomphalaria*	83	131	0.63285	0.126823	0.051698	-2.003619

Note: The analysis involved 56 and 83 nucleotide sequences representation of Kenya and Africa regions, respectively. There were a total of 207 positions in each of the final dataset m=number of sequences, S=number of segregating sites, ps=S/n, $\boldsymbol{\Theta}$=ps/a1, $\boldsymbol{\pi}$=nucleotide diversity, and D is the Tajima test statistic ($\boldsymbol{\pi}$ and S/a1 both estimate $\boldsymbol{\Theta}$, where E (expected) E [$\boldsymbol{\pi}$]=$\boldsymbol{\Theta}$, E [S]=a1 $\boldsymbol{\Theta}$), software default significant at $P < 0.10$. Evolutionary analyses were conducted in MEGA6.

4.5 Transmission levels of resistant traits in *B. pfeifferi* under laboratory conditions

4.5.1 Snails infection rates under laboratory conditions

The study results revealed that there exist some populations of *B. pfeifferi* snails that are resistant to *S. mansoni* infection in the in the field. There was observed enhancement of resistance traits in different generation of snails under laboratory conditions. The snails samples collected from field exhibited significantly high rate of infection at 76% shedding cercaria while 24% of the snails showed some resistance. However, when the resistant population were selected and bred through (F1 and F2) progenies and subjected to *S. mansoni* miracidia, the infection rates significantly differed with infections rates exhibited by field collected samples. In F1 generation, susceptible snails dropped to 4% while 90.8% were resistant. In F2 generation susceptibility decreased even further to only 0.8% and that of resistant group increased further to 95.1% (Figure 4.5.1).

