Current status and future predicted distribution patterns of bilharzia-transmitting snails under climate change and implications for vector-borne diseases in South Africa

Report to the
WATER RESEARCH COMMISSION

By

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EXECUTIVE SUMMARY

BACKGROUND

Schistosomiasis, commonly known as bilharzia, is an infection caused by the trematode parasites of the genus *Schistosoma* transmitted to humans through contact with water bodies that contain the molluscan vectors contaminated with the parasite. This disease is most prevalent in tropical and subtropical areas, particularly in poor and rural communities. Schistosomiasis is a serious public health issue in South Africa, with at least 4.5 million people infected with the disease annually and approximately 20 million people at risk of infection.

Climate change is predicted to severely affect aquatic ecosystems by changing rainfall patterns, air temperatures, and the severity and frequency of occurrence of natural disasters such as floods and droughts. This also poses a threat to human health as it may affect food security, air quality and the distribution and occurrence of infectious, food- and vector-borne diseases. This is particularly true for regions, such as sub-Saharan Africa, that are already burdened by environmental disasters such as floods and droughts and many tropical diseases including schistosomiasis. Invasive species also pose a significant risk to the distribution and survival of native biota. *Tarebia granifera* is a freshwater snail from south-east Asia that has become established as one of the most prolific invasive species in South African freshwater ecosystems. This invasive snail has been found in regions of South Africa where schistosomiasis is known to occur, but it is presently unknown whether *T. granifera* acts as an intermediate host to these parasites in South Africa. This snail also has the potential to outcompete native snails such as those that transmit schistosomiasis. It is therefore imperative to study not only the effects of climate change, but the distribution of *T. granifera* as these factors play an integral role in the distribution of schistosomiasis, and thus the risk of exposure to humans in South Africa.

Climate change is predicted to alter air temperature and rainfall and, along with continued human population growth, the range and distribution of the intermediate snail hosts and the parasites causing schistosomiasis could potentially change, increasing population exposure. With increased temperature, the distribution of both snails and parasites may potentially increase in cooler regions and decrease in hot areas, while changes in annual average precipitation will increase disease transmission in areas with higher rainfall and decrease transmission in locations with reduced rainfall. In addition to these climatic changes, increased frequency and intensity of heat waves may cause more outbreaks of the disease in regions that were previously low or zero transmission areas. Changes in flow and flooding regimes

may also lead to the establishment of snails in new regions and/or temporary or permanent removal of snails in endemic regions. It is thus crucial to gain an understanding of whether future predicted climate change may influence the transmission of this disease in order to protect public health and inform adaptation and mitigation strategies. Since schistosomiasis predominantly affects poor and rural communities, it is also imperative to understand how communities at risk of becoming infected with this disease experience climatic and environmental change, and how these changes impact their health, well-being and livelihoods.

RATIONALE

Schistosomiasis is a neglected tropical disease that has been severely understudied since treatment is available for humans and the negative effects are not considered severe, particularly in relation to other diseases such as malaria and HIV and AIDS. However, continuous re-infection of schistosomiasis in both humans and animals poses serious health threats, and an increase in the frequency of treatment-resistant schistosomiasis is also being observed. It is therefore imperative that this disease is continuously evaluated. Even though schistosomiasis affects millions of people in South Africa, little has been done to evaluate the current distribution of the vectors and their associated parasites and towards achieving the WHO targets for the elimination of schistosomiasis transmission by 2030, since the last comprehensive evaluation of the vectors occurred in the 1950s to 1960s as part of the collections for the National Freshwater Snail Collection (NFSC). At present, the status of the snails and the disease in South Africa is unknown. It is further unclear how environmental and climatic changes, and the presence of invasive species may alter the distribution of the vectors and, consequently, the disease. Given that temperatures and rainfall have already changed over the past 60 years, and several new invasive mollusc species have been introduced into South Africa, the distribution of vectors may have changed as well, particularly in regions that were on the distribution fringes of this disease. Additionally, only a few studies have been undertaken to date, and our present understanding of human exposure and vulnerabilities to this disease is incomplete. In order to fully comprehend the vulnerability of people to waterborne diseases such as schistosomiasis, it is necessary to gain an understanding of not only the physical environment, but also of the broader social environment in which people live and work. A better understanding of perceived health risk could also inform public health policies and actions, and influence mitigation and adaptive responses, since perceptions and actions drive health-related behaviours. Designing effective response programmes requires an understanding not only of changes in the distribution of schistosomiasis intermediate host snails, but also of existing knowledge, attitudes, perceptions and practices surrounding schistosomiasis in endemic communities.

The overarching aim of this research project was to determine whether the distribution ranges of the snail vectors and associated parasites of schistosomiasis have expanded in recent years, how affected human communities perceive and experience these changes, and whether changes in distribution may further expand and increase the potential prevalence of the disease in humans and animals in South Africa given future predicted climate change.

This was achieved through the specific aims listed below.

AIMS

Aim 1

Conduct field collection surveys of freshwater snail species, particularly those known to be schistosomiasis vectors, and their parasites in historic and potentially new suitable distribution sites.

Aim 2

Determine whether schistosomiasis infection areas have changed in the past half-century in South Africa with the use of historic water quality information and historic climatic modelling.

Aim 3

Determine potentially new suitable distribution ranges of schistosomiasis vectors in relation to future predicted climate change.

Aim 4

Assess community knowledge, attitudes, and perceptions of the past and present incidence of schistosomiasis in communities located in known schistosomiasis infection areas.

METHODOLOGY

The methodology of this study was divided into sections relating to each specific aim.

Methods to achieve Aim 1

Schistosomiasis vectors were collected from known historic and potentially new suitable distribution areas. Water, and habitat quality and suitability for the snail vectors and parasites, were evaluated in the field. Water quality was assessed by measuring various *in situ* water quality parameters including pH, temperature (°C), oxygen content (%) and conductivity ($\mu S.cm^{-1}$). A single, integrated subsurface water sample was also collected from each sampling site and kept frozen until analysis in the laboratory. Chemical nutrients were measured in the laboratory and included NO_3^- , NO_2^- , SO_4^{2-}, NH_4^+ ,NH_3, Cl^-, PO_4^{3-}, and total hardness. Habitat quality and suitability were determined by assessing the presence and approximate area covered by various riparian and aquatic vegetation as well as aquatic substrate and algal presence and water flow velocity, where applicable.

Molluscs were collected from both standing and flowing water habitats including rivers, streams, dams and wetlands. For the collection of molluscan species, all potential habitats of a sampling site were assessed for the presence of various molluscs. Molluscs were then collected from these various water bodies using a standard snail scoop with a stainless-steel mesh fitted inside a metal frame and by hand-picking from any surfaces including sediment, rocks and aquatic vegetation. All collected molluscs were placed in wide-mouthed polyethylene jars and incubated under artificial light intermittently to induce cercarial shedding after which snails were euthanised in 96% ethanol, dissected and examined for unshed parasites. Snail species and parasites were identified using both morphological and molecular methods.

Methods to achieve Aim 2

The historic snail dataset for the schistosomiasis-transmitting snails *Biomphalaria pfeifferi*, *Bulinus africanus* and *Bulinus globosus* were sourced from the National Freshwater Snail Collection (NFSC) in South Africa for three Provinces, namely Mpumalanga, Limpopo and Gauteng. Data were sourced for *Biom. pfeifferi* and *Bul. globosus* for the years 1955 to 1995 in Mbombela and Nkomazi municipalities (Mpumalanga Province), for *Biom. pfeifferi* and *Bul. globosus* from 1957 to 1964 in the Vhembe District Municipality (Limpopo Province) and for *Biom. pfeifferi* and *Bul. africanus* for the years 1970 to 2006, in the Tshwane and Johannesburg metropolitan municipalities (Gauteng). These snail species were selected for each Province due to their prevalence in each Province and the years chosen based on the most complete historic datasets available. In total, 19 bioclimatic variables were tested, and 13 predictor variables were used to describe the influence of climate on snail habitat suitability. Species distribution models (SDMs), including maximum Entropy (MaxEnt), generalised linear models (GLM) and Random Forest (RF) models, were employed to predict historic snail distributions in each of the selected Provinces. The findings were visualised on maps for each Province and utilised colours to indicate habitat suitability, and thus probability of occurrence, where red indicated high probability, yellow represented moderate probability, and shades of green indicated low probability. Variability among models was addressed by performing a Variance Inflation Factor (VIF) analysis to mitigate multicollinearity among selected variables. Five variables were retained for each species model. To assess model performance, Receiver Operating Characteristics (ROC) and the Area Under the Curve (AUC) values were employed.

Methods to achieve Aim 3

The snail sampling data were obtained from NFSC from 1950 to 2006, and 15 303 records were recorded. The locations close to water bodies (dams, rivers, reservoirs, lakes, and swamps) were digitised. Latitude and longitude coordinates were stored in decimal degrees

using a WGS84 datum. Nineteen bioclimatic and four soil variables were extracted for the years 1979 to 2018 using ERA 5 reanalysis data provided by the Copernicus Climate Change Service. The temporal aggregation was the mean climatological period pre-calculated over the 40-year reference period 1979-2018 with a spatial resolution of 0.5° x 0.5°.

Modelling future climate builds on four Representative Concentration Pathways (RCPs) adopted by the IPCC for its fifth assessment report (AR5) in 2014. All models were based on CMIP5, with RCP 4.5 representing a moderate scenario in which greenhouse gas (GHG) emissions are expected to increase up until 2040 and then to decline, and the RCP 8.5 scenario, in which GHG emissions are expected to increase throughout the 21st century, creating a 'high emission scenario'. The future periods (2040-2070 and 2070-2100) were derived from CMIP5 climate projections provided by the Copernicus Climate Change Service. The future period data were obtained from three GCMs created in a grid format of 0.5° x 0.5°. A group of variables was selected based on bioclimatic and soil factors to minimise collinearity in the dataset. Variance Inflation Factor analysis was used to measure the strength of the relationship between each predictor and the rest for the averages over 1970-2006. The process was repeated until none of the variables had a high correlation coefficient.

Three modelling algorithms were applied to overcome their limitations, namely GLM, MaxEnt and RF. A GLM was used to calculate the relationship between the response and the predictor, and logistic regression was used as the most widely used GLM form for environmental modelling. Maximum entropy was used to estimate the distribution within the investigated area based on the current locations' environmental conditions, and the Random Forest algorithm was used as a bagging approach (bootstrap) to incorporate multiple trees by repeatedly sampling the training data, resulting in fitting a tree to each sample by averaging the predictions and reducing the model variance.

Methods to achieve Aim 4

This part of the study employed a mixed-method sequential explanatory research design. Data on community knowledge, attitudes, perceptions and practices were collected through household questionnaires and in-depth interviews. The participants for household questionnaires were selected based on predetermined inclusion criteria aligned with the study's objectives. These questionnaires were administered to permanent residents of the HaNesengani community aged 18 years and above. In-depth interviews included individuals in higher risk groups (agricultural workers and caregivers of children under 15), healthcare providers, and those residing in HaNesengani for over 15 years, meeting the criteria of age and residency. In-depth interviews also included healthcare workers and traditional healers working within the community, regardless of their duration of stay or years of service.

The household questionnaires were conducted on August 28 and 29, 2021, over a two-day period, led by fieldworkers trained in ethical community engagement practices by the Rural Innovation Hub at the University of Limpopo. Quantitative data underwent analysis, and an interview guide for the in-depth interviews was designed to allow for a deeper exploration of interesting findings from the questionnaire data. The in-depth interviews, assisted by a translator, were conducted on April 12 and 13, 2022.

RESULTS AND DISCUSSION

Through morphological and molecular analysis of the molluscs, ten species were identified, namely *Bulinus africanus, Bul. depressus, Biomphalaria pfeifferi*, *Gyraulus costulatus, G. connollyi, Melanoides tuberculata, Radix natalensis*, *Pseudosuccinea columella, Physella acuta,* and *Tarebia granifera*. The identification of *Bul. depressus*, *G. costulatus* and *G. connollyi* resulted in additional sequences of the ITS2 and COI sequences generated for these species and *R. natalensis* formed four genetically diverse groups located at different isolated sites. This suggests the possibility that *R. natalensis* may move or be transported between the sampling sites through active dispersal by various animals, including birds, or by other natural mechanisms. The molecular analysis of the invasive snail *Pseudosuccinea columella* indicated that all the specimens collected at the various sites were genetically identical, with no base pair differences of the ITS2 and COI genes, indicating that the invasion of this species was the result of invasion by a single specimen or multiple invasions from a single location. All *T. granifera* were genetically identical females, thus supporting previous hypotheses that all *T. granifera* in South Africa come from few or even a single invasion event or a small population of introduced snails.

Although the cercarial shedding experiments were unsuccessful, immature or early-stage infections of several parasitic species were detected using molecular analyses of the hepatopancreas. The unsuccessful cercarial experiments were likely as a result of the naturally low prevalence of *Schistosoma* sp. in snails, and the life cycle complexity of digenean trematodes is potentially the reason why these species were not present within the collected vectors. The detection of *Neofibricola smiti* within *Bulinus* is the first detection of this trematode within a mollusc host of South Africa, producing new insight into the life cycle of *N. smiti.* The detection of *Petasiger variospinosus* in several species indicated that this trematode is not host-specific within the *Bulinus* species group. The detection of *Orientocreadium batrachoides* within specimens of *R. natalensis* is also the first report of this digenean trematode within a freshwater mollusc species. It is particularly concerning that no trematode parasites were detected in the invasive snails *Pseu. columella* and *T. granifera*, while the native snail, *Melanoides tuberculata*, was infected with two species of trematodes known to reduce snail population growth.

This study found that native snails have decreased in spatial distribution and abundance since historic collections took place, possibly due to anthropogenic and natural alterations to the physical habitat, through increased rural development and agricultural use. Such developments have likely also resulted in increased water use, causing changes in water quality and the presence of invasive mollusc species competing for the same ecological niche which has resulted in a decline of native mollusc species and their associated parasites, including those that transmit schistosomiasis. At sites where *T. granifera* were the dominant species, few other mollusc species were present and, when present, other species were in much lower densities than *T. granifera*. The population densities reached by *T. granifera* were much higher than those of native molluscs and exceeded the densities reported from its native range. The ability of *T. granifera* to rapidly spread through the aquatic ecosystem, resist unfavourable conditions that are detrimental to native mollusc species, and the lack of natural predators are of concern for native species and their associated parasites. However, this may indirectly be having a positive effect on diseases transmitted by native mollusc species such as schistosomiasis, although further investigation would be necessary to assess this theory.

The historical distribution of suitable habitats for *Bulinus globosus* and *Biomphalaria pfeifferi* in Mbombela and Nkomazi local municipalities was modelled using the MaxEnt and GLM models. Both models showed satisfactory performance based on the AUC scores. The MaxEnt model exhibited the highest AUC scores, indicating greater occurrence probability prediction for historical distribution. Model predictions generally aligned with true known distributions for the snail species, with few deviations. Both models revealed variations in the relative contributions of each variable for each species, emphasising the role of elevation, rainfall, and temperature as the main environmental factors affecting distribution. *Biomphalaria pfeifferi* thrived in both Mbombela and Nkomazi across all seasons, while the central areas of the two municipalities provided more suitable habitats for *Bulinus globosus*, particularly in the summer.

To assess the historical distributions of *Biom. pfeifferi* and *Bul. globosus* in the Vhembe District Municipality, the RF and MaxEnt models were used. The RF model outperformed the MaxEnt with AUC values indicating better performance. For both mollusc species, minimum cold month temperatures were determined to be one of the most important factors contributing to habitat suitability. Precipitation also played an important role, with *Biom. pfeifferi* particularly favouring the post-rainy season. The results are in line with existing studies on temperature, precipitation, and habitat preferences for *Biom. pfeifferi* and *Bul. globosus*.

The historical habitat suitability for *Biom. pfeifferi* and *Bul. globosus* in the Tshwane and Johannesburg metropolitan municipalities was assessed using the MaxEnt and RF models.

The models indicated that temperature was one of the most important factors affecting the habitat suitability for these snails, with *Biom. pfeifferi* faring better in warmer temperatures compared to *Bulinus africanus*, which is known to tolerate cold conditions better than *Biom. pfeifferi.* Precipitation also played an important role, with excessive rainfall negatively affecting habitat suitability for the snails. Overall, the models effectively depicted how environmental factors shape the distribution of these snail species, providing valuable insights into their habitat preferences for the future.

The potential future distribution of suitable habitat and distribution pattern models indicated that for *Bul. africanus*, temperature preferences are predicted to shift to tolerate higher temperatures while retaining reproductive suitability in cooler ranges. Rainfall is predicted to be an important factor impacting its distribution, with this species avoiding excessive moisture. Its suitability is predicted to be primarily influenced by precipitation, temperature, and changes in season. *Bulinus globosus* is currently known to thrive in warm temperatures and it is predicted to exhibit resilience to increased temperature and rainfall in the future. Its distribution is predicted to be affected by both increased and decreased rainfall levels in both wet and dry periods. *Biomphalaria pfeifferi* currently prefers warm, wet environments, and temperature tolerance and rainfall preferences are predicted to vary across different periods and scenarios. All the snail species will face shifts in habitat suitability, both increases into previously uninhabitable regions and decreases in previously habitable regions due to changing climate conditions. Despite the variations between models, scenarios, and periods, it is predicted that these snail species will maintain most of their current distribution patterns. They are projected to continue their current distribution in Limpopo, Mpumalanga, KwaZulu-Natal, North West, Gauteng, and Eastern Cape Provinces. However, their distribution will likely shift, with new areas previously not considered areas of distribution becoming suitable due to climate change. These include the Free State and Western Cape that have historically been unsuitable.

Regular piped water supply interruptions emerged as a primary driver of risky water behaviours and the persistent prevalence of schistosomiasis in the study community selected for Aim 4. While participants exhibited familiarity with the local name for the disease and its symptoms, a lack of understanding about the life cycle of schistosomiasis and its transmission was observed among participants. The research also found that there was a discontinuation of awareness campaigns and infection prevention activities for school children due to resource constraints. Results revealed that the youngest age group, aged five to 17 years, exhibited the lowest awareness levels and the highest vulnerability to schistosomiasis. These findings underscore the importance of awareness campaigns in local languages and a reliable piped water supply, especially in endemic rural areas. The role of traditional healers as essential healthcare providers also emerged from the study; yet their misconceptions about

schistosomiasis transmission, symptoms, and treatment highlighted a lack of collaboration between formal and traditional healthcare sectors. To address this gap, fostering cooperation and knowledge exchange among community healthcare providers is imperative. These findings highlight the need for comprehensive community-wide awareness campaigns, dispelling myths about the mode of transmission, and emphasising how to prevent infection. Ultimately, addressing these multifaceted challenges can contribute to the mitigation of schistosomiasis within endemic communities like HaNesengani.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Due to the fact that *T. granifera* serves as a host for diverse species of trematodes within and outside its native ranges, further investigation of its role as a host for trematodes within all invaded habitats across southern Africa is necessary. Parasites such as trematodes influence native snail population growth, but no parasitic infections have been reported in *T. granifera* in South Africa; this release from parasites enables *T. granifera* to reach higher densities and spread rapidly, possibly contributing to their advantage over native snails within the invaded ecosystems of South Africa. Without intervention, this invasive species has the potential to completely displace many of the native freshwater snail species, including those that transmit schistosomiasis and other water-borne diseases, and further investigation is needed to determine how *T. granifera* affects native aquatic biota and, potentially, human health.

The historic distribution models indicated that Mbombela had the most suitable distribution areas for *Biom. pfeifferi* and *Bul. globosus* in the Mpumalanga Province compared to Nkomazi Local Municipality. This finding is in agreement with previous studies, highlighting the role of temperature, precipitation, and elevation in snail distribution dynamics. Climate and bioclimatic changes could turn previously unsuitable areas into new infection “risk zones” for schistosomiasis transmission in the future. In the Vhembe District Municipality, the most suitable habitats for *Biom. pfeifferi* and *Bul. globosus* included areas with low precipitation and higher temperatures. In the Tshwane and Johannesburg metropolitan municipalities, models revealed that precipitation negatively correlated with historic habitat suitability for both *Biom. pfeifferi* and *Bul. africanus*, with *Biom. pfeifferi* favouring warmer temperatures than *Bul. africanus*. It is recommended that future research incorporate human activities and land-use activities into predictive models as these may affect the distribution of habitable regions.

The forecast distribution of schistosomiasis-transmitting snails in South Africa consistently revealed similar trends in snail distribution across diverse climate scenarios. Common trends included the emergence of newly habitable regions while some existing endemic regions would contract, although the distribution will remain largely consistent with their current distributions. Additionally, the models suggested the potential presence of these snails in

previously uninhabited regions of the Free State and Western Cape Provinces. Further research into the co-evolutionary history of the *Schistosoma*-transmitting snails and parasites may provide insight into the flexibility of the parasites to parasitise other species. Additionally, a deeper understanding of the resilience of snails and parasites to changes in environmental conditions will assist with future seasonal distribution predictions of the disease.

The community knowledge, attitudes, perceptions, and practices concerning schistosomiasis in the HaNesengani community indicated that the community's lack of access to safe and clean water is the primary factor causing unsafe water practices and thus exposure to schistosomiasis. Although participants are familiar with the local term "mutambotambo" for schistosomiasis, they have a limited understanding of its life cycle, increasing their susceptibility to infection, and indicates the need for awareness campaigns and access to a reliable water supply for vulnerable communities.

The local clinics have ceased awareness campaigns and infection prevention activities in schools due to resource shortages and this is a likely reason why younger age groups have a lower awareness level of this disease even though they are the most susceptible to infection. Misconceptions about transmission are also prevalent and respondents avoid the use of protective gear in water contact. Community-wide campaigns, focusing on accurate information dissemination and addressing misconceptions, are recommended as they are crucial in creating an awareness in the communities about schistosomiasis.

Traditional healers, while important healthcare providers, also exhibit misconceptions about schistosomiasis and currently there is only limited collaboration between formal and traditional healthcare sectors. Addressing this divide could promote collaboration and knowledge sharing among providers. There is a need for production and dissemination of awareness materials in local languages that are familiar to residents.

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LIST OF ABBREVIATIONS

ANOSIM	analysis of similarity
AUC	area under curve
bp	base pair
BRT	boosted regression tree
CCA	canonical correspondence analysis
CMIP	Coupled Model Intercomparison Project
COI	cytochrome c oxidase subunit I mitochondrial gene
DNA	deoxyribonucleic acid
DALY	disability adjusted life years
EC	electrical conductivity
ENM	ecological niche model
FAU	formazine attenuation unit
FGS	female genital schistosomiasis
GCM	general circulation model
GHG	greenhouse gases
GLM	generalised linear model
HIV-AIDS	human immunodeficiency virus – acquired immunodeficiency syndrome
HF	high flow
IPCC	Intergovernmental Panel on Climate Change
ITS2	internal transcribed spacer 2 of the nuclear ribosomal DNA
KAPP	knowledge, attitudes, perceptions, and practices
LF	low flow
MaxEnt	maximum entropy
mtDNA	mitochondrial DNA
NCBI	National Center for Biotechnology Information
NFSC	National Freshwater Snail Collection
nMDS	non-metric multidimensional scaling
NGR	Ndumo Game Reserve
NTD	neglected tropical disease
NWU	North-West University
PCA	principal component analysis
PCR	polymerase chain reaction
RCP	Representative Concentration Pathway
RF	Random Forest
ROC	Relative receeiver operating characteristic curve

rDNA	ribosomal deoxyribonucleic acid
rRNA	ribosomal ribonucleic acid
SD	standard deviation
SDG	Sustainable Development Goal
SDM	species distribution modelling
SRES	Special Report on Emissions Scenarios
TDS	total dissolved esolids
TSS	true skill statistic
USA	United States of America
VIF	variance inflation factor
WHO	World Health Organization
WRC	Water Research Commission
WWF	World Wildlife Fund

CHAPTER 1: INTRODUCTION AND PROJECT AIMS

Compiled by: *L.de Necker, M.H. Le Roux, J.J Pearson, and N. Sithole*

1.1 Background

1.1.1 Freshwater molluscs and their ecological importance

Freshwater molluscs, consisting of two main groups namely Bivalvia and Gastropoda, are one of the most diverse groups of aquatic invertebrates, with ~5 000 species inhabiting various temporary and permanent freshwater, and even estuarine, habitats globally (Oloyede et al. 2016). There are approximately 137 freshwater mollusc species native to southern Africa of which 111 are gastropods and 26 are bivalves (Appleton 2002; Bird et al. 2019). However, given the lack of attention given to this group of biota in Africa along with improved molecular identification techniques, the number of known species is likely much higher (Darwall et al. 2011).

Freshwater molluscs have relatively low mobility, specialised ecological niches and specific environmental preferences (Brown and Lydeard 2010; Covich 2010; Hoverman et al. 2011). Climate-related factors (such as temperature and precipitation) as well as environmental and hydrological aspects are essential influencers of the structure of freshwater mollusc assemblages (Oloyede et al. 2016). Flow velocity, habitat availability, changes in water chemistry, predation and the presence of invasive vectors also affect the diversity and abundance of native molluscs within aquatic systems (Lodge et al. 1987; Dillon 2000; Brown and Lydeard 2010; Covich 2010; Barton et al. 2022). Furthermore, instream microhabitat differences such as differences in substrate composition, riparian and instream vegetation, and land use contribute to variations among snail populations and species composition in freshwater (De Kock and Wolmarans 1998; Dillon 2000; Oloyede et al. 2016). These aquatic biota further constitute a large portion of freshwater biodiversity and fulfil an important role in the aquatic food web as primary consumers, playing a vital role in the breakdown of organic matter in the aquatic environment (Strong et al. 2007; Hoverman et al. 2011; Oloyede et al. 2016; Barton et al. 2022). In South Africa, freshwater molluscs function as an important food source for aquatic and terrestrial predators and are key in maintaining ecosystem function (Brown and Lydeard 2010). Freshwater molluscs are therefore not only reliable indicators of the ecological status of freshwater systems, but play an essential role in nutrient cycling, energy transfer through the food web and structuring of the aquatic community (Dillon 2000; Woodward and Hildrew 2002; Strong et al. 2007; Hoverman et al. 2011; Cordellier et al. 2012; Selbach and Poulin 2020; Barton et al. 2022).

1.1.2 Socio-economic importance of freshwater molluscs

Freshwater molluscs not only play an essential ecological role, but many have an important secondary economic and medical role as well. Although not many species of the southern African freshwater molluscs grow large enough for human consumption, rural communities have been known to harvest larger species from the family Ampullariidae and larger bivalve species such as *Chambardia* sp. and *Unio* sp. for food or as bait for fishing (Day and de Moor 2002; Darwall et al. 2011; Oloyede et al. 2016). More notably, numerous freshwater mollusc species are the vectors and intermediate hosts of parasitic diseases that can cause severe infections and negative health impacts in both humans and animals (Oloyede et al. 2016). Among these medically important molluscs are *Radix natalensis* that transmits the parasite *Fasciola gigantica* to humans and livestock, causing fascioliasis (liver-fluke disease), *Melanoides tuberculata* that transmits the parasite *Paragonimus westermani* to humans, causing paragonimiasis (lung-fluke disease), and gastropods of the genus *Biomphalaria* and *Bulinus* that transmit trematode parasites of the genus *Schistosoma* to humans and livestock, causing schistosomiasis (bilharzia) (Day and de Moor 2002).

1.1.3 Factors affecting the distribution of freshwater molluscs

Habitat structure and composition are some of the most important factors affecting the abundance and distribution of freshwater molluscs (Brown 1994). These biota are typically found between aquatic plants and mud rich in decaying organic matter, as well as on rocks, stones and concrete covered with algae and other organic debris (Tchakonte et al. 2014; Oloyede et al. 2016). Aquatic plants serve as substrates for breeding, feeding area for microflora and decaying plant matter of the aquatic plants, while also protecting molluscs from high water velocities and predators such as fish and birds (Oloyede et al. 2016). Water flow and velocity within habitats may also indirectly influence freshwater snail occurrence as it may inhibit sedimentation which has been known to alter soil chemistry, affect oxygen levels and, in areas with high silt, may foul the gills of prosobranchs (Brown 1994; Darwall et al. 2011; Dida et al. 2014).

Water temperature and dissolved oxygen are often the most important physicochemical variables influencing freshwater snail distribution (Lodge et al. 1987; De Kock and Wolmarans 2005). Water temperature determines the onset and termination of reproduction in most freshwater molluscs as well as developmental rates, fecundity and breeding patterns (Lodge et al. 1987). Mineral solubility and gas content (particularly oxygen) are also influenced by water temperature which may indirectly affect molluscan assemblages and general limnological conditions by altering photosynthesis and bacterial decomposition rates (Hoverman et al. 2011). The optimum temperature range for freshwater snail development

and growth in southern Africa is between 15 °C and 25 °C (Appleton 1978; De Kock and Wolmarans 2005). Higher water temperatures result in lowered oxygen conditions which reduces the mollusc population since dissolved oxygen plays an important role in snail breeding and egg development (Oloyede et al. 2016). Low oxygen levels may also impede the presence of some prosobranchs (gill-breathing snails) while the ability of pulmonates (air-breathing snails) to use atmospheric oxygen provides a clear advantage in such hypoxic conditions (Dillon 2000).

1.1.4 Threats to freshwater molluscs

Multiple factors threaten the natural biodiversity of freshwater species across the globe (Darwall et al. 2011; Collen et al. 2014; WWF 2020). Among the most important threats to freshwater molluscs are anthropogenic alterations to the environment, alien invasive species, and climate change (Darwall et al. 2011).

Anthropogenic alterations to the environment include deforestation and land-use change for mining, industry, agriculture, and urbanisation as well as dam construction to meet the growing demand for water, irrigation and hydro-electric power (Nel et al. 2007; Darwall et al. 2011). Such developments are more extensive on the African continent where most countries are still considered developing nations (Nel et al. 2007; Edwards et al. 2014). The risk to aquatic biota is therefore far greater in Africa than in most of the developed countries worldwide. These anthropogenic alterations not only impact on the physical habitat for aquatic biota but may lead to change in the water quantity available to aquatic biota as well as the quality of water as increased human developments often lead to water pollution with pesticides, metals, nutrient-rich agricultural runoff, and human waste (Darwall et al. 2011; Alvareda et al. 2020; Birk et al. 2020; Erasmus et al. 2021). Freshwater molluscs, as with most aquatic biota, have particular water quality, flow velocity and habitat needs with a relatively sedentary lifestyle and low mobility and dispersal mechanisms (Barton et al. 2022). They are therefore far more sensitive to alterations in the physical and abiotic environment of an ecosystem than most other biota with active dispersal mechanisms.

Invasive species

After habitat modification and pollution, one of the most important threats to freshwater biodiversity in southern Africa is the introduction of alien species (Snoeks et al. 2011; Weyl et al. 2020). Many rare and threatened species occur in southern Africa and, as a result, the species of the region are particularly vulnerable to the introduction of alien species. This is further exacerbated by anthropogenic developments already placing ecosystems under pressure and considerable international trade which increases the risk of the introduction of alien species (Snoeks et al. 2011; Faulkner et al. 2020).

Approximately 10 alien gastropod species occur in southern Africa and have been found to cause serious repercussions including direct environmental effects, such as reducing the biodiversity of the native snails, as well as indirect effects on the health of humans by affecting the distribution of certain diseases (Appleton and Miranda 2015a; Bird et al. 2019). Some of the most important invasive mollusc species that have been introduced in southern Africa include *Pseudosuccinea columella*, *Aplexa marmorata*, *Physella acuta*, and *Tarebia granifera* (De Kock et al. 1989; Appleton 2003; De Kock and Wolmarans 2008; Darwall et al. 2011; Weyl et al. 2020). These four species have become extremely successful invaders in southern Africa (De Kock et al. 1989; Miranda et al. 2010; Appleton and Miranda 2015a) as they are highly tolerant to alterations in environmental conditions and can reproduce rapidly, particularly in the absence of any natural predators (Ebbs et al. 2018). As such, they are able to outcompete native mollusc species and have done so successfully in several regions, including important conservation areas such as Kruger National Park and Ndumo Game Reserve (Appleton 2003; De Kock and Wolmarans 2008; de Necker et al. 2021). In addition, *Ps. columella*, *A. marmorata*, and *Ph. acuta* have been reported to harbour various parasites that may pose a threat to native mollusc species as well as other aquatic and terrestrial biota (Ebbs et al. 2018; Di Maggio et al. 2019; Malatji et al. 2019; Barton et al. 2022). The presence of *Tarebia granifera* is of particular concern as it poses a threat to native species; this invasive snail species was first discovered in KwaZulu-Natal in 1999 and has since become exceptionally abundant in invaded ecosystems, dominating the benthic environment, and ultimately displacing native snail species with unknown consequences for the aquatic food web (Miranda et al. 2010; Miranda and Perissinotto 2014). This snail has a much harder and thicker shell than most mollusc species, which deters most predators from feeding on them. It has also been suggested that the harder, thicker shell also provides this mollusc with better thermoregulation in high-temperature extremes, thus potentially giving this invader an advantage over native species in the face of climate change (Miranda et al. 2016; Albarrán-Mélzer et al. 2020). These molluscs are also parthenogenetic (reproducing without fertilisation) and can produce one juvenile every 12 hours; they are thus able to reach extremely high densities in invaded areas in a relatively short period of time (Appleton et al. 2009; Sirza et al. 2020; Majdi et al. 2022). Furthermore, no parasitic infections have been reported in *T. granifera* from South Africa which may give the invasive snail an additional advantage over native species (Miranda et al. 2011a; Weyl et al. 2020) while also potentially negatively impacting native parasite diversity.

Climate change

Climate change and its effects have only recently been added to the many existing threats that aquatic ecosystems and their associated services face (Davidson et al. 2012; UNU-EHS

2016; WWF 2020). Global surface temperatures have slowly increased since the instrumental recording of temperatures started in 1850. In 2007, the Intergovernmental Panel on Climate Change (IPCC) published a report stating that there has been an increase in surface temperatures of approximately 0.13 °C per decade since 1955 and this is likely to continue further. Climate change is predicted to alter freshwater ecosystems through changes in river flow, surface runoff, precipitation, water temperatures, sediment transport, and habitat structure. This, in turn, will have an impact on environmental factors such as water quantity and quality, which will have direct impacts on the biological factors such as biodiversity and distribution of aquatic and terrestrial species associated with these ecosystems (Döll and Zhang 2010; Darwall et al. 2011; WWF 2020). Freshwater ecosystems are one of the most at-risk ecosystems to these changes, specifically those that are already exploited by humans, as it imposes additional stressors to these habitats. This is particularly true in the developing and semi-arid countries of sub-Saharan Africa where freshwater ecosystems are already heavily exploited for subsistence and commercial use and polluted by agricultural, urban, and mining activities (Nel et al. 2007; Bates et al. 2008; Darwall et al. 2011; Nhamo and Muchuru 2019).

The most important direct effect of climate change on freshwater molluscs is the alteration of water temperature (Kalinda et al. 2017a; Stensgaard et al. 2019; Maes et al. 2021). Warmer water temperature is generally associated with faster growth and reproduction of molluscs and cooler temperatures with a reduction thereof, although this varies greatly between species and whether or not the species already lives at its upper or lower thermal limits (Maes et al. 2021). Changes in water temperature also affect water chemistry, particularly oxygen content which, as previously discussed, is a major contributing factor that affects the distribution of freshwater molluscs as most are prosobranchs (gill-breathing). Secondary effects of climate change include alteration in water quantity through increased water abstraction for anthropogenic use and greater and prolonged periods of droughts or floods, as this will affect the chemical composition of the water as well as habitat structure and permanence (De Kock and Wolmarans 2005; Quayle et al. 2010). Molluscs require particular water salinity, dissolved solids, calcium ion content and pH for shell production and growth and alteration thereof will have severe impacts on their success and rate of growth (Maes et al. 2021). Most freshwater molluscs are also vulnerable to desiccation as they cannot survive dry conditions for long periods of time, lack active dispersal mechanisms to move to a new location if their habitat dries out, and will be slow to re-colonise a water body once it is inundated with water again (Jeffries et al. 2016; de Necker et al. 2021). Habitat permanence therefore plays an important role in the distribution of molluscs, and changes in that respect as a result of climate change pose a severe threat to the distribution of these aquatic biota.

It has been predicted that the most likely effect of climate change on freshwater mollusc distribution will be a shift in their distribution and geographical range rather than complete extinction as a result of the effect climate change on temperature and precipitation (Lafferty 2009; Stensgaard et al. 2019; Maes et al. 2021). Within the interior of South Africa, mean annual air temperatures will likely increase by 3–3.5 °C while the coastal regions will experience an increase of 1.5–2.5 °C. In summer rainfall areas (central, northern and eastern South Africa), the mean annual rainfall will increase by 40-80 mm per decade. In contrast, in the winter rainfall areas (southwestern South Africa) the mean annual rainfall will decrease by 20–40 mm per decade, with less rainfall in winter and increased rainfall events from January onwards. Areas such as Limpopo, Mpumalanga, and KwaZulu-Natal (summer rainfall regions) will therefore experience hotter and wetter conditions in summer and autumn, while the Western Cape and southern parts of the Eastern Cape (winter rainfall regions) will experience hotter and drier winter conditions (Dallas and Rivers-Moore 2014). The distribution of freshwater molluscs in the country will therefore likely shift with these changes in temperature and rainfall patterns and there is a need to gain an understanding of how this might take place.

1.2 Schistosomiasis

1.2.1 Schistosomiasis in southern Africa

Schistosomiasis, commonly referred to as bilharzia, is a neglected tropical disease (NTD) caused by the trematode parasites of the genus *Schistosoma* (Adekiya et al. 2018; 2020). The disease is found in at least 78 countries worldwide with an estimated 220.8 million people requiring treatment for the disease in 2017 (Ogongo et al. 2022) and is most prevalent in tropical and subtropical areas, particularly in poor and rural communities (Hotez et al. 2014; Adenowo et al. 2015; Stensgaard et al. 2019).

Recent estimates indicate that approximately 652 million people are at risk of infection, with approximately 190 million people already infected in Africa alone; this results in approximately 200 000 deaths annually (Thétiot-Laurent et al. 2013; Verjee 2019; Dawoud and Abdou 2020). Schistosomiasis is ranked as the second-most important parasitic disease after malaria (Alemu et al. 2018; Nelwan 2019) and is the most widespread NTD in sub-Saharan Africa after hookworm infections (Adekiya et al. 2018). At least 20 million people experience negative effects from the disease as a result of chronic infection and it causes the loss of approximately 70 million disability adjusted life years (DALYs) annually, which is higher than the DALYs lost due to malaria or tuberculosis (Hotez and Fenwick 2009; Darwall et al. 2011; Ogongo et al. 2022). People most at risk of infection are those that use natural water bodies for domestic use, such as washing clothes and fetching water, agricultural activities, fishing, or recreational activities such as swimming, with women and children aged five to 15 years being more at risk

of infection than others (King 2010; Adenowo et al. 2015; Sacolo-Gwebu et al. 2019; Deol et al. 2019). The World Health Organization (WHO) has set goals for the elimination of schistosomiasis by 2030 in accordance with the Sustainable Development Goals (SDGs) adopted by the United Nations in 2015 (Nwoko et al. 2021; Ogongo et al. 2022). However, despite this goal and the fact that it is a serious public health problem in sub-Saharan Africa, schistosomiasis continues to persist largely as a result of the continued reliance only on preventative chemotherapy, through praziquantel, and a lack of suitable diagnostic methods (Nwoko et al. 2021). In South Africa, schistosomiasis is also not considered a reportable disease and there are currently no control programmes for this disease in the country (Appleton and Miranda 2015b).

The parasitic cercariae are transmitted to humans through contact with water bodies that contain the intermediate snail hosts contaminated with the parasite (Steinmann et al. 2006; Adenowo et al. 2015; McManus et al. 2020). This parasite has a complex life cycle with an intermediate invertebrate host (vector), in this case snails, and a warm-blooded definitive vertebrate host, such as humans (Figure 1.1; Appleton and Madsen 2012; Adenowo et al. 2015; McManus et al. 2020). The eggs of the parasites end up in water through the excreta (urine or faeces) of infected people and animals. These eggs hatch in the water into ciliated larvae, known as miracidia, that seek out and penetrate the tissue of their intermediate snail host (Appleton and Madsen 2012; Adenowo et al. 2015). The miracidia grow into cercariae, fork-tailed larvae with a long tail, that emerge from the snails once they have matured and seek out their definitive host. The cercariae penetrate the skin of their host, lose their tail and travel through the circulatory system of their host to the liver where the parasites mature into adults. Once mature, a male and female parasite pair and travel to the bladder or bowels of their host (depending on the species of *Schistosoma* sp.) where the adults release eggs that will be excreted, and the cycle will start again (Appleton and Madsen 2012; Adenowo et al. 2015; McManus et al. 2020).

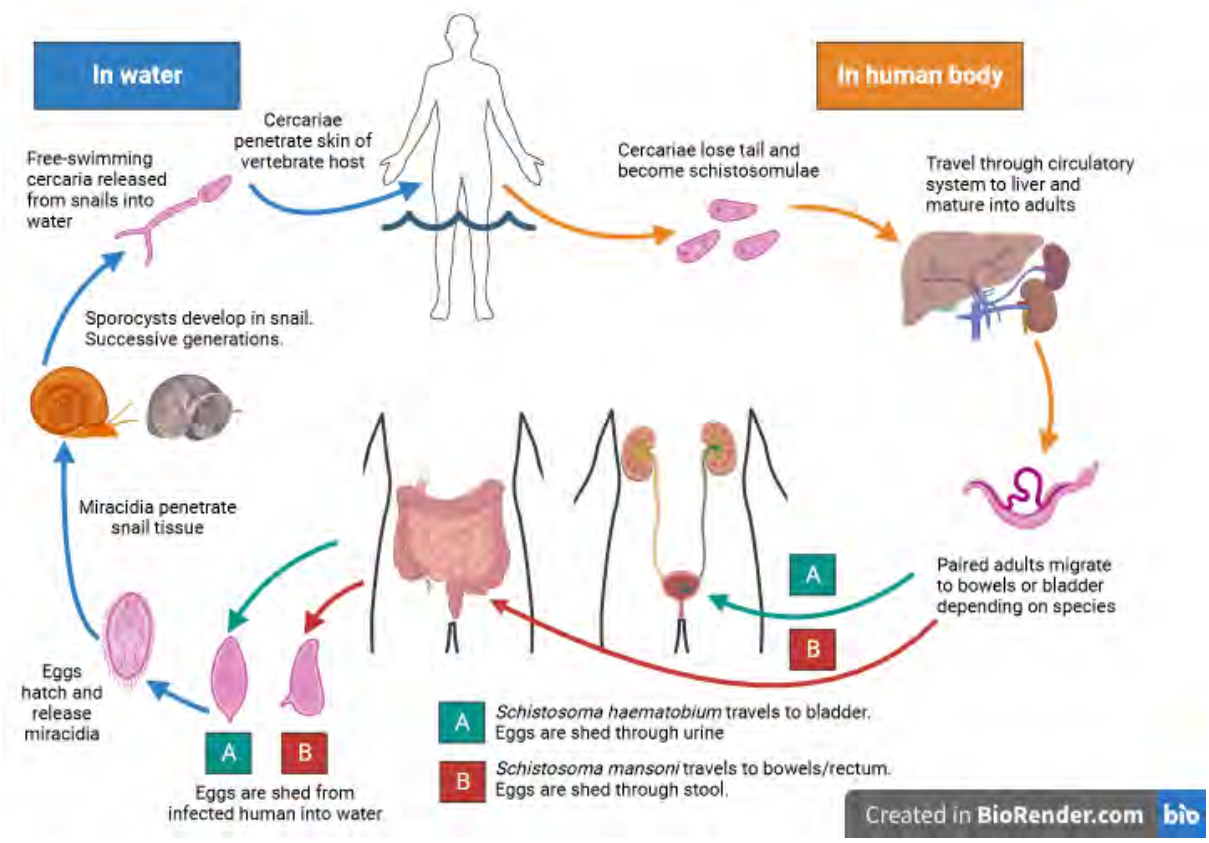

Figure 1.1: Life cycle of *Schistosoma haematobium and Schistosoma mansoni* in the human body. Created with BioRender.com.

Although there is effective treatment for schistosomiasis in the form of praziquantel, re-infection due to continued exposure to infected water occurs frequently in endemic regions (Kalinda et al. 2017a). Continued re-infection or manifestation of chronic schistosomiasis, as well as reported resistance to the praziquantel treatment, poses several long-term health issues. This is particularly true for rural, poverty-stricken areas where access to effective medical care and treatment is limited (King and Dangerfield-Cha 2008). Symptoms of schistosomiasis are predominantly caused by the movement of unshed eggs through the blood systems and tissue. Some of the symptoms that appear when people are initially infected include flu-like symptoms such as a fever, chills, fatigue, and a cough with more severe symptoms such as blood in urine and stool and an enlarged liver occurring over a longer period of time (Gryseels et al. 2006; Deol et al. 2019; Nelwan 2019). Chronic schistosomiasis in animals (from *Schistosoma mattheei*) include hepatic (liver) fibrosis, anaemia, and weight loss. Infection has shown to cause death in up to 90% of livestock with heavy infections, with more severe effects in sheep than cattle as sheep may die from infection up to four years after initial exposure (de Bont and Vercruysse 2008; Darwall et al. 2011). In humans, chronic infection with *S. haematobium* causes chronic granulomas predominantly in the bladder which may lead to bladder cancer in infected persons and, in the case of females,

to female genital schistosomiasis (FGS). Female genital schistosomiasis leads to increased susceptibility to sexually transmitted diseases along with other gynaecological symptoms and, if left untreated, to infertility, miscarriage, ectopic pregnancies and various other birth complications (Dyll-Myklebust and Zwane 2015; Kukula et al. 2019). *Schistosoma mansoni* may cause hepatic fibrosis, liver enlargement, anaemia, chronic bloody diarrhoea, malnutrition and growth stunting (particularly in children), and even death (King and Dangerfield-Cha 2008; Adekiya et al. 2020; Ogongo et al. 2022). Infected humans and animals also have reduced immune responses and are consequently more susceptible to other parasites and diseases as well, including malaria, tuberculosis, and HIV-AIDS (Ogongo et al. 2022). It has also been suggested that infections with helminthic worms reduces the efficacy of vaccines and increases the risk of mother-to-child HIV transmission, which poses severe public health risks (King 2010; Ogongo et al. 2022). Schistosomiasis is therefore a threat to both humans and livestock (De Kock and Wolmarans 2005) and it us thus essential for researchers to gain an understanding of the distribution of the disease and how climate change and human population growth may affect the distribution thereof.

Three aquatic snail species known to carry the trematodes causing schistosomiasis, and two of the five potential *Schistosoma* spp. that generally infect humans, are found in southern Africa. *Biomphalaria pfeifferi* (Kraus 1848) serves as the vector for *Schistosoma mansoni* which causes intestinal and rectal schistosomiasis. *Bulinus africanus* (Kraus 1848) and *Bulinus globosus* (Morelet 1866) are the vectors of *S. haematobium*, causing urinary schistosomiasis in humans, and *S. mattheei*, causing bovine schistosomiasis in cattle and sheep (Day and de Moor 2002; De Kock and Wolmarans 2005; Adekiya et al. 2020). The snails are considered tropical since they are found predominantly in areas with relatively mild to warm air temperatures (15-25 °C) and higher rainfall (300-900 mm/annum). Their distribution has therefore largely been in the northern and eastern parts of South Africa, including Limpopo, Mpumalanga, and the coastal parts of KwaZulu-Natal, with isolated populations found in the upper parts of North-West, Gauteng and the Eastern Cape (De Kock et al. 2004; Figure 1.2). As a result, schistosomiasis is most prevalent in these Provinces (De Boni et al. 2021; Figure 1.3). Temperature and season also play a large role in the transmission and abundance of the parasite as the intra-molluscan developmental stage occurs much faster in the summer, when water temperatures are warmer (Pitchford and Visser 1965; Appleton and Madsen 2012). The parasites also occur more prominently in areas with high rainfall (>800 mm/annum) and areas with high human activity as there is a direct correlation between human activities and the presence of *S. mansoni* and *S. haematobium* (Appleton and Madsen 2012; Adenowo et al. 2015). Since *S. haematobium* is transmitted from humans to water through urine, this parasite occurs most frequently in regions where people,

particularly children, play and swim in the water, which takes place most frequently in summer (Appleton and Madsen 2012). *Schistosoma mansoni* and *S. mattheei* are transmitted to water bodies through the faecal matter of humans and animals, respectively. These two parasites therefore most often occur in regions where there are farmlands (both subsistence and commercial) and rural and informal settlements close to water bodies. In many cases, people in these areas do not have access to adequate sewerage systems and are highly dependent on nearby water bodies for both domestic (drinking, irrigation, washing and fishing) and recreational (playing and swimming) activities (Wolmarans et al. 2006; Appleton and Madsen 2012; Adenowo et al. 2015). These two parasites are at their highest prevalence during high rainfall events as there is increased faecal contamination through surface runoff from farms and informal human settlements during these periods (Appleton and Madsen 2012).

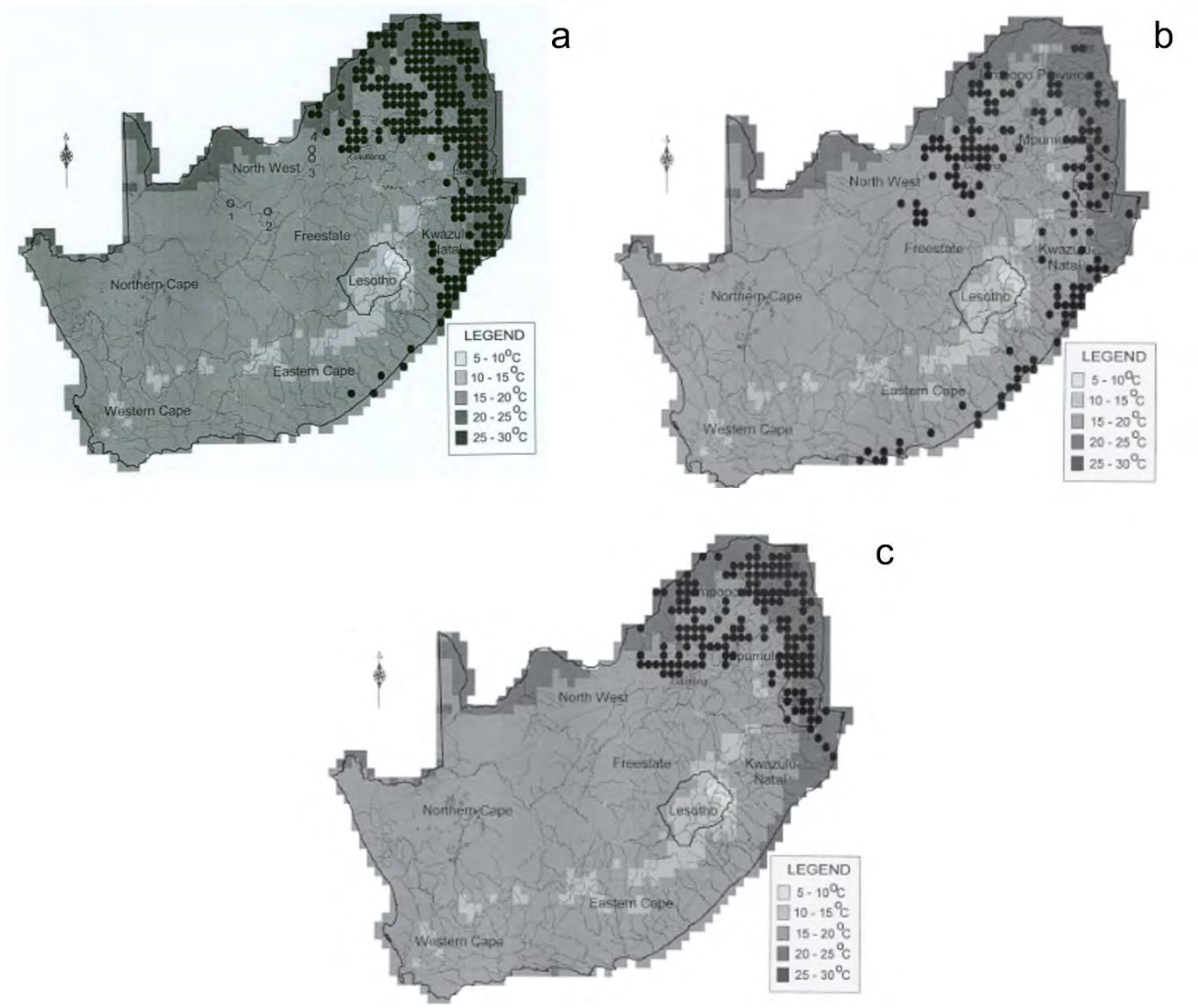

Figure 1.2: The historic geographic distribution of a) *Biomphalaria pfeifferi*, b) *Bulinus africanus*, and c) *Bulinus globosus* across South Africa based on collection records in the database of the National Freshwater Snail Collection (NFSC). Obtained from De Kock et al. (2004) and De Kock and Wolmarans (2005).

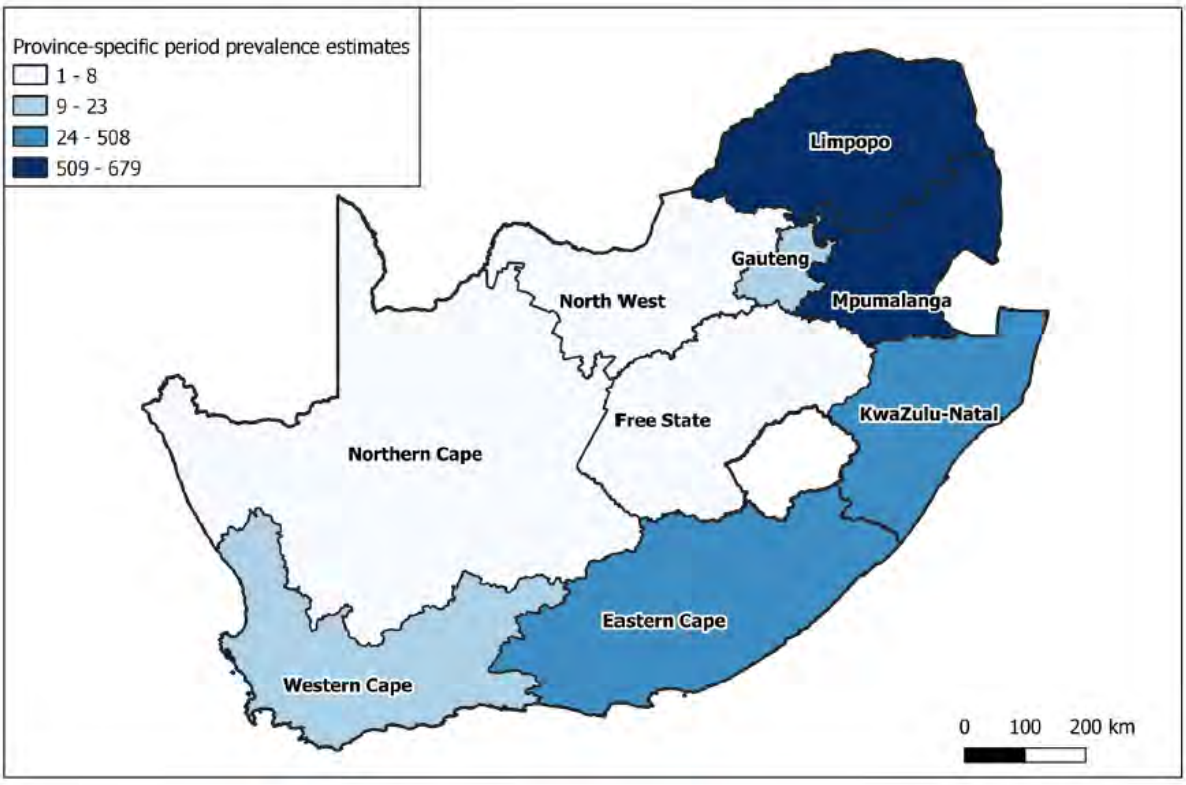

Figure 1.3: Prevalence estimation for *Schistosoma haematobium* across South Africa from 2011 to 2018 based on secondary data from the National Health Laboratory Services records. Obtained from De Boni et al. (2021).

1.2.2 Schistosomiasis and climate change

Climate change not only poses a significant threat to biodiversity and the natural environment, but to human health as well. Sub-Saharan Africa is projected to experience the greatest burden of mortality attributable to the impacts of climate change as the region is much more vulnerable to the impacts of climate change than other continents, and it is already burdened by many diseases that impact human health and wellbeing (Bates et al. 2008; WHO 2009; Adekiya et al. 2020). Climate change impacts human health either directly through increased severe weather and climate events such as flooding, droughts, and extreme temperatures, but also indirectly through environmental changes that affect food security, air quality, and the distribution and the occurrence of diseases. Climate change poses the risk of indirectly altering the distribution of waterborne and vector-borne diseases as a result of changes in environmental conditions (Dallas and Rivers-Moore 2014; Adekiya et al. 2020; Maes et al. 2021). The effect of climate change on vector-borne diseases such as schistosomiasis is complex and may vary greatly between regions, vector species and parasites. Given the future predicted changes in air temperature and rainfall, along with continued human population growth, a potential change in the range and distribution of the intermediate snail hosts and the

parasites causing schistosomiasis is possible, potentially increasing population exposure and/or altering the geographic distribution of this disease (Lafferty 2009; Stensgaard et al. 2019; Adekiya et al. 2020; Maes et al. 2021). Among several other factors, two of the most important effects of climate change that may affect the distribution of, and risk of exposure to, schistosomiasis are temperature and precipitation, as shown in Figures 1.4 and 1.5 (McCreesh and Booth 2013; Adekiya et al. 2020).

It has been suggested that changes in temperature will affect the population dynamics of schistosomiasis by altering the growth, distribution, survival, and fecundity of the snail vectors as well as the parasites themselves (Appleton 1977; Moodley et al. 2003; McCreesh and Booth 2013; Kalinda et al. 2017a; Adekiya et al. 2020). Increases in temperature to approximately 32 to 33 ºC have been found to increase the rate of infection of schistosomiasis as the warmer temperature accelerates the prepatent period of the parasite from up to 35 days to only 15 days (Gordon et al. 1934; Kalinda et al. 2017a; Stensgaard et al. 2019). Warmer temperatures also increase the risk of exposure to people, particularly children, through increased direct contact with water for recreational activities such as swimming (Lévesque et al. 2002; Soldánová et al. 2013). However, extended temperature increases above 34 ºC cause a decline in the development of both the mollusc vectors and parasites (Gordon et al. 1934; Pflüger 1980; Stensgaard et al. 2019). This is likely as a result of the optimum temperature range for the snail vectors being exceeded, resulting in decreased mollusc abundance, reduced development, and growth of the parasites and, ultimately, decreases in the infectious stages of schistosomiasis (McCreesh and Booth 2013; Stensgaard et al. 2019; Adekiya et al. 2020). Initial increases in temperature as a result of climate change may thus potentially increase infectivity in at-risk and exposed populations, whereafter the disease could decrease in those regions. Increased frequency and intensity of heat waves, particularly in naturally colder climates, may also cause more outbreaks of schistosomiasis in regions that were previously low or zero transmission areas (McCreesh and Booth 2013). This has already been demonstrated in some colder climates, where an outbreak of urogenital schistosomiasis occurred in southern Europe (Boissier et al. 2015; Stensgaard et al. 2019). There is thus the potential that the distribution of both snails and parasites may shift into previously non-transmission areas if temperatures increase, or decrease, to within the “optimum range” for the molluscs. It has already been suggested that it is most likely that the geographic distribution of schistosomiasis will shift in response to climate change rather than expand, and has already been predicted in some African countries, including Zimbabwe (Pedersen et al. 2014a; Stensgaard et al. 2019).

Rainfall plays a crucial, indirect role in the distribution of schistosomiasis by affecting the habitat availability and suitability for the snail vectors, as well as altering water volume and flow velocity in lotic systems (McCreesh and Booth 2013; Pedersen et al. 2014a; Manyangadze et al. 2021). Changes in annual average precipitation through climate change will therefore affect the distribution of schistosomiasis and the risk of exposure to humans and animals (McCreesh and Booth 2013; Adekiya et al. 2020; Manyangadze et al. 2021). As with changes in temperature, change in rainfall is predicted to have a complex effect on the distribution of the snail vectors and parasites causing schistosomiasis. In general, higher rainfall is predicted to increase the risk of exposure to schistosomiasis, while reduced rainfall has been associated with a lower prevalence of schistosomiasis (Adekiya et al. 2020; Manyangadze et al. 2021). Higher rates of precipitation increase habitat availability and create temporary and new habitats for the snail vectors to establish in. However, increased rainfall could also increase the flow velocity in an ecosystem, which has negative effects on snail abundance as well as parasite eggs and infectious stages (McCreesh and Booth 2013; Adekiya et al. 2020; Manyangadze et al. 2021). Increased precipitation also causes greater water turbidity, disturbing snail habitats, decreasing the survivability of the parasitic cercariae and the likelihood of locating a host (Adekiya et al. 2020; Manyangadze et al. 2021). The most important effect of reduced precipitation on schistosomiasis is the reduction in water availability and the risk of drought which negatively affects the snail vectors and, consequently, the parasites as well, thus reducing the risk of exposure to humans and animals (McCreesh and Booth 2013; Kabuyaya et al. 2018; Chimbari et al. 2020). Reduced rainfall also leads to reduced water velocity and flow, which may increase the suitability of certain ecosystems, such as rivers and streams, for the snail vector, thus potentially increasing risk of exposure. Changes in flow and flooding regimes may also lead to the establishment of snails in new regions and/or temporary or permanent removal of snails from known endemic regions (McCreesh and Booth 2013; Adekiya et al. 2020).

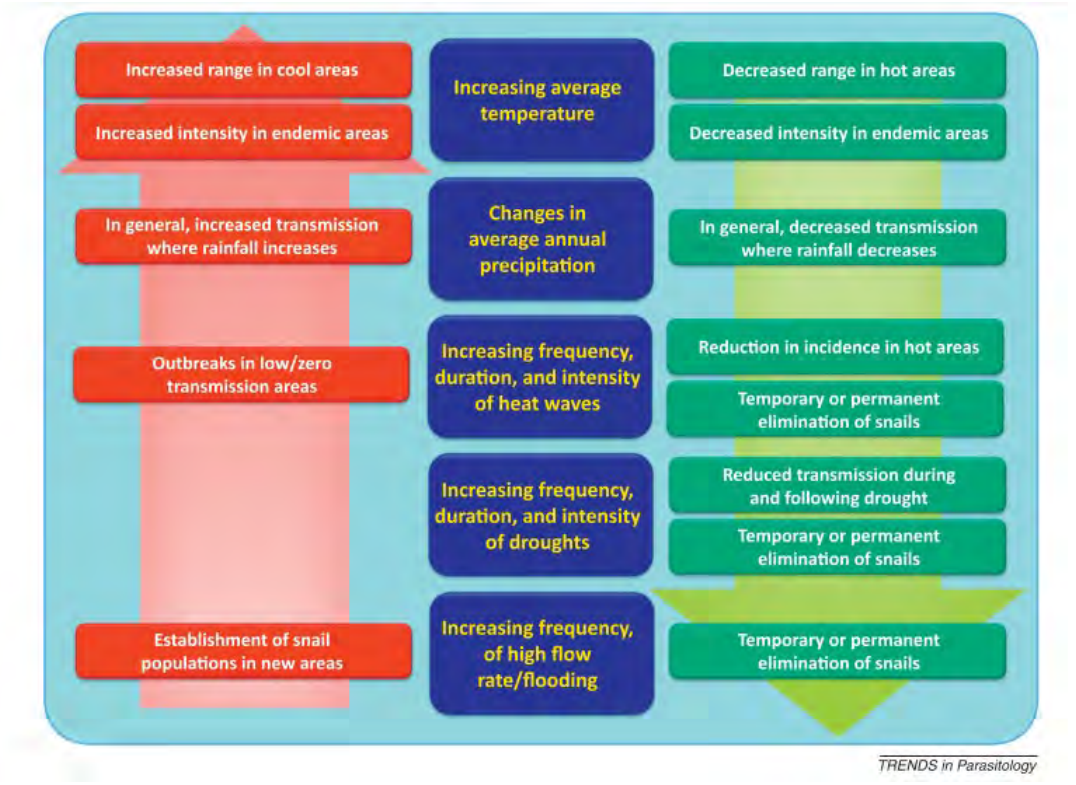

Figure 1.4: Potential effects of climate change on the distribution of schistosomiasis and the snail vectors that transmit it. Obtained from McCreesh and Booth (2013).

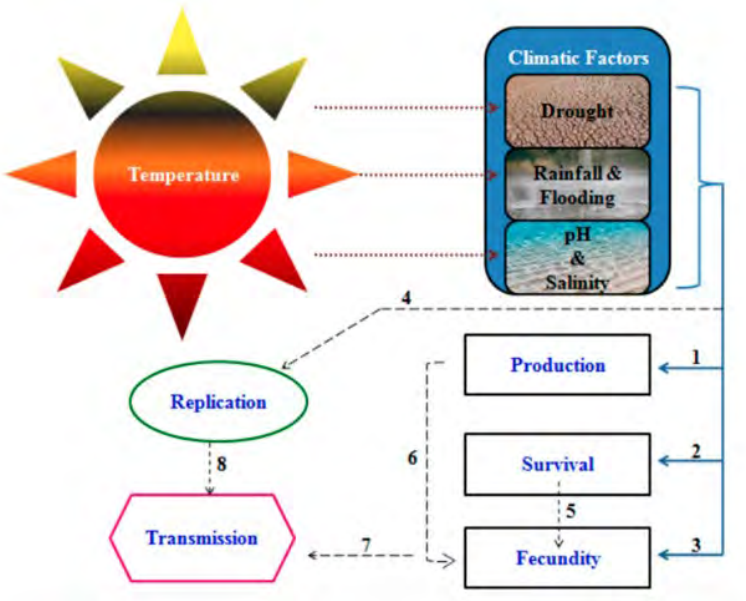

Figure 1.5: Proposed effects of climate change on the distribution of snail vectors transmitting schistosomiasis. Obtained from Adekiya et al. (2020).

1.2.3 Schistosomiasis and human populations

The life cycle of *Schistosoma* sp. relies on the presence of not only the intermediate hosts but the definitive hosts as well (Utzinger et al. 1997; Nelwan 2019). In humans, lack of access to appropriate sanitary facilities and potable water, along with poor sanitary practices are the most important contributing factors to the occurrence of schistosomiasis and the transmission thereof, since the disease life cycle relies on the presence of human urine and faeces in freshwater habitats infested with the snail vectors (Adenowo et al. 2015; Nelwan 2019; McManus et al. 2020; Ogongo et al. 2022). Additionally, human population growth in endemic schistosomiasis regions, and frequent use and reliance on infested freshwater ecosystems for domestic and agricultural use increases the risk of exposure to this disease (Utzinger et al. 1997; Manyangadze et al. 2016a; Ogongo et al. 2022) and may even increase the areas where this disease occurs. For this reason, the proximity and activity of people to infected water bodies, as well as population growth and movement, play crucial roles in the transmission of schistosomiasis and affects the risk of exposure to both human and animal communities. There us thus a need to provide communities in endemic schistosomiasis regions with appropriate sanitation facilities and access to safe drinking water as well as educating them regarding this disease and the risks involved regarding infection, as this will assist in interrupting the transmission of schistosomiasis (Kabuyaya et al. 2018; Maseko et al. 2018; Sokolow et al. 2018).

1.3 Project rationale and aims

1.3.1 Rationale

Climate change may either increase or decrease the distribution of schistosomiasis, and there is thus a crucial need to gain an understanding of whether and how future predicted climate change may influence the transmission of this disease in order to protect public health and inform adaptation and mitigation strategies.

Models that predict the potential effects of climate and schistosomiasis distribution can provide valuable information regarding areas that will become newly suitable for transmission in the future (McCreesh and Booth 2013). However, such models need to be carefully designed with specific parameters determined for the region and intermediate hosts and parasites in question as these can vary greatly. Although this has been attempted in several studies across the world (Stensgaard et al. 2013) and in some African countries including Ethiopia (Kristensen et al. 2001), Uganda (Stensgaard et al. 2006), and Zimbabwe (Pedersen et al. 2014a) no such model has been adapted or tested in a South African context. In addition, the last comprehensive snail distribution studies in South Africa took place in the 1950s to 1960s as part of the collections for the National Freshwater Snail Collection (NFSC) of South Africa.

More recently, several localised studies have assessed the prevalence and distribution of *S. haematobium* in humans in small rural communities in the Eastern Cape (Meents and Boyles 2010), North-West (Wolmarans et al. 2006), KwaZulu-Natal (Pillay et al. 2014; Manyangadze et al. 2016a; Kabuyaya et al. 2018) and Limpopo (Samie et al. 2010) Provinces. Although research provide valuable insight into the prevalence in humans in South Africa, not provide a clear indication of the distribution and abundance of the intermediate host snails and what the potential future distribution of these snails and their parasites may be.

Given that temperatures and rainfall have already changed in the past 60 years, the distribution of snails may have already changed, particularly in regions that were on the distribution fringes of the snail vectors transmitting schistosomiasis. Through predicted climate change, the distribution of this disease is likely to change, and it is of utmost importance that research determines how this may occur. Since schistosomiasis mainly affects poor, rural agricultural communities, it is also very important to gain an understanding of how affected communities experience climatic and environmental change and how these changes impact their health, well-being and livelihoods. Only a few studies have investigated adaptive capacity to either the individual or cumulative health risks associated with climate variability and change in southern Africa, and thus our understanding of vulnerabilities is incomplete (Boamah et al. 2017). An understanding of not only the physical environment, but also of the broader social environment in which people live and work is necessary in order to fully comprehend vulnerability to schistosomiasis (Kukula et al. 2019). Furthermore, a better understanding of the perceived health risk could inform public health policies and actions, and influence mitigation and adaptive responses, as perceptions drive health related behaviours. Designing effective response programmes requires an understanding not only of changes in the distribution of schistosomiasis intermediate host snails, but also of existing knowledge, attitudes, perceptions, and practices surrounding schistosomiasis in endemic communities (Sacolo et al. 2018).

1.3.2 Aims

The central aim of this research project was to determine whether the distribution ranges of the snail vectors and associated parasites of schistosomiasis have expanded in recent years, how affected human communities perceive and experience these changes, and whether changes in distribution may further expand and increase the potential prevalence of the disease in humans and animals in South Africa given future predicted climate change.

To achieve this, the study was divided into four specific aims targeting a particular element of the overarching aim. These specific aims were as follows:

Aim 1

Conduct field collection surveys of freshwater snail species, particularly those known to be schistosomiasis vectors, and their parasites in historic and potentially new suitable distribution sites.

Aim 2

Determine whether schistosomiasis infection areas have changed in the past half-century in South Africa with the use of historic water quality information and historic climatic modelling.

Aim 3

Determine potentially new suitable distribution ranges of schistosomiasis vectors in relation to future predicted climate change.

Aim 4

Assess community knowledge, attitudes, and perceptions of the past and present incidence of schistosomiasis in communities located in known schistosomiasis infection areas.

1.4 Layout of the report

Chapter 1: This chapter provides the background, rationale, and aims of the study.

Chapter 2: A description is given of the study area and the methodology followed throughout the study.

Chapter 3: Morphological and molecular identification of freshwater snail species in South Africa is carried out to address Aim 1 of the study.

Chapter 4: Parasites of freshwater snail species in South Africa are assessed to address Aim 1 of the study.

Chapter 5: The role of environmental and biological factors contributing to the distribution of native freshwater snails in South Africa is investigated to address Aim 1 of the study.

Chapter 6: Climate modelling is used to assess the historic distribution of snails transmitting schistosomiasis in South Africa and to map areas of vulnerability to schistosomiasis to address Aim 2 of the study.

Chapter 7: Future predicted climate change, historical distribution maps and current distribution of freshwater snails in South Africa are used to model and map the potential future distribution of schistosomiasis in South Africa to address Aim 3 of the study.

Chapter 8: The knowledge, attitudes, perceptions and practices of communities relating to schistosomiasis and environmental change in the Limpopo Province are assessed to address Aim 4 of the study.

Chapter 9: A summary is given of the conclusions and recommendations emanating from this study.

CHAPTER 2: STUDY AREA, SITE SELECTION AND GENERAL METHODOLOGY

Compiled by: *L. de Necker, L.F. Lindeque, J.J. Pearson, and M.H. Le Roux*

2.1 Introduction

Both human and animal schistosomiasis occurs in South Africa, most notably in the northern and eastern Provinces including North West, Limpopo and Mpumalanga (De Kock et al. 2004). As a result, sampling sites to achieve Aims 1 to 3 were based in these Provinces. Site selection to conduct field collection surveys of the schistosomiasis vectors and parasites in each of the Provinces (Aim 1) was based on the available historical distribution data of schistosomiasis vectors obtained from the NFSC database. Additional sites were selected in the Free State Province to assess whether any schistosomiasis vectors were located in the region and were hosts for any parasitic infections, including schistosomiasis. Due to the vast number of sampling sites in the NFSC database, the number of sites were then further reduced in each Province based on assessments at potential sampling locations including accessibility, land-use activities, and vegetation presence. Sites located in close proximity to human settlements and agricultural activities with aquatic vegetation present were selected for sampling as these factors increased the likelihood of collecting freshwater molluscs that were infected with schistosomiasis. In addition, the Limpopo and Phongolo River system (in northern KwaZulu-Natal) were targeted for sampling of invasive mollusc species, specifically *Tarebia granifera* as it is the most abundant invasive snail species in these Provinces and outcompetes native snails, including those that transmit schistosomiasis. Most sites overlapped between those targeted for native snail species and those targeting invasive snail species, although several were additionally sampled for the invasive mollusc species as this formed part of a separate research project. To achieve Aim 4, a single community in Limpopo Province was selected for the community KAPP surveys. This community was selected based on proximity to historic distribution sites, type of water-use activities in the region, and access to sanitation facilities and potable water sources. Limited access to such facilities, greater dependence on nearby water bodies, and water-use activities such as swimming and fishing put people at greater risk of contracting schistosomiasis (Utzinger et al. 1997; Manyangadze et al. 2016a; Ogongo et al. 2022).

2.2 Sampling sites

2.2.1 North West Province

The North West Province is situated on the flat interior plateau of South Africa, bordering Botswana to the north and stretching to the Vaal River in the south. This Province falls within the austral summer rainfall season (November to March) with an annual precipitation of 300-500 mm.

The temperature ranges from an average of 17 °C with maximum temperatures reaching approximately 30 °C during the summer season and an average of 3 °C to a maximum of 21 °C during the winter season (Winde and Erasmus 2011). The area falls within the Southern Temperate Highveld ecoregion with predominantly savanna and thornveld biomes and temporary pans (Galvin et al. 2008). Two economically important industries within the North West Province are agriculture and mining, with the latter being one of the largest employers within the region (Yager et al. 2012). The sites sampled during the current study were located close to Rustenburg, one of the largest cities within the Province, with the mining industry dominating the local landscape. The water used within the mining industry is predominantly discharged into the surface waters of surrounding natural rivers and dams, potentially harming the aquatic ecosystem within the discharge area (Erasmus et al. 2020). Sites LLF1-3 were sampled within the northern parts of the North West Province at the Hex River, Roodekopjes Dam and the Crocodile River (Figure 2.1; Figure 2.2a-c).

2.2.2 Limpopo Province

The Limpopo Province is the northernmost Province of South Africa, bordering Zimbabwe, Botswana, and Mozambique. The Limpopo River is one of the largest rivers (~1 750 km) in southern Africa (Mosase and Ahiablame 2018). The river rises as the Crocodile River in the Witwatersrand and is joined by the Marico River forming the Limpopo River at the border between South Africa and Botswana (SARDC 2003). The Limpopo River basin drains an area of ~415 000 km^2 in South Africa, Botswana, Zimbabwe, and Mozambique (Mosase and Ahiablame 2018). At the confluence of the Shashe River (the border between Botswana and Zimbabwe), the Limpopo River continues east to form the border between South Africa and Zimbabwe, before entering Mozambique at Pafuri (SARDC 2003). The Limpopo River system has 27 important watersheds, 12 of which are from South Africa (Mosase and Ahiablame 2018). Tributary rivers of the Limpopo River in South Africa sampled during this study included the Crocodile, Mogalakwena, Olifants, Luvuvhu, Shingwedzi, and Letaba rivers.

The average air temperature over the river system ranges between 0 and 36 °C from winter to summer (Mosase and Ahiablame 2018). The Limpopo River system consists of arid climate conditions with an average yearly rainfall of 200 mm in the west to 1 500 mm in the east; rainfall occurs between October and April during austral summer (Mosase and Ahiablame 2018). Rainfall in the river system differs between years, causing droughts during drier years and flood events during wetter years (Mosase and Ahiablame 2018). Increased agricultural activities, industrial development, and population growth place increased pressure on the already stressed water resources of the Limpopo River system (Van der Zaag et al. 2010; Bawden et al. 2014). The population is estimated to be nearing 23 million people in primarily rural settlements within the Limpopo Province, which relies heavily on the basin's natural water resources (Mosase and

Ahiablame 2018). There is thus already a great deal of stress on this aquatic ecosystem (Ubisi et al. 2017). Research further indicates that climate change will likely lead to rises in temperature and changes in rainfall patterns across the world and this could have a detrimental effect on aquatic ecosystems in South Africa, increasing the frequency of flooding and drought events (Mwenge Kahinda et al. 2016; Mosase and Ahiablame 2018).

Field collection took place during the high-flow period (April/May 2021) and the low-flow period (August 2021). The sites sampled for native mollusc species during the high-flow period were all located in the north to north-eastern parts of the Limpopo Province, labelled LHF1 to LLF9 (Figure 2.1). All the sites were located within the Limpopo River catchment with little to no marginal and emergent vegetation and a primarily sandy substrate with exposed bedrock (Figures 2.3-2.4). In some cases, no floating vegetation was present during this survey. The sites sampled during the low-flow sampling period, labelled LLF1 to LLF15, were located more inland than those sampled during the high-flow sampling period within the Limpopo River catchment and had a greater diversity of habitat types, with varying substrates and vegetation present.

A single village in the area was also targeted for the community surveys. Community surveys were conducted in HaNesengani, a rural village in the Makhado Local Municipality, Limpopo Province. It is located at 23° 5' 45.95" S and 30° 23' 43.69" E, covering 11.02 km^2 with 3 603 households and a population of 13 951 (Statistics South Africa 2011). The area has access to basic amenities such as schools and primary health care services, but many residents still collect wood and water from natural sources for domestic and other purposes (Bornman et al. 2012). According to Statistics South Africa (2011), only 19% of the community in HaNesengani had access to piped water near their homes and only 3.2% had toilet facilities connected to a sewerage system. Formal employment opportunities are also scarce, resulting in large numbers of subsistence farmers in the area (Bornman et al. 2012).

The study area was selected based on the high prevalence of schistosomiasis reported in the region (see Anyanwu et al. 2020; Samie et al. 2010). Samie et al. (2010) found that *Schistosoma haematobium* infections were widespread in the region, with a high risk of infection, especially among young adults, primary school children and females. Water bodies in the area, particularly the Luvuvhu River that flows through HaNesengani, contain intermediate host snails that carry schistosomiasis (Dickens et al. 2020). Figure 2.5 shows the location of the village and sites where intermediate host snails of the *Schistosoma* genus: *Bulinus globosus*, *Bulinus africanus* and *Biomphalaria pfeifferi* (in green, red, and yellow, respectively) were found in the water bodies of the area and in the Luvuvhu River (Figure 2.5).

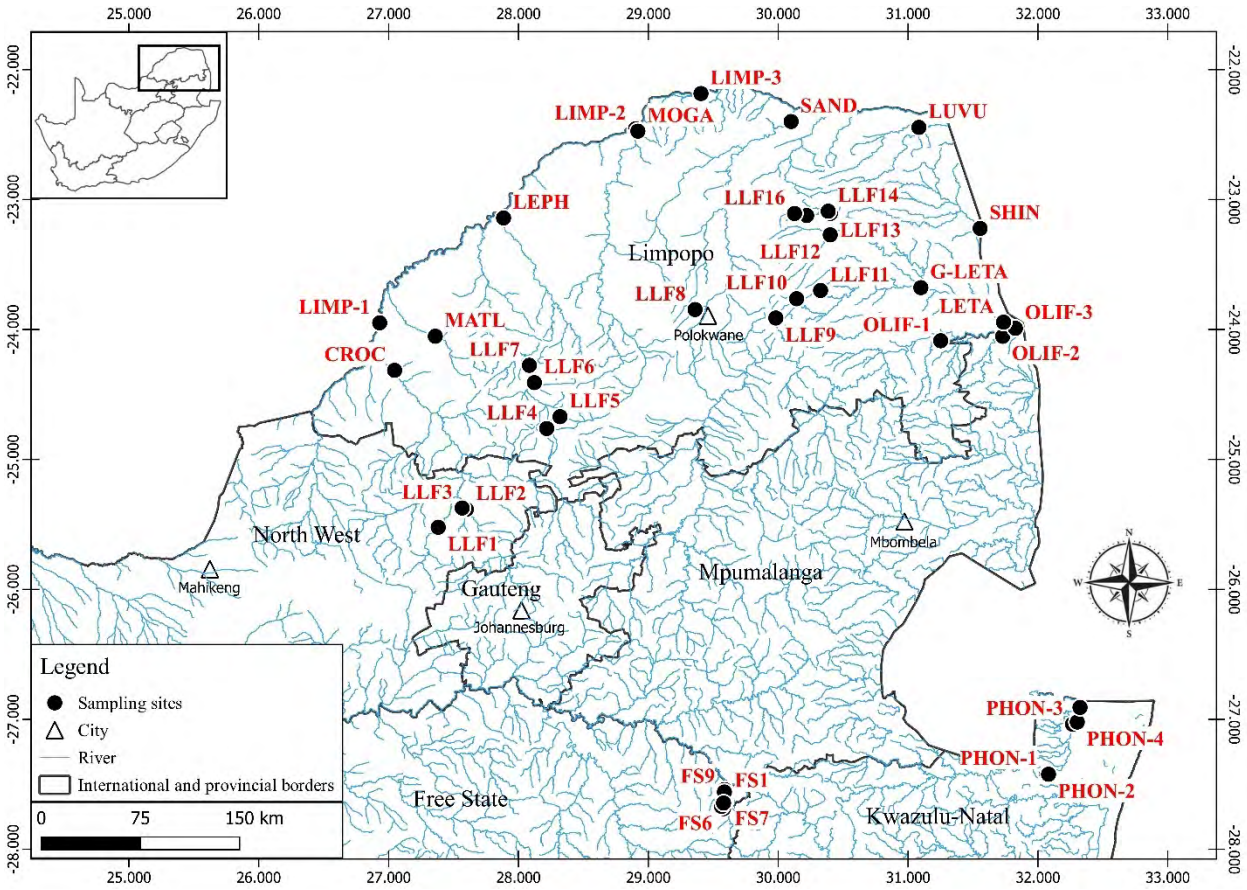

Figure 2.1: Map indicating locations sampled during the high-flow (HF) and low-flow (LF) seasons in 2021 in the North West and Limpopo Provinces, South Africa as well as all sites sampled in the Phongolo River (PHON-1 to 4) and Free State Province (FS1 to 9) (L – Limpopo; HF – high flow; LF – low flow).

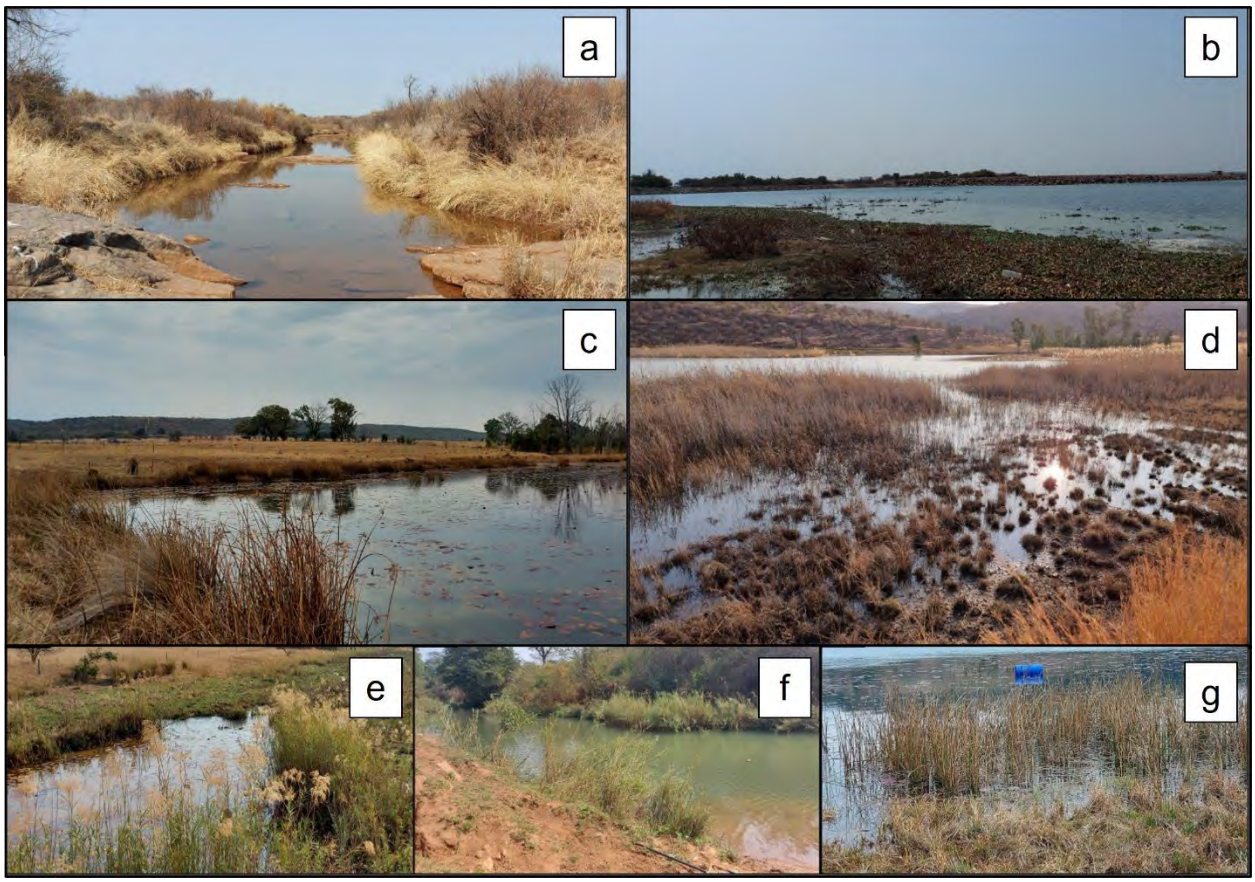

Figure 2.2: Photographs of the sites sampled during the low-flow sampling period in the North West and Limpopo Provinces. (a) Site LLF1, isolated stream near the Hex River; (b) Site LLF2, farm dam near the Crocodile River; (c) Site LLF3, located on the Crocodile River; (d) Site LLF4, located on the Buffelspruit; (e) Site LLF5, located at the Donkerpoort Dam; (f) Site LLF6, located on the Klein Sand River; and (g) Site LLF7, located on the Mokolo River.