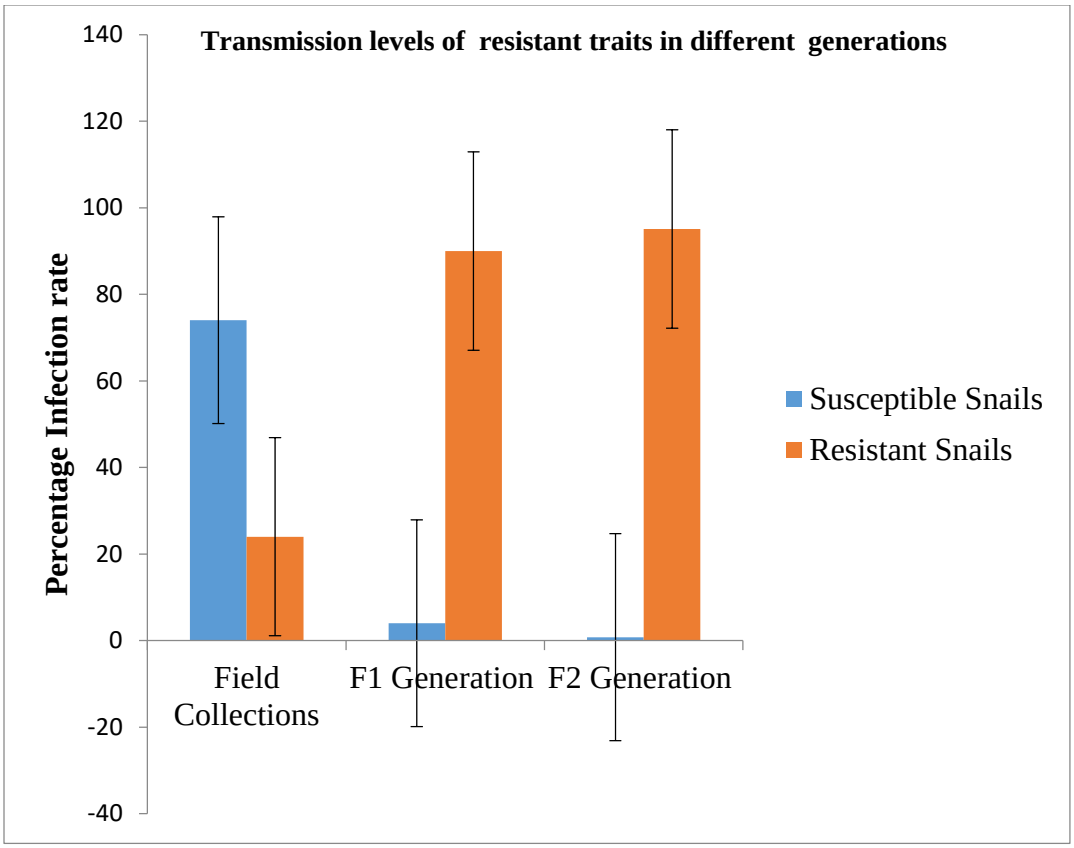

Figure 4.5.1: percentage of infected and non-infected *B. pfeifferi* snails in field sample population through F1 to F2 generation

The F-test statistics of *B. pfeifferi* on infection rates revealed highly significant differences between the Field collected snails, F1 and F2 generations. The mean value for the Field, F1 and F2 snail samples were 36.6 ± 3.72, 1.93 ± 1.46, 0.36 ± 0.049 respectively. Comparing the infected snails, the Field samples and F1 generation for the infected snails, F = 6.48 and P = 0.00 at 0.05 confidence level, the two populations variations were found to be significantly different. The Field and F2 snail samples for the infected snails, F = 57.59 and P = 0.00 at 0.05 confidence level, the two populations variations were found to be significantly different. Lastly, the F1 and F2 generation of infected were found to have

an F = 8.88 and P = 0.00 and at 0.05 confidence level were found to be significantly different (Table 4.6.1).

Comparing non-infected snails, the Field samples and F1 generation snails, F = 2.54 and P = 0.01 at 0.05 confidence level, the two population's variations were found to be significantly different. The field and F2 snail samples for the non-infected snails, F = 3.13 and P = 0.00 and at 0.05 confidence level, the two populations variations were found to be significantly different. Lastly, the F1 and F2 snail samples of non-infected were found to have an F = 1.23 and P = 0.058 and at 0.05 confidence level were found to be significantly different(Table 4.5.1). Since there was significant difference between the field, F1 and F2 generations, this suggests there might be enhancement of resistant gene through selection of resistant population progressively from field samples, F1 and F2 generations.

Table 4.5.1: Means infection rates for the Susceptible and resistant snails for the Field, F1 and F2 snails

Snail samples		N	Mean	Variance	F	Prob > F
Infected Snails	Field snails	30	36.6± 3.7	13		
	F1 Snails	30	1.93±1.5	2.1	6.48	2.76E−06
	Field snails	30	36.6±3.7	13.8		
	F2 Snails	30	0.37±0.1	0.24	57.6	1.48E−06
	F1 Snails	30	1.93±1.5	2.13		
	F2 Snails	30	0.37±0.5	0.24	8.9	7.78E−06
Non-Infected snails	Field snails	30	11.6±4.0	16.06		
	F1 Snails	30	43.6±2.5	6.32	2.5	1.40E−06
	Field snails	30	11.07±4.0	16.06		
	F2 Snails	30	45.67±2.3	5.13	3.1	2.95E−06
	F1 Snails	30	43.6±2.5	6.32		
	F2 Snails	30	45.67±2.3	5.13	1.2	5.77E−06

4.5.2 Snails Mortality Rate under lab conditions

The observation of experimental snails revealed there were statistically no significant differences in mortality rates between snails samples from Field, F1 and F2. The mean values for the number of dead snails in the Field, F1 and F2 snail samples were 6.0 ± 2.0; 2.5 ± 1.6 and 3.1± 2.0 respectively. Comparing the number of the dead snails for the Field and F1 samples, F = 1.62 and p = 0.22 at 0.05 confidence level, the two populations variations were not significantly different. In the case of Field and F2 snail samples, F = 1.06 and P = 0.87 at 0.05 confidence level, the two populations variations were not significantly similarly to comparison of F1 and F2 at F = 0.65 and P = 0.26 were not significantly different (Table 4.5.2). The observed percentages mortality rates, as well for Field samples, F1 and F2 snails were determined at 15%, 6% and 7.6% respectively and were not statistically significant differences (Figure 4.5.2).