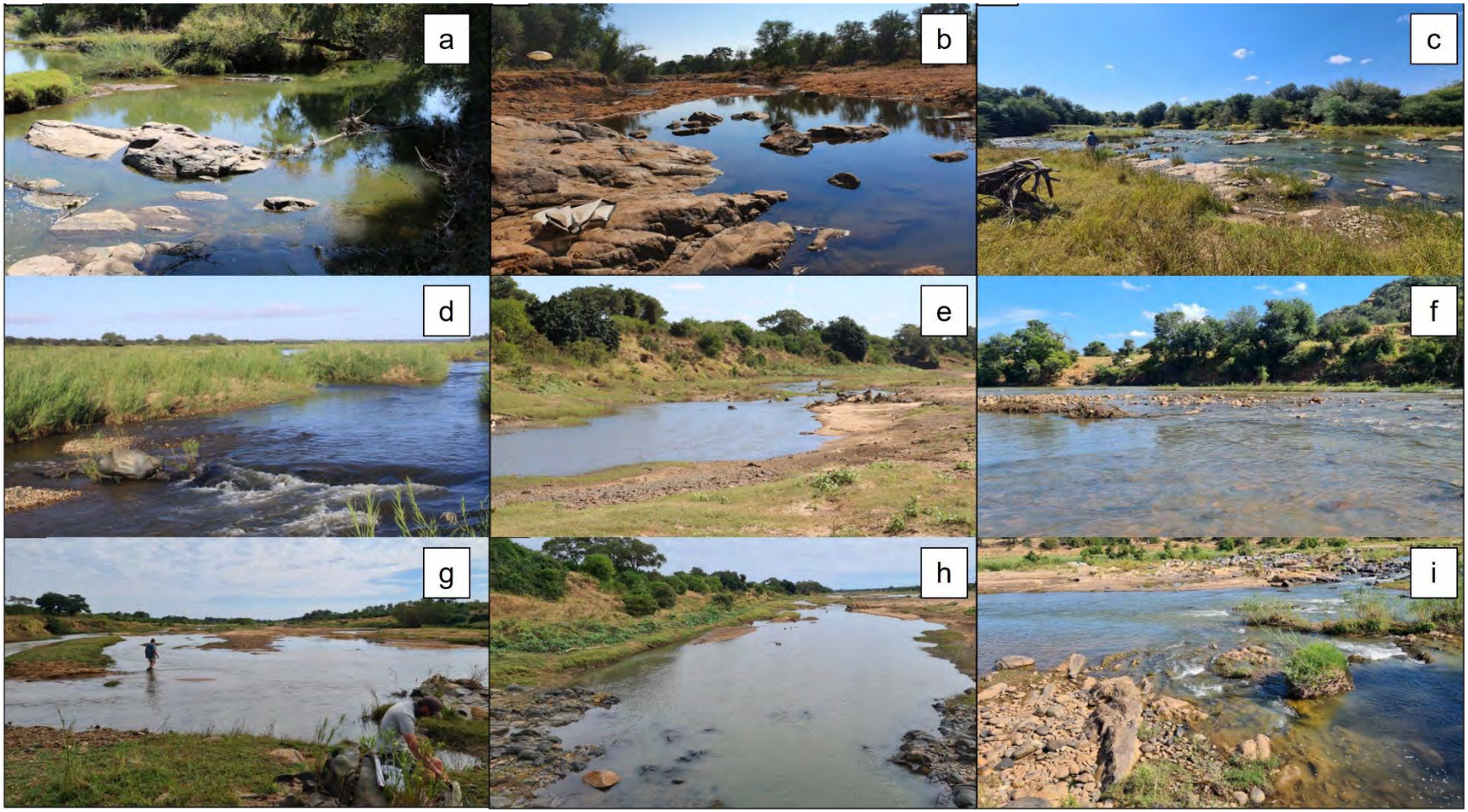

Figure 2.3: Photographs of the sites sampled during the high-flow sampling period in the Limpopo Province. The first three sites, namely (a) Site LHF1, (b) Site LHF2, and (c) Site LHF3 were located on the Limpopo River; (d) Site LHF4 is located on the Olifants River; (e) Site LHF5 is located on the Shingwedzi River; (f) Site LHF6 is located on the Luvuvhu River; (g) Site LHF7 is located on the Groot Letaba River; (h) Site LHF8 is located on the Letaba River; and (i) Site LHF9 is located on the Olifants River.

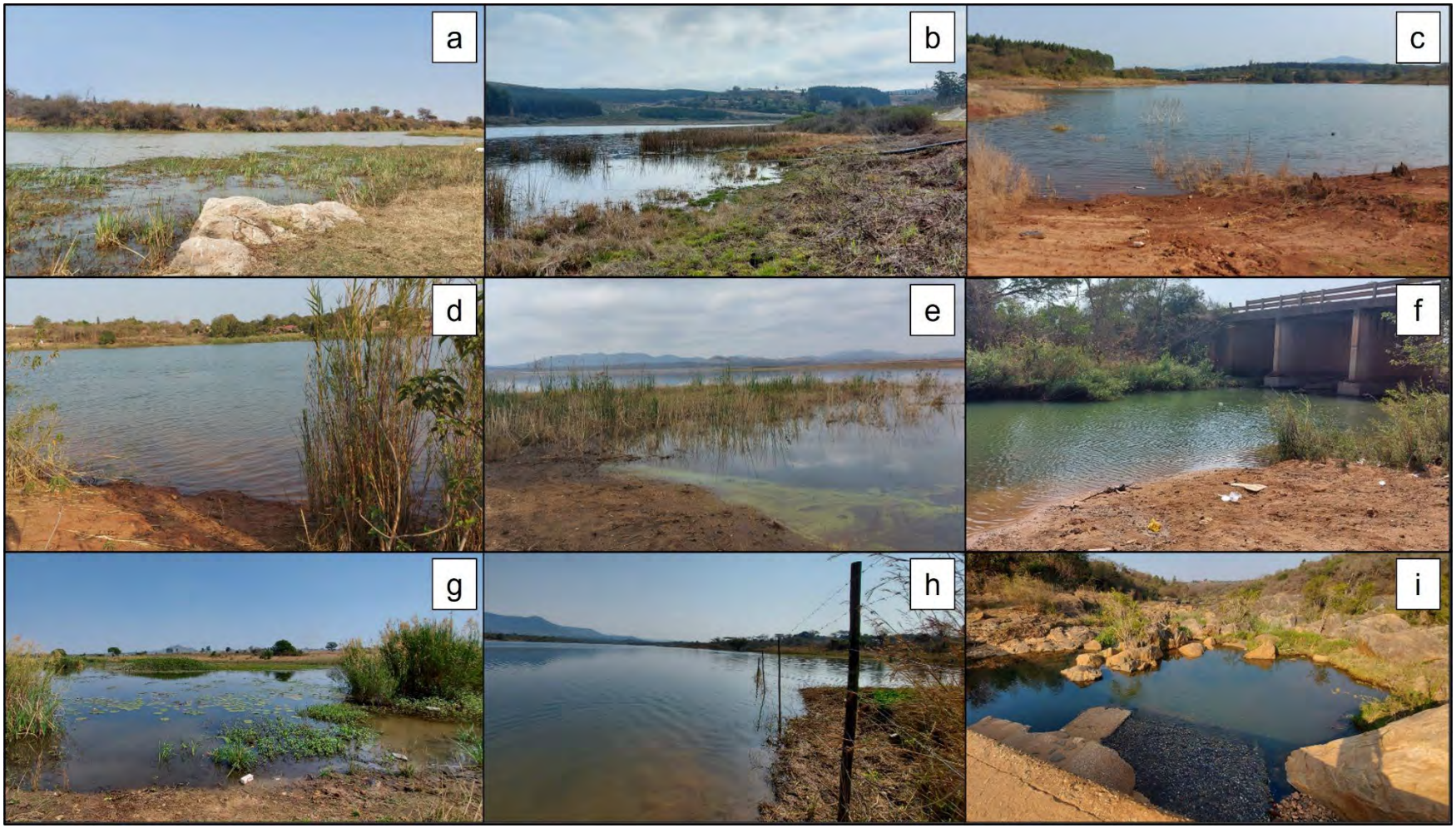

Figure 2.4: Photographs of the sites sampled during the low-flow sampling period. (a) Site LLF8 is located on the Bloed River; (b) Site LLF9 located at the Stanford Lake; (c) Site LLF10 is located at the Tzaneen Dam; (d) Site LLF11 located at the Mothomeng Dam; (e) Site LLF12 is located at the Middle-Letaba River; (f) Site LLF13 located on Luvuvhu River; (g) Site LLF14 located on the Magic Dam near Tshivhazwaulu town; (h) Site LLF15 located on an unnamed dam near Valdezia town; and (i) Site LLF16 located below Albasini Dam on the Luvuvhu River.

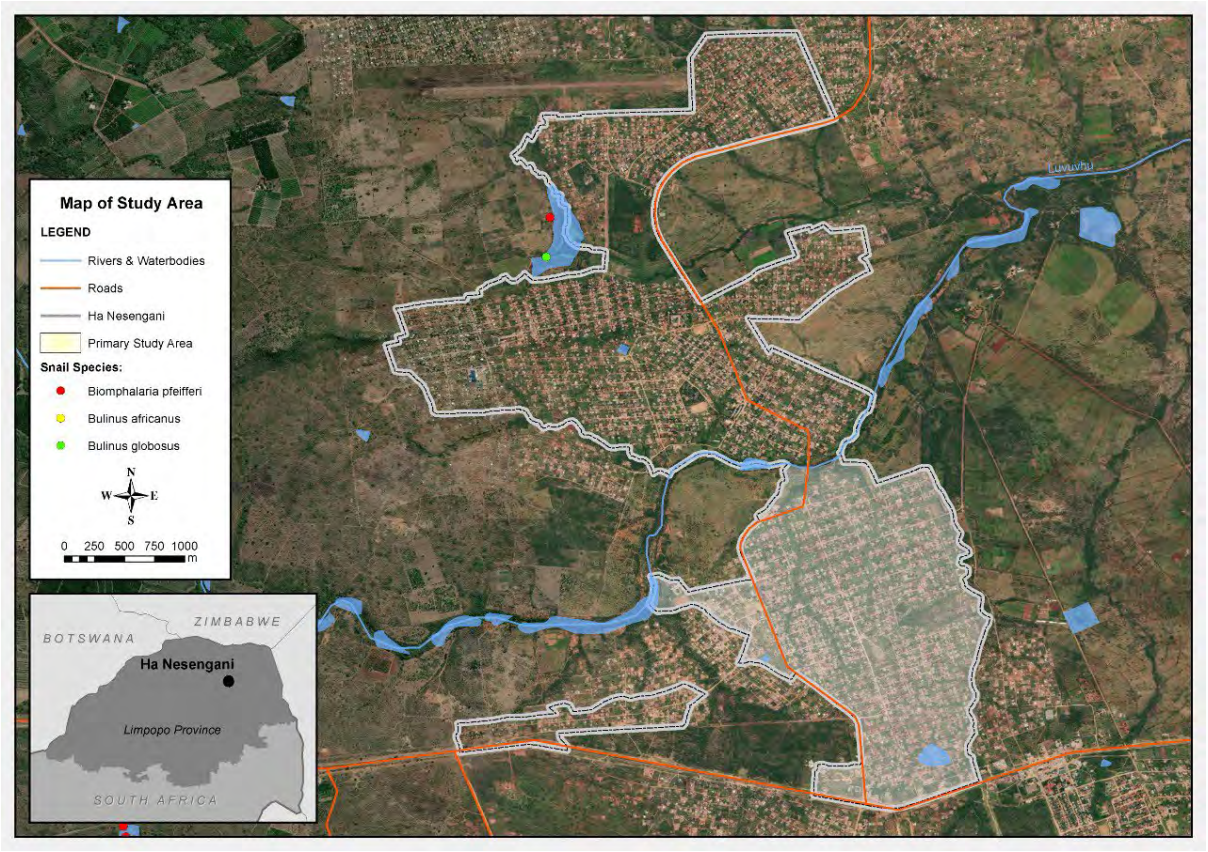

Figure 2.5: Map of HaNesengani showing villages, geographic setting and previously detected infectious snail species in nearby water bodies.

Additional sites were sampled on the Limpopo River and its tributaries specifically targeting invasive snail species. The first site is located on the Crocodile River (CROC, Figure 2.6 (a-b), the largest tributary of the Limpopo River, next to the Rooibokkraal Road. This site is located just above the confluence with the Marico River (the start of the Limpopo River). High water flow conditions were noted, marginal vegetation was limited to grass and reeds and the substrate consisted of gravel beds, sand, and mud. Flow conditions were also high at the LIMP-1 site on the Limpopo River (Figure 2.6 c-d). This site is located 30 km downstream of the CROC site. Marginal vegetation was diverse with herbaceous plants, shrubs, grasses, and sedges observed. Deep and shallow riffles, rapids, runs, large pools, backwater pools and eddies made up the hydraulic biotopes, and substrates included bedrock, boulders, gravel, sand, mud, and silt. The MATL site on the Matlabas River had marginal vegetation dominated by reeds, with only a few types of grasses and sedges present and the substrate was dominated by sand, gravel, and mud. The Lephalala River, the fourth site, LEPH, is situated downstream of a weir approximately 15 km upstream of the confluence with the Limpopo River (Figure 2.6 e-f). Dominated by reeds, the marginal vegetation was also made up of very few types of grasses and sedges. The hydraulic biotopes consisted of shallow and intermediate riffles and rapids and shallow pools. Downstream of the weir, substrates were made up of bedrock, boulders, and cobbles with sand-mud and sometimes gravel dominating further downstream. The second site on the main stem of the

Limpopo River, LIMP-2, is located inside the Limpokwena Nature Reserve, ~300 km downstream of the LIMP-1 site (Figure 2.7 a-b). Due to bank scouring and high sand-mud deposition, marginal vegetation was limited, while the opposite riverbank had reeds, grasses and sedges, and substrates included bedrock, boulders, cobbles, gravel, and sand-mud. Located on the Mogalakwena River, the MOGA site is the final site inside the Limpokwena Nature Reserve, two km upstream of the confluence with the Limpopo River (Figure 2.7 c-d). The site was devoid of marginal vegetation due to recent bank scouring during high-flow events. Due to a lack of flow, hydraulic biotopes were limited to isolated pools and substrates were limited to bedrock, cobbles, gravel, and sand. Inside the Mapungubwe National Park, the final site on the Limpopo River (LIMP-3) is located about 110 km downstream of the LIMP-2 site (Figure 2.7 e-f). There was a low variety of depth-velocity classes and limited substrates. The site on the Sand River (SAND) falls within the Musina Nature Reserve, next to a high-water bridge on the R508 road (Figure 2.7 g-h). The site is located ~19 km from the confluence with the Limpopo River, creating the border between South Africa and Zimbabwe. Little marginal vegetation was present, mostly limited to reeds. Cobbles, large boulders, and sand-mud-silt were located in the slower flowing areas, with fine gravel and coarse sand dominating most of the substrates. The LUVU site is on the Luvuvhu River, ~1 km from the Outpost Lodge inside the Kruger National Park (Figure 2.7 i-j). Grasses dominated marginal vegetation and cobbles and large boulders primarily represented the substrate.

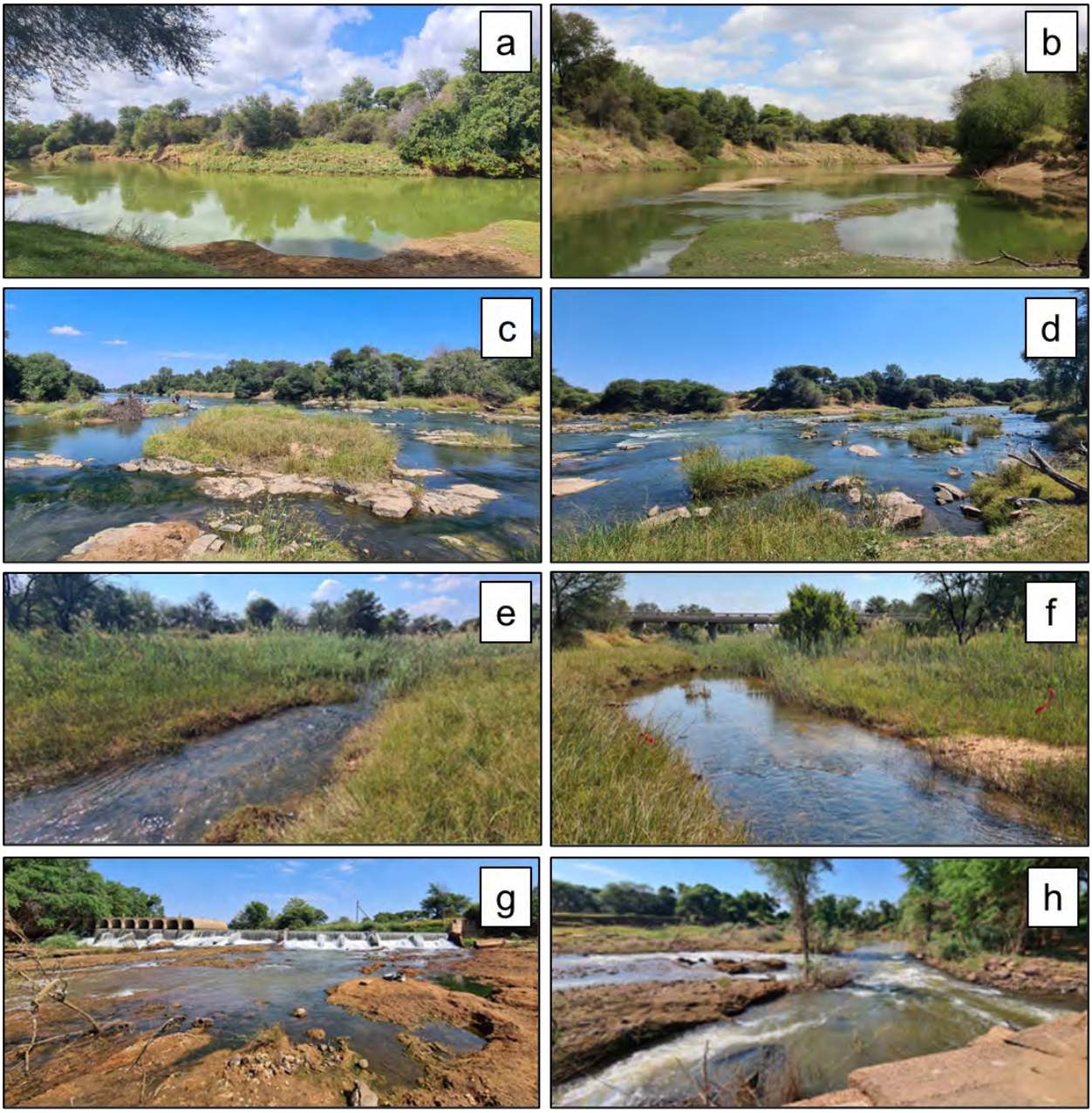

Figure 2.6: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the CROC site sampled on the Crocodile River; (c-d) the LIMP-1 site on the Limpopo River; (e-f) the MATL site on the Matlabas River; and (g-h) the LEPH site sampled on the Lephalala River. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

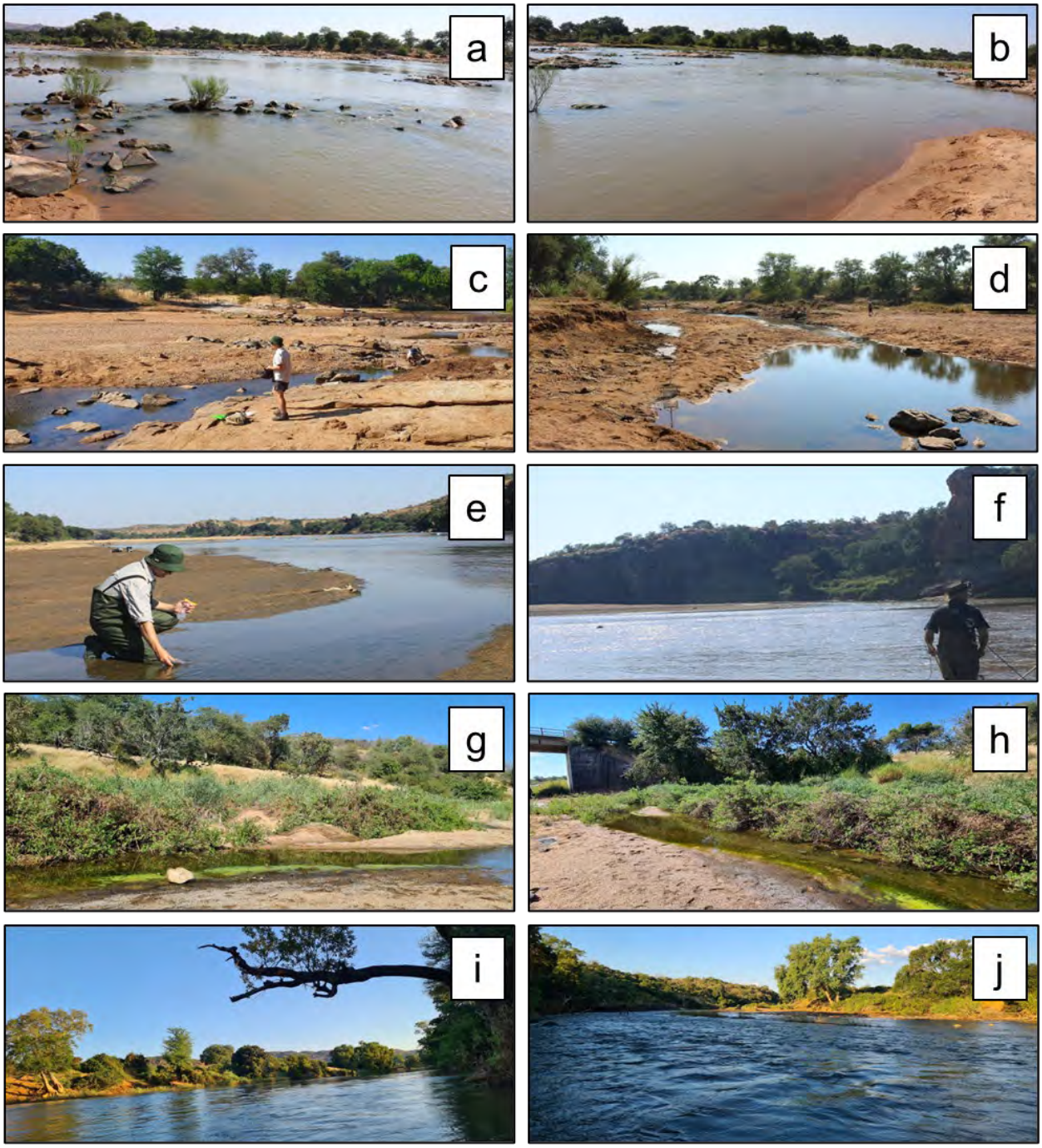

Figure 2.7: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the LIMP-2 site on the Limpopo River inside the Limpokwena Nature Reserve; (c-d) the MOGA site sampled on the Mogalakwena River inside the Limpokwena Nature Reserve; (e-f) the LIMP-3 site on the Limpopo River inside the Mapungubwe National Park; (g-h) the SAND site sampled on the Sand River in the Musina Nature Reserve; and (i-j) the LUVU site on the Luvuvhu River inside the Kruger National Park. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

The Olifants River rises near Trichardt (Mpumalanga Province, South Africa), flowing north-east through the Kruger National Park across the Lowveld, and is joined by the Letaba River inside the park (Nkhonjera 2017). It continues through the Lebombo Mountains in the Olifants Gorge, becoming the Rio dos Elefantes River in Mozambique, flowing into the Massingir Dam and finally joining the Limpopo River further downstream (SARDC 2003; Nhassengo et al. 2021). The Olifants River is one of the most polluted and threatened river systems in South Africa due to the deteriorating water quality caused by mining, industrial, and agricultural activities (de Villiers and Mkwelo 2009; Van Vuuren 2009; Kemp et al. 2016; Gerber et al. 2015; Addo-Bediako 2020). The first site on the Olifants River (OLIF-1) is found within the Kruger National Park (KNP), located 2.5 km downstream of the Mamba gauging weir and 500 m downstream of the Olifants confluence with the Klaserie River (Figure 2.8 a-b). Water flow was low during low-flow surveys (September 2020), with algae being very dominant and marginal vegetation consisting of reeds and grasses. Sand and gravel were the dominant substrates, but bedrock, boulders and cobbles were also present. During the second survey in May 2021, the OLIF-1 site had a very high flow (Figure 2.9 a-b). Marginal vegetation was dominated by grasses with some reeds present as well. Bedrock, cobbles, gravel, and sand were the dominant substrates. The second site on the Olifants River, OLIF-2, is located at the Balule low-water bridge on the secondary S90 road between the Olifants and Balule rest camps inside KNP (Figure 2.8 c-d). The site is 70.6 km downstream from the first site and 5.7 km from the Olifants Rest Camp. The Balule low-water bridge forms an impoundment during flooding events, causing deposition of sand and gravel upstream of the bridge. Loose gravel and cobbles on a deep bed of sand were the dominant substrates, with bedrock, mud, and silt present. Upon the second survey of the OLIF-2 site in May 2021 (Figure 2.9 c-d), reeds and grasses dominated the marginal vegetation. Substrate included bedrock, cobbles, gravel, and sand. The last site on the Olifants River (OLIF-3), is approximately 200 m upstream of the Olifants-Letaba confluence, 18 km downstream of the OLIF-2 site, and ~9 km from the Olifants Rest Camp inside KNP (Figure 2.8 e-f). The Olifants-Letaba confluence joins at an angle that results in an upstream inundation of the Olifants River, leading to sand, gravel, and cobble deposition. During the first field survey at the site in October 2020, most hydraulic biotopes were fast-flowing, with several freshly inundated areas due to rainfall events. Large boulders, cobbles, gravel, and sand were the dominant substrates on a deep bed of sand with embedded cobbles and gravel. Mud and silt were limited to the inputs of intermittent streams. The site was not revisited during the second survey in 2021 due to safety reasons (presence of Nile crocodiles and hippopotami). The Great Letaba River site (G-LETA) is located inside the Great Letaba Wildlife Reserve, ~7 km from the confluence with the Letaba River (Figure 2.9 e-f). Marginal vegetation was made up of grasses and reeds. Cobbles, bedrock, and large

boulders were limited, while sand and gravel were the dominant substrates. The second site on the Letaba River (LETA) is located approximately 10 km upstream of the Olifants-Letaba confluence, inside KNP (Figure 2.9 g-h). Grasses dominated marginal vegetation. Bedrock, large boulders, and sand-mud were well represented in the substrate, while sand and gravel were dominant. Situated on the Shingwedzi River, the SHIN site is ~1 km from the Mozambique border in KNP (Figure 2.9 i-j). The site is 140 km from the Shingwedzi-Elefantes River confluence and 240 km from the Elefantes-Limpopo confluence in Mozambique. Marginal vegetation was limited to reeds and few types of grasses. The surface water was mostly restricted to isolated pools, deficient in hydraulic biotopes and depth-velocity classes.

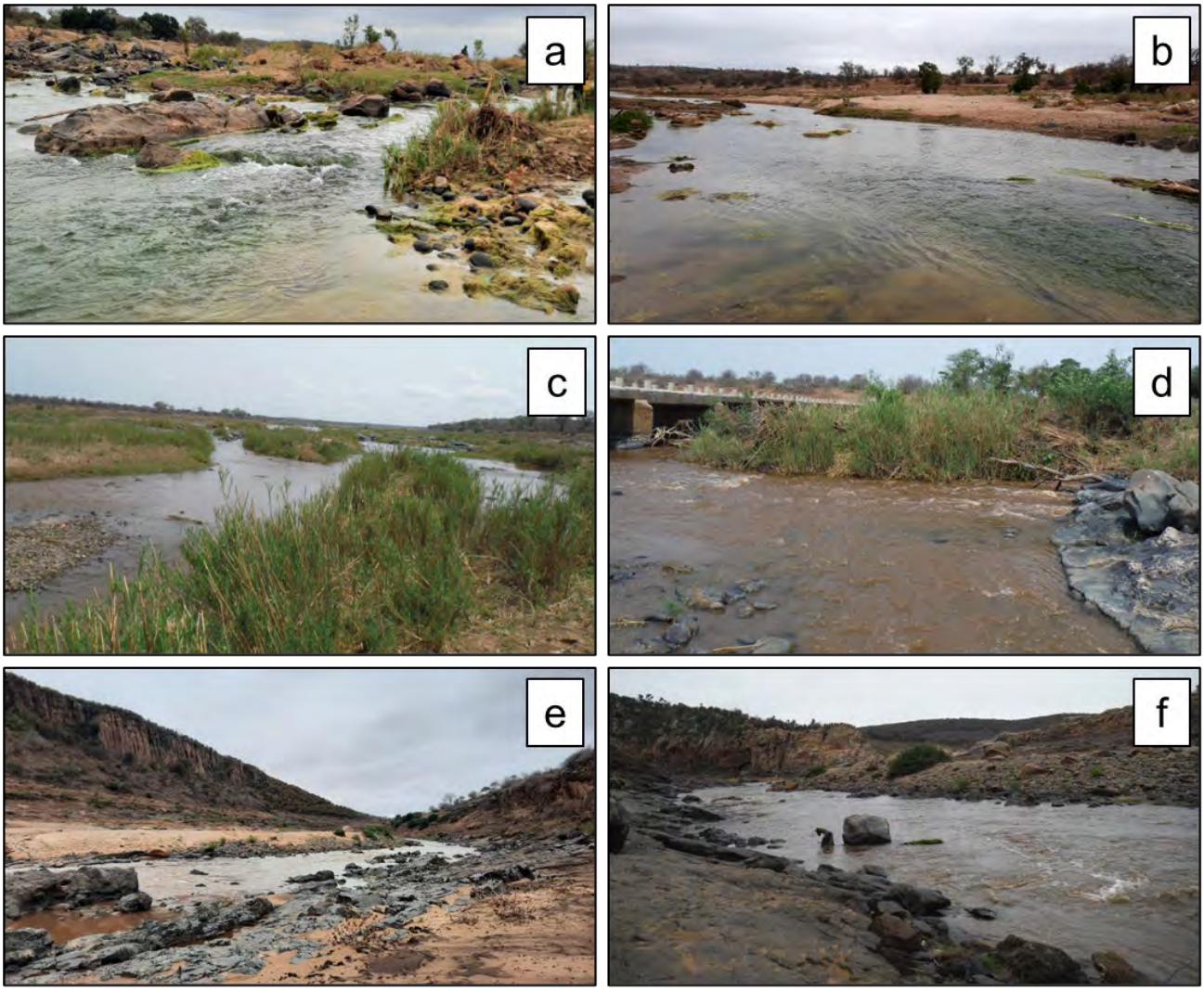

Figure 2.8: Photographs of sites on the Olifants River sampled from September to October 2020 in the Kruger National Park: (a-b) the OLIF-1 site downstream of Mamba weir; (c-d) the OLIF-2 site at Balule low-water bridge; and (e-f) the OLIF-3 site upstream of the Olifants-Letaba confluence. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

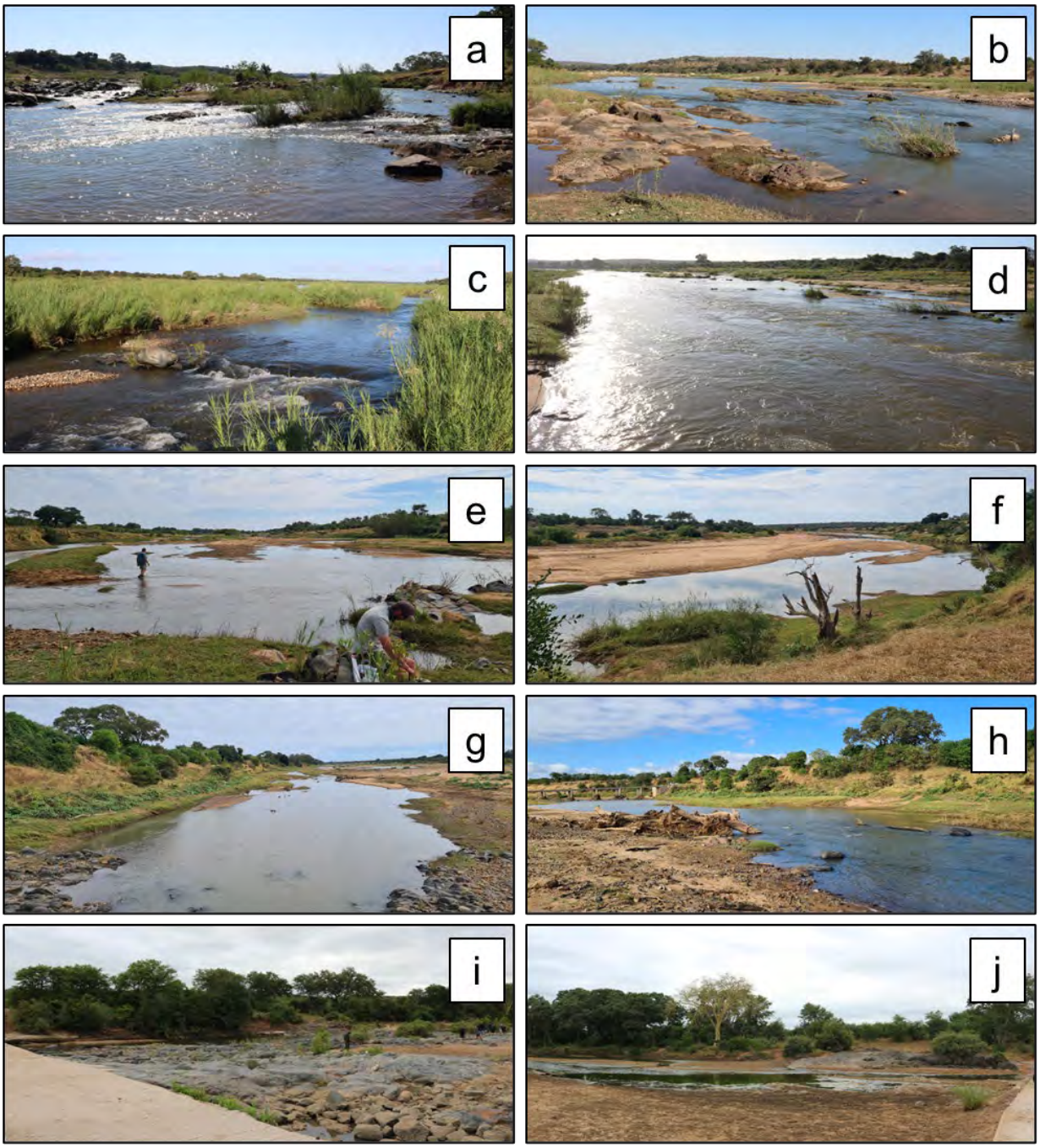

Figure 2.9: Photographs of sites on the Limpopo River system sampled from April to May 2021: (a-b) the OLIF-1 site downstream of Mamba weir; (c-d) the OLIF-2 site sampled on the Olifants River at Balule low-water bridge; (e-f) the G-LETA site on the Great Letaba River inside the Great Letaba Wildlife Reserve; (g-h) the LETA site sampled on the Letaba River; and (i-j) the SHIN site on the Shingwedzi River. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

2.2.3 Free State Province

The Seekoeivlei Nature Reserve was declared a nature reserve in 1978 and is located near the small town of Memel, Free State Province, South Africa (Figure 2.1 and 2.10). The reserve is found within the more extensive Seekoeivlei Wetland and covers an estimated 4 754 ha and was declared a Ramsar wetland of international importance in 1997 (FSDTEEA 2005). This wetland consists of more than 200 oxbows that have formed over centuries by the meandering flow of the Klip River (Tooth et al. 2002; McCarthy et al. 2010) and are regarded as part of a floodplain depression providing essential water purification and flood mitigation functions (McCarthy et al. 2010; Ollis et al. 2015). The Klip River flows through the nature reserve and forms part of the upper Vaal River catchment and is considered one of the important tributaries supplying approximately 46% of the surface water flow in the upper Vaal River catchment (DWAF 2004). The Seekoeivlei Nature Reserve is the only protected area in the Free State Province that covers the Amersfoort Highveld Clay Grassland and the Eastern Temperate Freshwater Wetland veld type (McCarthy et al. 2010). The annual precipitation of this area is 700 -1 200 mm, with most of the yearly rainfall occurring from November to March. Annual mean temperatures range from 13 °C to 25 °C during the summer season and between -1 °C and 15 °C during the winter season (McCarthy et al. 2010). Ten sampling sites were selected in the reserve and field collection surveys occurred in February 2022 with all sampling sites located within and surrounding the Seekoeivlei Nature Reserve (Figure 2.10). Extensive amounts of vegetation were present at all the sites (marginal, emergent, and floating vegetation; Figure 2.11).

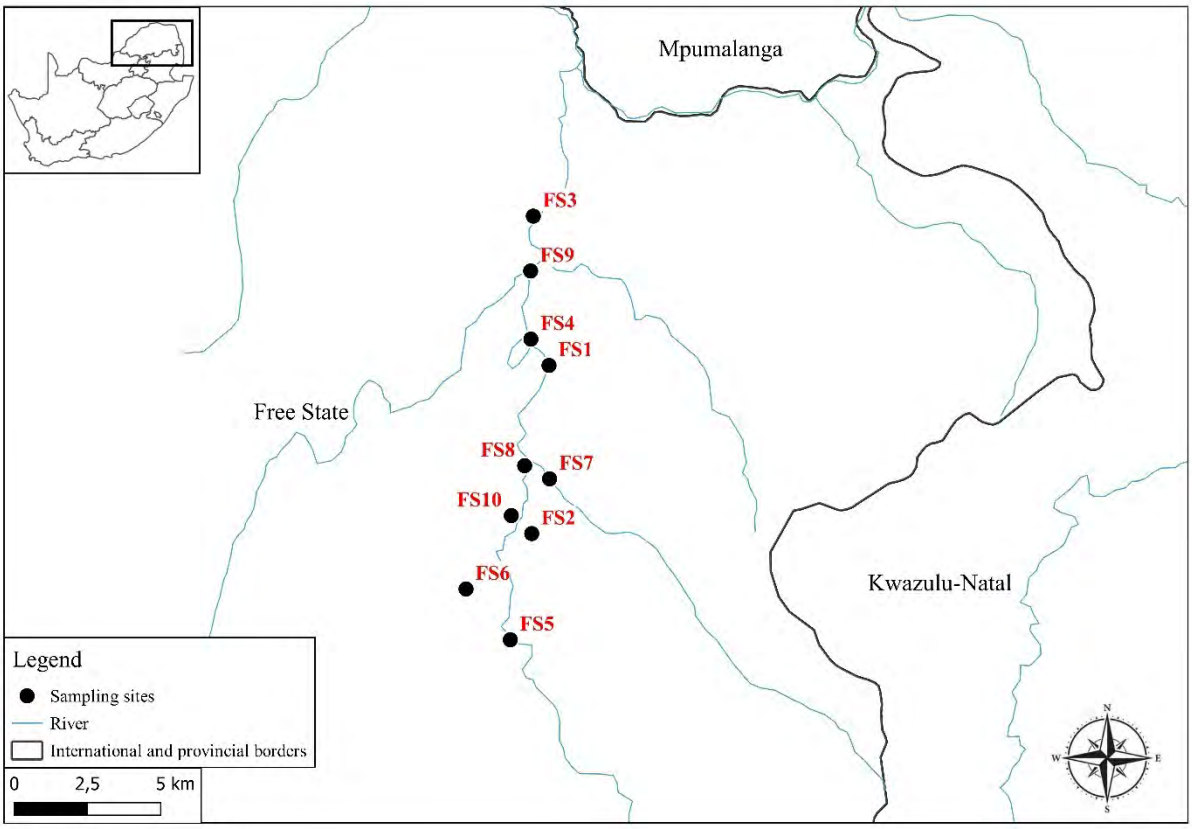

Figure 2.10: Map indicating selected sites sampled on the Klip River, Wasgoedspruit and Pampoenspruit located near the Seekoeivlei Nature Reserve, Memel, Free State Province, South Africa (FS – Free State).

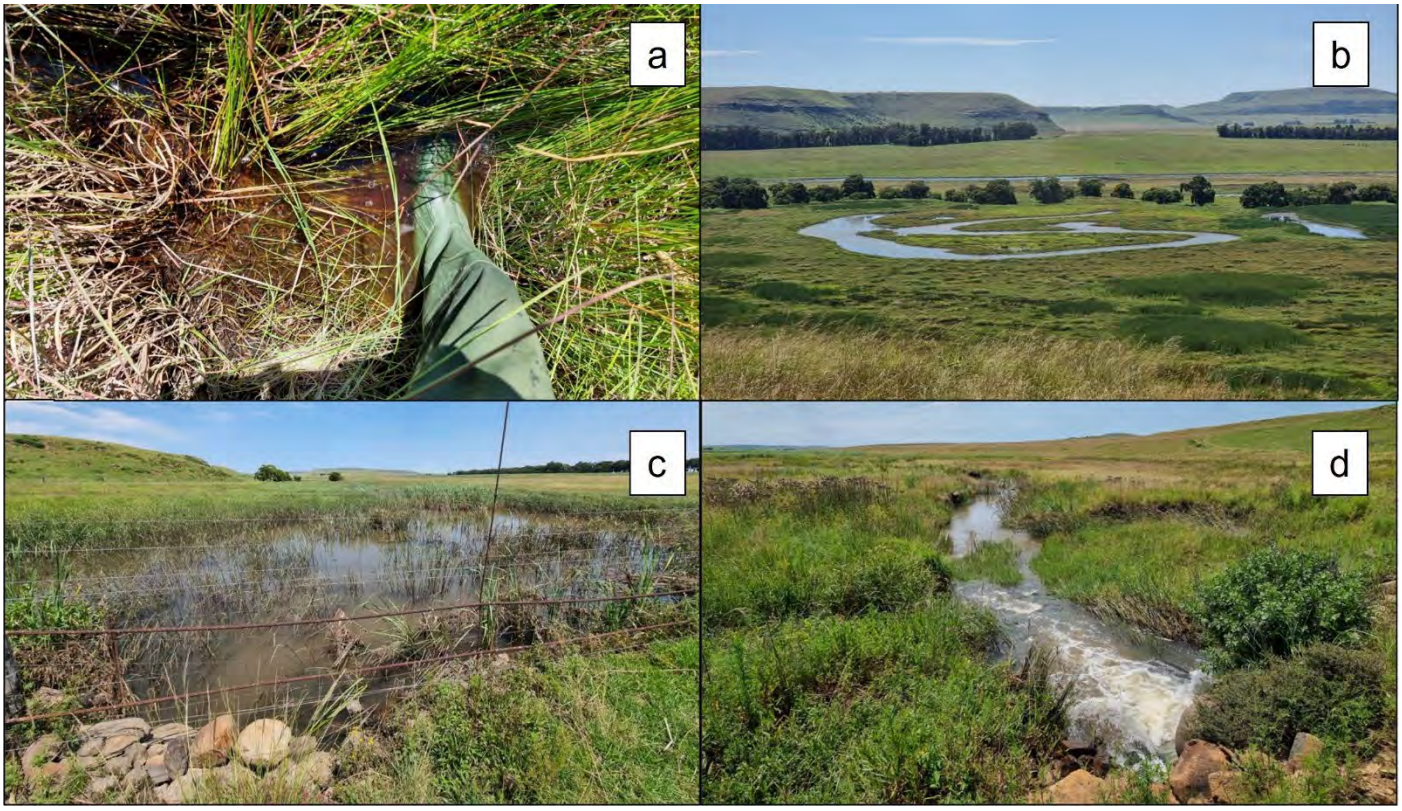

Figure 2.11: Photographs of the Seekoeivlei Nature Reserve indicating (a) the thick vegetation cover surrounding all of the sites sampled along the Klip River; (b) the oxbows formed by the Klip River; (c) flooding of the perimeter fence of the Seekoeivlei Nature Reserve; and (d) the high flow velocity of the Klip River as it flows out of the Seekoeivlei Nature Reserve.