Table 4.5.2. Mortality rates of the experimental snails for the Field samples, F1 and F2 progenies

Snail Samples	N	Mean	Variance	F	Prob> F
Field collections	30	6.0±2.0	4.1	1.62	0.2
F1 snails	30	2.5±1.6	2.5		
Field collections	30	6.0±2.0	4.1	1.06	0.87
F2 nails	30	3.1±2.0	3.9		
F1 nails	30	2.5±1.6	2.5	0.65	0.26
F2 nails	30	3.1±1.9	3.9		

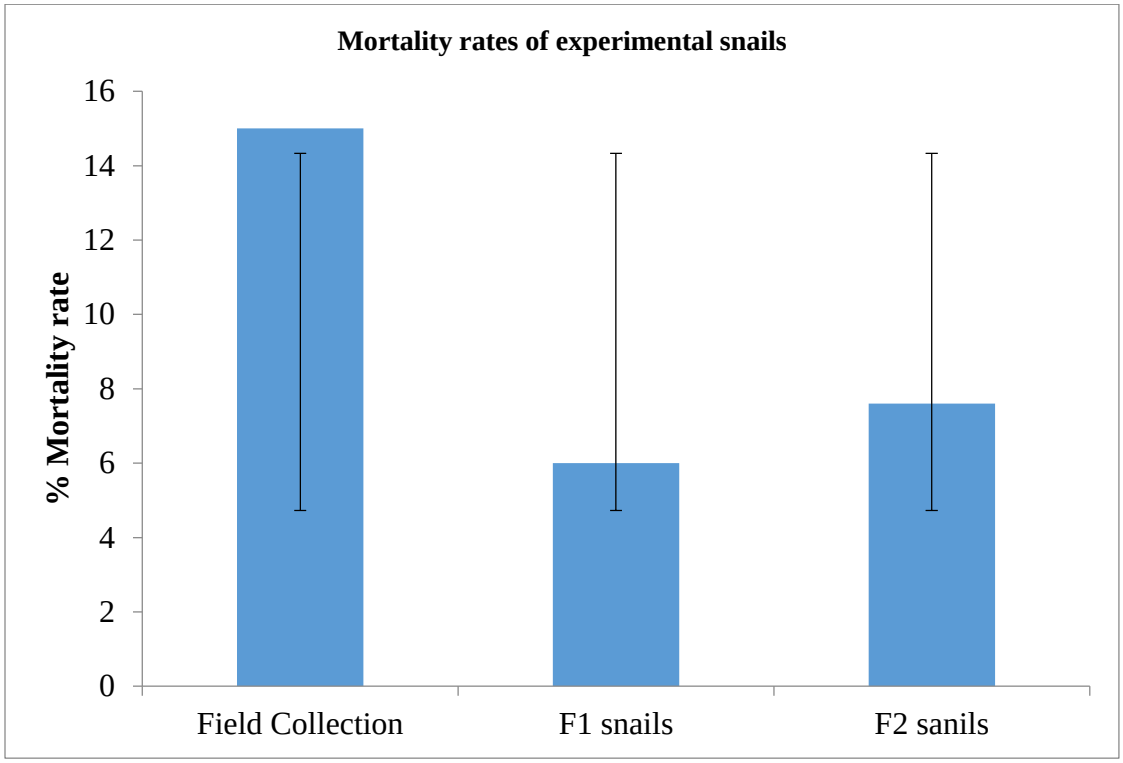

Figure 4.5.2: Mortality rate in *B. pfeifferi* under laboratory conditions

CHAPTER FIVE

DISCUSSION

5.1 Resistant and Susceptible populations of *Biomphalaria sp* against Schistosomes miracidia

Infections by *S. mansoni* in the study area were found to be endemic in the region while other helminth water waterborne diseases were sporadic. Identifying schistosomes' larvae resistant snails' populations is an important ingredient in schistosomiasis control strategies. The present study recorded an important aspect where 24% proportion of the *B. pfeifferi* snails samples collected from the study site were refractory to *S. mansoni* infection. This is as important natural aspect that can be exploited in expanding vector snails biological control methods. This finding concurs with other previous studies by Stothard, *et al*., (2002); Coelho, *et al.*, (2004) and Rosa, *et al*., (2006) that had reported that not all strains within the snail species transmit schistosomiasis. In the genus *Bulinus africans* certain strains of snails are resistant to Schistosomes transmission. Similarly, *S. mansoni* miracidia infects certain species of *Biomphalaria* whereas other strains appear to be refractory to infection (Stothard, *et al*., 2002). Where the resistant trait is a dominant phenotype and crosses with a susceptible population, this offers potential for biological control of schistosomiasis (Coelho, *et al.*, 2004; Rosa, *et al*., 2006). The population of snails from the study area is however a certainly important vector for transmitting schistosomiasis where an average of 76 % of the snails were positive in shedding cercaria, indicating an effective invertebrate stage sporocyst cycle as had been reported earlier by(Neva & Brown, 1994; Hamburger, *et al*., 1998). This observation supports the observed hospital records showing

S. mansoni as endemic in the study area. The rapid human population growth and erratic weather patterns prompting the continued expansion of irrigated agricultural schemes are expected to enhance transmission of schistosomiasis in endemic regions and these findings are similar to what was reported by (Wilson& Coulson, 2009). With the concerns that presently there is no effective vaccine against schistosomiasis (Bergquist & Colley, 1998; You, *et al*., 2017),it is important that various concerted measures are put in place to disrupt transmission of schistosomiasis.