2.2.4 KwaZulu-Natal Province

The Phongolo River rises near Wakkerstroom (Mpumalanga Province, South Africa), flowing eastwards towards the Lebombo Mountains (KwaZulu-Natal) and into the Pongolapoort Dam (Lankford et al. 2011). The Phongolo River Floodplain lies downstream of the dam all the way up to the junction of the Phongolo and Usuthu rivers. The floodplain is South Africa's largest (13 000 ha when fully inundated) and most diverse floodplain ecosystem, with ~50 fish species, >40 frog species, and >430 bird species (SAWCP 1996; Smit et al. 2016; Acosta et al. 2020). The floodplain has an area of 120 km^2 and approximately 90 floodplain depressions (commonly referred to as pans in South Africa), of which all are ecologically and economically important (Lankford et al. 2011; Dube et al. 2015; 2020; de Necker et al. 2020; 2022). The climate consists of subtropical (hot and humid) conditions, where temperatures can reach well over 40 ºC with an average seasonal summer rainfall of 634 mm from December to March (Kyle and Marneweck 1996; Lankford et al. 2011). The Ndumo Game Reserve, located at the junction of the Usuthu and Phongolo river systems, is the only protected area (10 117 ha) found within the floodplain (Kyle and Marneweck 1996). The Ndumo Game Reserve was also placed on the list of Wetlands of International Importance, under the Ramsar convention in 1997, due to the wide range of unique wetlands and biodiversity (Kyle and Marneweck 1996). The Phongolo system is dammed (Pongolapoort Dam) directly upstream of the floodplain; mismanagement and untimely flows have resulted in a disconnection between the river and its floodplain, as it is only flooded after controlled water is released from the dam, when it nears full capacity (Lankford et al. 2011; Dube et al. 2015; Welicky et al. 2017; de Necker et al. 2020). The disconnection from the river has been exacerbated due to supraseasonal droughts in the area and as a result, no controlled flood releases have occurred since 2014 (de Necker et al. 2020). The lack of water flow has resulted in almost total desiccation of the floodplain, with only two of the approximately 90 floodplain pans containing water (de Necker et al. 2020). The Phongolo River was selected as it is known to have *T. granifera* (Smit et al. 2016; Dube et al. 2017; 2019). Sampling sites were selected within the Phongolo River system, stretching downstream of the Pongolapoort Dam to just before the confluence with the Usuthu River inside the Ndumo Game Reserve. Site selection was based on study sites used as part of previous studies in the region (Dube et al. 2017; de Necker 2016; 2019; de Necker et al. 2020; 2022).

The first site (PHON-1) is located on Pongolapoort Dam at Tiger Lodge (Figure 2.12; Figure 2.13 a-b). The marginal vegetation was dominated by grasses and some herbaceous plants and sedges. The substrate was made up of gravel, sand, and mud. The second site (PHON-2) is immediately below the dam wall (Figure 2.13 c-d), approximately 5 km downstream of

the first site. Water flow was visually rated low and algae were dominant. Reeds dominated the marginal vegetation, with a few types of grasses present. Water was mostly shallow, and sand-mud dominated the substrate. The third site (PHON-3) is located approximately 50 km further downstream of PHON-2 at a high-water bridge (Figure 2.13 e-f). The marginal vegetation consisted of shrubs, grasses, reeds, and herbaceous plants. Hydraulic biotopes consisted of deep and shallow runs. Sand, mud, and fine gravel dominated the substrate. Domestic waste was observed along the banks of the river, most likely from a village located close to the site. Local people at the site were also collecting water for household use. The most downstream site (PHON-4) is located 5 km downstream of PHON-3 at a walkway bridge for foot traffic (Figure 2.13 g-h). The common water hyacinth (*Eichhornia crassipes*), an invasive aquatic macrophyte, was found floating on the water surface, while reeds and shrubs dominated the marginal vegetation. Hydraulic biotopes consisted of deep and shallow runs. The substrate consisted of large boulders, cobbles, and sand-mud.

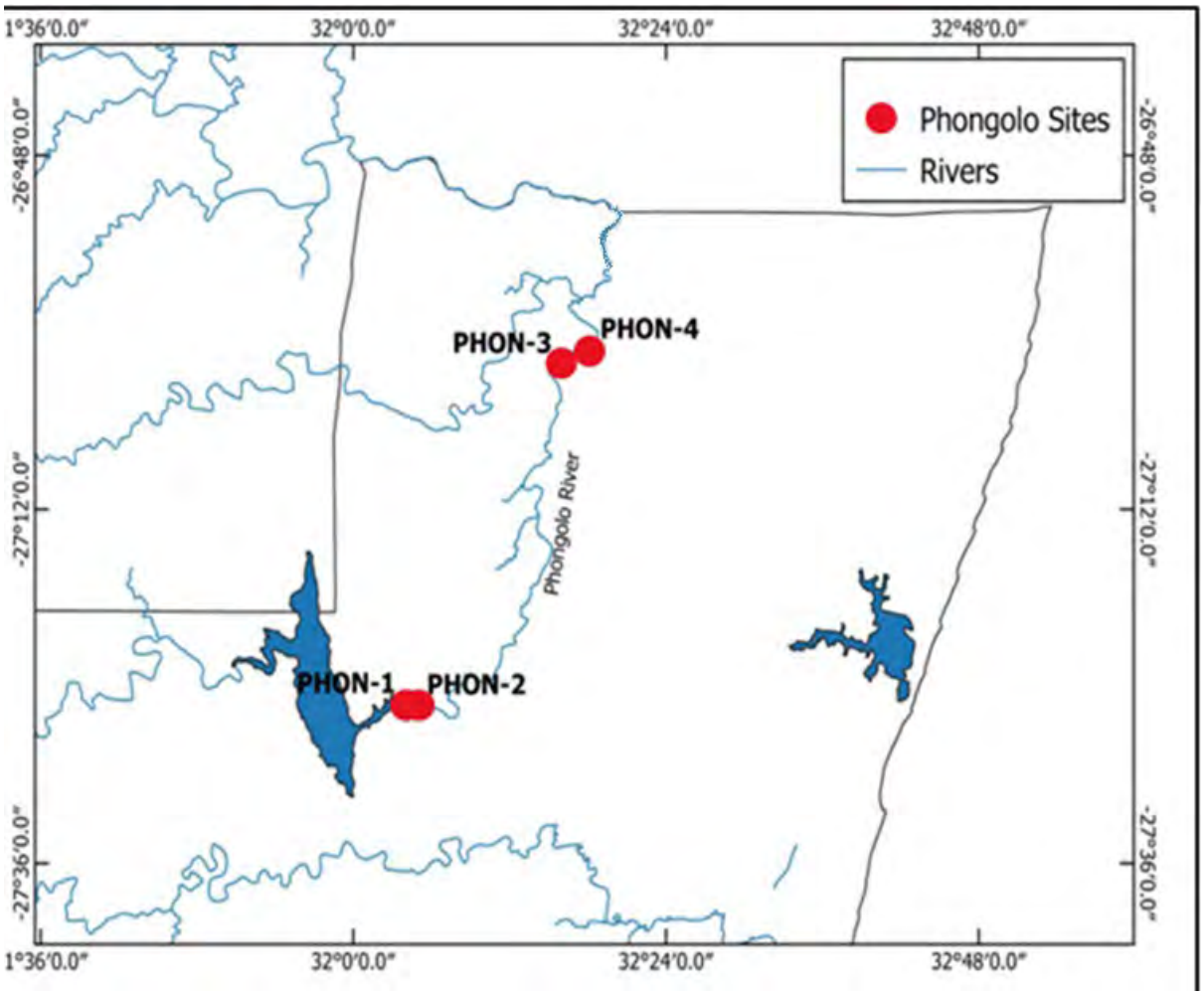

Figure 2.12: Map indicating all selected sites on the Lower Phongolo River system (red dots), South Africa.

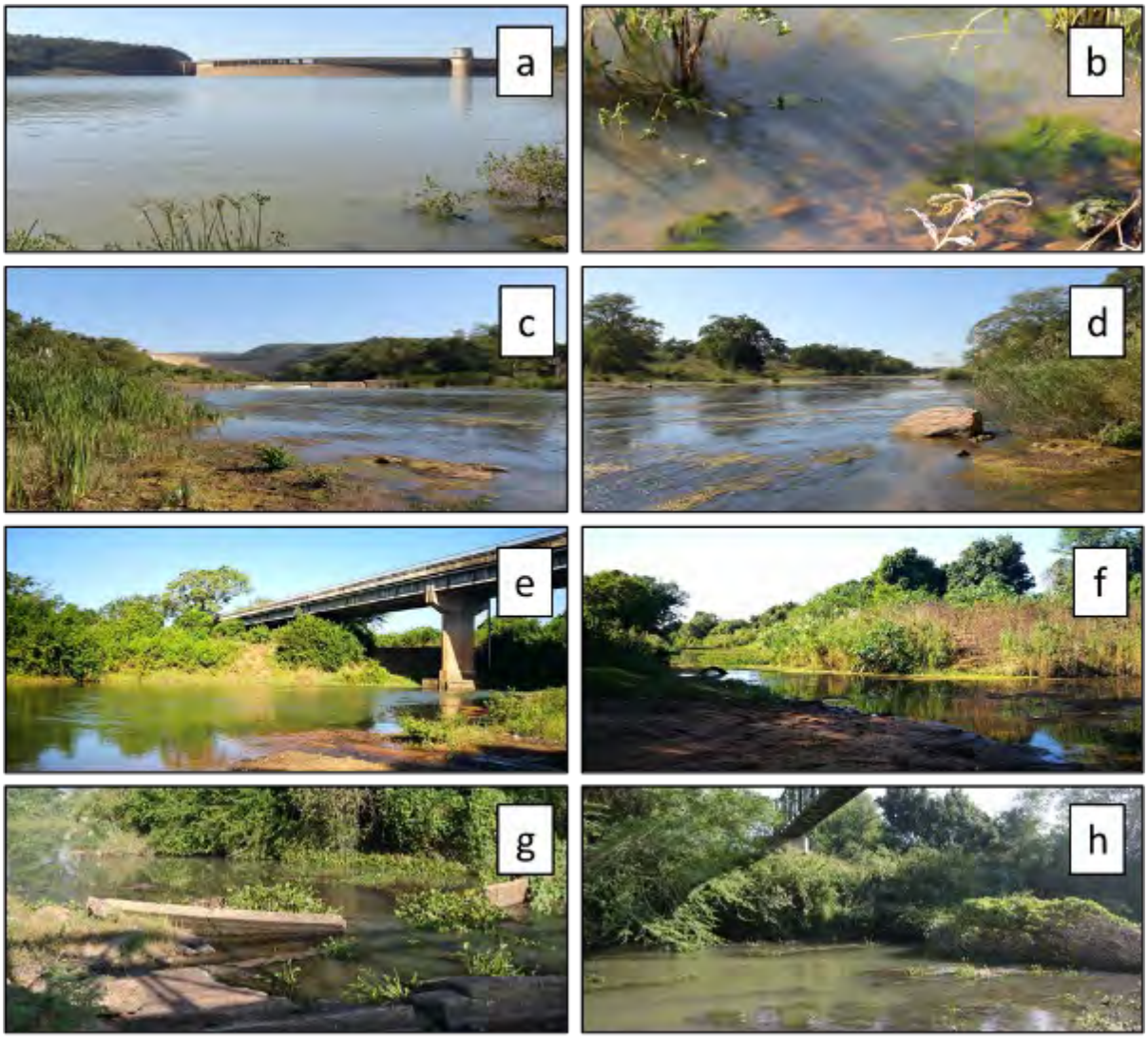

Figure 2.13: Photographs of the sites sampled on the Phongolo River from August to September 2017: (a) PHON-1 site on Pongolapoort Dam at Tiger Lodge, and (b) a typical biotope at this site; (c-d) the PHON-2 site below the dam wall; (e-f) the PHON-3 site located at a high-water bridge; and (g-h) the PHON-4 site. Photographs on the left indicate an upstream view, and those on the right indicate a downstream view of the sites.

2.3 Methods

2.3.1 Water quality

Physicochemical water quality variables pH, temperature (°C), electrical conductivity (EC) (µS.cm^{-1}), total dissolved solids (TDS) (mg/L), dissolved oxygen (mg/L) and oxygen concentration (%) were measured in situ at each site using Extech EC500 pH/electrical conductivity meter and Extech DO600 dissolved oxygen meter. Water samples (250 mL) were also collected from each site and frozen (-4 ˚C) on-site in a mobile freezer until analysis could be conducted in the laboratory at the North-West University (NWU). In the laboratory, unfiltered water samples were allowed to defrost and come to room temperature. The following chemical variables were analysed: turbidity (FAU), ammonium (NH_4^+), sulphates (SO_4^{2-}), nitrites (NO_2^-), nitrates (NO_3^-) chlorides (Cl^-), phosphates (PO_4^{3-}) and total hardness using the appropriate test kits and a Merck Spectroquant® Pharo 300 Spectrophotometer (Merck KGaA 2014; www.merck-chemicals.com).

2.3.2 Habitat assessment

A qualitative habitat assessment of the riparian and aquatic vegetation was performed at each site to determine the habitat suitability for freshwater snail species (Figure 2.13). The habitat type (river, wetland, isolated pool), as well as the presence of marginal, floating, emergent, and submerged vegetation were determined along with algae presence; the weather conditions at the time of sampling, and the site coordinates were recorded on the day of collection. Any anthropogenic activity within or near the collection area was also noted, as this could impact the habitat suitability for the freshwater mollusc species (Appleton and Miranda 2015b).

Data sheet for Site:			
Date:		Weather:	
Time:		Habitat type (e.g. pool, wetland, river)	
Habitat assessment			
	In current		Out of current
Marginal vegetation			
Floating vegetation			
Emergent vegetation			
Submerged vegetation			
Algae			
Stones			
GSM			
Additional notes:			

Figure 2.14: Example of the datasheet used during the qualitative habitat assessment.

2.3.3 Freshwater mollusc collection and morphological identification

All sites were sampled for all available aquatic benthic molluscs (including snails and bivalves/clams), using a snail sampler, a standard D-frame dip net and hand-picking depending on the type of habitat that was present (Figure 2.15). The snail-sampler is made of an aluminium flat frame with a sieve (30 x 30 cm, 2 mm mesh size) and handle, and was used to scoop up sediment and sieve through the benthic zone of the site. The dip net (30 cm x 30 cm; 500 µm mesh size) was used to sample under and between floating and aquatic vegetation, while hand-picking was used to collect molluscs from vegetation and structures such as rocks and boulders. Each site was sampled for a maximum of 30 minutes with the number of scoops counted at each site.

All specimens were preserved in 90% ethanol and identified morphologically in the field with the aid of standard identification guides (Appleton 2002; Day and de Moor 2002; Fry 2021) after which samples were stored in a mobile freezer and transported back to the laboratory (NWU) for further identification. In the laboratory, microscopy was done for further morphological identification to a lower taxonomic level, particularly for specimens of the mollusc genera *Bulinus, Biomphalaria,* and *Gyraulus* as molluscs are particularly difficult to identify. These specimens were also photographed using a Nikon AZ100M microscope with the aid of a Nikon Digital Sight DS-F12 Camera and associated NIS-Elements Software (Nikon Instruments, Tokyo, Japan) and edited using Adobe Photoshop (Adobe Inc. 2019). All snail samples were collected and processed with the necessary permits (Permit No. ZA/LP/107276) from the Limpopo Department of Economic Development, Environment and Tourism, Ezemvelo KZN Wildlife (Permit No. OP 864/2021), and ethical clearance from the North-West

University AnimCare Animal Research Ethics Committee (Ethics No. NWU-01539-20-A9; Ethics No. NWU-00452-20-A1).

2.3.4 Cercarial shedding experiment

All collected samples were screened for parasitic infection using the cercarial shedding method (Webber et al. 1986). This method entails alternating eight-hour periods of dark and light intervals over the course of 48 hours. Light exposure is done to induce shedding of the cercarial life stage of trematodes, potentially infecting the sampled freshwater molluscs (Wolmarans et al. 2002). Shedding examination was conducted by placing individual mollusc specimens in containers filled with double-distilled water (ddH_2O) under an artificial light source, out of direct sunlight, to induce a stress response within the host mollusc species and artificially stimulate cercarial shedding. During the eight-hour window of light exposure, samples were examined at 20-minute intervals with the aid of a Nikon stereomicroscope (Nikon Instruments, Tokyo, Japan) for signs of cercarial shedding. Afterwards, the specimens were placed in containers that prevent light penetration, thus simulating night-time conditions and reducing stress (Wolmarans et al. 2002; Schwelm et al. 2020). Feeding was withheld during the light exposure and restored afterwards. When the 48-hour shedding experiment was completed, specimens were euthanised, given a specific sample code, and preserved in separate Eppendorf tubes with 98% molecular-grade ethanol. Samples were placed in a freezer (-4° C) until further laboratory analyses.

Figure 2.15: Photographs illustrating the different methods of collection used: (a-b) the use of a snail sampler; (c) screening each sample taken with the snail sampler for the presence of molluscs; and (d) hand-picking from vegetation present within the sampling area.

2.3.5 Molecular analysis of schistosomiasis-transmitting molluscs

To validate the morphological identification of collected mollusc species known to transmit schistosomiasis, molecular characterisation and identification were conducted, particularly for *Bulinus* species, which are known to be difficult to identify morphologically (Day and de Moor 2002). The mollusc was carefully removed from its shell using forceps and small tongs, with the shell being crushed and the tissue removed if the specimen could not be extracted without damaging the shell or soft tissue. Subsequently, a piece of hepatopancreas was cut with a sterile scalpel blade and placed in a newly labelled Eppendorf tube with 98% molecular-grade ethanol. The specimen was then preserved in a freezer at -20 °C until further analyses. Cross-contamination was prevented by sterilising all forceps and tongs using bleach and 98% ethanol between specimens (Hoogendoorn et al. 2019; Schols et al. 2019).

Identification of potential trematode infection was established using molecular identification by extracting the trematode 28S rRNA gene from the molluscan tissue. The larger samples of each mollusc species identified were utilised to test for prepatent trematode infections molecularly. Larger, more mature mollusc specimens have a higher chance of trematode

infection (Levri et al. 2005; Giannelli et al. 2016). Therefore, larger specimens were used for molecular analyses.

Molecular analyses were conducted by extracting DNA from the stored sample using a combination of hepatopancreas and tissue collected from the foot of the snail specimen. After removing as much ethanol as possible, the residual ethanol within the Eppendorf tube was evaporated by placing the open tube in a heat block at 56 °C. Total genomic DNA was extracted from the snail tissue and hepatopancreas using the Chelex®100 method and following the protocol developed by Walsh et al. (1991) for DNA extraction. The supernatant of each extraction was transferred to sterile tubes and frozen (-20 °C) to prevent DNA degradation.

The DNA samples were amplified using the appropriate primers by polymerase chain reaction (PCR) protocols for the target gene fragments. Primers were selected based on the preliminary identification of mollusc species and recommendations from previous studies (Folmer et al. 1994; Tkach et al. 2001; Veeravechsukij et al. 2018a; 2018b; Hoogendoorn et al. 2019; Schols 2019; Schols et al. 2019). All the primers used in this study are summarised in Table 2.1. A PCR master mix created containing the appropriate PCR reagents was prepared to reduce pipetting, cross-contamination risk, and possible mixing errors (West and Sawyer 2006; Hoogendoorn et al. 2019; Schols et al. 2019). The polymerase chain reaction amplifications were visualised by 1% agarose gel electrophoresis, and unpurified samples were sent to Inqaba Biotechnical Industries (Pty) Ltd. in Pretoria, South Africa, for purification and sequencing.

Geneious v.11 bioinformatics software (Biomatters Ltd., Auckland, New Zealand) was used to assemble the newly generated sequences. Sequences from the present study were compared with relevant sequences of trematode species available in GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) by using the BLAST tool to determine genetic similarities to identify trematode species present. Sequences were aligned using MUSCLE (Edgar 2004) as implemented in Geneious v. 11, under the default parameter values. Aligned sequences were analysed with the aid of the jModelTest v. 2.1.2 software (Posada 2008) to determine the best substitution model according to each dataset's Akaike information criterion (AIC). Bayesian inference (BI) analysis was performed using the MrBayes plug-in (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) for Geneious v. 11, and maximum likelihood analysis was carried out with the aid of MEGA11 (Tamura et al. 2021). The nodal support (for maximum likelihood and Bayesian inference analysis) was validated by applying 1 000 bootstrap pseudo replicates. The phylogenetic tree was visualised using the MrBayes plug-in within Geneious v.11. Pairwise genetic distance matrices as a percentage

difference (p-distance) and the number of base pair (bp) differences between sequences used for the phylogenetic analysis, were calculated with Geneious v. 11 software.

Infection RD-PCR (rapid diagnostic multiplex PCR) and *Schistosoma* RD-PCR for *Schistosoma* species detection and identification were used to simultaneously evaluate for schistosomiasis within emollusc species known to be an intermediate host for the neglected tropical diseases (De Kock 1995; Wolmarans et al*.* 2002; De Kock et al*.* 2004; Schols et al*.* 2019). Molluscan DNA already extracted by the Chelex®100 method was used for the *Schistosoma* Multiplex-PCR protocol as described by Schols et al*.* (2019). The list of primers used for the Multiplex-PCR for specific *Schistosoma* detection and identification is described in Table 2.2.

Table 2.1: Genetic markers used during polymerase chain reaction (PCR) amplification of COI gene and ITS2 region of mollusc species. Table adapted from Schols et al*.* (2019).

Genetic marker	Primer	Primer Sequence (5'–3')	Reference
28S rRNA	Digl2	AAG CAT ATC ACT AAG CGG	(Tkach et al. 2001)
	1500R	GCT ATC CTG AGG GAA ACT TCG	(Tkach et al. 2001)
	ECD2	CTT GGT CCG TGT TTC AAG ACG GG	(Tkach et al. 2001)
	300F	CAA GTA CCG TGA GGG AAA GTT G	(Tkach et al. 2001)
ITS2 rDNA	3S	GGTACCGGTGGATCACGTGGCTAGTG	(Bowles et al. 1993)
	ITS2.2	CCTGGTTAGTTTCTTTTCCT CGC	(Cribb et al. 1998)
COI mtDNA	LCO1490	GGTCAACAAATCATAAAGATATTGG	(Folmer et al. 1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	(Folmer et al. 1994)

Table 2.2: Genetic markers used in the Infection RD-PCR and *Schistosoma* RD-PCR. Adapted from Schols et al. (2019).

Genetic marker	Primer	Primer Sequence (5'–3')	Reference
Infection RD-PCR	18S_SNAIL_F	AGTATGGTTGCAAAGCTGAAACTTA	(Carolus et al. 2019)
	18S_SNAIL_R	TACAAAGGGCAGGGACGTAAT	(Carolus et al. 2019)
	18S_Digenea_F	CAGCTATGGTTCCTTAGATCRTA	(Schols et al. 2019)
	18S_Digenea_R	TATTTTTCGTCACTACCTCCCCGT	(Schols et al. 2019)
	ITS2_Schisto_F	GGAAACCAATGTATGGGATTATTG	(Gryseels et al. 2006)
	ITS2_Schisto_R	ATTAAGCCACGACTCGAGCA	(Gryseels et al. 2006)
Schistosoma RD-PCR	Asmit1	TTTTTTGGTCATCCTGAGGTGTAT	(Littlewood et al. 1997)
	Sh.R	TGATAATCAATGACCCTGCAATAA	(Webster et al. 2010)
	Sman.R	TGCAGATAAAGCCACCCCTGTG	(Van den Broeck et al. 2011)
	Smat.R	CACCAGTTACACCACCAACAGA	(Schols et al. 2019)
	Sb.R	CACAGGATCAGACAAACGAGTACC	(Webster et al. 2010)

2.3.6 Molecular analysis of invasive mollusc Tarebia granifera

A piece of each of the snail foot tissue samples was cut with sterile scalpel blades and placed in separate sterile Eppendorf tubes for analysis. The hepatopancreas samples were divided in two with sterile scalpel blades, one half of each sample was crushed and placed in separate sterile Eppendorf tubes for further analysis. After removing as much of the ethanol from the tubes as possible, the remaining ethanol from each sample was evaporated in a heat block at 56 ˚C. The total genomic DNA was extracted from both the snail foot tissue and hepatopancreas samples with the use of the PCRBiosystems Rapid DNA Extraction Kit (PCRBiosystems available from Analytical Solutions, Randburg, South Africa). The manufacturer's protocol was followed: 20 µL of lysis buffer, 10 µL of proteinase K buffer and 70 µL of molecular grade water was added to each sample. Samples were then incubated at 75 ˚C for 5 minutes (mixing the samples twice by vortex during the 5 minutes); then at 95 ˚C for 10 minutes. After incubation, samples were diluted with 150 µL of molecular grade water instead of 900 as recommended, in order to obtain DNA at a higher concentration. The samples were then centrifuged at 15 596 RCF (relative centrifugal force) for one minute. Finally, 150 µL of the supernatant from each sample was transferred to sterile tubes and frozen (-20 ˚C) to prevent DNA degradation.

The DNA samples were amplified by polymerase chain reaction (PCR) protocols for target gene fragments using the relevant primers. These primers were selected based on preliminary identification of snail species or trematodes as recommended by previous studies (Folmer et

al. 1994; Tkach et al. 2001, 2003; Veeravechsukij et al. 2018a, b; Schols 2019; Lopes et al. 2020; Vermaak 2021). All the primers used for DNA amplification during this study are summarised in Table 2.3. Three genetic markers were targeted for DNA amplification (one for snails and two for trematodes). For the amplification of the cytochrome c oxidase subunit 1 (COI) mitochondrial gene, LCO1490 forward and HCO2198 reverse primers (Folmer et al. 1994) were used. The COI genetic marker was used to identify snails due to its high evolutionary speed (Hebert et al. 2003) and it is well represented in sequences found on GenBank (NCBI, https://www.ncbi.nlm.nih.gov/) (Schols 2019). The remaining two genetic markers were used to identify trematode infections in the snails. The Internal Transcribed Spacer 2 region (ITS2) was amplified with the 3S forward primer (Bowles et al. 1993) and ITS2.2 reverse primer (Cribb et al. 1998). For amplification of the 28S ribosomal DNA, the Digl2 forward (Tkach et al. 2001) and 1500R reverse (Tkach et al. 2003) primers were used. Additionally, two internal primers, 300F (forward) and ECD2 (reverse), were used to sequence the 28S rRNA genetic marker.

For each targeted genetic marker, a PCR master mix containing the appropriate PCR reagents was prepared. By using a master mix, the amount of pipetting is reduced, the risk of contamination is minimised, possible mixing errors are prevented, and it also saves time (West and Sawyer 2006). The COI PCR master mix was made up of 8 µL of double-distilled water (ddH_2O), 1.25 µL of both the forward (LCO1490) and reverse (HCO2198) primers, and 12.5 µL of Kapa Hifi Hot Start Taq DNA polymerase per sample. The master mix (23 µL) and DNA template (2 µL) were added to the Eppendorf tubes and the samples were amplified in a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) machine following PCR conditions as illustrated in Figure S2.1a. For the ITS2 genetic marker, the PCR master mix consisted of 7 µL of ddH_2O, 1.25 µL of both the forward (3S) and reverse (ITS2.2) primers and 12.5 µL of Dream Taq DNA polymerase per sample. The master mix (22 µL) and DNA (3 µL) were added to the Eppendorf tubes and the samples were amplified following PCR conditions as illustrated in Figure S2.1b. Finally, the 28S PCR master mix contained 8 µL of ddH_2O, 1.25 µL of both the forward (Digl2) and reverse (1500R) primers, and 12.5 µL of Dream Taq DNA polymerase per sample. The master mix (23 µL) and DNA (2 µL) were added to the Eppendorf tubes and the samples were amplified under PCR conditions as illustrated in Figure S2.1c. The success of the PCR amplification was determined using agarose gel (1%) electrophoresis; the gel was stained with SafeView Classic DNA dye. Successful PCR amplicons were sent to Inqaba Biotechnical Industries (Pty) Ltd. in Pretoria, South Africa for purification and sequence generation.

Geneious v.11 bioinformatics software (Biomatters Ltd., Auckland, New Zealand) was used for sequence assembly, trimming and alignment of the obtained sequences. Trematode (ITS2

and 28S) and *T. granifera* (COI) sequences obtained in the present study were compared with relevant sequences of trematode and snail species available in GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) by using the Basic Local Alignment Search Tool (BLAST), to determine genetic similarities for species identification.

Tarebia granifera sequences (COI) from the present study were compared with one another as well as the available *T. granifera* sequences in GenBank to determine genetic similarities between *T. granifera* from different regions (sites and countries). The alignments were constructed using MUSCLE (Edgar 2004) implemented in Geneious v.11 under default parameter values. The COI alignment included eight sequences from the present study (one *T. granifera* sequence per site) and ten sequences from GenBank, with *M. tuberculata* selected as an outgroup based on the phylogenetic analysis by Von Gersdorff Sørensen et al. (2005) and Harding et al. (2019). Only one *T. granifera* sequence per site was used due to all *T. granifera* sequences from the present study being 100% identical (see section 3.3.5 for findings). The sequences from the present study and GenBank sequences used for the phylogenetic analysis during this study are given in Table S2.1.

Prior to further analysis, the best fitting model for each alignment was determined with the use of jModelTest v.2.1.2 software (Guindon and Gascuel 2003; Darriba et al. 2012) according to the Akaike information criterion (AIC). The HKY + I model was the best fitting model for the *T. granifera* COI dataset. Bayesian inference was performed by MrBayes v.3.2.6 software (Ronquist et al. 2012) and maximum likelihood analysis was carried out by RaxML-HPC v.8 on XSEDE (Stamatakis 2014) run on CIPRES Science Gateway v.3.3 (Miller et al. 2010) (available at https://www.phylo.org/). The Markov chain Monte Carlo (MCMC) algorithm was used for the Bayesian inference analysis, the chains were run for 10 000 000 generations and sampled trees every 1 000 generations. The “burn-in” parameter was set to disregard the first 25% of the sampled trees, using only the remaining 75% of sampled trees to create the consensus trees (Hoogendoorn et al. 2019). For all alignments, the nodal support (from the maximum likelihood analysis) was validated by applying 100 bootstrap pseudoreplicates. The phylogenetic tree was then visualised by using FigTree v.1.4 software (Rambaut 2012). Pairwise genetic distance (p-distance) matrices as percentage similarity (%) and the number of base pair (bp) differences between the sequences used for phylogenetic analysis (Table S2.2) were calculated in Geneious v.11 software.

Table 2.3: Primers used for DNA amplification and sequencing during this study. The table was adapted from Vermaak (2021).

Genetic marker	Primer	Sequence	Reference
COI	LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer et al. (1994)
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al. (1994)
ITS2	3S	5'-GGT ACC GGT GGA TCA CGT GGC TAG TG-3'	Bowles et al. (1993)
	ITS2.2	5'-CCT GGT TAG TTT CTT TTC CTC CGC-3'	Cribb et al. (1998)
28S	Digl2	5'-AAG CAT ATC ACT AAG CGG-3'	Tkach et al. (2001)
	1500R	5'-GCT ATC CTG AGG GAA ACT TCG-3'	Tkach et al. (2003)
(internal)	ECD2	5'-CTT GGT CCG TGT TTC AAG ACG GG-3'	Tkach et al. (2003)
(internal)	300F	5'-CAA GTA CCG TGA GGG AAA GTT G-3'	Tkach et al. (2003)

2.3.7 Historical distribution of Biomphalaria pfeifferi *and* Bulinus globosus *in Mbombela and Nkomazi local municipalities*

Historic snail data collection

The historic snail datasets for *Biomphalaria pfeifferi* and *Bulinus globosus* were obtained from the National Freshwater Snail Collection (NFSC) of South Africa for the Mbombela and Nkomazi local municipalities from 1955 to 1995. These snail species were selected because they are prevalent in the eastern parts of Mpumalanga, which is the north-eastern part of South Africa where transmission rates are high (Joubert et al. 1990). The 1955 to 1995 period was selected as the most complete dataset for these species for the Mpumalanga Province was available for these years. The data were stored in quarter-degree grids and to refine the sampling points, they were manually digitised to obtain the X and Y coordinates. After digitising, a total of 962 *Biom. pfeifferi* and 1193 *Bul. globosus* snail points were recorded across the Mbombela and Nkomazi local municipalities (Table 2.4).

Table 2.4: Digitised snail records of *Biomphalaria pfeifferi* and *Bulinus globosus* from sample sites within the Mbombela and Nkomazi local municipalities.

Snail species	Mbombela eMunicipality	Nkomazi Local Municipality
Biomphalaria pfeifferi	755	207
Bulinus globosus	1038	155

Environmental variables

Climatic indicators from 1950 to 2020 were obtained using ERA 5-Land data provided by the Copernicus Climate Change Service. Bioclimatic indicators as in WORLDCLIM were also obtained using the Copernicus Climate Change Service as ERA 5 reanalysis data from 1970 to 2018. In total, 19 bioclimatic and 13 climatic variables were downloaded for this study. These indicators were used to describe the influence of climate on the species' habitats.

A principal component analysis (PCA) was performed on the climatic variables of occurrences using the XLSTAT package in Excel to define independent axes in the environmental space (Dray and Dufour 2007). Climate, elevation and bioclimate data were used with the snail data to determine which variables were highly correlated to the snail sampling points. This was done to reduce multicollinearity in the climate and bioclimate datasets. Variables that had a correlation >0.8 were excluded. Table 2.5 shows the selected variables used in the species distribution models for each host snail.

Table 2.5: Suitable bioclimatic and climatic variables from principal component analysis used to model the historical distribution of *Biomphalaria pfeifferi* and *Bulinus globosus* within Mbombela and Nkomazi local municipalities.

Species	Environmental variables	Description
Biomphalaria pfeifferi	BIO 1	Annual mean temperature
	BIO 2	Mean diurnal range [mean of monthly (max-min) temperature]
	BIO 3	Isothermality
	BIO 8	Mean temperature of wettest quarter
	BIO 13	Wettest month
	BIO 14	Driest month
	SWV1 layer	Soil water volume
	SRO layer	Surface runoff
Bulinus globosus	BIO 1	Annual mean temperature
	BIO 2	Mean diurnal range [mean of monthly (max-min) temperature]
	BIO 3	Isothermality
	BIO 4	Seasonality (standard deviation)
	BIO 8	Mean temperature of wettest quarter
	BIO 13	Wettest month
	Elevation	Elevation
	SWV1 layer	Soil water volume
	SRO layer	Surface runoff
	TP layer	Total precipitation

Modelling procedures and data analysis

Species distribution models, MaxEnt and the generalised linear model (GLM), were used to predict and model the historical distribution of *Biom. pfeifferi* and *Bulinus globosus* in Mbombela and Nkomazi local municipalities. To model the historical distribution, R Studio was used to access GLM and MaxEnt modelling packages. To visualise the modelling results, colours were used to indicate predicted historical distribution. Red indicates a high probability of suitable conditions for the species, yellow indicates conditions typical of those where the species were found, and shades of blue indicate a low predicted probability of suitable conditions.

Kriging interpolation was used to determine the historical seasonal distribution of *Biom. pfeifferi* and *Bul. globosus* within the Mbombela and Nkomazi local municipalities. The digitised data were divided into different seasons (summer, autumn, winter and spring), using the dates that were recorded for each snail species.

2.3.8 Historical distribution of Biomphalaria pfeifferi *and* Bulinus globosus *in the Vhembe District Municipality*

The occurrence data of *Biom. pfeifferi* and *Bul. globosus* were obtained from the National Freshwater Snail Collection (NFSC) for the years 1957 to 1964 (Table 2.6). The two snail species used in this model were selected because of their prevalence in the Limpopo Province

and the period 1957 to 1964 was selected as the most complete data records for these species in this Province were available for these years. Data were digitised using the same method as described in section 2.3.7 (Chapter 2).

Table 2.6: Digitised snail records of *Biomphalaria pfeifferi* and *Bulinus globosus* from sample sites within the Vhembe District Municipality.

Snail	*Biomphalaria pfeifferi*	*Bulinus globosus*
Record	261	276

Environmental variables

The 19 bioclimatic variables used in the models were obtained from the WorldClim version 2.1 climate data for 1970-2000. The spatial resolution of the data was 30 arc seconds (~1 km^2) (Çoban et al. 2020). The data in TIFF files were uploaded to ArcMap, and clipped for the Vhembe District Municipality. The variance inflation factor (VIF) was used to detect and remove multicollinearity between bioclimatic variables. In a multiple linear regression model, VIF detects multicollinearity in the predictors (Murray et al. 2012). Multicollinearity is the correlation of values, how far or near they are to each other. A VIF value that was greater than 10 has a collinearity that is problematic because it is too high and will not show independent explanatory ability (Pradhan 2016). In this study, five variables were selected of the 19 variables after VIF was completed. These variables were BIO 2, BIO 3, BIO 6, BIO 13 and BIO 15 for both species (Table 2.7).

Table 2.7: Suitable bioclimatic variables used to model the historical distribution of *Biomphalaria pfeifferi* and *Bulinus africanus* in the Vhembe District Municipality.

Name	Environmental variables	Description
Mean temperature	BIO 2	Mean diurnal range [mean of monthly (max-min temperature)]
	BIO 3	Isothermality
	BIO 6	Minimum temperature of the coldest month
Precipitation	BIO 13	Wettest month
	BIO 15	Seasonality (coefficient of variation)

Modelling procedures and data analysis

The species distribution models (SDMs) combine the points of the species that are present in the data, with the environmental variables (Beaumont et al. 2008; Santana et al. 2017). Using an algorithm such as RF or MaxEnt, it produces maps for the geographical extent of the data, variable importance as well as response curves. Species distribution models use changing times because with time, the climate and environment change, while the study area remains

the same throughout the modelling (Jiménez-Valverde et al. 2008). A study conducted in China by Zhao et al. (2022) used MaxEnt and Random Forest (RF) to predict potential distribution. The MaxEnt is a species distribution model that predicts the distribution in a study area (Elith et al. 2011). The MaxEnt model was first published in 2006 and has been applied more than a thousand times in different environmental and distribution patterns (Merow et al. 2013). The MaxEnt software builds a relationship between the two sets of data inputs, namely snail presence points and the bioclimate data to determine the distribution patterns of the Vhembe District Municipality. Furthermore, the model not only allows the use of different climatic factors but also allows other factors to be incorporated into it, such as the pH and water temperature, to make the data more reliable and accurate. For this study, the MaxEnt model needs to be supported by facts, to ensure accurate results (Beaumont et al. 2008).

In an RF, each tree depends on the values of a random vector that was sampled separately and with the same distribution across all of the trees in the forest (Breiman 2001). It is an algorithm that is also used in RStudio as an SDM using species presence data and bioclimate data as background samples. A variety of classification or regression trees (CART) make up an RF (Valavi et al. 2021). Recursive partitioning techniques are the foundation of CARTs. Recursive partitioning divides the data into potentially high-dimensional rectangular covariate (predictor) space divisions repeatedly, selecting those for which the response data are largely homogeneous. A collection that includes multiple trees generalises better. In general, RF fits hundreds to thousands of distinct trees and makes a compilation for their predictions (Valavi et al. 2021).

The RStudio version RStudio-2023.03.1-446 and R workspace version R.4.3.0 were used to model the historical distribution using USDM and SDM. For MaxEnt and RF, 5 000 random points were generated for the *Biom. pfeifferi* and *Bul. globosus*. Because absent data is not available for this study which would have been used as the random points, the random points will be extracted from the present data; 30% of the data will be used as test data. The test data are less sensitive to the parameters, or the environmental features, whereas 70% of the data, referred to as the training data, are highly sensitive to the parameters. Therefore, the difference between training data (70%) and test data (30%) is the change that has occurred in the model (Lissovsky and Dudov 2021).

With MaxEnt, the model can be run several times to conveniently average the outcomes of all the models that were generated after running the model several times. The ability to evaluate the model's performance while utilising all available data without having an independent dataset is made possible by using this feature in conjunction with withholding a specific amount of the data for testing. The model's level of variability may be measured by carrying out several runs (Young et al. 2006; Kogo et al. 2019). In this study, the number of replicates

was 15. Receiver operating characteristic (ROC) curve is a 2D plot that shows a curve used in identifying the area under the (ROC) curve (AUC) (Shengping and Gilbert 2017). The average AUC for an ROC curve is 0.5, the most useful AUC is ranked any value above 0.75, and an AUC score of 1 indicates a perfectly accurate test (Phillips and Dudík 2008).

2.3.9 Historical distribution of Biomphalaria pfeifferi *and* Bulinus africanus *in the Tshwane and Johannesburg metropolitan municipalities*

The NFSC was used to extract the occurrence data for *Biom. pfeifferi* and *Bul. africanus* in the Tshwane and Johannesburg metropolitan municipalities from 1970 to 2006 (Table 2.8). These species were selected as they are the dominant species in the Gauteng Province (De Kock et al. 2004) and period 1970 to 2006 was selected as the most complete dataset was available from the NFSC for these species in Gauteng for these years. The occurrence dataset was digitised and stored as quarter-degree grids and the digitised snail points were assigned WGS98 as a coordinate system.

Table 2.8: Digitised snail records of *Biomphalaria pfeifferi* and *Bulinus africanus* from sample sites within the Tshwane and Johannesburg metropolitan municipalities.

Species	Tshwane Metropolitan Municipality	Johannesburg Metropolitan Municipality
Biomphalaria pfeifferi	524	No records
Bulinus africanus	64	374

Environmental variables

Bioclimatic variables were extracted from the WorldClim website for the years 1970 to 2006 and consisted of a range of environmental climatic variables. The spatial resolution ranged between 30 seconds (~1 km^2) and 10 minutes (~340 km^2) and consisted of 19 bioclimatic variables (BIO 1 to BIO 19; Table 2.9). These were available as ZIP files which entailed 19 Geo Tiff (.tif) files. These indicators were used to make inferences about the influence of climate on the ecosystems and their components such as biodiversity at various spatial resolutions.

Table 2.9: Suitable bioclimatic variables used to model the historical distribution of *Biomphalaria pfeifferi* and *Bulinus africanus* in Tshwane and Johannesburg metropolitan municipalities.

Environmental variable	Description
BIO 1	Annual mean temperature
BIO 2 .	Mean diurnal range [mean of monthly (max-min) temperature]
BIO 3	Isothermality
BIO 4	Temperature Seasonality (standard deviation x100)
BIO 5	Maximum temperature of the warmest month
BIO 6	Minimum temperature of coldest month
BIO 7	Temperature annual range
BIO 8	Mean temperature of wettest quarter
BIO 9-	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 11	Mean temperature of coldest quarter
BIO 12	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 15	Precipitation seasonality
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
BIO 18	Precipitation of warmest quarter
BIO 19	Precipitation of coldest quarter

Data analysis for the Tshwane and Johannesburg metropolitan municipalities

The methodology utilised in this section to perform data analysis was adopted from the methodology approach used in section 2.3.8 (Chapter 2).

2.3.10 Future climate modelling of schistosomiasis-transmitting snails

Occurrence data

The snail sampling data were obtained from the historical data available in the NFSC database from 1950 to 2006, and 15 303 records were recorded. The occurrence dataset for *Bul. africanus*, *Bul. globosus* and *Biom. pfeifferi* is illustrated in Table 2.10. The original sampling points were stored in quarter-degree grids. To overcome this, the snail sampling points were refined by digitising based on the descriptions of the sampling points on a 1:50 000 scale topographical map using ArcGis 10.8.2. The locations were digitised close to water bodies (dams, rivers, reservoirs, lakes, and swamps). Latitude and longitude coordinates were stored in decimal degrees using a WGS84 datum.

Table 2.10: The number of historical data points for snail vectors of schistosomiasis species recorded in South Africa from 1950 – 2006.

Species	Occurrence points
Bulinus africanus	3 051
Bulinus globosus	3 153
Biomphalaria pfeifferi	9 166

Climate data

Baseline data

From 1979-2018, 19 bioclimatic and four soil variables were extracted using ERA 5 reanalysis data provided by the Copernicus Climate Change Service. The temporal aggregation was the mean climatological period pre-calculated over the 40-year reference period 1979-2018 with a spatial resolution of 0.5° x 0.5°.

Future period

Modelling future climate builds on four Representative Concentration Pathways (RCPs) adopted by the IPCC for its fifth assessment report (AR5) in 2014 (IPCC 2013). All models were based on CMIP5 with RCP 4.5, which represented a moderate scenario in which greenhouse gas (GHG) emissions are expected to increase up until 2040 and then to decline, and the RCP 8.5 scenario, in which GHG emissions are expected to increase throughout the 21st century, creating a ''high emission scenario''. The future periods (2040-2070) and (2070-2100) were derived from CMIP5 climate projections provided by the Copernicus Climate Change Service. The future period data were obtained from three GCMs created in a grid format of 0.5° x 0.5° (Table 2.11). The baseline and future data were downloaded as NetCDF-4 files and were converted to a raster using multidimensional tools in ArcGIS 10.8.2. The data were prepared in ArcGIS and RStudio 4.2.1.

Table 2.11: The three general circulation models used in this study.

General circulation model	Characteristics	Development centre
access1-0	Based on the UK Met Office UM atmosphere model, the GFDL MOM4p1 ocean model, the LANL CICE4.1 sea-ice model and the MOSES2 land surface model.	CSIRO & Bureau of Meteorology, Australia
bcc-csm-1-m	This model has coupled BCC−AGCM2.0 with the land surface process model CLM3, the global ocean circulation model POP, and the global dynamic/thermodynamic sea ice model CISM.	Beijing Climate Center Climate System Model
hadgem2-cc	A configuration of the HadGEM2 model is an atmosphere-only simulation with other component interfaces replaced with ancillary file input.	UK Met Office Hadley Centre

Selection of climate variables

A group of variables was selected based on bioclimatic and soil factors to minimise collinearity in the dataset. The USDM R package calculated the VIF. This analysis measures the strength of the relationship between each predictor and the rest. In VIFs, the multiple correlation coefficients (R^2) are obtained by performing a regression analysis of each predictor variable against the other variables in the model. As a rule of thumb, a VIF >10 indicates a collinearity problem (Chatterjee and Hadi 2015). All the variables >10 were removed from the dataset using a threshold (th) of 0.85. This is calculated by excluding highly correlated variables through a stepwise procedure. During the VIF calculation process, the algorithm looks for variables with a linear correlation exceeding the predefined threshold value (th). This was done for the averages over the period 1970-2006. The process was repeated until none of the variables had a high correlation coefficient (r). To determine VIF, a linear regression model was employed, where the numerical variable of interest was used as the response variable in Equation (1). The final set of bioclimatic predictors is illustrated in (Table 2.12). It must be noted that, due to the nature and distribution of the species, each species has a unique set of environmental variables.

$$\mathrm{VI} = \frac{1}{1-R_i^2} \quad (1)$$

Where R^2 represents the linear model's regression coefficient.

Table 2.12: The selection of bioclimatic variables for the ecological models based on their VIF values.

Species	Variable	Description	VIF
Bul. africanus	BIO 3	Isothermality	9.1
	BIO 5	Max. temperature of the warmest	4.0
	BIO 8	month	4.1
	BIO 13	Precipitation of the wettest month	5.5
	BIO 15	Precipitation seasonality	6.6
	BIO 16	Precipitation of the wettest quarter	7.4
	Soil water volume for the wettest quarter		9.0
	Soil water volume for the coldest quarter		6.3
	Soil water for the warmest quarter		5.5
Bul. globosus	BIO 4	Temperature seasonality	3.9
	BIO 7	Temperature annual range	3.1
	BIO 8	The mean temperature of the wettest quarter	3.8
	BIO 15	Precipitation seasonality	3.2
	BIO 16	Precipitation of the wettest quarter	5.8
	BIO 17	Precipitation of the driest quarter	5.7
	Soil water for the warmest quarter		1.7
Biom. pfeifferi	BIO 4	Temperature seasonality	6.9
	BIO 8	The mean temperature of the wettest quarter	3.2
	BIO 12	Annual precipitation	7.3
	BIO 16	Precipitation of the wettest quarter	9.2
	BIO 18	Precipitation of the warmest quarter	3.1

Ecological niche modelling

The present study is based on a multi-model SDM approach (Naimi and Araújo 2016) implemented in the SDM package, and *dismo* was used to examine and model species distribution (Naimi and Araújo 2016). This R package unifies different implementations of SDM into one object-oriented framework that is reproducible and extensible. Based on the methodology of Zuza et al. (2021), the occurrence data were divided into two groups. Models were evaluated using 70% training data and 30% test data. The analysis was conducted with 5 000 random pseudo-absence points. To assess the stability and accuracy of the models, the number of maximum iterations was increased to 5 000 iterations using subsets to reduce underestimations and overestimations. By doing so, the model will have adequate time to

converge. The regularisation remained at 1 to reduce model overfitting (Young et al. 2006; Merow et al. 2013).

Higher values in the area under the curve (AUC), receiver operating characteristic (ROC) curve and true skill statistic (TSS) indicated a better performance (Allouche et al. 2006) in evaluating the model's predictions. Known as the Hanssen Kuipers Discriminator, TSS compares the number of actual positive forecasts to the number of correct hypothetical projections. It is pertinent to note that TSS includes omissions and commissions. A value of +1 indicates accurate classification. In contrast, a value of -1 indicates no better performance than random. Random predictions are represented by 0.5, and predictions >0.5 are better than the random model (Anderson and May 1979; Wouyou et al. 2022). There are four values of AUC: 0 (unsuitable), 0.7-0.8 (suitable), 0.8-0.9 (highly suited) and >0.9 (exceptionally suited) (Allouche et al. 2006).

Based on the results of the three ecological models, an ensemble model was developed to model the distribution of schistosomiasis in SA. Ensemble models combine all algorithms, which improves performance (Allouche et al. 2006; Mushi et al. 2022). Based on this, to create an ensemble, all three models were combined using "weighted averaging" by using the TSS that was >0.75 (Allouche et al. 2006). The weighted averaging gives more weight to the model with higher accuracy than other techniques.

Visual and statistical analyses of suitable habitat patterns

A classification system based on the IPCC classification method created thresholds for ecological model outputs. These thresholds are as follows: non-suitable habitats (0.00 to 0.33), low suitability habitats (0.33-0.66), moderate suitability habitats (0.66-0.90), and high suitability habitats (0.90-1.00) (IPCC 2013; Gong et al. 2023). To create suitability and change composite maps, the baseline and future periods for suitable habitats were evaluated to determine the changes in the distribution of *Bul. africanus, Bul. globosus* and *Biom. pfeifferi*. To reclassify suitability change outputs for each climate model and period, we used the reclass tool in ArcGIS. The natural-break classification method (Jenks) was used to identify three categories of change in suitability: decline in suitability (-0.5), no change (0), and increase in suitability (0.5).

2.3.11 Assessment of community knowledge, attitudes, perceptions and practices

The study employed an explanatory sequential mixed-methods approach to explore community beliefs concerning the cause, transmission, symptoms and treatment of schistosomiasis. The study collected quantitative data via household questionnaires to assess knowledge, attitudes, and practices related to schistosomiasis, as well as community perceptions on the correlation between environmental conditions and the prevalence of the

disease. To provide a more nuanced and contextualised understanding, qualitative data were then gathered through in-depth interviews. This approach facilitated the integration of both datasets, contributing to a deeper understanding of the community's knowledge, attitudes, perceptions and practices concerning schistosomiasis. Importantly, this strategy mitigated the limitations inherent in relying solely on either quantitative or qualitative data sources. Permission to conduct the study was obtained from the Nesengani Tribal Authority, and ethical clearance was obtained from the Turfloop Research Ethics Committee (TREC/337/2021:PG) at UL and the NWU Health Research Ethics Committee (NWU-00452-20-A1).

Sampling and data collection procedures

The sampling frame for this study was the total number of households (3 603) in HaNesengani as reported during the last census by Statistics South Africa in 2011. Inclusion and exclusion criteria, sampling methods and sample sizes for both data collection methods are summarised below.

Household questionnaires: Individuals aged 18 or older, permanently residing in HaNesengani, were considered eligible. Participant selection was achieved through the straightforward process of simple random sampling. Sample size was initially calculated at 271 using Cochran’s formula; however, the sample size was increased and 342 household questionnaires were administered during the data collection campaign. Household questionnaires were administered by a team of 18 fieldworkers over a two-day period on August 28 and 29, 2021. Fieldworkers were 4th year level students from the Department of Geography and Environmental studies at the University of Limpopo, trained in ethical community engagement practices prior to the data collection.

In-depth interviews: People in high-risk groups (agricultural workers, primary caregivers of children under the age of 15 years), health care providers, and people who had been residing permanently in HaNesengani for more than 15 years were included in the in-depth interviews. Purposive sampling was used to select participants for in-depth interviews based on the study's inclusion and exclusion criteria. Participants were recruited during the questionnaire administration phase and through the recommendations of other participants. Sample size for the in-depth interviews employed a qualitative methodology driven by the principle of informational redundancy. A total of 15 interviews were conducted, guided by the concept of data saturation as proposed by Vasileiou et al. (2018). The interview guide was developed after an analysis of the quantitative data, to probe deeper into insights gained during the first phase of data collection. Interviews were conducted over a two-day period, with the assistance of a translator.

Data collection instruments

(i) Household questionnaire

A structured questionnaire with closed-ended questions was used to elicit information on schistosomiasis knowledge, attitudes and practices as well as the relationship between environmental conditions and schistosomiasis. It comprised (i) multiple-choice questions, (ii) the Likert rating scale, and (iii) dichotomous questions.

Multiple-choice questions required only one response, and the option was used for socio-demographic questions. The Likert scale (five-point scale) question type was used to assess participant schistosomiasis related attitudes and practices, as well as their perspectives on the relationship between environmental change and the occurrence of schistosomiasis. This is because, unlike multiple-choice questions, they have an inherent quantitative value that allows responses to be meaningfully ordered from low to high. Dichotomous questions were the simplest form of closed-end questions as respondents were provided with two options to select from, i.e., yes or no. These were used for questions pertaining to knowledge of the cause, transmission and symptoms of schistosomiasis (Struwig and Stead 2001). The questionnaire was divided into six sections as summarised in Table 2.13 below.

Table 2.13: Description of different sections of the household questionnaire administered to participants.

Section A	Inclusion/exclusion criteria to determine whether the respondents were suitable for participation in the study. If they did not meet the inclusion criteria, they were not allowed to complete the questionnaire.
Section B	Socio-demographic information (gender, level of education, occupation and access to safe water and sanitation).
Section C	Sought to determine the level of knowledge the respondents had regarding the cause, transmission and symptoms of schistosomiasis.
Section D	Sought to elicit the attitudes of the participants regarding the prevention and treatment of the disease.
Section E	Aimed at determining the practices that the participants engaged in that put them at risk of getting infected with schistosomiasis.
Section F	Questions for caregivers of children under the age of 15 years. Aimed to identify high-risk activities of children in the community.
Section G	Questions for participants who had been residing at HaNesengani for a period of over 15 years at the time of the study. Aimed to determine their views on the relationship between environmental change and the occurrence of schistosomiasis.

(ii) In-depth interview guide

The in-depth interview guide was developed following the administration of the household questionnaire and subsequent analysis of the data. The questions in the interview guide followed up on the questions from the household questionnaire and sought to further explain concepts and information that were gathered during the administration of the household questionnaire. The in-depth interview guide was semi-structured and was developed to allow flexibility in the manner and order in which questions were asked to provide the opportunity for dialogue during the interviews (Bartlett and Vavrus 2016). The interview guide contained two main sections: Section A focused on socio-demographic information, which sought to obtain respondents' details such as gender, level of education, occupation and access to water and sanitation. Section B included interview questions at two levels: main questions and probes.

All the participants were asked the first three main questions, in varying orders, depending on the respondents' answers. Following this, questions were asked depending upon the criteria that were used to select them for participation. For example, following the three main questions and their probes, healthcare providers were asked questions that pertained to the examination of patients, treatment options available and groups that are most likely to visit the healthcare

centre with schistosomiasis-related issues. This same structure was followed for caregivers of children under the age of 15 years, agricultural workers and participants who had been residing permanently at HaNesengani for more than 15 years at the time of the study

Data analysis

Data collected for the household questionnaires were captured using Google forms and exported into Excel Workbook format (.xlsx) for data pre-processing and analysis. Data were analysed with the Statistical Package for the Social Sciences (SPSS) version 26, using descriptive statistics such as central tendency (mean) to present findings in the form of tables and graphs. Pearson's Chi-Square Test of Independence was performed to establish whether relationships exist between participants' age, gender, and level of education and their level of knowledge. This test was also used to establish whether participants had positive or negative attitudes and practices regarding schistosomiasis. The results were described based on the selected significance level ($\alpha = 0.05$). Where the calculated p-value was greater than the significance level, it was concluded that there was no association (statistical significance) between the variables and vice versa.

Data from in-depth interviews were transcribed verbatim and then checked and verified for consistency against the audio files to ensure the transcription accuracy. The checked transcriptions were read several times to ensure familiarity with the data, which served as the foundation for data analysis. The transcripts for each of the respondents were further examined manually to identify relevant insights that would aid in explaining the results from the household questionnaires. A narrative analysis of relevant data from the interviews was included in the discussion of quantitative results, to provide a deeper understanding of the context.

CHAPTER 3: MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF MOLLUSC SPECIES IN SOUTH AFRICA

Compiled by: *L. de Necker, J.J. Pearson, M.H. Le Roux, N.J. Smit and V. Wepener*

3.1 Introduction

Aquatic molluscs are crucial primary consumers in freshwater ecosystems, serving as the foundation of the food web (Dudgeon et al. 2006). With the phylum Mollusca being second in number of species only to Arthropoda, identifying shared traits among its many taxa is daunting (Telford and Budd 2011). Taxonomic identification of freshwater molluscs can be challenging and may require specialised training to identify species based on shell morphology and internal anatomy (Pfenninger et al. 2006). This is particularly evident within species that are closely related. Identification using only morphological traits is sufficient in many cases to group specimens into particular morphological groups or genera, but it may be difficult to identify these specimens to species level due to cryptic differences in shell morphology. For example, morphological identification is typically used to classify *Bulinus* sp. into one of four morphological groups, three of which are found in southern Africa and act as hosts for schistosomiasis (Day and de Moor 2002), with identification to specific species level on morphology alone remaining a challenge. Furthermore, several distinct morphs of the highly invasive snail *Tarebia granifera* exist worldwide, with a high degree of variation in shell morphology (Veeravechsukij et al. 2018b). Appleton et al. (2009) reported two distinct morphological variants of *T. granifera* in KwaZulu-Natal, South Africa; one with a pale brown whorl and a dark spire, while the entire shell of the other is dark brown to almost black (Appleton et al. 2009). Veeravechsukij et al. (2018b) found two genetically different clades within the *T. granifera* population of Thailand. Due to these morphological variations found in *T. granifera*, molecular phylogenetic and phylogeographical analyses are needed to distinguish between the different morphotypes (Veeravechsukij et al. 2018b).

Failure to correctly identify native and invasive freshwater mollusc species is a cause for concern (Pfenninger et al. 2006). This is particularly true for medically, economically, and ecologically important species since incorrect identification may result in inaccurate parasitological studies and inhibit the effective assessment and implementation of control measures for various vector-borne diseases, including schistosomiasis (Hastein et al. 2005). Therefore, combining morphological and molecular analyses may offer a solution to the challenge of identifying freshwater molluscs, particularly those of medical and economic importance (Pfenninger et al. 2006; Lawton et al. 2015). The assessment of the internal transcribed spacer 2 (ITS2) nuclear ribosomal DNA and cytochrome c oxidase subunit 1 (COI) mitochondrial gene regions as species identification markers indicate a higher phylogenetic resolution. The COI genetic region also

provides new insight into the evolutionary relationships of mollusc species (Lawton et al. 2015). Therefore, the use of morphological identification and molecular analysis of the ITS2 and COI genetic regions is necessary to accurately identify mollusc species and contribute to the continued debate regarding the evolutionary relationships of mollusc species. Molecular studies on the phylogenetic diversity of *T. granifera* are lacking (Veeravechsukij et al. 2018b; Yin et al. 2022) and the genetic diversity of *T. granifera* in South Africa remains unexplored. Since both parasites and snail hosts can show a high degree of cryptic diversity, and since many host-parasite associations are highly species-specific, it is important to accurately identify the lineages of *T. granifera* that could serve as potential trematode hosts in invaded regions. Therefore, studies on the genetic diversity of *T. granifera* populations in South Africa are needed and phylogeographical analysis may shed some light on where this invader came from, and which potential trematodes may be able to infect *T. granifera* in South Africa.

The aims of this chapter were as follows: 1) to accurately identify native freshwater mollusc species known to transmit schistosomiasis in South Africa using a combination of morphological identification from published literature and molecular analyses of the conserved genetic region; and 2) to determine the genetic diversity of *T. granifera* from the selected sites on the Limpopo River system and Phongolo River with the use of molecular and phylogenetic analyses.

3.2 Methods

3.2.1 Field collection and laboratory assessment

Freshwater molluscs were collected and identified using morphological characteristics (see Chapter 2, section 2.3.3). All collected snails were assessed for potential trematode infections using a cercarial shedding experiment (see Chapter 2, section 2.3.4) after which they were sacrificed and the tissue visually assessed for any parasitic infections. Thereafter, tissue samples were collected from the snails to accurately identify the snails to species level using molecular techniques (see Chapter 2, sections 2.3.5 and 2.3.6).

3.2.2 Data analysis

Detailed data analyses are indicated in Chapter 2, section 2.3.5 and 2.3.6

3.3 Results

3.3.1 Bulinidae

A total of 225 specimens were collected and morphologically identified to be members of the family Bulinidae, specifically species of the genus *Bulinus*. It is not easy to distinguish between *Bulinus* species due to the many overlapping morphological similarities between species of this genus. The *Bul. africanus/globosus* group and the *Bul. truncatus/tropicus* group are the two

groups native to South Africa that have the most overlapping morphological features, as both groups have a globose shape with a short spire. Specimens belonging to *Bul. africanus/globosus* were identified by their globose shape, short spire, and large basal whorl. Both *Bul. africanus* and *Bul. globosus* have a fold on the ventral surface, a distinguishing characteristic of this group. The *Bul. truncatus/tropicus* group is identifiable by having a globose shape shell, moderately developed spire, and rounded columella. A total of 115 specimens collected belonged to the *Bul. africanus/globosus* group (Figure 3.1; A-B), and 110 specimens were identified to belong to the *Bul. truncatus/tropicus* group (Figure 3.1).

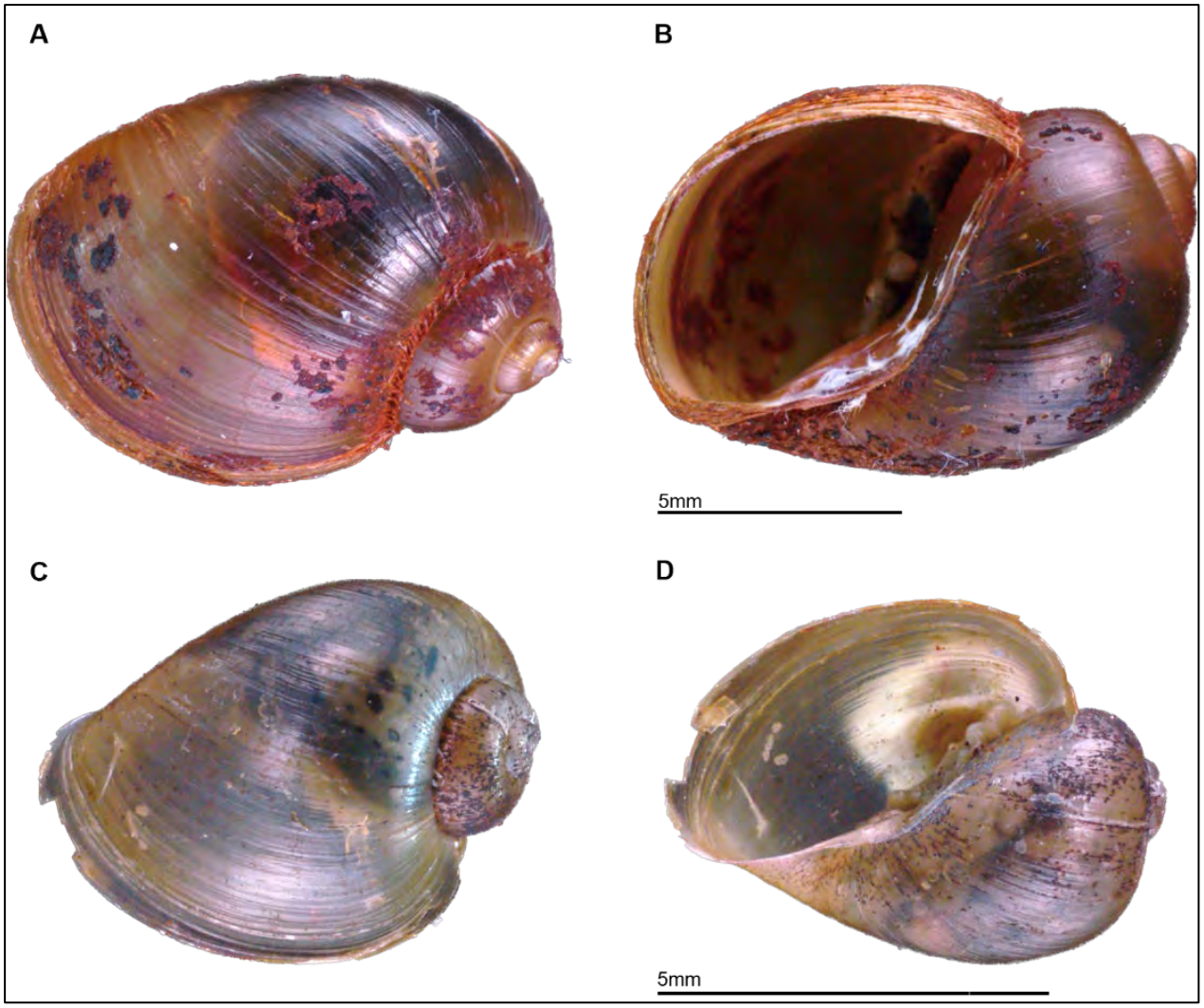

Figure 3.1: Bulinidae specimens collected during the current study. Morphologically identified as (A-B) *Bulinus africanus*: (A) the ventral view of *Bulinus africanus*; and (B) the dorsal view. (C-D) Identified as *Bulinus depressus*: (C) the ventral view of *Bulinus depressus;* and (D) the dorsal view. (A-D scale bar = 5 mm).

The molecular analyses resulted in 24 novel sequences. Phylogenetic trees were generated based on the ITS2 (601 base pairs; Figure 3.2; Table S3.1 and S3.2) and COI (491 base pairs; Figure 3.3; Table S3.3). Phylogenetic analysis of the novel ITS2 sequences showed that the specimens from this study fell within two distinct clades (Figure 3.2). The first group of six sequences formed a clade with available sequences of the *Bul. africanus/globosus* group and ten sequences grouped with the *Bul. truncatus/tropicus* group. The six sequences all grouped with published *Bul. africanus* sequences retrieved from GenBank (AM921970), confirming the morphological identification of these species as *Bul. africanus*. The ten sequences in the *Bul. truncatus/tropicus* group were similar to available sequences of *Bul. truncatus*, providing a positive molecular identification of these specimens.

The COI mtDNA analysis indicated a separate phylogenetic group within the *Bul. truncatus/tropicus* group (Figure 3.3). Morphologically these species were identified in the field as *Bul. tropicus* and some as *Bul. depressus*. It is clear from the phylogenetic analysis that these novel gene sequences did not clade with *Bul. tropicus* or *Bul. truncatus*, forming a separate clade. The separation but close phylogenetic relationship between *Bul. tropicus* and the distinct clade formed by the samples generated during this study were identified as *Bul. depressus.*

All the Bulinidae species collected were within the Limpopo Province, South Africa. Members of the *Bul. africanus/globosus* group were collected at more sites than *Bul. truncatus/tropicus* (Figure 3.4). Only four locations where both *Bulinus* groups were collected had more than one of the two groups present (LLF7, LLF8, LLF11, LLF14). Morphological identification was accurate to the specific *Bulinus group*. However, the combination of morphological analysis and phylogenetic analysis supported the designation of *Bul. africanus* and *Bul. depressus.*

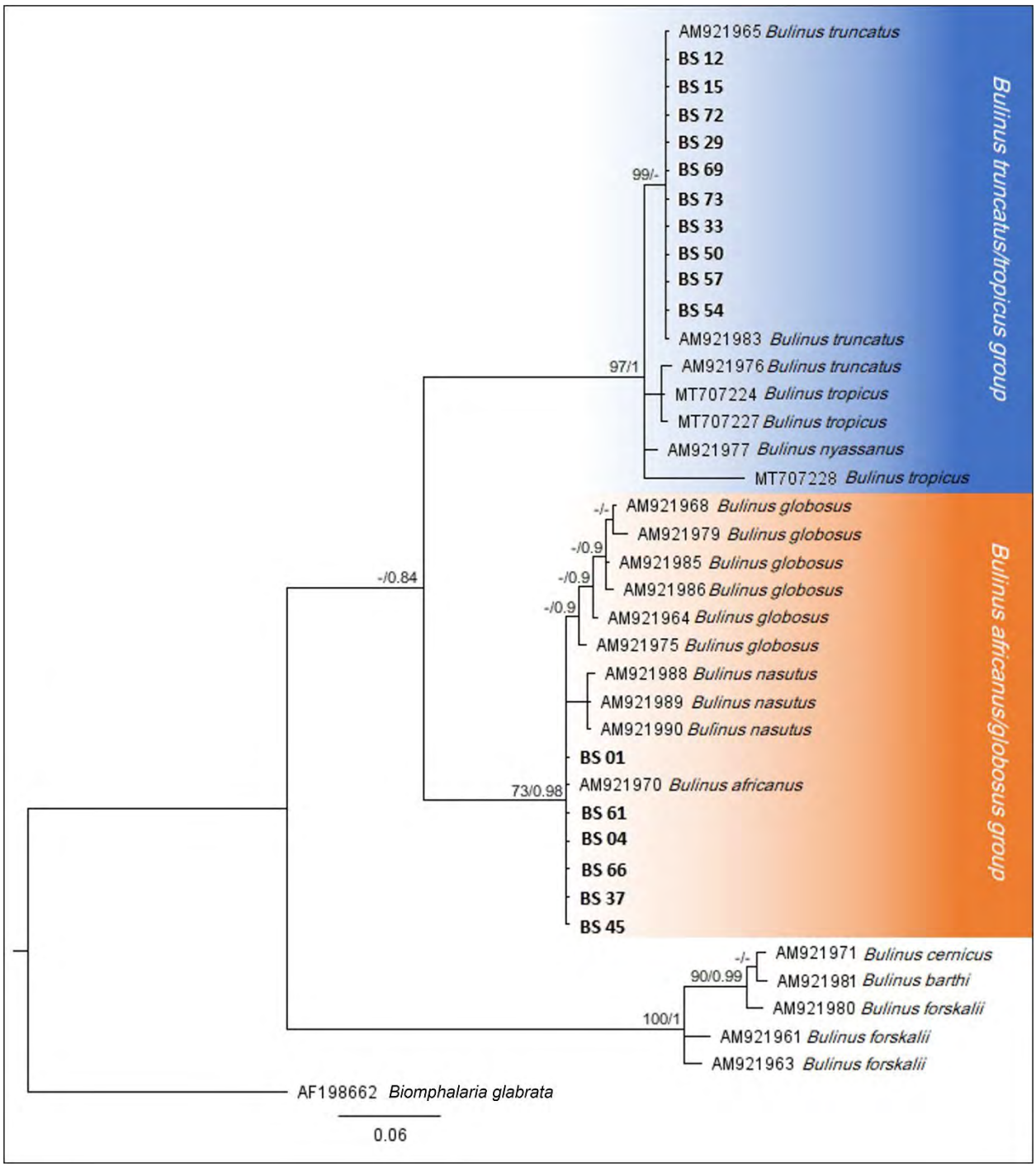

Figure 3.2: Bayesian inference phylogram of *Bulinus* genus based on partial sequences of the ITS2 rDNA region. New sequences are in bold. Posterior probability followed by bootstrap support values are indicated next to the branches (posterior probability < 70 and bootstrap < 0.80 not shown). *Biomphalaria glabrata* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site. Sequences generated during the current study are represented in bold. See Tables S3.1 and S3.2 for base pair differences.

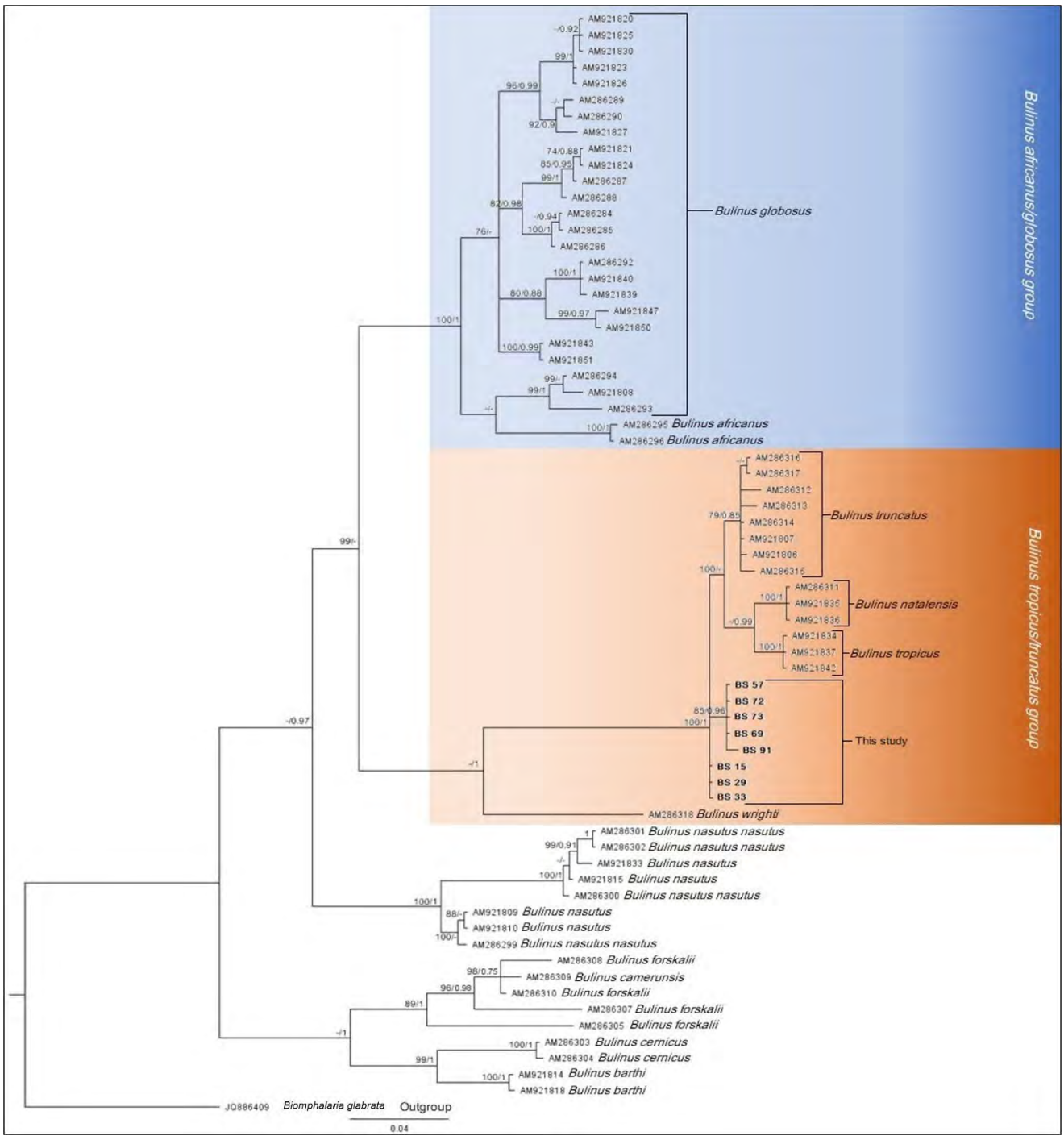

Figure 3.3: Bayesian inference phylogram of *Bulinus* genus based on partial sequences of the COI mtDNA gene. New sequences are in bold. Posterior probability followed by bootstrap support values is indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Biomphalaria glabrata* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site. Sequences generated during the current study are represented in bold. See Table S3.3 for base pair differences.

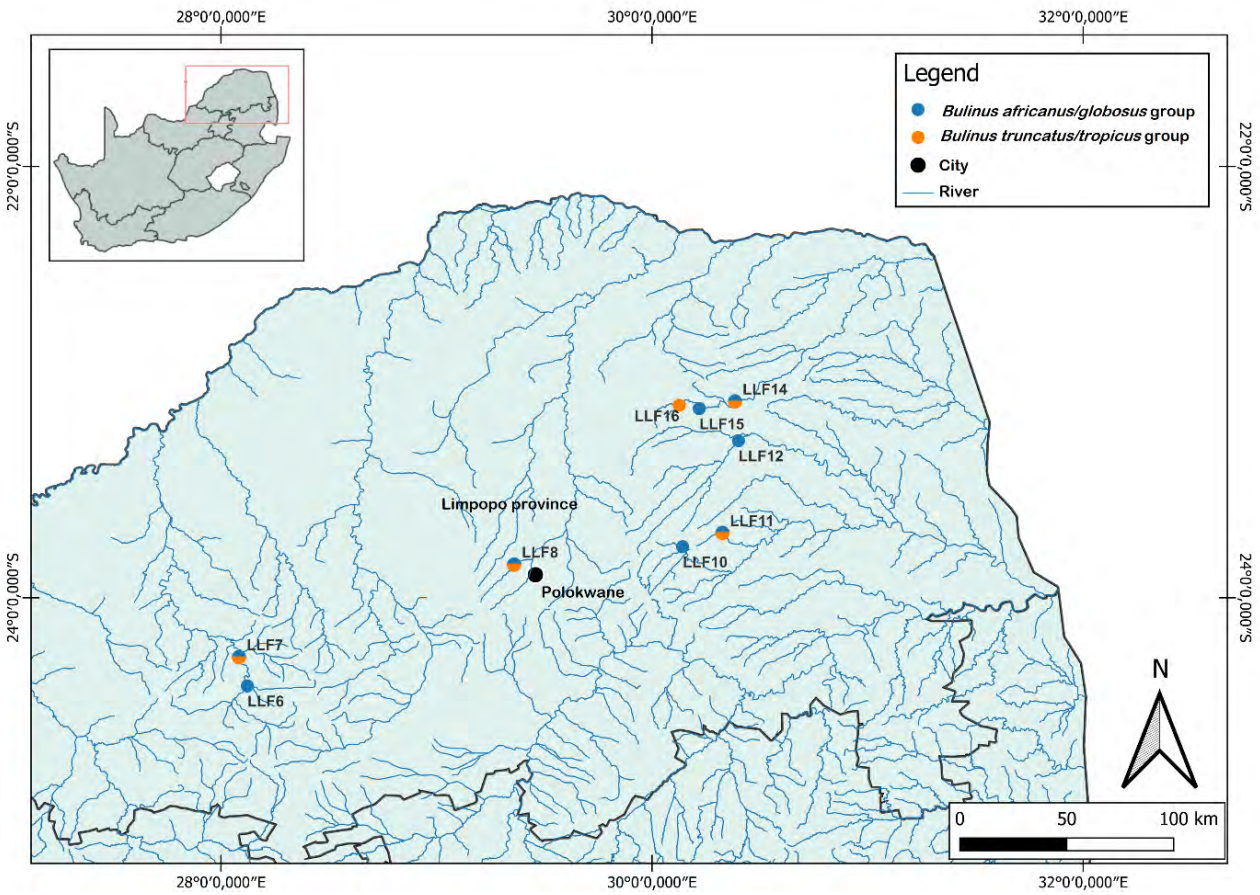

Figure 3.4: Map indicating locations where *Bulinus* specimens were collected and identified using molecular techniques. *Bulinus africanus/globosus* group is indicated with a blue dot on the map, and *Bul. truncatus/tropicus group* is indicated with an orange dot. Sites where both groups were collected are indicated with a blue and orange dot.

3.3.2 Planorbidae

A total of 294 specimens were collected in this study and were morphologically identified, based on their shell morphology, as members of the Planorbidae family. Specimens collected during this study were identified as species of two of these, the *Biomphalaria* and *Gyraulus*. Originally, based on morphological characteristics, the specimens were identified in the field as *Biomphalaria pfeifferi* based on the circular shape of the umbilicated shell and the even rounded basal whorl (Figure 3.5; A-B). A second species of *Biomphalaria*, *Biomphalaria glabrata,* was identified morphologically in the field. However, in the laboratory, all these specimens were again analysed and re-identified as *Gyraulus costulatus*. The morphological similarities of *Bio. glabrata* and *G. costulatus* resulted in the mistakenly identified specimens since both species have an angular basal whorl and are almost similar in size. Further identification under a dissection microscope confirmed the identification of *G. costulatus* with the observation of strong, regularly spaced transverse ribs (Figure 3.5; C-D). The third Planorbidae species collected was identified as a member of the *Gyraulus* genus in the field and confirmed to be *G. connollyi* (Figure 3.5; E-F) in the laboratory by observing the presence of fine growth lines, the rounded shoulder of the basal whorl and the aperture deflecting downwards that are characteristic of this species.

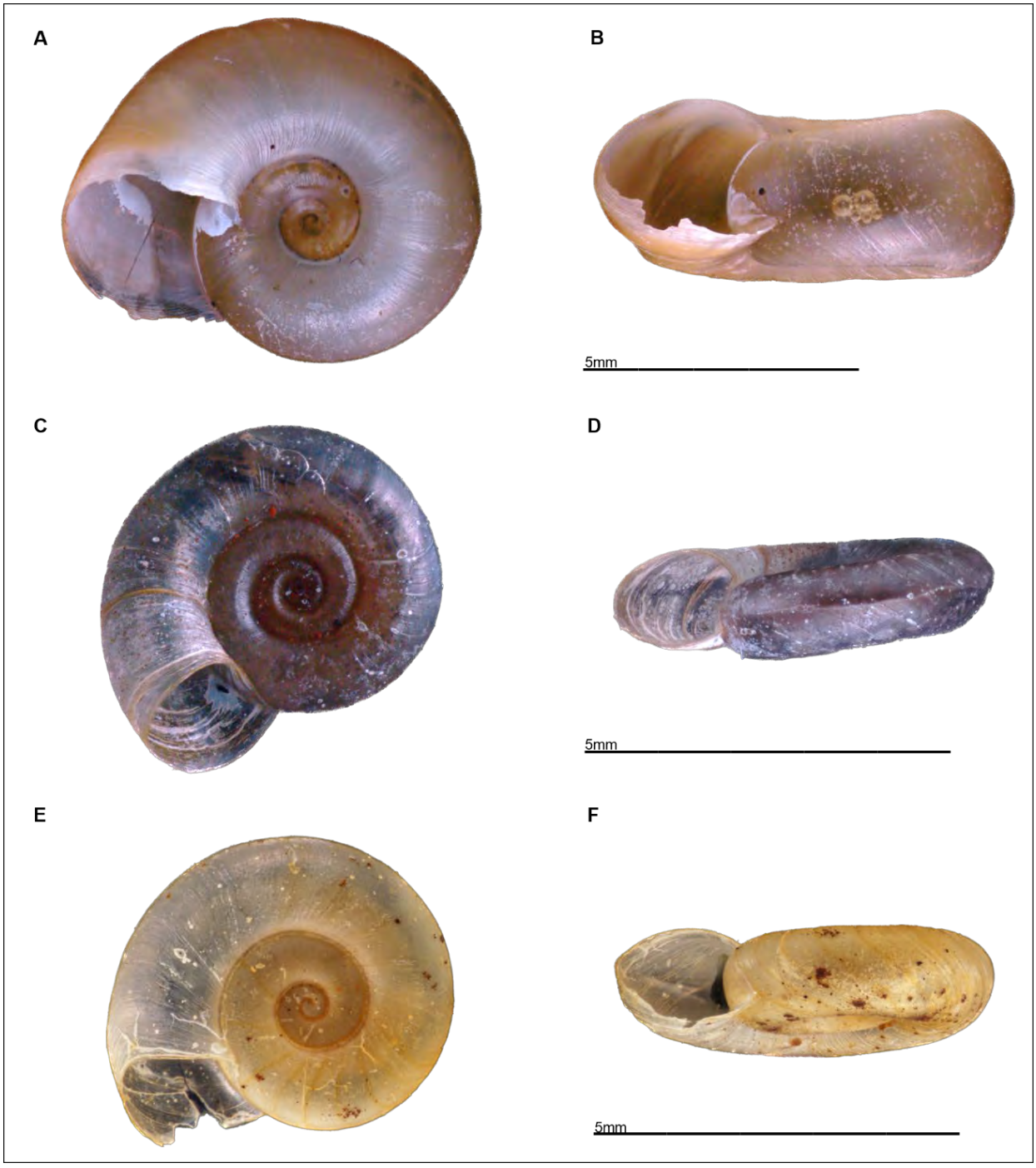

Figure 3.5: Planorbidae specimens of *Biomphalaria* and *Gyraulus* species collected during the current study. Morphologically identified as *Biomphalaria pfeifferi* (A-B): (A) the ventral view of *Biomphalaria pfeifferi;* and (B) the dorsal view. (C-D) Identified as *Gyraulus costulatus*: (C) the ventral view of *Gyraulus costulatus*; and (D) the dorsal view. (E-F) Identified as *Gyraulus connollyi*: (E) the ventral view of *G. connollyi;* and (F) the dorsal view. (A-F scale bar = 5 mm).

The molecular analyses resulted in 12 novel sequences for these species of Planorbidae. Phylogenetic trees were generated using sequences of ITS2 (544 bp; Figure 3.6) and COI (527 bp; Figure 3.7) genetic regions. The phylogenetic tree generated for the ITS2 rDNA gene indicated that the five novel sequences morphologically identified to belong to *Biom. pfeifferi* were confirmed by the phylogenetic analysis with good nodal support. The base pair differences between the sequences generated during this study and the sequences of *Biom. pfeifferi* published on GenBank, indicated zero to one base pair difference (Table S3.4). The interspecific variation for *Biomphalaria* species in this dataset is 0% (0–1 nt).

Gyraulus sp. (BP11 and BP19) collected in the North West Province, South Africa, grouped with good support with *G. convexiusculus.* The identification of *G. convexiusculus* is improbable as this species is primarily found in the Middle East and, to date, has not been recorded in South Africa or on the African continent. These findings indicate a close evolutionary relationship between these two species. The base pair differences between the sequences generated during this study and the sequences of *Gyraulus* published on GenBank, indicated 10 to 11 base pair differences (Table S3.5). The interspecific variation for *Gyraulus* species in this dataset was 0-2% (10-11 nt). No sequences of the ITS2 region were available on GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) for *G. costulatus* to compare the sequences generated during this study. Only COI gene sequence analysis was used for samples collected in the Seekoeivlei Nature Reserve.