In this study the PCR primers franking Schistosomes rDNA ITS1 were specific and highly sensitivity, at femtoMole concentrations similar to detection levels obtained by (Lu, *et al*., 2016). This indicated that probably much higher sensitivity can be achieved by designing nested primers for the target DNA fragment and this can provide an efficient Schistosomes infection detection PCR kit for epidemiological survey of schistosomiasis transmission, which is endemic in the present study area and Kenya in general (Stothard, *et al*., 2017). At molecular level, experiments with PCR primers targeting *Biomphalaria sp* rDNA ITS region to detect the pre-patent infections of the snails by cercaria is important. The results in present study show that not all the snail samples that had been exposed to *S. mansoni* miracidia sustained infections and the titres of the pathogen at DNA were different. Some snails had positive amplifications of Schistosome DNA in the tissue biopsy at approximately 400 bp. Other snails tissue biopsy were miracidia/cercaria free or with blurred amplifications. The recorded observations indicate that some variant snails in every sampling unit resist Schistosome infections. Similar such observation had earlier been reported by Rosa, *et al*., (2006).

The freshwater *Biomphalaria* snails are known to be important vector for *S. mansoni* and its therefore imperative that all strategies to interrupt the disease at vector stage need more attentions (Campbell, *et al*., 2018). In this study, the reported variability in susceptibility to transmit the human infective larva stage, cercaria have also been recorded in some other previous studies and were attributed to macrophage-like hemocytes cellular immunity for the resistance to infection (Campbell, *et al*. 2018; Plows, *et al.,* 2006 and Coelho, *et al*., 2004 Coustau, *et al.*, 2015). A necessary aspect in rapid determination of infection cases is provision of molecular tools to detect infection of snails by cercaria. Traditionally, this has been demonstrated by assessment of snails infection through shedding of cercaria method under direct light illumination. This method though easy and inexpensive but requires long and variable pre-patent periods from three weeks to two months for snails to readily shed cercaria. Positive Molecular detection of snail infection by cercaria as was demonstrated in this study could provide basis for a rapid and accurate epidemiological diagnostic tools. This finding can be collaborated by other study of PCR amplification targeting Cytochrome c-oxidase sub-unit 5 gene and rDNA ITS regions as reported by Webster, *et al.,*(2010) and Stothard, *et al*., (2017).