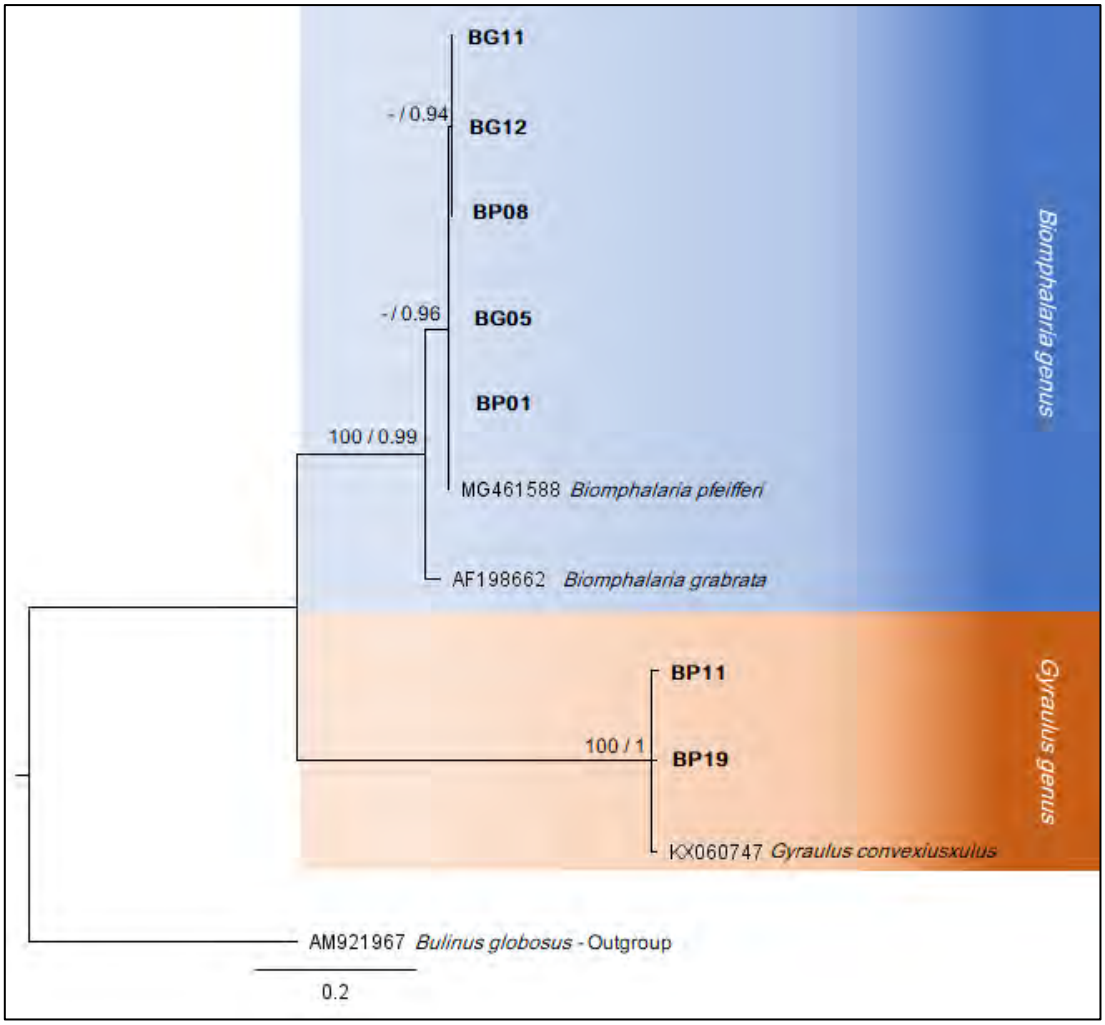

Figure 3.6: Bayesian inference phylogram of species of the genera *Biomphalaria* and *Gyraulus* based on partial sequences of the ITS2 rDNA region. New sequences are in bold. Posterior probability followed by bootstrap support values are indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Bulinus globosus* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site. Sequences generated during the current study are represented in bold.

The phylogenetic tree for the COI gene supported the morphological identification of *Biomphalaria pfeifferi* with the base pair differences between the sequences generated during the current study and the sequences of *Biom. pfeifferi* published on GenBank, indicating one to six base pair differences (Figure 3.7; Table S3.6). The interspecific variation for *Biomphalaria* species in this dataset was 0-7% (1-7 nt). Molecular analysis in the case of *Gyraulus* was only able to positively confirm these specimens to genus level and confirm that two different species are present. The two species of *Gyraulus* collected during the current study indicated considerable interspecific variation based on the base pair differences and difference in percentage identity between species collected in the North West Province (BP19) and the specimens collected in the Seekoeivlei Nature Reserve (SX1,15,17, JX12) within the Free State Province (Table S3.7). The samples collected at the Seekoeivlei Nature Reserve grouped phylogenetically with a published sample of *G. chinensis* (AF199086). As there are no available sequences of these two species in GenBank and the morphological identification confirmed them as *G. costulatus* and *G. connollyi,* respectively, this study constitutes the first genetic data for these two species (Figure 3.5). The distribution of *Biom. pfeifferi* is expected in the north-eastern parts of South Africa, as confirmed during the current study (Figure 3.8). *Gyraulus costulatus* was collected at the sites sampled in the Limpopo Province of South Africa, and *G. connollyi* was identified at sites sampled in the Seekoeivlei Nature Reserve within the Free State Province of South Africa (Figure 3.8).

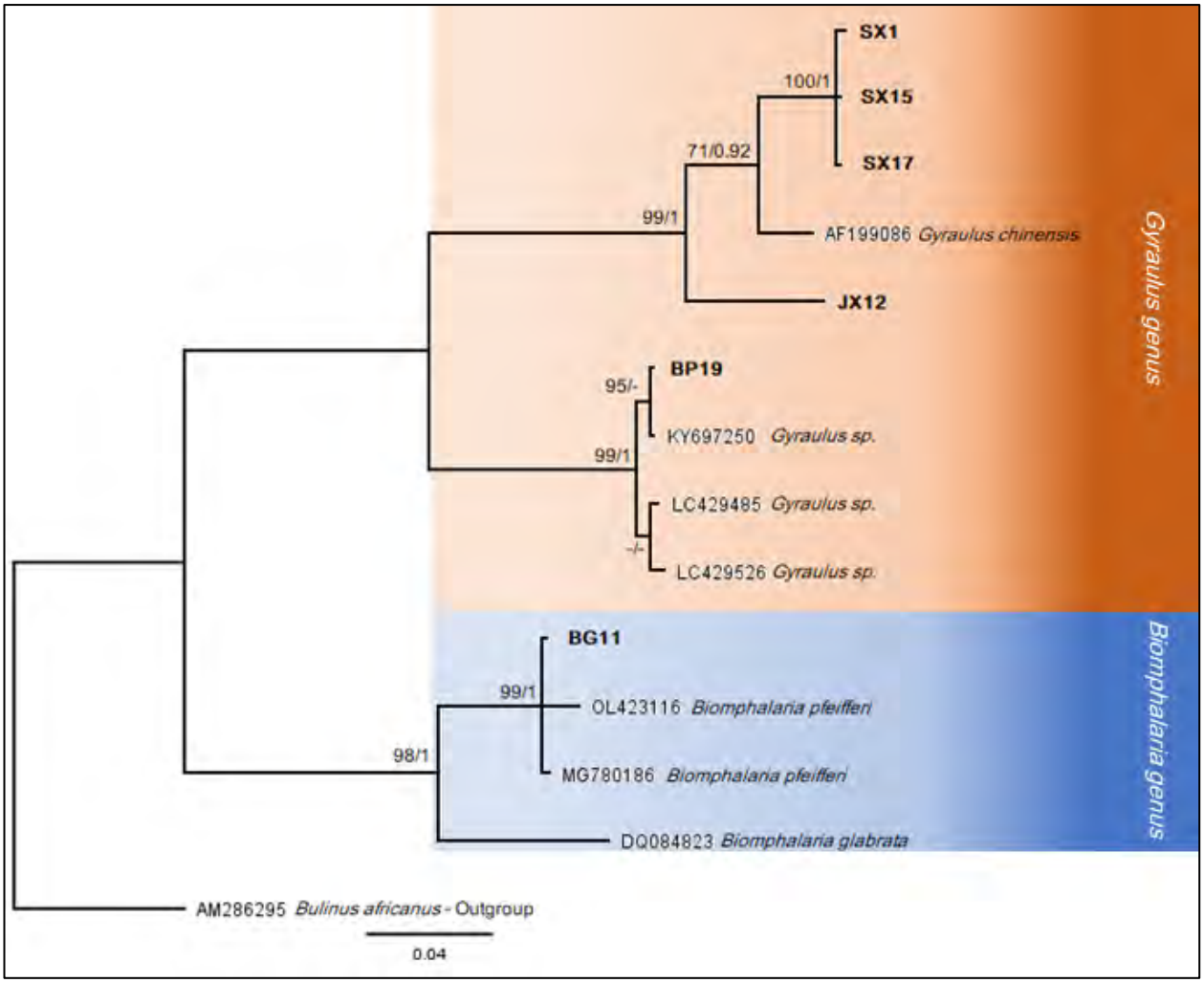

Figure 3.7: Bayesian inference phylogram of species of the genera *Biomphalaria* and *Gyraulus* based on partial sequences of the COI mtDNA gene. New sequences are in bold. Posterior probability followed by bootstrap support values is indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Bulinus africanus* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site. Sequences generated during the current study are represented in bold.

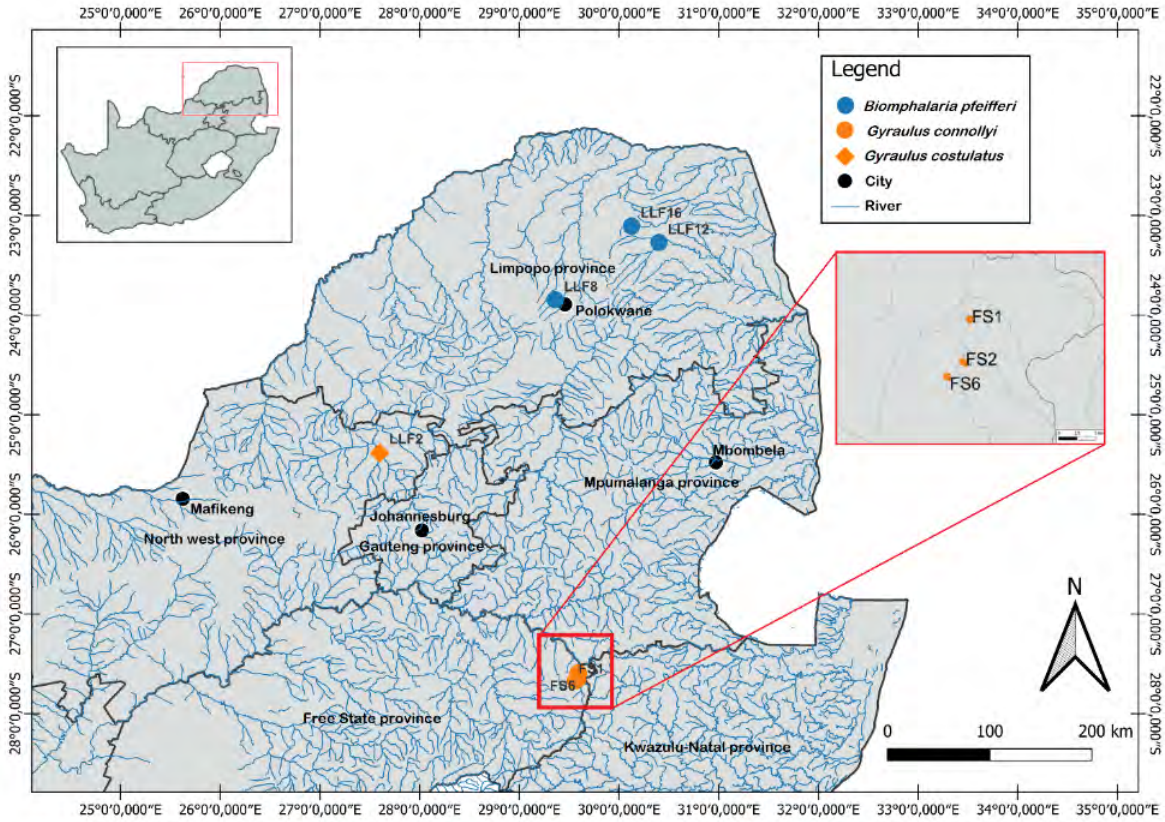

Figure 3.8: Map indicating sites where specimens belonging to the *Biomphalaria pfeifferi* (indicated by the blue dots), *Gyraulus connollyi* (indicated by the orange dots) and *Gyraulus constulatus* (indicated by the orange diamond-shaped icon) were collected. The area within the red square is the site sampled within the Seekoeivlei Nature Reserve. Enlargement of the area within the red square indicates the three sites sampled within Seekoeivlei Nature Reserve, where specimens of *G. connollyi* were collected.

3.3.3 Lymnaeidae

In total, 264 mollusc specimens were collected from 13 sites in South Africa's Limpopo and North West Provinces and easily morphologically identified as members of the Lymnaeidae family. Specimens were morphologically identified to be species of *Radix* and *Pseudosuccinea*. A total of 221 of the specimens were morphologically identified to be *R. natalensis* based on the remarkably swollen basal whorl and the distinctly visible growth lines. The remaining 43 specimens were morphologically identified as *Pseudosuccinea columella* based on the swollen basal whorl, but less swollen than the basal whorl of *R. natalensis* and the presence of growth and ridge lines. The molecular analyses resulted in 36 novel sequences of *R. natalensis* and *Pseudosuccinea columella*. Phylogenetic trees were generated using sequences of ITS2 (477 bp; Figure 3.9) and COI genetic regions (426 bp; Figure 3.10).

The ITS2 sequences obtained confirmed the morphological identification of *R. natalensis* with good nodal support (Figure 3.9). The base pair differences between the sequences generated during this study and the sequences of *R. natalensis* published on GenBank, indicated zero to two base pair differences, except for specimens RN58 and RN02 collected at sites LLF14 and LLF04 during the current study (Table S3.8). The sequences showed a 16 to 18 base pair difference between all of the *R. natalensis* sequences published, as well as the sequences generated during the current study when compared. However, the phylogenetic tree generated visually indicated that these two specimens clade with the *R. natalensis* species (Figure 3.9). The interspecific variation for *Radix* species in this dataset was 6% (0–18 nt). The genetic analysis of the ITS2 region confirmed the identification of *Pseudosuccinea columella* clade with good nodal support (Figure 3.9). The base pair differences between the sequences generated during this study and the sequences of *Pseu. columella* published on GenBank, indicated mostly zero base pair difference, with one exception of one sequence (JN614470; Table S3.9). This was collected at Rionegro, Colombia by Correa et al. (2011) and indicated a three base pair difference from the other sequences in the dataset. The interspecific variation for *Pseudosuccinea* species in this dataset was 1% (0–3 nt).

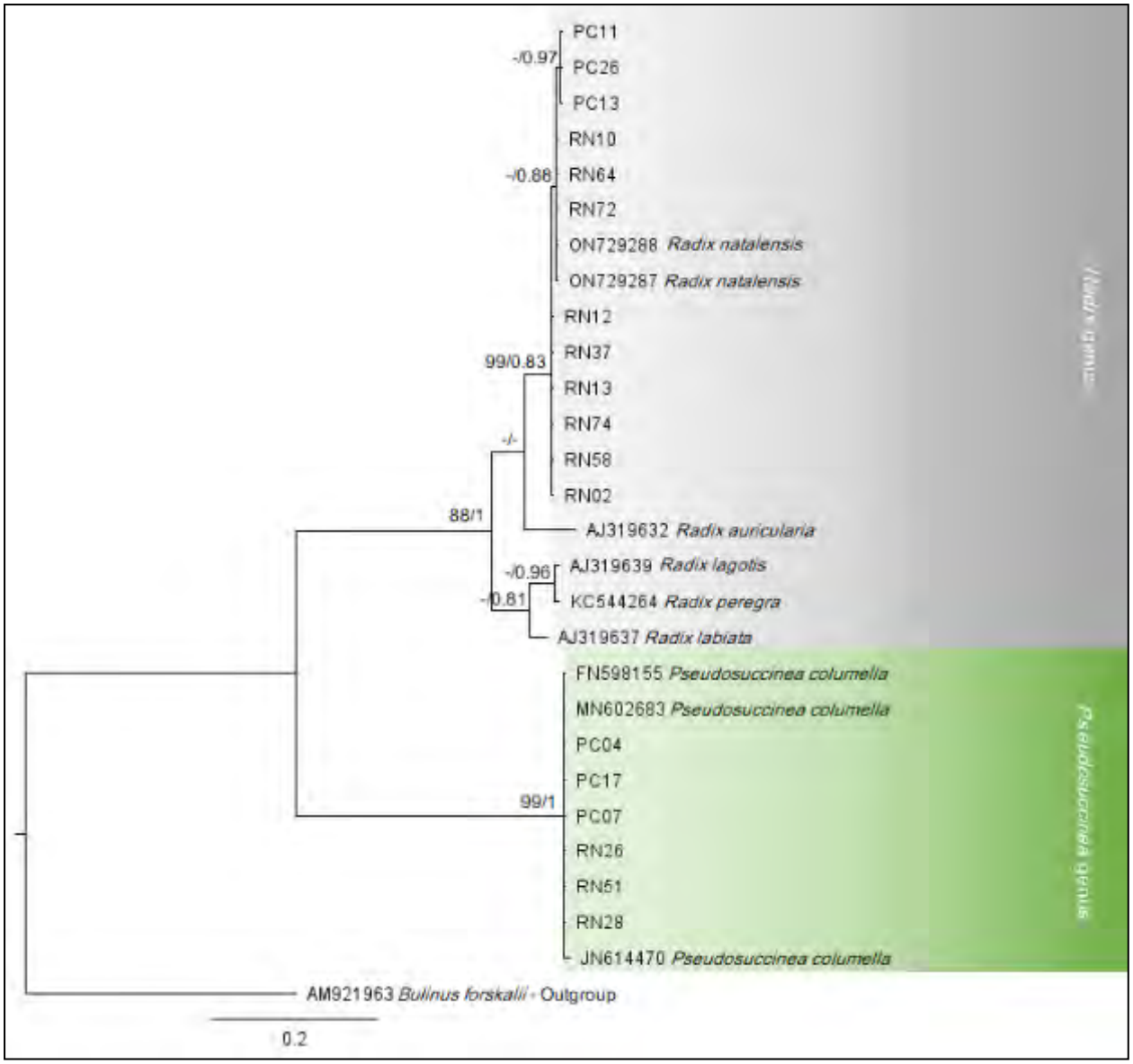

Figure 3.9: Bayesian inference phylogram of species of the genera *Radix* and *Pseudosuccinea* based on partial sequences of the ITS2 region. New sequences are labelled PC and RN. Posterior probability followed by bootstrap support values are indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Bulinus forskalii* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

Phylogenetic analysis of *R. natalensis* based on the COI gene indicated that the species were grouped into four monophyletic clades within the *R. natalensis* species group. Genetically, the base pair variations between the COI gene of the novel sequences were limited, with only 2-17 base pair differences between *R. natalensis* COI gene sequences (Table S3.10). The groups identified by the phylogenetic analysis were geographically isolated (Figure 3.11), and the sites are colour coded to relate to Table S3.10. The locations where different monophyletic clades were collected are separated from each other, as seen on the map (Figure 3.11), and the sites where the same monophyletic clades were collected are not in close proximity. The only location with an overlap of species is site LLF13 that contained species of both groups 2 and 3. The COI gene sequences generated confirmed the morphological identification of *Pseu. columella*. No genetic variance was indicated between *Pseu. columella* specimens based on the *Pseudosuccinea* dataset, with 100% sequence similarity and zero base pair differences (Table S3.11). The *Pseu. columella* specimens were mainly collected in the Limpopo Province of South Africa, as indicated in Figure 3.12.

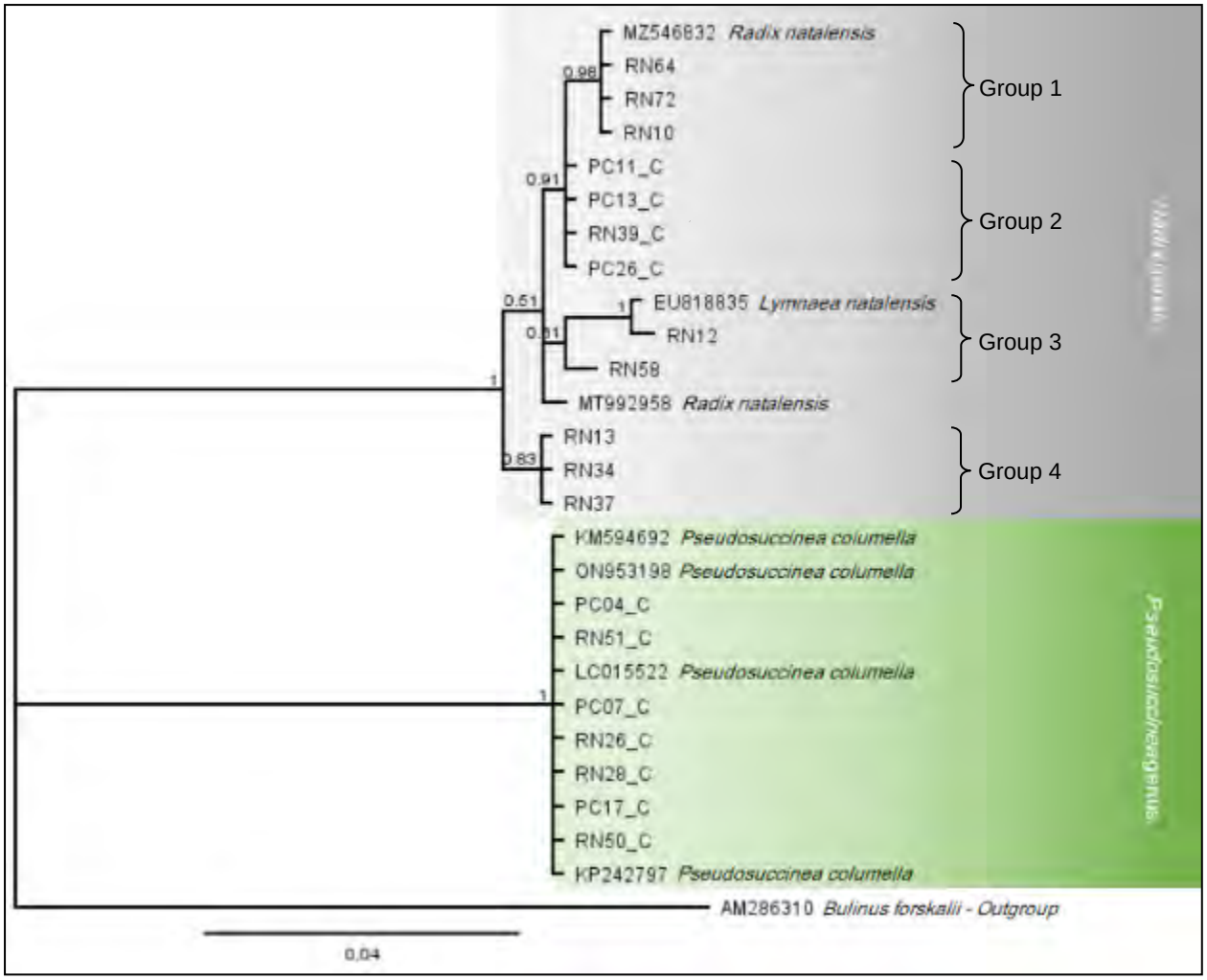

Figure 3.10: Bayesian inference phylogram of species of *Radix* and *Pseudosuccinea* based on partial sequences of the COI mtDNA gene. New sequences are labelled PC and RN. Posterior probability followed by bootstrap support values are indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Bulinus forskalii* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

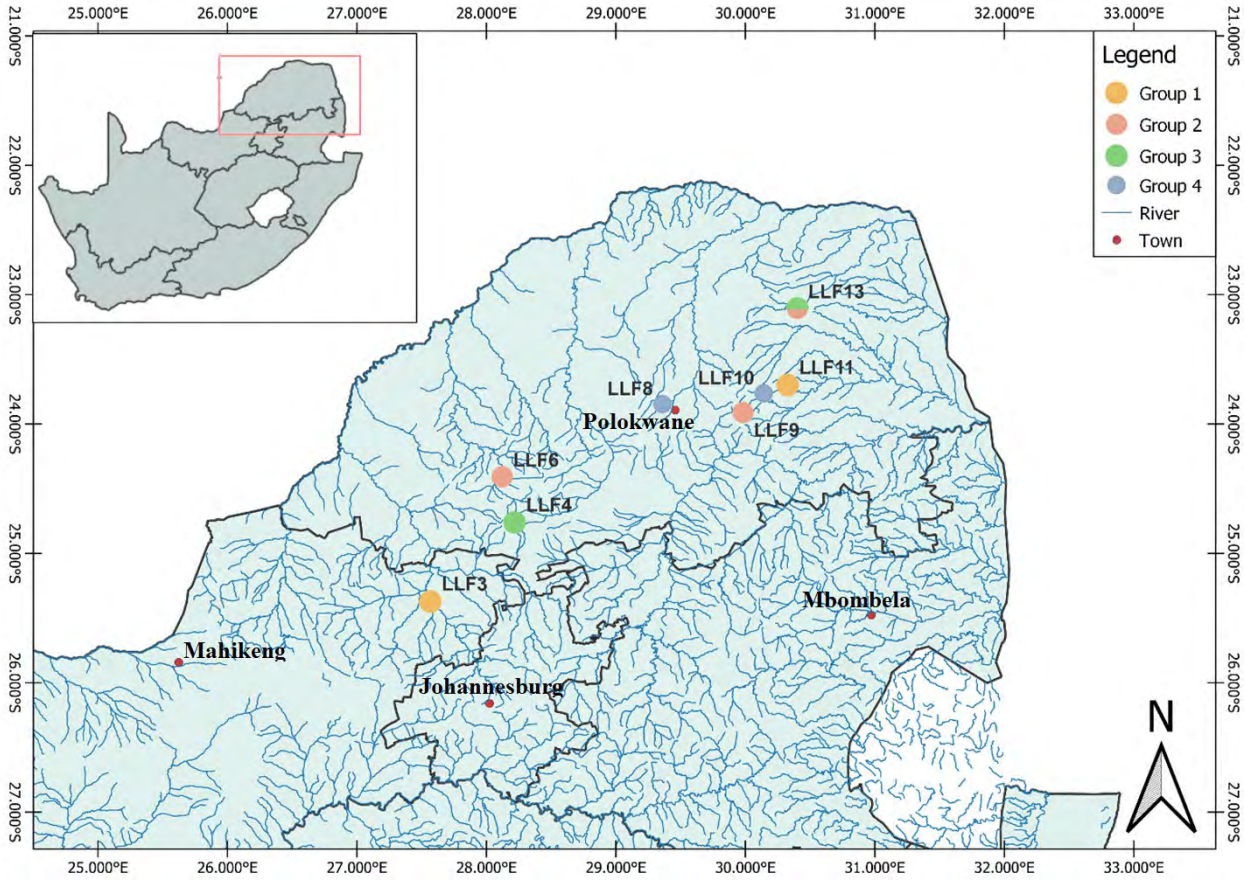

Figure 3.11: Map indicating the sites with *Radix natalensis* that phylogenetically grouped between locations based on the COI gene mutations. A yellow dot characterises areas with monophyletic group 1 present, pink for group 2, green for group 3 and purple for group 4. The only site with two of the monophyletic groups present was site LLF13 with a green and pink dot on the map, indicating that monophyletic groups 2 and 3 were present.

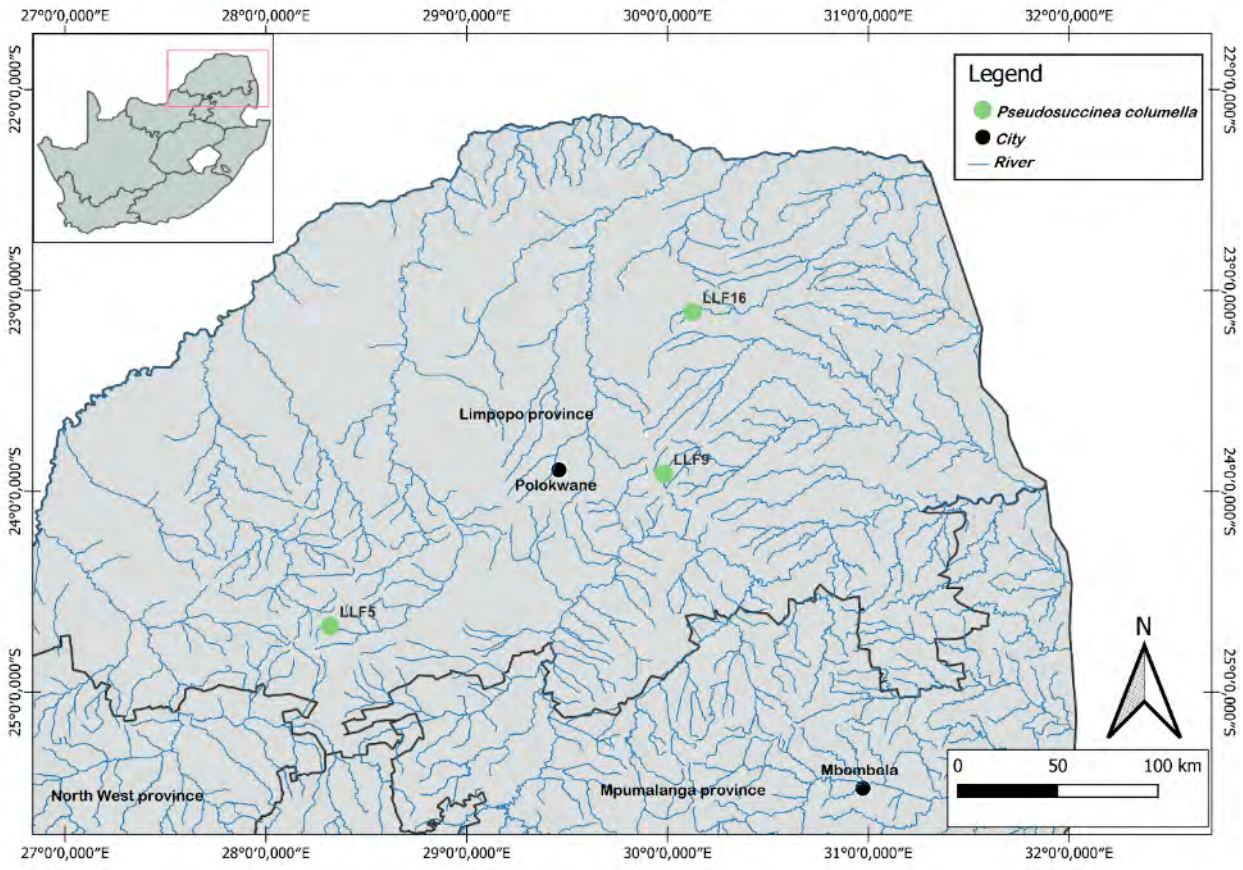

Figure 3.12: Geographical location of the three sites (LLF5, LLF9 and LLF16) where specimens identified as *Pseudosuccinea columella* were collected during the present study in Limpopo Province.

3.3.4 Physidae

A total of 140 specimens belonging to the Physidae were collected at three sites within the Limpopo Province, South Africa. The specimens collected were morphologically identified as *Physella acuta*. The morphological identification was based on the characteristics of the ovate shell shape, the spire being sharply pointed and the glossy texture of the shell. Molecular analyses were conducted, and a single novel sequence of *Phy. acuta* was generated from the specimens collected. Phylogenetic analysis was based on sequences of the *Physella* COI genetic dataset (Figure 3.13). The *Physella* specimen collected was identified as belonging to African clade 3 as defined by Lawton et al. (2018). The specimen used for genetic analysis was collected at site LLF4, as indicated on the map below (Figure 3.14). The sequences showed a zero to 15 base pair difference between *Phy. acuta* published sequences when compared to the sequences generated during the current study (Table S3.12). In this dataset, the interspecific variation for *Phy. acuta* species within African clade 3 was 0-2% (0-15 nt).

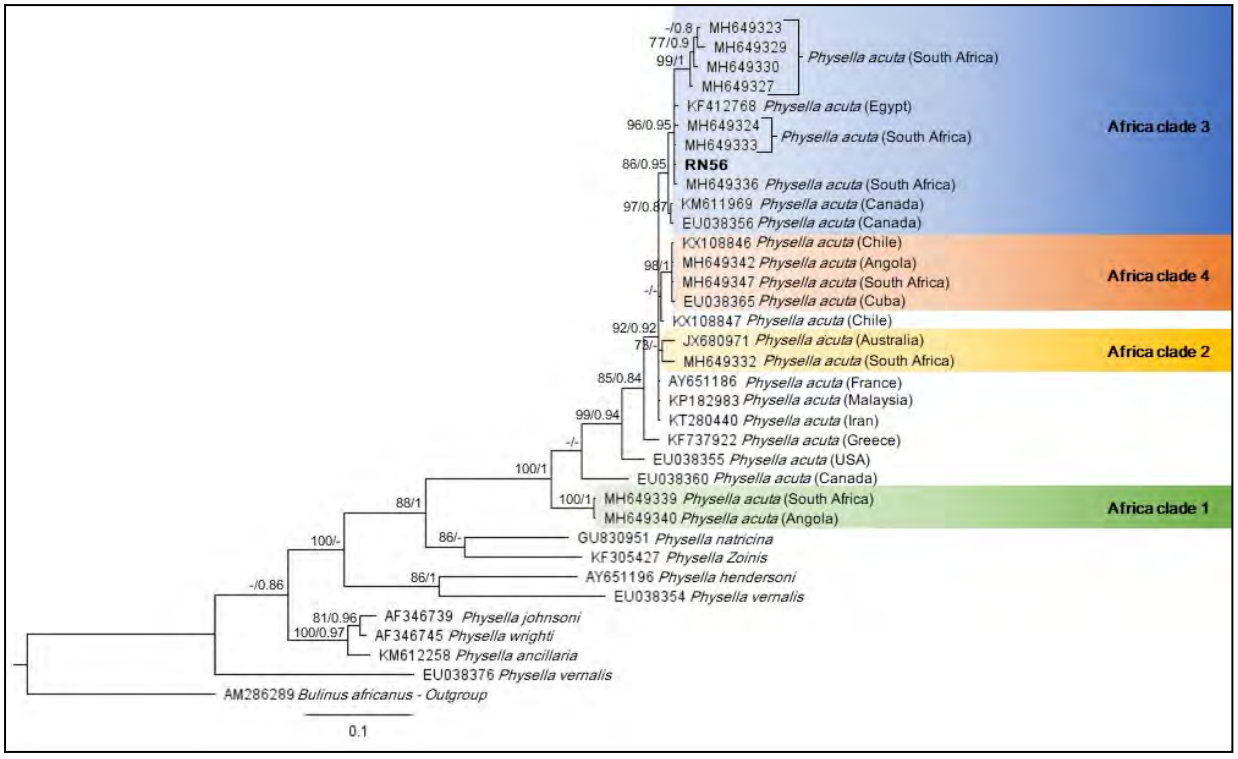

Figure 3.13: Bayesian inference phylogram of species of *Physella* based on partial sequences of the COI mtDNA gene. New sequences are in bold. Posterior probability followed by bootstrap support values are indicated next to the branches (posterior probability <70 and bootstrap <0.80 not shown). *Bulinus africanus* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

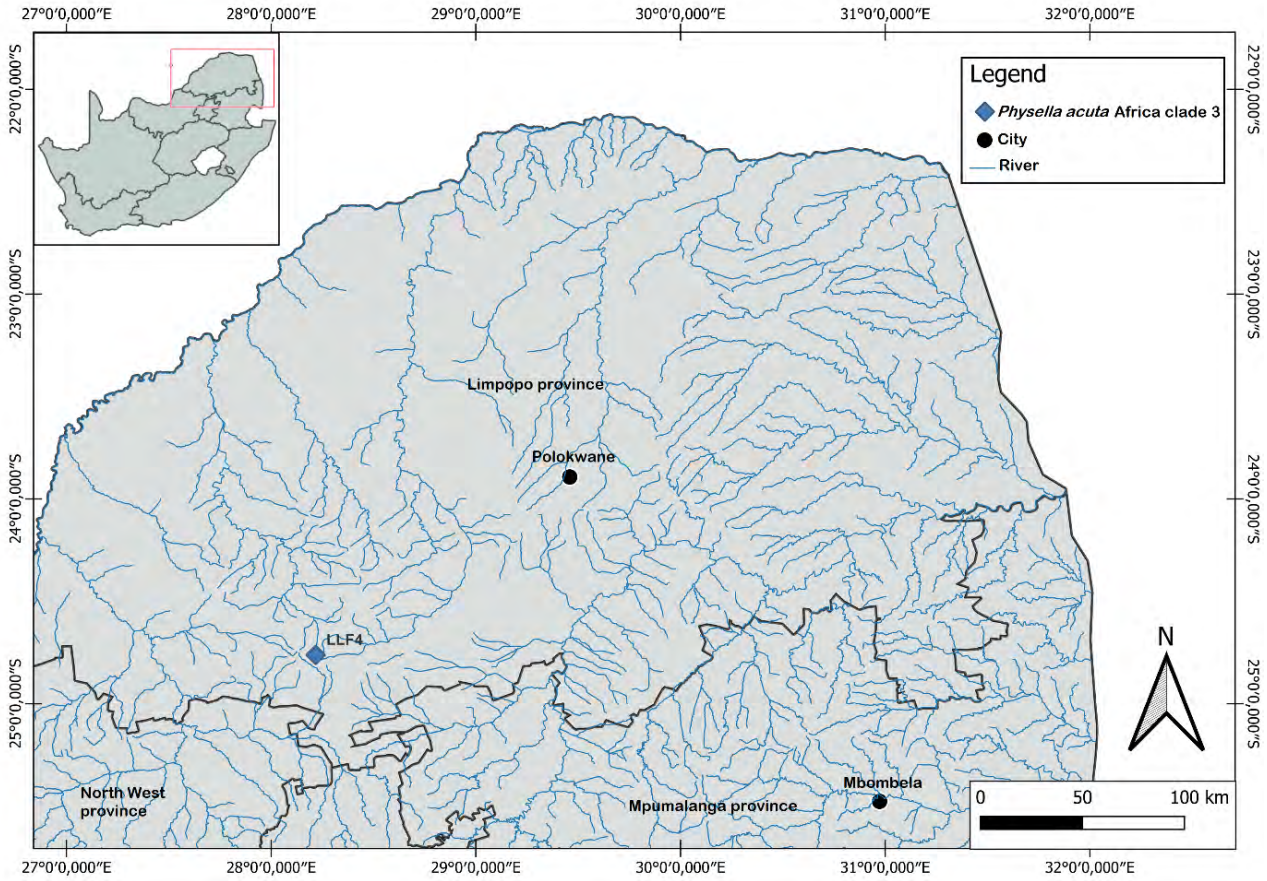

Figure 3.14: Geographical location of site LLF4, where the specimen identified as *Physella acuta* used for genetic analysis was collected and identified to belong to African clade 3.

3.3.5 Tarebia granifera

All *T. granifera* COI sequences generated in the present study were found to be genetically identical (100% similar), thus only one *T. granifera* sequence per site was used for the phylogenetic analysis. Within the phylogenetic tree based on the COI dataset (Figure 3.15), *T. granifera* sequences (657 bp) generated in the present study formed a highly supported clade with sequences of *T. granifera* from China (GenBank accession No. MZ662113; Table S3.13) and Thailand (GenBank accession No. MK000375). No intraspecific divergence was observed between the aforementioned COI sequences of *T. granifera* within this clade, thus the sequences generated in the present study were 100% identical to those from China and Thailand. The overall intraspecific variation between the *T. granifera* COI sequences generated in the present study and those of the other reference sequences used for analyses was between 1.8 to 3.6% (11-21 bp), with *T. granifera* from the USA (GenBank accession No. MT671805) and Thailand (GenBank accession No.MK000294) indicating the lowest divergence, while *T. granifera* from Singapore (GenBank accession No. AY958762) indicated the highest intraspecific divergence. For the COI sequences, the interspecific divergence between *T. granifera* from the present study was lowest for *Tarebia lineata* (Gray 1828) from

Malaysia (GenBank accession No. MW591171) with 14.2% (93 bp), while the highest interspecific sequence divergence was observed for *Tarebia lineata* from India (GenBank accession No. KY511306) with 16.1% (106 bp).

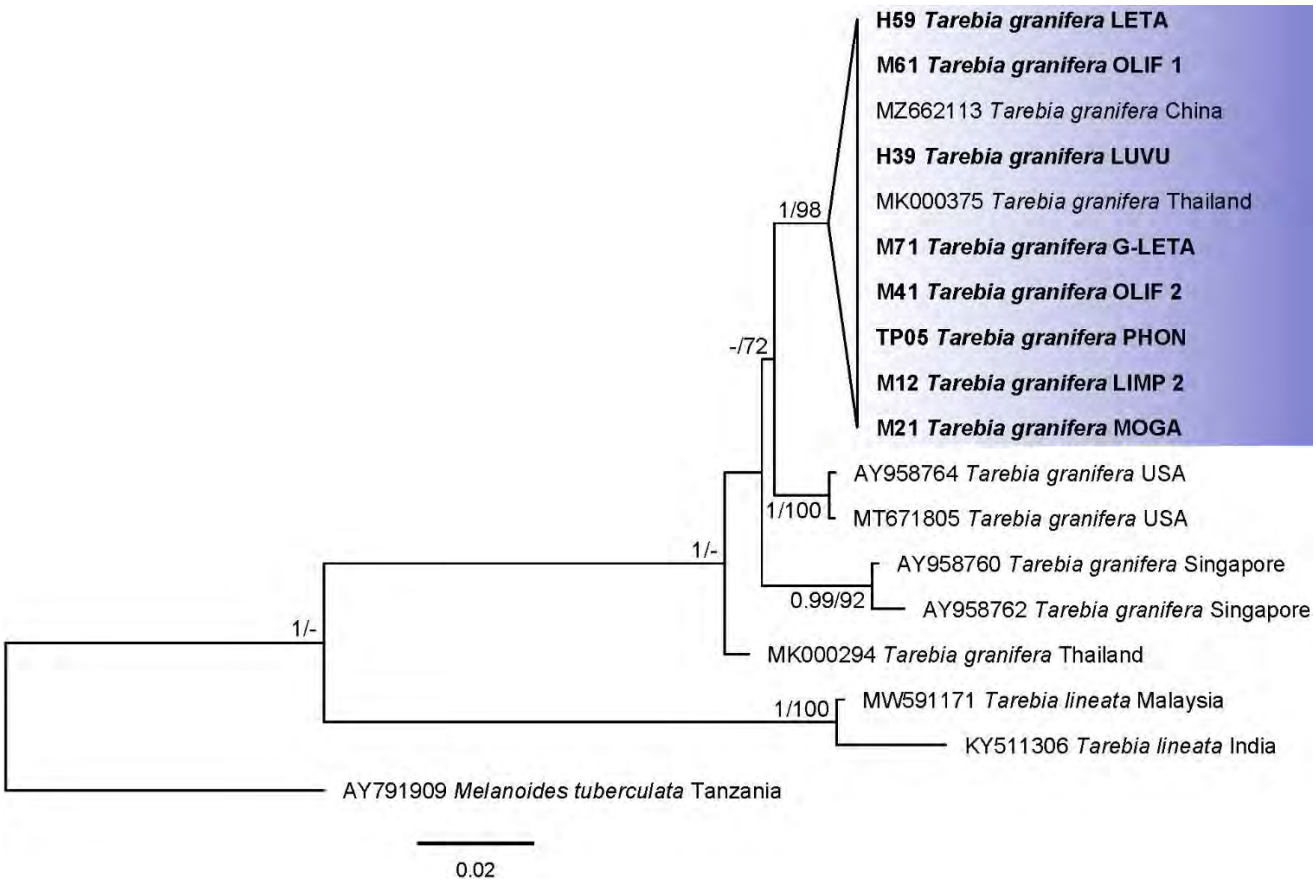

Figure 3.15: Bayesian inference (BI) phylogenetic tree for representatives of Thiaridae based on eight COI sequences obtained from *T. granifera* during the present study and ten sequences obtained from GenBank (see Table S3.13). Support values are indicated on the nodes as BI/ML (maximum likelihood). Values lower than 0.9 (BI) and 70 (ML) were excluded. The expected number of substitutions per site are indicated by the scale bar. Sequences from the present study are highlighted in bold with the sample number followed by the snail species and site code. Reference sequences include the GenBank accession number followed by the snail species and country of origin.

3.4 Discussion

The aim of this chapter was to identify freshwater mollusc species, particularly those known to transmit schistosomiasis in South Africa using morphological and molecular analyses and to determine the genetic diversity of *T. granifera* from the selected sites on the Limpopo River system and Phongolo River. The molecular analyses provided support for the morphological identification of the snails, resulting in the identification of eight distinct species of which six were native and three invasive. All *T. granifera* samples from the selected sites were found to be genetically identical, suggesting that all *T. granifera* in South Africa come from a few or even a single invasion event.

3.4.1 Bulinidae

Morphological identification of specimens of the *Bulinus* genus to species level is problematic due to the similarities in the shell structure of many of them. Thus, morphological identification requires the observation of shell micro-sculpture morphology and the shape and size of reproductive organs (Raahauge and Kristensen 2000; Jørgensen et al. 2007). The latter can only distinguish between certain species within the *Bul. africanus/globosus* group, namely the *Bul. africanus*, *Bul. globosus* and *Bul. nasutus* members (Raahauge and Kristensen 2000). However, to achieve this, specialised training is required. Inaccurate identification of *Bulinus africanus* and *Bul. nasutus* is possible due to the lack of significant differences in the shell morphology and reproductive organs of these two species (Raahauge and Kristensen 2000). Therefore, a combined approach of shell morphology and genetic analysis is an efficient method of establishing an accurate identification at species level (Jørgensen et al. 2007). The morphological identification of snails during the current study revealed that the snails sampled included two of the four globally recognised species groups of *Bulinus* sp., as well as three groups previously found in southern Africa (Raahauge and Kristensen 2000; Day and de Moor 2002; Jørgensen et al. 2007). The two groups identified were the *Bul. africanus/globosus* group and the *Bul. tropicus/truncatus* group.

Genetic analyses were conducted using the ribosomal ITS2 region and mitochondrial COI gene to distinguish between different *Bulinus* members of the specimens collected as the phylogenetic relationships between members of the four species groups of Bulinidae remain obscure. Therefore, a combination of conservative genes was used to resolve the inconsistencies within these species groups and explain the evolutionary origin. Sequence analysis of the ITS2 region of the *Bul. africanus/globosus* group confirmed that the specimens collected belong to this group and species level with all six of the novel sequences generated grouping with good nodal support to the genetic sequence of *Bul. africanus* (AM921970) published on GenBank.

The ITS2 data of the *Bul. tropicus/truncatus* group indicated that specimens that were morphologically identified to belong to the *Bul. tropicus/truncatus* group were genetically identified as *Bul. truncatus* specimens. However, the COI gene sequence data contradicted the findings obtained based on the ITS2 region analysis. These results indicated that the group in question still clustered within the *Bul. tropicus/truncatus* group, but not with the published sequences of *Bul. truncatus*, *Bul. tropicus*, or *Bul. natalensis* available on GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi). These findings indicate that, in the case of the *Bul. truncatus/tropicus* group, the ITS2 genetic region cannot accurately identify specimens to species level and should be used to complement the results of the phylogenetic analysis of the COI genetic region (Yao et al. 2010). Therefore, the use of morphological traits and multiple genetic markers (ITS2 region and COI gene), especially the COI gene, is required to accurately identify species of Bulinidae (Jørgensen et al. 2007; Kane et al. 2008).

Using multiple lines of evidence, the results of the ITS2 dataset confirmed the morphological identification of several additional specimens (BS15, -29, -33, -57, -72, -73, -91) to the *Bul. tropicus/truncatus* group. Based on morphological traits and the phylogenetic analysis, these specimens were positively identified to be members of *Bul. depressus*. The COI gene sequence data further identified that, phylogenetically, these specimens formed a separate clade within the *Bul. tropicus/truncatus* group. However, the identified specimens did not group with published sequences of *Bul. truncatus*, *Bul. tropicus* or *Bul. natalensis*. Therefore, these sequences could be positively identified as *Bul. depressus*. There is still some dispute regarding the latter clade as, in some cases, *Bul. depressus* is not included within the *Bul. tropicus/truncatus* group (Day and de Moor 2002; De Kock and Wolmarans 2005). However, Brown (1994) considered that *Bul. depressus* should be a part of the *Bul. tropicus/truncatus* group. The novel sequences generated during this study topologically formed part of the clade with *Bul. tropicus/truncatus* group and the sister group *Bul. wright* (Jørgensen et al. 2011). The results of the phylogenetic analyses the COI mtDNA gene presented here support the idea that *Bul. depressus* does indeed belong within the *Bul. tropicus/truncatus* group. The geographical area of collected specimens during this study is within the historically collected ranges *of Bul. africanus* and *Bul. depressus* (De Kock and Wolmarans 2005; 2006a), corroborating the identification of *Bul. africanus* and *Bul. depressus.*

3.4.2 Planorbidae

Specimens collected from the Planorbidae family were successfully morphologically identified as *Biom. pfeifferi* by their umbilicated (depressed navel) and evenly rounded periphery region (Day and de Moor 2002; De Kock et al. 2004). The phylogenetic analysis, with the aid of the amplification of the ITS2 and COI genetic regions, supported the morphological identification of these specimens. Two species of *Gyraulus* known to be native to South Africa are *G.*

costulatus and *G. connollyi* (Appleton 2002; Day and de Moor 2002). According to Brown and Van Eeden (1969), the morphological differentiation between the *Gyraulus* species collected was established by viewing the conchological characteristics. However, this was not the case, as *G. costulatus* and the invasive species *Biomphalaria glabrata* share similarities when it comes to in-field identification. Based on ITS2, the phylogenetic analyses of *Gyraulus* species collected in the North West Province indicated that species were correctly identified as belonging to the *Gyraulus* genus. The newly generated sequences grouped with good nodal support to a published sequence of *G. convexiusculus*. However, *G. convexiusculus* has only been identified in the Middle East and Western parts of India (Ayyagari and Sreerama 2020). The phylogenetic identification of *Gyraulus* species collected was more challenging, with no gene sequences for *G. connollyi* and *G. costulatus* available on GenBank. The phylogenetic analysis based on COI indicated a concerning result with specimens collected at the Seekoeivlei Nature Reserve grouping with a published sequence of *G. chinensis*, a known invasive that has been reported at a facility supplying fish and aquatic plants for the aquarium trade in the KwaZulu-Natal Province, South Africa (Appleton et al. 2015b). However, the specimens were morphologically identified to not be *G. chinensis* but rather the native species *G. costulatus*. All *Gyraulus* specimens were identified utilising their presence within South Africa by reviewing published literature and morphological traits of the shell (Day and de Moor 2002).

3.4.3 Lymnaeidae

The morphological identification of *R. natalensis* was confirmed with molecular analyses and morphological characterisation, but the formation of monophyletic groups within the study area with similarities between isolated populations was of interest. The identification of *Radix* species using shell morphology is regarded as inaccurate by Pfenninger et al. (2006) due to the variability of largely overlapping morphological traits between species and phenotypical plasticity to environmental conditions. This is confirmed by the map indicating the localities of the phylogenetically grouped specimens and assuming that the environmental conditions to which these specimens were exposed were identical (Pfenninger et al. 2006). The other possibility for the isolated population of genetically similar species may be from lateral dispersal between water bodies with the aid of migratory avian species, which has been previously reported (Kappes and Haase 2012). The dispersal of mollusc species depends on a wide range of factors (geographical, vector used and climate) and further research into this topic will be required.

The identification of the invasive Lymnaeidae *Pseu. columella* was confirmed by morphological identification and supported by the phylogenetic analysis based on the ITS2 region and the COI gene. All the sequences generated by this study and published sequences

from GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated no genetic variation. This result suggests that the method *Pseu. columella* primarily uses to reproduce is self-fertilisation (Alba et al. 2019). This suggests that in the Limpopo Province, South Africa, the population of the invasive *Pseu. columella* is from a single invasion or multiple invasions from the same region (Nicot et al. 2008).

3.4.4 Physidae

The invasive *Phy. acuta* has been reported in several freshwater habitats across South Africa and is considered the second most widespread invasive mollusc species (De Kock and Wolmarans 2007; Lawton et al. 2018). *Physella acuta* has not been identified as any vector for snail-borne diseases within South Africa (Lawton et al. 2018). However, the rapid dispersal of *Phy. acuta* throughout a freshwater ecosystem can harm the native mollusc species present (Dobson 2004). At the locations where the invasive *Physella* species were present, a decrease in the native populations of medically important mollusc vectors has been observed, suggesting that *Physella* could be used as a potential biological control agent to reduce the risk of infection in locations identified as high transmission areas for medically important digenean trematodes (Dobson 2004). The use of *Phy. acuta* as a possible biological control agent must be accompanied by accurate identification and strict monitoring to determine the ecological impact and mitigate any undesired impacts on the local ecology. According to Lawton et al. (2018), the use of morphological identification and molecular analysis based on the COI gene has been widely adapted to identify *Phy. acuta* at the species level and can be used to determine the geographical origin of the invasion. In a study conducted by Lawton et al. (2018), four distinct African clades were identified within samples collected from the Vaal River in South Africa, Angola, and Burundi. The four African clades indicated that *Phy. acuta* resulted from multiple invasions (Lawton et al. 2018). During the current study, the application of the COI gene for phylogenetic analysis accurately identified the invasive *Physella acuta* to be a member of the African clade 3 based on populations sampled within South Africa and published on GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi). This clade contains haplotypes from Mexico, the USA, Greece, Egypt, Iran, Macedonia, and South Africa (Lawton et al. 2018). The identification of species belonging to all four clades within South Africa was recorded by Lawton et al. (2018) within the Vaal River catchment in the North West Province. This finding indicates that *Phy. acuta* from different African clades can be located within the same area (Lawton et al. 2018) and suggests that more than one of the *Phy. acuta* African clades could be present at a single site during the current study.

3.4.5 Tarebia granifera

Phylogenetic analysis conducted in the present study indicated that *T. granifera* from the Limpopo River system and the Phongolo River were all genetically identical based on the mitochondrial DNA gene (COI). This was expected, as invasive snails usually have considerably lower genetic diversity within their invasive ranges compared to their native ranges (Facon et al. 2003; Hanson et al. 2013; Ebbs et al. 2018). Furthermore, male *T. granifera* were found to be absent from the samples during the present study, thus the snail populations rely on parthenogenic reproduction (clones of their female parents) that results in low genetic diversity. Although Chaniotis et al. (1980a) reported male *T. granifera* from its invasive ranges in Puerto Rico, Veeravechsukij et al. (2018b) did not find any evidence of males within the populations in its native ranges in Thailand. In addition, all other studies in South Africa have also demonstrated that no males were present in any of the studied populations (Appleton and Nadasan 2002; Appleton et al. 2009; Miranda et al. 2011a; 2011b). Thus, new-born *T. granifera* are genetically identical (clones) to their female parent (Appleton et al. 2009). Parthenogenic reproduction may have some advantages for *T. granifera*, as only a single individual is able to start a new population (Jokela et al. 1997; Gerritsen 1980; Ben-Ami and Heller 2007; Miranda et al. 2011a), driving its success as an invader (Appleton et al. 2009). Furthermore, asexual populations have been found to have a reduced risk of parasitic infection and fewer predators that feed on them (Lively 2001; Ben-Ami and Heller 2005; Miranda et al. 2011a). According to the “Genetic Paradox of Invasions” hypothesis, asexual reproduction and high reproductive output of invasive species enable them to avoid the risk of reducing genetic drift and inbreeding (Schrieber and Lachmuth 2017). Clonal reproduction also gives *T. granifera* another advantage that contributes to the successful invasion and expansion of the snail. Since all sexually mature individuals can contribute to population growth, *T. granifera* is able to rapidly colonise new environments and can quickly reach high densities (Baker 1955; Veeravechsukij et al. 2018b; Makherana et al. 2022).

Although Appleton et al. (2009) reported two distinct morphological variants of *T. granifera* in KwaZulu-Natal, it is possible that they may be genetically identical. However, this will need to be confirmed with further analyses, as the present study did not assess different morphological variants. Aquatic snails have been found to have morphological variability due to different environmental conditions across different habitats that can produce considerable shell modifications (Dillon 2000; Glaubrecht 2004; 2009; 2011; Veeravechsukij et al. 2018b). Variations in features such as shell colour, size and shape do not always allow for precise identifications and do not always indicate different species and/or morphs (Davis 1994; Michel 1994; Von Gersdorff Sørensen et al. 2005; Veeravechsukij et al. 2018b). A study by Veeravechsukij et al. (2018b) found that morphometric data of *T. granifera* from Thailand were

insufficient in distinguishing between different morphs of *T. granifera*. In addition, the aforementioned study also found that two distinct morphs, based on molecular analysis, did not indicate any observable differences in shell morphology. This highlights the importance of using molecular techniques rather than only morphological techniques to identify genetic differences between individuals in order to distinguish genotypical diversity (Von Gersdorff Sørensen et al. 2005; Veeravechsukij et al. 2018b).

Results indicated that the *T. granifera* sequences from the present study were found to be identical to sequences of *T. granifera* from China and Thailand. This suggests that *T. granifera* was likely introduced to South Africa from China or Thailand, which further confirms as well as narrows down previous reports that it was introduced from Southeast Asia (Appleton et al. 2009; Appleton and Miranda 2015a). The results of the present study are in support of the statements of Appleton and Nadasan (2002), that *T. granifera* were likely introduced to South Africa via the aquarium trade from Hong Kong. The likelihood that aquatic invasive species may be introduced is increased through the aquarium trade (Walker 1978; Madsen and Frandsen 1989; Cowie 1998; Pointier 1999; Letelier et al. 2007; Strayer 2010; Preston et al. 2022). This is particularly important since the South African Revenue Service data indicated that imports from Asia have doubled during the period 1998 to 2011 (Appleton and Miranda 2015a), with particularly strong links between the aquarium trade and Hong Kong as a source of supply (Appleton and Nadasan 2002). After the first collection of *T. granifera* in 1999 from South Africa, Appleton and Miranda (2015a) reported two additional Asian freshwater snail species introduced into South Africa, *Radix rubiginosa*, first collected in 2004, and *Gyraulus chinensis*, first collected in 2006.

Interestingly, the phylogenetic analysis during the present study indicated that COI sequences of *T. granifera* from China and Thailand were identical. This may be as a result of introductions of invasive morphs between the two counties; however, this requires further investigation. A better understanding of the genetic diversity and phylogeny of aquatic snails will be useful in future studies to consider host and parasite relationships as well as their invasion dynamics (Ebbs et al. 2018; Veeravechsukij et al. 2018b). For example, in Lake Malawi, Genner et al. (2008) found that a highly invasive morph of *M. tuberculata* introduced from Southeast Asia was free from trematode infections and that this invader was resistant to native trematodes that were found to infect the native morphs. In addition, the invasion of Asian morphs of *M. tuberculata* in Lake Malawi has been found to threaten the native thiarid species diversity in the region (Genner et al. 2004; Von Gersdorff Sørensen et al. 2005; Genner et al. 2007). In another example, the invasion of *T. granifera* in the Caribbean islands has led to the extinction of some thiarid species while others have become scarce (Pointier et al. 2010). These findings emphasise the importance of not only understanding the interactions between invasive and

native snail species, but also understanding the genetic diversity of these species which drives their ecological interactions and their relationships with parasites. These interactions can have severe effects on ecosystem function and community structures of native communities (Johnson et al. 2009), especially since trematodes from snail hosts pose a threat to human and animal health (Sodeman 1991; McKoy et al. 2011; Veeravechsukij et al. 2018a; 2018b; Yin et al. 2022).

3.5 Conclusion

The aims of this chapter were to identify native freshwater mollusc species, particularly those known to transmit schistosomiasis in South Africa, using both morphological and molecular techniques to investigate the genetic diversity of *T. granifera*. Molluscs were identified using morphological traits, and, for all but *T. granifera*, by molecular analysis using the ITS2 region of rDNA and the COI mitochondrial gene compared to published sequences available by using phylogenetic analysis, while the COI gene was used for the molecular identification of *T. granifera.* To a large degree, the phylogenetic analysis based on the ITS2 region and the COI gene supported the morphological identification, identifying the presence of *Bul. africanus, Bul. depressus, Biom. pfeifferi*, *G. costulatus, G. connollyi, R. natalensis*, *Pseu. columella, Phy. acuta*. The identification of *Bul. depressus*, *G. costulatus* and *G. connollyi* resulted in additional sequences of the ITS2 region and COI gene being generated for these species. Molecular analysis also indicated that *R. natalensis* formed four genetically diverse groups located at different isolated sites. This suggests the possibility that these mollusc species may move or be transported between the sampling sites through active dispersal by various animals, including birds, or by other natural mechanisms. The molecular analysis of the invasive *Pseu. columella* indicated that all specimens collected at various sites were genetically identical, with no base pair differences of the ITS2 and COI genes. This finding indicates that the invasion of *Pseu. columella* was the result of a single specimen or multiple invasions from a single location.

All the *T. granifera* specimens collected at the selected sites were found to be genetically identical females, thus supporting previous hypotheses that all *T. granifera* in South Africa come from a few or even a single invasion event or a small population of introduced snails. Furthermore, this study indicated that *T. granifera* were likely introduced from China or Thailand which narrows down previous reports that it was introduced from Southeast Asia. These findings support the view that *T. granifera* were likely introduced from Hong Kong via the aquarium trade. Due to the fact that *T. granifera* serves as a host for diverse species of trematodes within and outside its native ranges, it would be wise to further investigate its role as a host for trematodes within all invaded habitats across southern Africa.

CHAPTER 4: PARASITES OF FRESHWATER MOLLUSCS IN SOUTH AFRICA

Compiled by: *L. de Necker, J.J. Pearson, M.H. Le Roux, N.J. Smit and V. Wepener*

4.1 Introduction

Freshwater molluscs function as an important intermediate host (vector) for most trematode parasites affecting human and animal health worldwide (Schols et al. 2020). Most trematode parasites have a multi-stage life cycle that includes the presence of a suitable mollusc vector species and the presence of a suitable definitive human or vertebrate host (Schols 2019). Many of these trematode species are of veterinary, economic and medical importance, substantially affecting the local population and economic stability of the region where they are located (King 2010; Schols 2019). Trematodes of medical and veterinary importance include members of the genera *Schistosoma, Fasciola*, and *Echinostoma,* which infect humans and livestock. Until recently, a low infection of trematode parasites was thought to have little to no effect on the health of their hosts and did not require medical treatment. Recent studies, however, have indicated that the presence and intensity of parasitic infection increase the risk of morbidity due to the increased risk of chronic disease development (King 2010; Lacorcia and Prazeres da Costa 2018).

Moreover, immunological research has indicated a correlation between chronic trematode infection and reduced protective responses against unrelated bacterial and viral infections (Malhotra et al. 1999; LaBeaud et al. 2009; King 2010; Lacorcia and Prazeres da Costa 2018). Therefore, undiagnosed trematode infections directly affect food security and decrease the quality of life and life expectancy of populations living near the affected area (King 2010; Lacorcia and Prazeres da Costa 2018). For this reason, research has focused on the above-mentioned three trematode species as they cause such severe diseases in humans and animals, while there has been a lack of research on other parasite species that are found in aquatic ecosystems (Colley et al. 2014; Adenowo et al. 2015). It is thus important to analyse the current distribution of trematode parasites that infect mollusc species, as climate change and anthropogenic factors can alter the availability of (1) suitable vectors, and (2) allow vectors to spread to areas that historically were unsuitable for the vectors and associated trematode species.

Thiaridae, or trumpet snails, such as *Tarebia granifera* and *Melanoides tuberculata*, pose a severe threat to public health and are of veterinary importance as they have been known to transmit trematode parasites of fishes, birds, and mammals, including humans (Sodeman 1991; Mitchell et al. 2000; Veeravechsukij et al. 2018a). Both *T. granifera* and *M. tuberculata*

are known to be first intermediate hosts for diverse and prevalent species of trematodes that have been reported to affect the intestinal, respiratory, and hepatic systems of animals and humans (see Tables S4.1 and S4.2). *Tarebia granifera* is well known as a first intermediate host for several trematode species that cause human diseases, particularly in their native ranges (Veeravechsukij et al. 2018a; 2018b). Classical hypotheses such as the “spillover” and “spillback” hypotheses are important to consider with regard to invasive host species (Chalkowski et al. 2018). Spillover of parasites occurs when parasites from invasive host species are introduced into a new environment and subsequently infect native species, therefore posing a health risk to native species (Daszak et al. 2000; Lymbery et al. 2014; Iglesias et al. 2015; Chalkowski et al. 2018). Parasite spillback occurs when native parasites from a native host infect an invasive host, therefore increasing the risk to infect other native species (Hoberg et al. 2002; Kelly et al. 2009; Chalkowski et al. 2018).

Intestinal trematodes such as *Haplorchis pumilio* (Looss 1896)*, Haplorchis taichui* (Nishigori, 1924) and *Centrocestus formosanus* (Nishigori 1924) that infect both *T. granifera* and *M. tuberculata* have been reported to infect people in China, Thailand, and Korea (Veeravechsukij et al. 2018a). These trematodes use fish as second intermediate hosts and infect mammals and humans that consume infected fish (Krailas et al. 2011; 2014; 2016). The oriental avian eye fluke *Philopthalmus gralii* (Mathis and Ledger 1910) also uses both *T. granifera* and *M. tuberculata* as first intermediate hosts (Tables S4.1 and S4.2). *Tarebia granifera* was also reported to serve as a host for exotic parasites in its invasive ranges in Jamaica (McKoy et al. 2011) and Texas (Tolley-Jordan and Owen 2008). *Tarebia granifera* from Texas (invasive) have been found to be infected with *P. gralii* (Tolley-Jordan and Owen 2008) while ostriches (*Struthio camelus* Linnaeus 1758) from farms in Zimbabwe have been found to be infected with *P. gralii* from *M. tuberculata* (Mukaratirwa et al. 2005). Furthermore, studies on *M. tuberculata* as hosts for trematodes in South Africa have been neglected in recent years, since the last reports describing trematodes from *M. tuberculata* in South Africa date back to 1921 and 1938 (Faust 1921; Porter 1938, respectively).

It may be possible that *T. granifera* and *M. tuberculata* share the same species of trematodes in South Africa or that the invasive snail harbours exotic parasites that might be infecting fish, birds, mammals and, potentially, humans. Appleton et al. (2009) suggest that due to the rapid spread of the invasive *T. granifera* and resultant replacement of native *M. tuberculata*, such problems may become worse in the future. To date, no trematode species have been reported in *T. granifera* from South Africa (Miranda et al. 2011a; 2011b; Weyl et al. 2020). However, this situation could change, and it needs to be closely monitored in the future. This is particularly true since *T. granifera* may serve as a host for invasive parasites that could be brought in via migratory birds, or eventually serve as a host for native parasite taxa, such as

schistosomiasis, that spillover from native hosts. Consequently, studies on thiarid species and their parasitic diversity in South Africa will contribute to a better understanding of the biodiversity, biology and evolutionary relationships of the snail hosts and their trematodes.

The aims of this chapter were to 1) assess the parasitic trematode species and parasitic load within mollusc species collected from the north-eastern Provinces of South Africa and 2) investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes. This was achieved through tests for natural emergence of cercariae from the snails, snail dissections and molecular analyses.

4.2 Methods

Throughout this chapter, shedding experimentation as well as molecular analyses were conducted following methods described by Schols (2019), Schols et al. (2019) and Hoogendoorn et al. (2019). All analyses were done using the methodology explained in Chapter 2 sections 2.3.4 to 2.3.6.

4.3 Results

4.3.1 Cercarial shedding

In total, 910 specimens of freshwater mollusc members of the *Bulinus, Biomphalaria, Radix* and *Pseudosuccinea* genera were used to screen for signs of cercarial shedding. Additionally, a total of 294 *T. granifera* and 62 *M. tuberculata* were collected from the Limpopo River system and 44 *T. granifera* were collected from the Phongolo River for the examination of trematode infections. No cercarial shedding was observed during the shedding experiments. After the negative results were obtained from the cercarial shedding experiment, specimens were euthanised and dissected, and the mollusc species' soft tissue was inspected for signs of infection. No visible cysts were observed during the inspection of soft tissue. Samples were therefore prepared for molecular analyses to determine if any prepatent trematode infection(s) were present.

4.3.2 Parasitic infections in schistosomiasis-transmitting snails

Molecular analyses of the tissue of representatives of *Biomphalaria* and *Bulinus* were negative for *Schistosoma* infection. The presence of *Fasciola hepatica* was not detected within species of *Pseudosuccinea* and *Radix*, its known mollusc intermediate hosts, either. None of the known vectors for these diseases indicated signs of infection with the cercarial shedding, nor could these parasitic species be detected by molecular methods. However, three trematode infections were detected within samples of *Bulinus* spp., namely *Neofibricola smiti*, detected by molecular methods in one specimen of *Bul. globosus* collected at site LLF14 during the

low-flow sampling event in August 2021. Two infections of *Petasiger variospinosus* were also detected in two specimens of *Bul. depressus* collected at site LLF6 and LLF7 also sampled during the low-flow sampling event of August 2021. In addition, *Orientocreadium batrachoides* was detected by molecular method in two specimens of *R. natalensis* collected at site LLF6 during the low-flow season.

Neofibricola smiti Achatz, Martens, Kudlai, Junker, Boe and Tkach, 2022

The 28S rDNA sequence isolated from the *Bul. africanus* specimen collected at site LLF14 (Chapter 2, Figure 2.1) was identical to be that of *N. smiti* when compared to sequences published on GenBank. The phylogeny of the *Neofibricola* dataset (Figure 4.1) was validated by good nodal support with Bayesian inference of 0.85 out of a maximum score of 1 and a maximum likelihood of 96 out of a maximum score of 100.

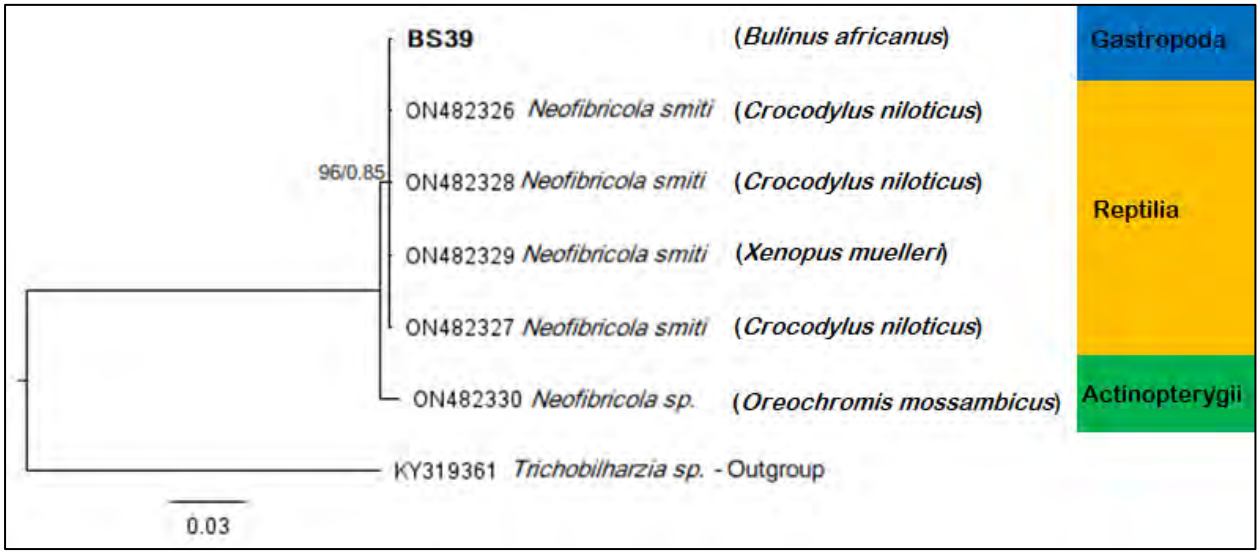

Figure 4.1: Bayesian inference phylogram of selected *Neofibricola* taxa based on partial sequences of the 28S rDNA gene. Newly sequenced species are highlighted in bold. The host/vector species are indicated in brackets, and the hosts class is given. Posterior probability followed by bootstrap support values is indicated next to the branches (posterior bootstrap <70 and probability <0.85 not shown). *Trichobilharzia* sp. was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

The identification of *N. smiti* was supported by the pairwise genetic distance (p-distance) of 0% pairwise difference (Table 4.1). The base pair differences between the sequence generated during this study and the sequences of *N. smiti* published on GenBank indicated zero base pair differences. The interspecific variation for *Neofibricola* species in this dataset is 0–1% (0–12 nt).

Table 4.1: Nucleotide genetic divergence among *Neofibricola* included in the molecular phylogenetic analysis of the 28S rDNA. Values above the diagonal are expressed in the percentage pairwise genetic distance (p-distance), while values below the diagonal represent the number of base pair differences. The analysis involved seven nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1 209 positions in the final dataset.

Species name		Sequence number	1	2	3	4	5	6	7
1	*Neofibricola smiti*	BS39		0%	0%	0%	0%	1%	28%
2	*Neofibricola smiti*	ON482326	0		0%	0%	0%	1%	28%
3	*Neofibricola smiti*	ON482328	0	0		0%	0%	1%	28%
4	*Neofibricola smiti*	ON482329	0	0	0		0%	1%	28%
5	*Neofibricola smiti*	ON482327	0	0	0	0		1%	28%
6	*Neofibricola sp.*	ON482330	12	12	12	12	12		29%
7	*Trichobilharzia sp.*	KY319361	320	320	320	320	320	322	

Petasiger variospinosus (Odhner, 1910) Yamaguti, 1933

The 28S rDNA sequence isolated from the *B. depressus* specimens collected at sites LLF6 (sample BS62) and LLF7 (Sample BS92) was identical to that of *Petasiger* sp. 5 when compared to sequences published on GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi; Figure 4.2). Following its submission to GenBank, *Petasiger* sp. 5 was morphologically identified to be *Petasiger variospinosus* ((Odhner, 1910) Yamaguti, 1933) by Hammoud et al. (2022). The phylogeny of *P. variospinosus* dataset (Table 4.2) indicates good nodal support for Bayesian inference and maximum likelihood analyses, with Bayesian inference of 1 out of a maximum score of 1 and a maximum likelihood of 99 out of a maximum score of 100.

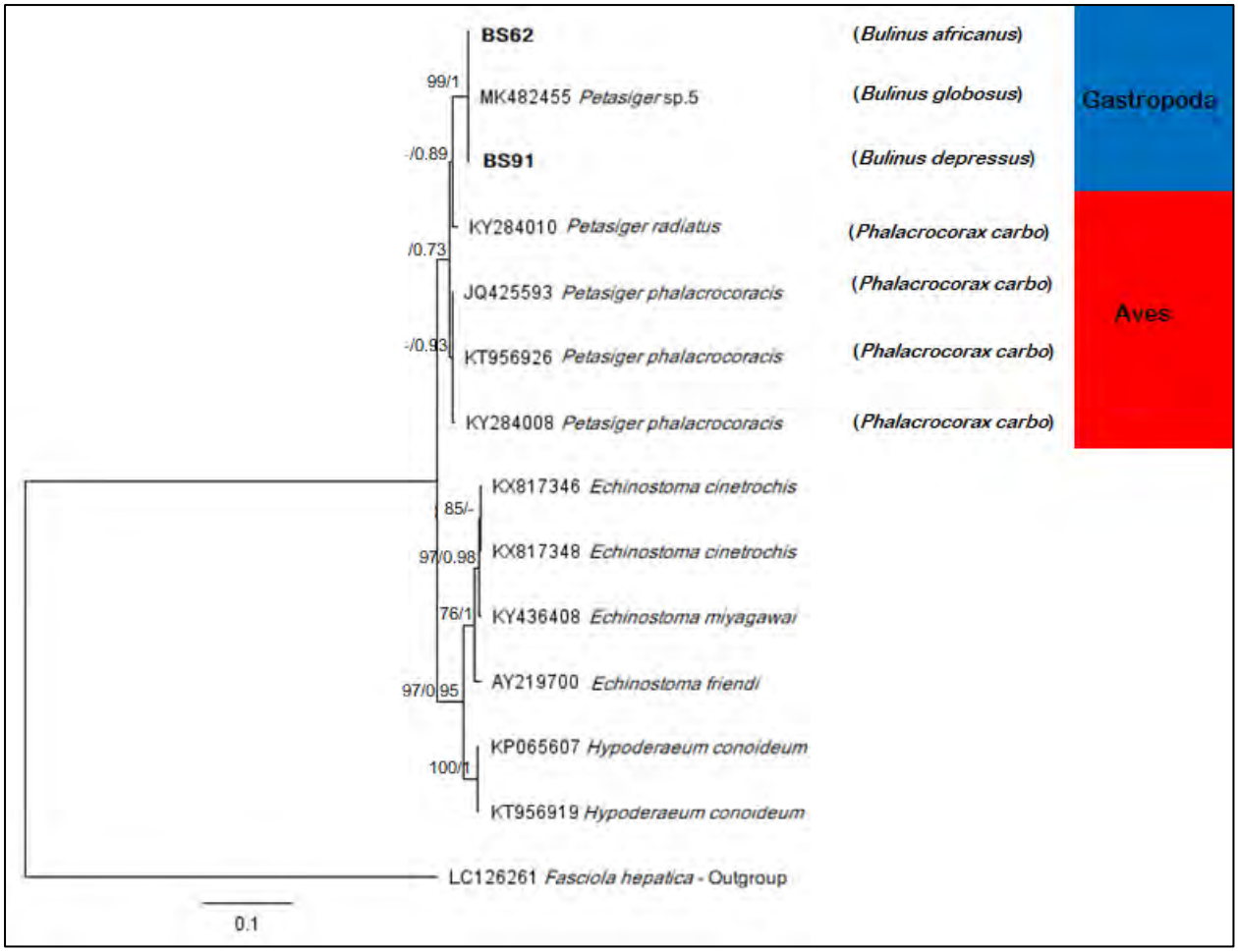

Figure 4.2: Bayesian inference phylogram of selected *Petasiger*, *Echinostoma* and *Hypoderaeum* taxa based on partial sequences of the 28S rDNA gene. Newly sequenced species are highlighted in bold. The host/vector species are indicated in brackets, and the hosts class is given. Posterior probability followed by bootstrap support values is indicated next to the branches (posterior bootstrap <70 and probability <0.85 not shown). *Fasciola hepatica* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

The identification of *Petasiger variospinosus* was supported by the pairwise genetic distance (p-distance) of 0% pairwise difference (Table 4.2). The analyses indicate zero base pair differences between specimen BS62 collected at site LLF6 and *Petasiger* sp. 5, and only one base pair difference between specimen BS91 collected at site LLF7 and *Petasiger* sp. 5. The newly generated sequences differed by one base pair. The overall interspecific variation of *Petasiger* species is 0-3% (0–26 nt).

Table 4.2: Nucleotide genetic divergence among *Petasiger, Echinostoma* and *Hypoderaeum* taxa included in the molecular phylogenetic analysis of the 28S rDNA. Values above the diagonal are expressed in the percentage pairwise genetic distance (p-distance), while values below the diagonal represent the number of differences. The analyses involved 14 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1 014 positions in the final dataset.

Species name		Sequence number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Echinostoma friedi*	AY219700		1%	1%	1%	4%	4%	9%	9%	9%	7%	7%	7%	7%	97%
2	*Echinostoma cinetorchis*	KX817346	7		0%	0%	4%	4%	9%	9%	9%	7%	7%	7%	7%	97%
3	*Echinostoma cinetorchis*	KX817348	7	0		0%	4%	4%	9%	9%	9%	7%	7%	7%	7%	97%
4	*Echinostoma cinetorchis*	KY436408	6	2	2		4%	4%	9%	9%	9%	7%	7%	7%	7%	97%
5	*Hypoderaeum conoideum*	KP065607	18	32	32	32		0%	8%	8%	9%	7%	7%	7%	7%	97%
6	*Hypoderaeum conoideum*	KT956919	18	32	32	32	0		8%	8%	9%	7%	7%	7%	7%	97%
7	*Petasiger variospinosus*	BS62	40	72	72	72	68	68		0%	0%	3%	3%	3%	2%	96%
8	*Petasiger* sp. 5	MK482455	40	72	72	72	68	68	0		0%	3%	3%	3%	2%	96%
9	*Petasiger variospinosus*	BS91	40	73	73	73	69	69	1	1		3%	3%	3%	2%	96%
10	*Petasiger phalacrocoracis*	JQ425593	34	63	63	63	62	62	24	24	25		0%	0%	1%	94%
11	*Petasiger phalacrocoracis*	KT956926	34	63	63	63	62	62	24	24	25	0		0%	1%	94%
12	*Petasiger phalacrocoracis*	KY284008	34	65	65	65	64	64	26	26	27	2	2		1%	94%
13	*Petasiger phalacrocoracis*	KY284010	35	66	66	66	65	65	21	21	22	11	11	13		94%
14	*Fasciola hepatica*	LC126261	369	786	786	788	784	784	777	777	776	776	776	776	775	

Orientocreadium batrachoides Tubangui, 1931

Based on the phylogenetic analysis of the 28S rRNA sequence isolated from the *R. natalensis* specimens collected at site LLF6 (Chapter 2, Figure 2.1), the species was identified as *Orientocreadium batrachoides* based on comparisons with sequences published on GenBank (Figure 4.3). The phylogenetic analysis of the *O. batrachoides* dataset (Table 4.3) indicated good nodal support for Bayesian inference and maximum likelihood analysis, with Bayesian inference of 0.85 out of a maximum score of 1 and a maximum likelihood of 96 out of a maximum score of 100.

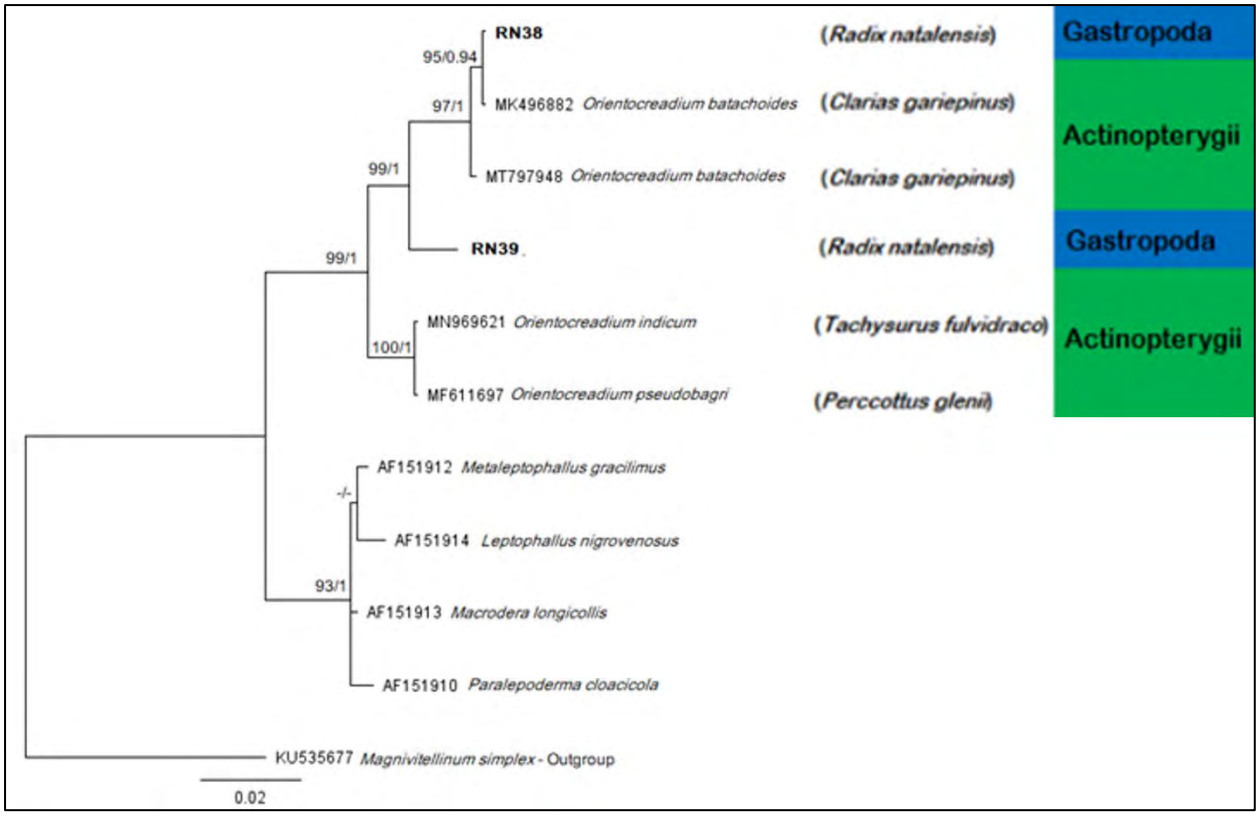

Figure 4.3: Bayesian inference phylogram of selected Orientocreadiidae and Plagiorchiidae taxa based on partial sequences of the 28S rDNA gene. Newly sequenced species are highlighted in bold. The host/vector species are indicated in brackets, and the host class is given. Posterior probability followed by bootstrap support values is indicated next to the branches (posterior bootstrap <70 and probability <0.85 not shown). *Magnivitellinum simplex* was used as an outgroup. The branch length scale bar indicates the number of substitutions per site.

The identification of *O. batrachoides* was supported by the pairwise genetic distance (p-distance) of 0% pairwise difference (Table 4.3) above the diagonal for the specimen RN38 collected at site LLF4, identifying it as a sample of *O. batrachoides*. The molecular 28S rDNA sequences generated matched and phylogenetically grouped with sequences published by Dumbo et al. (2019) with acceptable branch supports of maximum likelihood and Bayesian inference. Specimen RN39 collected at site LLF6 had a 2-3% percentage pairwise genetic distance to the published sample of *O. batrachoides* (MK496882; MT797948) and 26-27 base pair differences between the samples of *O. batrachoides*.

Table 4.3: Nucleotide genetic divergence among Orientocreadiidae and Plagiorchiidae taxa included in the molecular phylogenetic analysis of the 28S rDNA. Values above the diagonal are expressed in the percentage pairwise genetic distance (p-distance), while values below the diagonal represent the number of differences. The analyses involved 11 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1 014 positions in the final dataset.

Species Name		Sequence number	1	2	3	4	5	6	7	8	9	10	11
1	*Orientocreadium indicum*	RN38		3%	0%	0%	3%	3%	6%	7%	6%	7%	14%
2	*Orientocreadium indicum*	RN39	26		3%	2%	3%	3%	6%	6%	6%	6%	13%
3	*Orientocreadium batrachoides*	MK496882	0	26		0%	3%	3%	6%	7%	6%	7%	14%
4	*Orientocreadium batrachoides*	MT797948	6	27	6		3%	3%	6%	7%	6%	6%	14%
5	*Orientocreadium indicum*	MN969621	38	31	38	32		0%	5%	5%	5%	5%	13%
6	*Orientocreadium pseudobagri*	MF611697	38	31	38	32	0		5%	5%	5%	5%	13%
7	*Metaleptophallus gracillimus*	AF151912	71	61	71	65	53	53		1%	1%	1%	12%
8	*Leptophallus nigrovenosus*	AF151914	70	62	70	65	55	55	8		1%	1%	12%
9	*Macrodera longicollis*	AF151913	70	60	70	65	54	54	5	9		1%	11%
10	*Paralepoderma cloacicola*	AF151910	72	60	72	67	57	57	8	12	7		12%
11	*Magnivitellinum simplex*	KU535677	137	128	137	132	122	122	111	116	113	114	

4.3.3 Parasitic infections in Thiaridae snails

All sampled *T. granifera* and *M. tuberculata* were mature adults. All dissected *T. granifera* were found to be females, with embryos in their brood pouches. Table 4.4 indicates the number of snails collected and screened for cercariae with the average snail length sampled from the selected sites. Neither of the two snail species shed cercaria during any of the shedding experiments. However, the molecular study investigating snail trematode infections resulted in the detection of two species of trematodes in *M. tuberculata* from the LIMP-1 site on the Limpopo River, while all *T. granifera* from all sites were found to be uninfected.

Table 4.4: Number of Thiaridae snail specimens, shell length (average ± SD), and length range (mm) sampled at the selected sites.