This study presents evidence that the water canals draining the irrigated farming fields contained vector snail susceptible to *S. mansoni.* This depicted that Schistosomiasis incidences were persistent in Mwea area, similar to the findings by Mutuku, *et al*., (2017). The transmission in such zones of less developed countries are presumably due to lack of comprehensive efforts to curtail the parasites transmission. The local hospital records in the study site as well depicted an active transmission of the disease to the local community, albeit at low persistence incidences. These data sets may seem to suggest that

schistosomiasis MDA campaigns and awareness initiatives for better public hygiene may be needed in line with recommendations by Knopp, *et al*., (2019) study.

Schistosomiasis infections and presence of vector snails in the study area showed that the disease was in active transmission mode in Mwea irrigation agriculture scheme in Kirinyaga County. A comprehensive public health intervention program is critical. To eliminate or reduce schistosomiasis transmission integrated relevant interventions are needed. These may include mass drug administration using praziquantel, public health education, provision clean water, improved sanitation, and vector snails control strategies. Towards this end, locally adapted *Biomphalaria sp* snails that are refractory to transmission of *cercaria* could be propagated and tested for purposes of biological control against schistosomiasis.

5.2 Morphological characteristics of *S. mansoni* resistant and susceptible vectors snails

Snails shell morphometric identification was used to determined populations dominating the Mwea irrigation scheme in Kenyan highland, which was identified as *B. pfeifferi*. This finding concurs with other previous works by (Mutuku, *et al*., 2017). In previous studies (Loker,*et al*., 1993; Brown, 1994), it had been reported that in Africa, transmission of intestinal schistosomiasis caused by *S. mansoni* was enabled by 12 species of *Biomphalaria sp* of which *B. pfeifferi* was one of the most prominent intermediate hosts, just as this study has recorded. The species is reported to be widely distributed in the Lake Victoria tributaries, rivers in central Kenya, small impoundments, and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the Kenya coast.

However, comparing *B. pfeifferi* physical characteristics in the shell morphometric parameters in *S. mansoni* resistant and susceptible snails from Mwea irrigation scheme did not show significant differences. The average means of shell height, shell width, aperture height, Aperture width and spiral length for resistant and susceptible snails were not significantly different. These findings demonstrated that the exhibited resistant traits may not be as a result of snails morphological characteristics but probably due to snail internal defense mechanisms. Similar observations had been reported by Negrao-Correa, *et al*., (2007) and Nacif-Pimenta, *et al*., (2012). Elsewhere it had been reported that susceptibility of *Biomphalaria sp.* to *S. mansoni* infection could be attributed to inherited characters(Newton, 1953; Webster & Woolhouse, 1999).

Morphological symmetries determination using shell dimensions showed that the determined taxonomical key parameter on shell length to shell width and aperture length to aperture width had close linearity indicating all the snail samples were the same species *B. pfeifferi.* These findings are similar to earlier reported studies (Wullschleger & Jokela, 2002; Schnabel, *et al*., 2013). Species and strains identification using the snail shell may not be conclusive hence the need for a revised nomenclature combining both morphological features and molecular markers as suggested by Dejong, *et al.*, (2003) study.

5.3 Phylogenetic analysis of *S. mansoni* vectors snails from Central Kenya region

Phylogenetic analysis is important to enriches understanding of how a species gene, genome, molecular sequences evolve. In this study, phylogenetic analysis of *Biomphalaria sp* isolates using rDNA ITS sequences reported three monophyla lineages associated with clusters of closely related populations, within Kenya with some lineages that are

monophyletic over long evolutionary time. Additionally, other populations reflected closely related clonally progenies as indicated in that are offshoots from these monophyletic lineages. These finding suggest that *Biomphalaria sp* is of a common ancestry and thus monophyletic. This characteristic was similarly observed in the composite phylogenetic analysis of *Biomphalaria sp* from across Africa drainages suggesting that some field isolates were likely to be monophyla lineages while other clades were clonally or very closely related isolates. These observations are similar to the findings by Daniels, *et al*., (2015).

The Kenyan isolates under this study, showed diverse genetic structure representative of six major isolates from across Africa comprising of *B. pfeifferi, B. sudanica*, *B. choanomphala*, *B. stanleyi*, *B. alexandria* and *B. glabrata*. This is possibly due to Kenya's geographic location in relation to the main drainages in Africa: the Lake Victoria and the Nile basin, river Congo basin and drainages from Eastern and Southern Africa Highlands to Indian Ocean. The disruption and redistribution of river systems with the formation of East African Rift Valley in Miocene period may provide a guide to tropical Africa fauna Phylogeographic affinities in this region (Daniels, *et al*., 2015; Schulthei, 2014). The observed population structure of *Biomphararia sp*, could likely be due to the isolated wetlands separated by geographic barriers.

Using schistosome rDNA sequences the present study showed that snails collected from the central highlands of Kenya are closely related isolates of *B. pfeifferi* a significant vector of *S. mansoni*. This could explain the observed active transmission of schistosomiasis in the study region. In earlier studies, it had been reported that major snails vectors that

transmit human schistosomiasis in Kenya include *Biomphalaria sp,* for *S. mansoni* in the central and western parts of the country; the *Bulinus sp,* for *S. haematobium* in the coastal region and the adjoining eastern regions. However, there are intermixtures of both species in some regions that have been reported before (Rollinson, *et al*., 1998; Dejong, *et al*., 2003).

In this study, Tajima's neutrality test for *Biomphalaria sp* performed using the rDNA gene sequencing to assess the snail population drift from equilibrium recorded a significant negative values (-1.681654 and -2.003619) for Kenyan and Africa regional isolates, respectively. This is an important observation to establish if *Biomphalaria sp* was still evolving randomly (naturally) or under balancing selection. A population at gene frequencies equilibrium, its DNA segregating sites and nucleotides diversity is expected to be the same with a Tajima-D test value = 0. In an earlier similar study, Tajima, F. 1989, it was reported that Tajima-D values close to zero indicate that the nucleotide diversity is near neutrality and the population from which samples were drawn is almost at equilibrium with respect to drift and mutation.

As reported in this study, with Tajima -D value which was less the zero depicted that *Biomphalaria sp* has excess of rear alleles and therefore it was still undergoing population expansion. This implies purifying selection against certain deleterious alleles or population expansion where new selected-for alleles are still in low frequency resulting in low heterozygosis. As reported by Tamura, *et al*., 2013) that given population is normally at constant size and natural mutation with no effects on its fitness and survival when it reaches equilibrium of gene frequencies, the current study indicated the population was still expanding.

5.4 Transmission levels of resistant traits in *B. pfeifferi* under laboratory conditions

B. pfeifferi is an essential link in the Schistosome life cycle, its population dynamics and its natural attributes are important to addressing Schistosome transmission. This could form a basis for planning and control measures against this snail intermediate hosts in the control of schistosomiasis. These study findings provide an important basis of selecting existing resistant population of *B. pfeifferi*. Over the experimental period, after the cultured snails were exposed to miracidia infection, the resistant snails became the dominant population under the Laboratory bred populations. This was consistent with previous works that indicated that isolates of *Biomphalaria sp* and *Bulinus sp* snails vary in their ability to transmit Schistosomes based on their susceptibility or resistance traits that is sometimes ascribed to the known hemocytes immune cells (Hanington, *et al*., 2012; Zahoor, *et al*., 2014; Pila, *et al*., 2016). It has been reported that, *S. mansoni* miracidia infects some strains of *B. glabrata* and *B. Ugandae* while some other strains are refractory to infection. Similarly, it has been noted that not all species within the genus *Bulinus Africans* act as intermediate host for *S. haematobium* (Stothard, *et al*., 2002).

From the field collections of snails through to F2 generation, this study recorded successfully isolation of desired resistant population of *B. pfeifferi* under laboratory conditions. This finding suggested that there was successful transmission of resistant traits from one generation to the other. The infections rates recorded under laboratory conditions for *B. pfeifferi* against *S. mansoni* were consistent with the previously works that suggested there could be enhancement of resistant genes in individual snails through selection pressures over generations. In previous studies, similar trends have recorded on determination of phenotypic resistance of snails in F1 and F2 progeny, genic flux of the

resistance traits in F1 progeny obtained by crossbreeding susceptible and resistant strain of the snails of *B. glabrata* species, where it was observed that resistant individuals predominantly occurred in all groups (Lewis*et al.*, 2008; Iman, *et al*., 2010). Previously, investigations by Richard and Merritt (1972), had documented dominant resistance heritability in *B. glabrata* snails. In Rosa, *et al*., (2005) work, two dominant genes determining resistance *in B. tenagophila* were reported. This has been further collaborated by the others studies that found that reported factors that influence both the resistance and susceptibility to *Schistosome* infection could be genetically heritable and may be determined by the activities of the Snails internal defense system (Negrao-Correa, *et al*., 2007; Webster, & Woolhouse, 1999; Hoffmann, *et al*., 2002).

In this study, snails recorded mortality rates were not significantly different among the experimental groups. The mortality observed could have been as result of natural causes as similar to mortality rates in snails were reported (Mangal, *et al*., 2010; Alvine, *et al*., 2018,).While in previous work Olasehinde & Oyerinde (2007) reported challenges in rearing *B. pfeifferi* under laboratory conditions, the present study demonstrated that these snails can be propagated from one generation to the other. These findings provide an important basis that can be consider for mass rearing of resistant snail for biological control trials.

The findings generated in this study under laboratory condition points to suggest that *S. mansoni* resistant populations of *B. pfeifferi* which was the predominant species can be isolated and mass reared with a view of diversifying biological control measures of the vector of schistosomiasis disease. These findings posit that desirable biological control of

schistosomiasis is possible using resistant sub-populations. While various vector snails control measures have been tried with varied success rates, the current study suggests it is possible to select and rear resistant or the less susceptible strains to infection, which is an ecologically safer means of breaking transmission cycles in schistosomiasis endemic areas in Kenyan highlands.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i) Population proportions of resistant and susceptible snails from Mwea Irrigation scheme

In the natural habitats, there exist population proportions of *B. pfeifferi* snails that are susceptible and resistant to transmission of *S. mansoni* cercaria. These traits could be explored for possible use in biological control of these vector snails.

ii) Morphological characteristics of *S. mansoni* resistant and susceptible *Biomphalaria sp* snails

There are no morphological characteristics differences between the observed populations of *S. mansoni* resistant and susceptible *B. pfeifferi* snails.

iii) Evolutionary development of vector *Biomphalaria sp*

Biomphalaria sp from this region is of a common ancestry with a genetic lineage that is monophyletic with clusters of closely related isolates or clonal expansion. The species has excess of rear alleles and therefore it was still undergoing population expansion through natural mutation.

iv) Transmission of resistant traits in *Biomphalaria sp* snails under laboratory conditions

Under laboratory conditions, there is enhancement and active transmission of resistant traits of *B. pfeifferi* snails from one generation to the next. The snails that are refractory to transmission of cercaria can be isolated and mass reared.

6.2 Recommendations

i) More studies are needed to cross breed the identified resistant and susceptible populations under laboratory conditions;

ii) Mass rearing of snails that are refractory to transmission of cercaria for possible field trials of biological method by population displacement which is an ecologically safer mean of breaking transmission cycles in schistosomiasis endemic areas;

iii) Further studies are needed to establish the mechanisms that confer resistance to Miracidia in snails;

iv) Detailed studies on phylogenetic and systematic classification for the *Biomphalaria sp* vector snails as an important prerequisite in defining isolates and strains that are susceptible or resistant to *S. mansoni.*

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APPENDICES

Appendix I: Similarity Report

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