Site Code	Sampling Date	River	Snail species	Number of snails sampled	Average snail length (mm) ± SD	Snail length range (mm)
PHON-4	16/11/2021	Phongolo	*Tarebia granifera*	44	12.17 ± 2.61	18 – 7.5
LIMP-1	22/04/2021	Limpopo	*Melanoides tuberculata*	62	18.49 ± 5.02	11.5 – 29.3
LIMP-2	25/04/2021	Limpopo	*Tarebia granifera*	67	16.76 ± 2.3	12.2 – 22.6
MOGA	26/04/2021	Mogalakwena	*Tarebia granifera*	24	14.53 ± 2.49	11 – 19.4
LUVU	29/04/2021	Luvuvhu	*Tarebia granifera*	24	15.01 ± 1.76	12.5 – 19.7
OLIF-1	05/05/2021	Olifants	*Tarebia granifera*	30	15.57 ± 2.28	12.1 – 21
OLIF-2	03/05/2021	Olifants	*Tarebia granifera*	33	16.95 ± 2.5	10.9 – 22.3
G-LETA	06/05/2021	Great Letaba	*Tarebia granifera*	78	15.47 ± 1.86	11.8 – 20
LETA	04/05/2021	Letaba	*Tarebia granifera*	38	16.28 ± 2.11	12.5 – 24.1

The sequences (ITS2 and 28S) from the two trematode species found in *M. tuberculata* were compared to the available sequences in GenBank to identify the trematode species. Sequences from the present study revealed that five of the ten *M. tuberculata* were infected with a *Haplorchis* sp. and one with a *Centrocestus* sp. A comparison of the ITS2 sequences (626 bp) obtained during the present study from the *Haplorchis* sp., with the best (highest percent similarity) available sequences in GenBank revealed that the *Haplorchis* sp. from the present study is 98.36% similar to *H. pumilio* from Peru (Lopes et al. 2020; GenBank accession No. MT829204). The genetic divergence between the *Haplorchis* sp. from South Africa (present study) and *H. pumilio* from Peru was 1.64% (6 bp). Comparison of the 28S sequences (1 182 bp) obtained during the present study from the *Haplorchis* sp., with the best

available sequences in GenBank revealed that the *Haplorchis* sp. from the present study is 98.89% similar to *H. pumilio* from Thailand (Thaenkham et al. 2010; GenBank accession No. HM004173), Vietnam (Le et al. 2017; GenBank accession No. KX815125 and KY369156), Peru (Pulido-Murillo et al. 2018; GenBank accession No. MG738252), and Brazil (Lopes et al. 2020; GenBank accession No. MT840091). The genetic divergence between the *Haplorchis* sp. from South Africa (present study) and *H. pumilio* from the aforementioned countries was 1.11% (13 bp).

A comparison of the ITS2 sequences (626 bp) obtained during the present study for *Centrocestus* sp., with the best available sequences in GenBank revealed that the *Centrocestus* sp. from the present study is 98.45% similar to a *Centrocestus* sp. from Israel (Dzikowski et al. 2004; GenBank accession No. AY245699). The genetic divergence between the *Centrocestus* sp. from South Africa (present study) and *Centrocestus* sp. from Israel was 1.55% (7 bp). A comparison of the 28S sequences (1 182 bp) obtained during the present study from the *Centrocestus* sp., with the best available sequences in GenBank revealed that the *Centrocestus* sp. from the present study is 96.86% similar to *C. formosanus* from Peru (Pulido-Murillo et al. 2018; GenBank accession No. MG738251), and Vietnam (Le et al. 2017; GenBank accession No. KY351633 and KY369154). The genetic divergence between the *Centrocestus* sp. from South Africa (present study) and *Centrocestus* sp. from the aforementioned countries was 3.14% (37 bp).

4.4 Discussion

The aim of this chapter was to assess trematode parasites of native and invasive snail species in north-eastern areas of South Africa. Findings of this study included that no cercarial shedding occurred and no parasites of the genus *Schistosoma* sp. were detected. However, five trematode parasites were detected in native freshwater snail species while no parasitic infections were present in any invasive snail species.

4.4.1 Parasitic infections in schistosomiasis-transmitting snails

Mollusc species subjected to shedding experiments were unsuccessful and indicated no signs of cercarial shedding of parasitic digenean trematodes. The complex life cycle of species-specific trematodes that are native to the north-eastern South African region and known to infect mollusc species could be absent from mollusc vectors due to their complex life cycle (Sacolo-Gwebu et al. 2019). It is known that schistosomiasis is caused by members of the genus *Schistosoma* and is transmitted by the species-specific intermediate hosts (vectors) of the genera *Bulinus* and *Biomphalaria* (De Kock et al. 2004; De Kock and Wolmarans 2005). As mentioned in Chapter 3, a total of 476 members of the *Bulinus* and *Biomphalaria* genera were collected during the Limpopo low-flow (LLF) sampling event during the August 2021 field

collections with no signs of *Schistosoma* infection detected by both molecular methods and shedding experiments. This may be as a result of the natural low prevalence of *Schistosoma* sp. parasites in freshwater snail species (Sacolo-Gwebu et al. 2019; Nwoko et al. 2023). In separate studies of schistosomiasis vectors in KwaZulu-Natal, Sacolo-Gwebu et al. (2019) found a prevalence of between 0.9 and 1% of *S. mansoni* and *S. haematobium* in *Bulinus* sp., respectively, while Nwoko et al. (2023) found a prevalence of 0.9 and 3.5% in *Biomphalaria pfeifferi* and *Bul. africanus*, respectively. It is therefore highly likely that the parasites do indeed occur in this region but were simply not detected in the snail species collected. An additional reason may be as a result of environmental conditions at the time of sampling. An experiment conducted by De Kock (1973) measured the ideal temperature for reproduction habits and survival of *Bul. africanus* under different environmental conditions and determined that survival and reproduction rates of *Bul. africanus* were decreased in freshwater ecosystems with an ambient water temperature below 20 °C. This concurs with the negative results of this study regarding the infection prevalence of *Schistosoma* intermediate host species during the August 2021 sampling event at the end of the South African winter season when water temperatures were cooler (~15.1 °C; see Chapter 5 for further information). Monitoring of trematode transmission infections becomes less effective as infection rates decrease among definitive hosts in contact with the selected area of study (Abbasi et al. 2010). Therefore, in addition, molecular PCR tests were used to detect the presence of immature infections, resulting in the detection of three trematode species: *Neofibricola smiti* ex *Bul*. *africanus*, *Petasiger variospinosus* ex *Bul. depressus*, *O. batrachoides* ex *R. natalensis*.

Neofibricola smiti is a digenean trematode of which the adult life stage has been collected from the Nile crocodile (*Crocodylus niloticus* (Laurenti, 1768)) and metacercariae have been collected from the African clawed frog (*Xenopus muelleri* (Peters, 1844)) (Achatz et al. 2022). The identification of *N. smiti* within *Bul. depressus* is the first report of the infection of this trematode species within a freshwater mollusc of South Africa, thus providing valuable information on the life cycle and hosts of this recently described species. This study also provides the first molecular characterisation of the life stages of *N. smiti* from freshwater mollusc specimens of the genus *Bulinus*.

An additional trematode species collected from the Limpopo Lowveld Ecoregion was molecularly identified as *Pet. variospinosus*, detected in samples of *Bul. depressus* and *Bul africanus*. As mentioned, the sequences of what was labelled as *Petasiger* sp. 5 on GenBank were morphologically identified to be *Pet. variospinosus* ((Odhner, 1910) Yamaguti, 1933) by Hammoud et al. (2022), infecting *Bul. globosus, Bul. ugandae and Bul. truncatus* collected from Kenya (Laidemitt et al. 2019; Hammoud et al. 2022). In the current study, it has now also been detected by molecular method in *Bul. depressus and Bul. africanus* from the Limpopo

Lowveld Ecoregion of South Africa. The life cycle of *Pet. variospinosus* was experimentally completed by King and Van As (2000) and it was identified that trematode cercariae of *Pet. variospinosus* infect a mollusc host as the first intermediate host and cercariae released by the mollusc vector infected larvae of *X. laevis* as second intermediate host, before infecting the definitive avian host. The detection of *Pet. variospinosus* in these two *Bulinus* species suggests that the parasite is not host-specific in the *Bulinus* group, although further analysis would be necessary to support this theory completely.

The third trematode species molecularly identified from snails collected during the study, is *O. batrachoides* from *R. natalensis. Orientocreadium batrachoides* is known to use the African sharp-tooth catfish (*Clarias gariepinus*; (Burchell, 1822)) as a definitive host. Little further information is available on the life cycle of *O. batrachoides* in South Africa. However, it has been reported in Turkey that the first intermediate host for this species is an aquatic snail (*Lymnaea viridis*), with the second intermediate hosts ranging from mosquito larvae, dragonfly nymphs, tadpoles, and even young fish (Tepe et al. 2013), all of which are also located in the aquatic ecosystems of South Africa where the snails are found. This is the first known molecular characterisation of larval *O. batrachoides* from the freshwater molluscs *Bul. depressus* in South Africa and suggests that there may be several other mollusc species that act as intermediate hosts for this parasite.

4.4.2 Tarebia granifera *and* Melanoides tuberculata *as hosts for trematode parasites*

During the present study, all *T. granifera* specimens were found to be females and free from trematode infection. Miranda et al. (2011b) reported similar findings in *T. granifera* from KwaZulu-Natal. This invasive snail may be free from trematodes, as exotic parasites may not have migrated with the host or the parasites could have been unable to survive within the newly invaded environment due to a lack of suitable intermediate or definitive hosts (Keane and Crawley 2002; Torchin et al. 2003; Genner et al. 2008). The invasive snail may also be immune to native trematode infections due to evolutionary adaptations (Ebert 1994; 1998; Prenter et al. 2004; Fromme and Dybdahl 2006). As trematodes co-evolved with their hosts, invasive species may have resistance or immunity against native parasites (Genner et al. 2008). Therefore, trematodes that are native to South Africa have co-evolved with and are specialised to infect native snails and *Tarebia granifera* might be an unsuitable host for these native trematodes.

A review study by Preston et al. (2022) found that invasive snails had up to 80% lower parasite diversity within invaded habitats compared to their native ranges and this may also be a reason why other invasive mollusc species collected during the study, namely *Pseudosuccinea columella*, were free from parasites. Invasive species may have an advantage over native

species when they have no parasites and/or no predators within invaded habitats (Genner et al. 2008). Trematodes play a vital role in host snail populations as they can affect changes in survivorship, behaviour, morphology and cause reduced fecundity and growth of their snail hosts (Sørensen and Minchella 2001; Lafferty and Kuris 2009; McKoy et al. 2011). Trematodes can act as parasitic castrators and cause reduced population growth within snail populations (Genner et al. 2008; Weyl et al. 2020), thus *T. granifera* that are free from trematode infections in South Africa likely have an advantage in terms of reproduction and contribute to rapid population expansion (Weyl et al. 2020). This correlates with the population densities of *T. granifera* in South Africa, with several studies reporting densities of several thousand individuals/m^2 (Appleton et al. 2009; Miranda et al. 2011a; Miranda and Perissinotto 2014; Jones et al. 2017; see Chapter 5 for further information). Such advantages can have considerable effects on the population dynamics and competitive interactions among invaders and native species (Byers and Goldwasser 2001; Genner et al. 2008).

For the first time in South Africa, this study found the native thiarid, *M. tuberculata*, to be infected with *Haplorchis* sp. and *Centrocestus* sp. trematodes. This indicates that the trematode diversity from thiarid snails in South Africa is higher than previously reported in the literature. Trematodes of the family Heterophyidae, which includes *Haplorchis* sp. and *Centrocestus* sp., have been reported to cause gigantism and castrations in infected *M. tuberculata* with considerable effects on snail population growth and abundances (Genner et al. 2008). Although *M. tuberculata* is native to South Africa (Connolly 1939; Brown 1994; Appleton 2002), invasive morphs and/or hybrids from Asia may be present (Pointier et al. 2003; Genner et al. 2004; Raw et al. 2015). Therefore, it may be possible that invasive morphs of *M. tuberculata* may host parasites that are exotic in South Africa. However, the genetic diversity of *M. tuberculata* will first need confirmation with molecular analysis (Appleton and Miranda 2015a). Although no *T. granifera* have been found with trematode infections in South Africa, this may become a problem in the future as *T. granifera* are known to be the first intermediate hosts for several species of zoonotic trematodes within and outside of their native ranges (see Table S4.1 and 4.2); this includes species of *Haplorchis* and *Centrocestus* that were found to infect the native thiarid during the present study. Considering the parasite "spillback" hypothesis (Hoberg et al. 2002; Kelly et al. 2009; Chalkowski et al. 2018), it may be possible that parasites from native thiarid snails could infect (spillback into) *T. granifera* populations in the future, particularly in view of the fact that several studies have reported native parasite spillback into invasive/introduced species (Hemmingsen et al. 2005; Krakau et al. 2006; McAllister and Bursey 2016; Chalkowski et al. 2018). This could have severe risks for wildlife and humans in the future due to the rapid spread and high population densities reached by *T. granifera* (Appleton et al. 2009).

Veeravechsukij et al. (2018b) found that Thai *T. granifera* with trematode infections had lower reproductive output than uninfected individuals. According to the "Enemy Release" hypothesis, the absence of enemies such as parasites and predators within invaded ecosystems contribute to the invasion success of introduced species (Chalkowski et al. 2018). Thus, having no trematode parasites that influence their population growth in South Africa and the several other characteristics of *T. granifera* (see Chapter 1 section 1.1.4) enable them to quickly become the dominant species and displace native thiarids (such as *M. tuberculata*) within invaded ecosystems. Several studies have indicated that *T. granifera* displaces native snail species, especially *M. tuberculata* in South Africa (De Kock and Wolmarans 2008; Appleton et al. 2009; Miranda et al. 2011b; Miranda and Perissinotto 2012; 2014; Jones et al. 2017). Although the mechanisms responsible for this displacement are not fully understood (Raw et al. 2013; 2015), the present study highlights the importance of trematode infections that play a role in the competitive interactions among invasive and native freshwater snail species as well as snail population control agents (i.e., as castrators; see Chapter 5 for more information).

Despite the numerous negative effects and threats posed by *T. granifera* to our native biota, as outlined throughout the present study, the snail may prevent the spread of the lung fluke disease (paragonimiasis) in KwaZulu-Natal (Appleton 2014). *Melanoides tuberculata* is known to be the first intermediate host for *Paragonimus westermani* (Kerbert, 1878) in Asia (Nakagawa 1917; Davis et al. 1994; Blair et al. 1999). According to Appleton (2014), the first intermediate host for the trematodes causing the disease must be *M. tuberculata* or *Tomichia natalensis* (Connolly 1939). Chaniotis et al. (1980b) previously suggested that *T. granifera* was also an intermediate host for *Par. westermani*; however, this theory was disproved by Michelson (1992). Thus, as *T. granifera* displaces *M. tuberculata* and *Tom. natalensis*, this may bring an end to the spread of paragonimiasis in KwaZulu-Natal (Appleton 2014). *Tarebia granifera* has also been used in several countries as biological control agents for snail hosts of *Schistosoma* trematodes leading to the successful eradication of schistosomiasis in some of these regions (Prentice 1983; Perez et al. 1991; Sodeman 1991; Pointier and Jourdane 2000; Appleton et al. 2009; Pointier et al. 2010; Hewitt and Willingham 2019). As will be further discussed in Chapter 5, *T. granifera* seems to be replacing native mollusc species in sites sampled during the current study, several of which are known to be hosts for *Schistosoma* trematodes (Day and de Moor 2002; Tumwebaze et al. 2019). It is therefore also possible that the invasive *T. granifera* acts as an unanticipated biological control agent for schistosomiasis within the invaded ecosystems of South Africa; however, further research would have to be conducted on the topic.

4.5 Conclusion

The aim of this chapter was to examine freshwater mollusc vectors for infections of digenean trematodes and to investigate the role of the invasive thiarid (*T. granifera*) versus the native thiarid (*M. tuberculata*) as hosts for digenean trematodes within the Limpopo River system and Phongolo River. The cercarial shedding experiments were unsuccessful indicating that mollusc species, which tested positive for the presence of digenean trematodes, had an immature or early-stage infection. Positive trematode parasites were identified using molecular analyses of the molluscan tissue (hepatopancreas). The natural low prevalence of *Schistosoma* sp. in snails, and the declining survival and reproduction rate of native mollusc species during colder seasons, as well as the life cycle complexity of the *Schistosoma* and *Fasciola* digenean trematodes are possible reasons why these species were not present within the vectors collected. The confirmed presence of *N. smiti* within specimens of *Bulinus* is the first detection of this digenean trematode within a mollusc host of South Africa, indicating *Bul. depressus* and *Bul. africanus* as possible vectors for *N. smiti*, and producing new insight into the life cycle of *N. smiti. Petasiger variospinosus* detected within specimens suggests that *Pet. variospinosus* is not host-specific within the *Bulinus* species group. The addition of the newly generated data on these parasites gives a broader insight into the geographical range of digenean trematodes as well as much-needed insight into the complex life cycles of these parasitic species. The detection of *O. batrachoides* within specimens of *R. natalensis* is also the first report of this digenean trematode within a freshwater mollusc species. It was further found that invasive snails *Pseu. columella* and *T. granifera*, did not act as a host for trematode parasites within the study area, while the native snail, *M. tuberculata*, was found to be infected with two species of trematodes known to reduce snail population growth. This release from parasites that influence snail population growth enables *T. granifera* to reach higher densities and spread rapidly, possibly contributing to their advantage over native snails within the invaded ecosystems of South Africa.

CHAPTER 5: THE ROLE OF ABIOTIC AND BIOTIC FACTORS ON NATIVE MOLLUSC DISTRIBUTION IN SOUTH AFRICA

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5.1 Introduction

Freshwater molluscs are regarded as some of the world's most important grazers and fulfil an essential role in the food web of freshwater ecosystems as primary consumers that feed on peri- and epiphytic aquatic biota (Strong et al. 2007). This makes them a necessary part of the natural nutrient cycle and energy transfer to higher trophic levels within freshwater ecosystems (Strong et al. 2007). Despite their ecological importance and wide distribution and diversity globally, freshwater snails are one of the most endangered groups of species (Bogan 2007) for a variety of reasons including climate change, anthropogenic alterations to the environment and invasive species (see Chapter 1 for detailed information). This not only poses a threat to the native freshwater mollusc diversity, but various diseases as well since many freshwater mollusc species act as intermediate hosts for many parasites causing a number of diseases that are of economic interest as they infect humans and animals (Hoverman et al. 2011; Oloyede et al. 2016). As a result, knowledge of the current status of mollusc species diversity, distribution and abundance has become essential not only for ecological reasons but for protection of human health (Wolmarans et al*.* 2002; King 2010; Adekiya et al. 2020). In South Africa, this is of particular importance as only limited information has been available in recent years regarding the diversity and abundance of freshwater mollusc species across the country.

The occurrence and abundance of freshwater snails in aquatic habitats are influenced by a range of abiotic and biotic factors (Oloyede et al. 2016). These factors include climate-related elements such as temperature and precipitation, hydrological characteristics, physical habitat features, and various physicochemical parameters (Lodge et al. 1987; Dillon 2000; Brown and Lydeard 2010; Covich 2010; Barton et al. 2022). Moreover, differences in microhabitat conditions within water streams, such as variations in flow patterns, substrate composition, vegetation along the banks and within the water, and land use, contribute to differences in snail populations and species composition in freshwater environments (De Kock and Wolmarans 1998; Dillon 2000; Oloyede et al. 2016). It is thus crucial to consider these factors when assessing the distribution of freshwater molluscs.

The interaction between native and invasive species is key to understanding their effect on native species populations, yet this is not always measured (Riley et al*.* 2008). Invasive mollusc species have a detrimental effect on the function and services of an ecosystem they

are introduced to (Appleton 2003). The lack of natural predators, reproductive success and, often, the lack of parasitic burden likely allows invasive species to outcompete native species for the same ecological niche (Riley et al. 2008). This disrupts the food web and can lead to a bottom-up effect on the aquatic system where invasive species are present by not being palatable to predators or being better at evasion than native species. In South Africa, four invasive freshwater molluscs were identified in the north-eastern Provinces that require monitoring and have the potential to become a threat to native species, including *Pseudosuccinea columella, Physella acuta, Aplexa marmorata* and *Tarebia granifera* (Appleton 2003; De Kock and Wolmarans 2008; Weyl et al. 2020). *Tarebia granifera* is the most notorious of these invasive species as it has numerous adaptations to make it the most successful invader, including having a shell that is exponentially harder than that of most native species, making it inedible for most predators (Strayer 1999; Appleton et al. 2009; Covich 2010). This invasive snail has been found at an extremely high abundance in nature conservation areas including the Kruger National Park (De Kock and Wolmarans 2008; Majdi et al. 2022) and the Ndumo Game Reserve (Acosta et al. 2020; de Necker et al. 2021). This finding is of particular concern as it indicates not only the high level of success this invasive mollusc has accomplished from its first collection in 1999 from KwaZulu-Natal (De Kock and Wolmarans 2008), but also that conservation areas are at great risk of being invaded. The exponential spread of invasive mollusc species most likely harms native species diversity and causes numerous negative consequences for aquatic ecosystems that become invaded (De Kock and Wolmarans 2008; Riley et al. 2008). Therefore, continuous monitoring of native species and the spread of invasive species is of major importance in assessing the current status of South Africa's freshwater molluscs.

In South Africa, many economically important freshwater snails that transmit diseases, such as schistosomiasis and fascioliasis, are located in the tropical and subtropical regions including KwaZulu-Natal, Limpopo and Mpumalanga (De Kock and Wolmarans 2005; Appleton and Madsen 2012). At the same time, the highly invasive *T. granifera* is located in these regions as well (Appleton 2003; Pointier et al. 2003; Wolmarans and De Kock 2006; Appleton et al. 2009; Miranda et al. 2010; 2011b; Dube et al. 2017; Malherbe 2018). As *T. granifera* are usually the dominant invertebrate species in invaded aquatic communities (Pointier et al. 1998; Canete et al. 2004; Pillay and Perissinotto 2008; Appleton et al. 2009; Weyl et al. 2020), they have a high probability of causing environmental harm due to their ability to reproduce rapidly (Parker et al. 1999; Keller et al. 2007; Rumi et al. 2010; Nash and Hoffmann 2012; Kesner and Kumschick 2018). To determine the interactions of invasive species, such as *T. granifera*, with their environment and other aquatic biota, and to develop a suitable control strategy, it is important to understand their population dynamics (Burlakova

et al. 2010; Miranda et al. 2011a). The density (number of individuals/m^2) of a population describes the distribution and abundance of a species within a habitat (Ruehl and Trexler 2011). Population size structures describe the size of individuals in a population and provide information on the growth rate, age at maturity, as well as reproductive status of individuals (Peters 1983; Brown et al. 2004; Ruehl and Trexler 2011).

The central aim of this chapter was to assess the current distribution of native and invasive freshwater gastropod species within the north-eastern Provinces of South Africa, based on sites of historical importance from the NFSC database of South Africa. Additional aims were to 1) assess the role of environmental factors and the invasive mollusc *Tarebia granifera* on the distribution of native freshwater molluscs, and 2) investigate the current distribution, densities, and population size structures of *T. granifera* at selected sites on the Limpopo River system and the Phongolo River.

5.2 Methods

5.2.1 Field collection and laboratory assessment

Water samples were collected (see Chapter 2, section 2.3.1) and a qualitative habitat assessment was conducted (see Chapter 2, section 2.3.2) at each sampling site. All molluscs were then sampled at each site as described in Chapter 2, section 2.3.3.

To determine the population density of *Tarebia granifera*, additional samples were collected from specific sites on the Phongolo and Limpopo rivers. A snail sampler was used to scoop up sediment from the river's benthic zone and sieve it following the method by Pearson (2022). At each site, three sets of ten scoops per set (30 scoops in total) were collected. Only live snails were included in the analysis. The number of sampled individuals was counted to calculate the species density per site, measured as the number of individuals sampled per square metre. Next, the population size structures of both the invasive thiarid (*T. granifera*) and the native thiarid (*Melanoides tuberculata*) were determined following the method of Pearson (2022). Due to the high densities and small sizes of *T. granifera,* manual measurements were not possible. Instead, a precise size determination method was used. The snail samples were dried in an oven at 50 ˚C for 48 hours. Using a King Test sieve shaker with Clear Edge Test sieves, the snails were sorted into specific size classes. The sieves were stacked in descending order of mesh size, with a collection pan at the bottom. The snails were placed in the sieves and shaken for 10 minutes, causing them to pass through the sieves based on their shell width, thus dividing them into size classes. The length of snails in each size class was then measured by hand using a digital calliper with an accuracy of 0.1 mm. The count and length measurements allowed the creation of size classes corresponding to each sieve size.

All *T. granifera* specimens from the sampled sites with a width >4 mm (length >10.39 mm) were measured using a digital calliper, from the anterior to the posterior end of the shell. Snails with a width ≤4 mm (length <10.39 mm) were categorised into size classes based on their length (Table 5.1). The size classes for *Melanoides tuberculata* were determined in a similar manner to *T. granifera*. In this study, size classes were used to represent different age groups, based on the size at which sexual maturity is attained in the populations, as reported in published literature. For *T. granifera*, the size classes included mature adults (>10.39 mm), capable of reproducing; adults (5.28 - 10.39 mm), considered to be approaching sexual maturity and starting reproduction; juveniles (1.92 - 5.27 mm), unable to reproduce; and newborn snails (<1.9 mm) that were recently born. Size classes for *Melanoides tuberculata* consisted of older adults (>12.64 mm) capable of reproduction; mature adults (6.25 – 12.64 mm) also capable of reproduction; adults (2.33 - 6.24 mm) in the process of reaching sexual maturity; and juvenile snails (<2.33 mm) unable to reproduce. The average length, length range, and standard deviation were calculated for mature adult *T. granifera* (>10.39 mm) and older adult *M. tuberculata* (>10.39 mm).

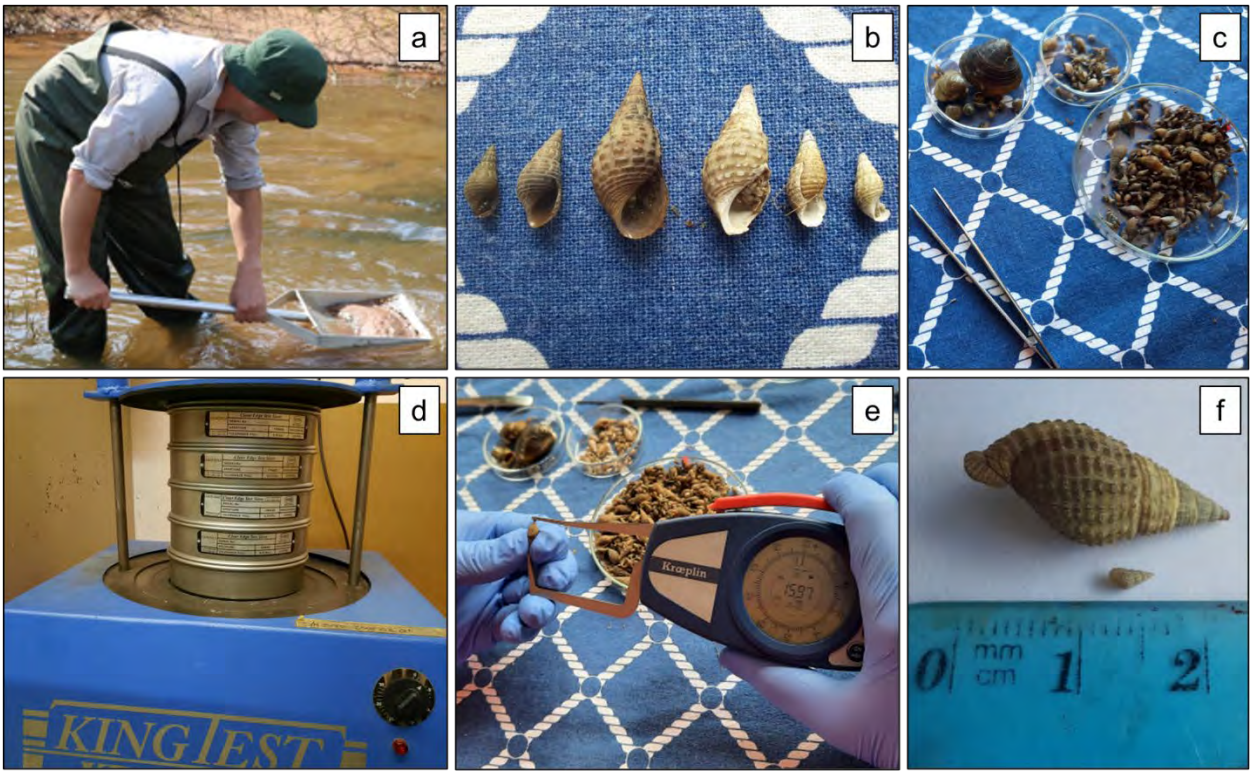

Figure 5.1: Methods used to collect and process aquatic snails: a) collecting snails with an aluminium snail sampler; b) comparison of live snails (left) and dead snails (right); c) snails being identified to species level and counted; d) the King Test (VB 200 300) sieve shaker with Clear Edge Test sieves used to sort snails into size classes; e) measuring snail length in mm with a digital calliper; and f) size comparison of a mature adult and a juvenile *Tarebia granifera.*

Table 5.1: Method used to group *Tarebia granifera* with a width ≤4 mm (length ≤10.39 mm) into size classes according to length.

Shaker sieve size (µm)	4 000	2 000	1 000	500
Snail width range (mm)	>4	4-2	2-0.5	< 0.5
Minimum snail length (mm)	10.04	5.28	1.92	<1.9
Maximum snail length (mm)	>10.04	10.39	5.27	1.9
Snail length size class (mm)	>10.39	10.39-5.28	5.27-1.92	<1.92
Snail age group	Mature adults	Adults	Juveniles	New-borns

The birth rate of *Tarebia granifera* was also calculated from the three sites sampled on the Olifants River between September and October 2020. Adult *T. granifera* snails gave birth while they were housed to investigate the possibility of parasitic infection (Chapter 4). The new-born snails were collected, and the birth rate was calculated: Birth rate = *Nb*/*Na*/*Nd*, where *Nb* is the number of juvenile snails born, *e* is the number of adult snails housed, and *Nd* is the number of days the snails were housed.

5.2.2 Data analysis

Data regarding the historical distribution of freshwater snails in the study regions were obtained from the NFSC to compare the historical distribution and diversity of snail species in the sampling areas to those collected during the current study. These data could only be compared qualitatively as the historical data only indicates the species at sampling sites with no quantitative information available.

The number of individuals and the number of taxa were determined for the various sites using untransformed data. Mollusc species data were then transformed by way of square root ($\sqrt{}$) to mitigate the effect of dominant taxa (de Necker et al. 2021). Collected environmental data, excluding pH, were log-transformed [$y = \log(x + 1)$] to improve data normality, before further parametric tests. The number of taxa collected at each site was compared between the two sampling surveys (high flow and low flow) using "Flow" as the a priori factor to test the impact of seasonal flow for both. Due to its high density at sampling sites, the presence or absence of the invasive mollusc *Tarebia granifera* was also used as an a priori factor "*Tarebia granifera"* to assess potential differences between sampling sites as well. Results were visualised using non-metric multidimensional scaling (nMDS) ordinate plots and dendrograms using Primer v7 and a Bray-Curtis similarity matrix (Clarke et al*.* 2014; Clarke and Gorley 2015; de Necker et al. 2021). The factors "Flow" and "*Tarebia granifera"* were tested for significant differences using the analysis of similarities (ANOSIM). Two values are produced by the ANOSIM, an R-value (from 0 to 1) and a significance-level percentage value. The closer the R-value is to 1, the more critical the selected factor is. The significance level indicates whether the element being tested is significant (≤5%) or not (≥5%; (Clarke and Warwick 1994; Clarke and Gorley 2015). A canonical correspondence analysis (CCA) was further conducted using Canoco v5 software to assess the mollusc abundance and diversity compared to water quality parameters between sampling sites and seasons.

To determine *T.* granifera population density (m^2) and snail population size contributions, GraphPad Prism 7 software was used to create graphical representations as well as test for significant differences. Differences in snail density sampled from the selected sites were tested for significance using a Kruskal-Wallis test for non-parametric data and Dunn's post hoc test to determine specific significant ($p<0.05$) pairwise differences between the population densities of *T. granifera* and the other snail species sampled (Stoline 1981; de Necker et al. 2019). Pearson's correlation tests were completed to determine whether *T. granifera* population density were significantly correlated to water quality variables (water quality variables were grouped for analysis).

5.3 Results

5.3.1 Water quality analysis

Water temperature was generally higher in the high-flow sampling season (22.94±0.52 °C) compared to the low-flow season (19.97±2.49 °C) with the lowest measured water temperature at site LLF4 during the low-flow sampling season (15.1 °C; Table 5.3), and the highest temperature measured at LHF4 in the high-flow sampling season (26 °C) (Table 5.2). The pH was similar between all the sites with a mean of 8.3 (± 0.52) during the high-flow sampling period and a mean of 8.4 (± 0.56) in the low-flow season. The highest pH measured was 9.27 at site LLF4 during the low-flow season and the lowest pH (7.5) was measured at site LHF3 during the high-flow season. The conductivity was much higher in the high-flow sampling period (994.50± 585.47 µS/cm) than in the low-flow season (346.54± 296.65 µS/cm) with the highest values measured during the high-flow season at site LHF3 (2415 µS/cm) compared to the lowest measurements during the low-flow season at site LLF9 (30.6 µS/cm). Dissolved oxygen saturation was higher in the high-flow season (121.78 ±0.98%) than in the low-flow season (106.17 ± 18.74%), ranging from 63.7% (6.79 mg/L) at site LLF4 in the low-flow season, to 154% (12.8 mg/L) at site LHF3 in the high-flow season. Turbidity was generally higher in the low-flow (13.13±6.38 FAU) than in the high-flow season (12.52±3.84 FAU) with the lowest values measured at site LLF7 (5 FAU) in the low-flow season, and the highest measured at site LLF2 (31 FAU) in the low-flow season. The concentration of chemical variables measured in the laboratory also varied between the two sampling seasons, with NH_4^+ (0.08±0.05), NO_2^- (0.02±0.02), Cl^- (104.93±177.83) and SO_4^{2-} (51.63±63.1) measured highest in the high-flow sampling season and NO_3^- (1.51±1.52) and PO_4^{3-} (3.9±2.21) measured highest in the low-flow sampling season.

Table 5.2: The *in situ* water and chemical water quality parameters obtained during the high-flow sampling season (April-May 2021) at the selected sites within the north-eastern Provinces. The standard deviation between all the sites is indicated in the last row labelled SD*.

Date	Site Code	In situ measurements						Chemical water quality parameters					
		Temperature (°C)	pH	Dissolved oxygen (mg/L)	Oxygen saturation (%)	Turbidity (FAU)	Conductivity (us/cm)	NH_4^+ (mg/L)	NO_3^- (mg/L)	NO_2 (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)
22-Apr-21	LHF1	22.40	9.20	10.8	121.4	9.67	781.50	0.06	1.77	0.08	0.24	53.00	14.00
25-Apr-21	LHF2	21.60	7.60	9.4	106.8	8.00	857.00	0.14	0.93	0.00	0.16	603.00	216.67
26-Apr-21	LHF3	23.60	7.50	12.8	154	20.00	2415.00	0.04	1.33	0.02	0.04	81.33	58.00
29-Apr-21	LHF4	26.00	8.80	11.4	131.9	12.00	422.00	0.12	1.97	0.01	0.11	32.67	62.67
03-May-21	LHF5	24.90	8.30	10	118.5	17.67	381.00	0.18	0.87	0.01	0.05	87.33	12.00
04-May-21	LHF6	20.30	8.60	10.2	109.5	8.00	547.00	0.10	0.41	0.00	0.10	13.37	4.00
05-May-21	LHF7	22.70	8.40	9.7	113.2	11.67	804.50	0.05	0.20	0.01	0.03	19.33	14.67
06-May-21	LHF8	22.70	8.80	11	125.5	12.67	1285.50	0.02	0.27	0.01	0.02	28.33	13.00
06-April-May-21	LHF9	22.30	8.30	10.2	115.2	13.00	1007.00	0.01	1.00	0.01	0.01	26.00	69.67
Mean ±SD*	-	22.94 ± 0.52	8.39 ±13.57	10.61 ±3.84	121.78 ±0.98	12.52 ±3.84	944.50 ±585.47	0.08 ±0.05	0.97 ±0.59	0.02 ±0.02	0.08 ±0.07	104.93 ±177.85	51.63 ±63.1

Table 5.3: The *in situ* water and chemical water quality parameters obtained during the low-flow sampling season (August 2021) at the selected sites within the north-eastern Provinces. The standard deviation between all the sites is indicated in the last row labelled SD*.

Date	Site code	*In situ* measurements						Chemical water quality parameters					
		Temperature (°C)	pH	Dissolved oxygen (mg/L)	Oxygen saturation (%)	Turbidity (FAU)	Conductivity (us/cm)	NH_4^+ (mg/L)	NO_3^- (mg/L)	NO_2 (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)
23-Aug-21	LLF1	18.30	9.25	11.72	122.60	14.00	-	0.03	2.00	0.01	3.74	33.00	48.00
23-Aug-21	LLF2	18.70	9.09	13.38	145.40	31.00	665.00	0.02	1.20	0.01	5.37	21.00	68.00
23-Aug-21	LLF3	19.40	9.04	10.45	114.30	13.00	-	0.01	1.60	0.01	6.15	10.00	91.00
24-Aug-21	LLF4	15.10	9.27	6.79	63.70	7.00	916.00	0.01	0.70	0.01	3.19	10.00	8.00
24-Aug-21	LLF5	17.50	9.08	8.14	85.00	7.00	-	0.01	0.50	0.01	7.11	10.00	7.00
24-Aug-21	LLF6	23.00	8.48	9.11	117.70	20.00	-	0.01	0.50	0.01	3.32	10.00	4.00
24-Aug-21	LLF7	21.60	8.57	8.75	91.20	5.00	208.00	0.01	0.50	0.01	0.82	10.00	10.00
25-Aug-21	LLF8	17.50	7.93	9.07	97.10	17.00	278.00	0.01	1.20	0.01	5.31	7.00	304.00
25-Aug-21	LLF9	18.20	8.10	8.57	88.80	7.00	30.60	0.01	1.80	0.01	3.57	10.00	4.00
25-Aug-21	LLF10	21.90	7.93	10.59	124.40	8.00	48.50	0.01	6.90	0.01	4.62	10.00	40.00
25-Aug-21	LLF11	23.60	7.72	10.48	123.30	12.00	171.20	0.01	1.70	0.01	3.12	9.00	37.00
26-Aug-21	LLF12	19.40	7.77	10.19	109.70	19.00	214.00	0.01	1.60	0.01	0.97	10.00	21.00
26-Aug-21	LLF13	18.50	7.76	10.49	110.30	10.00	394.00	0.01	2.40	0.01	0.81	10.00	42.00
26-Aug-21	LLF14	24.20	7.63	9.30	101.90	16.00	151.60	0.01	0.50	0.01	8.88	10.00	17.00
26-Aug-21	LLF15	20.70	7.82	8.98	97.20	14.00	184.60	0.01	0.50	0.01	1.99	10.00	2.50
26-Aug-21	LLF16	21.90	7.73	9.61	106.10	10.00	897.00	0.01	0.50	0.01	3.49	10.00	2.00
Mean ±SD*	-	19.97 ± 2.49	8.3 ± 0.61	9.73 ± 1.48	106.17 ± 18.74	13.13 ± 6.38	346.54 ± 296.65	0.01 ± 0.01	1.51 ±1.52	0.01 ± 0.00	3.9 ± 2.21	11.88 ± 6.14	44.09 ± 71.69

5.3.2 Mollusc species identification

Historical distribution of molluscs compared to currently collected species

The number of species collected in the current study was lower compared to historically collected specimens, with only ten different species collected compared to the 15 species previously recorded at the same sites. The results indicated that the diversity of mollusc species declined from 15 different species historically collected, with two being invasive species and the rest native, to 10 species collected during the current study with three being invasive species and the remaining six being native. A total of 4 557 invasive freshwater snail individuals were collected, while only 1 629 individuals of native freshwater snail species were collected during the Limpopo Lowveld Ecoregion sampling event (see Tables S5.1 and S5.2). Among these invasive species, a newly observed invasive species was identified that was not present historically, namely *T. granifera*, but detected at most sites during the current study. In the past, seven different species were identified at four of the sampled sites (LLF13-16). However, in the current study, the diversity of species across these sites was reduced to only four different species, all four of which were collected at LLF16.

Table 5.4: Species historically identified within the selected study area by data available from the NFSC database from the NWU-Potchefstroom campus, compared to species collected during this study and morphologically and molecularly identified from the sites located in the Limpopo Lowveld Ecoregion. Presence indicated by X.

Species:	**Species historically collected in the NFSC**	**Species collected during the current study**
Bulinidae		
Bulinus africanus	X	X
Bulinus depressus	X	X
Bulinus forskalii	X	
Bulinus globosus	X	
Bulinus natalensis	X	
Bulinus tropicus	X	
Lymnaeidae		
Pseudosuccinea columella	X	X
Radix natalensis	X	X
Physidae		
Physella acuta	X	X
Planorbidae		
Biomphalaria glabrata	X	
Biomphalaria pfeifferi	X	X
Gyraulus connollyi	X	X
Gyraulus costulatus	X	X
Thiaridae		
Melanoides tuberculata	X	X
Melanoides victoriae	X	
Tarebia granifera		X

High-flow season sampling

During the high-flow sampling period, 2 993 mollusc individuals from two genera were collected from eight sites within the Limpopo, North West and Mpumalanga Provinces. The most abundant species present was the invasive *T. granifera* (n = 2 059 individuals, totalling 68.8% of specimens collected during the high-flow sampling period) collected at all the high flow sites. The invasive species *Phy. acuta* (n = 2, 0.1%) was only found at one of the high-flow sites (site LHF7), and *Melanoides tuberculate* (n = 932, 31.1%) was collected at a single site (LHF3) where few *T. granifera* were present (five individuals). The high-flow sites were all river sites with little to no marginal, emergent, or submerged vegetation present. Each site had a single dominant taxon present, with six of the eight high-flow sites (LHF1, LHF2, LHF4, LHF5, LHF6, LHF8) having only one molluscan taxon present, namely *T. granifera*. The LHF3 and LHF7 sites had a second additional taxon present, namely the native species *M. tuberculata*, at site LHF3, and invasive *Phy. acuta*, at site LHF7 in addition to *T. granifera*. A higher abundance of *M. tuberculata* (932 individuals) was observed at site LHF3, compared to *T. granifera* (five individuals), while a higher abundance of *T. granifera* (139 individuals) was observed at site LHF7, compared to *Phy. acuta* (two individuals).

Low-flow season sampling

A total of 910 mollusc individuals from six different genera were collected during the low-flow sampling period. Species were identified with the aid of morphological and molecular methods (discussed in Chapter 4) and were identified to be members of the *Bul. africanus/globosus* group (containing *Bul. globosus* and *Bul. africanus*), *Bul. tropicus/truncatus* group (containing *Bul. tropicus, Bul. truncatus, Bul. natalensis* and *Bul. depressus*)*, Phy. acuta, Biom. pfeifferi, R. natalensis, Pseu. columella, G. costulatus* and *T. granifera*. Of these, species from three genera were considered invasive (*Phy. acuta*, *Pseu. columella* and *T. granifera*) and five were considered native (*Bul. africanus, Bul. depressus, G. costulatus, Biom. pfeifferi, R. natalensis*). The most abundant native mollusc species collected across all the low-flow sites were identified to be *R. natalensis* (n = 221 individuals, 24% of total specimens collected in the low-flow sampling period) collected at sites LLF1, LLF3, LLF4, LLF5, LLF6, LLF7, LLF8, LLF9, LLF10, LLF11, and LLF14, with *Bulinus* sp*.* being the least abundant native mollusc species collected in the same period (n = 110, 12% of total specimens collected in the low-flow sampling period) at sites LLF 6, LLF7, LLF11, LLF12, LLF13, and LLF14. The highest species diversity was recorded at site LLF16 with four different genera collected of which two are known invasive species (*Pseu. columella* and *Phy. acuta*) and two native species (*Bulinus* spp*.* and *G. costulatus).* Site LLF1 had the lowest species diversity, with only two individuals of *R. natalensis* present. Only one mollusc genus was recorded at Sites LLF3, LLF4, and LLF5, namely *R. natalensis,* but in higher abundance than at site LLF1, with an average of 34

specimens at these three low-flow sites. The highest number of individuals was collected at site LLF2, with 121 individuals of *Phy. acuta* and 111 individuals of *Biom. pfeifferi.*

The invasive mollusc species *T. granifera* was collected only at two sites (LLF12 and LLF13) during the low-flow sampling period, with only 16 individuals detected at each site. In addition to *T. granifera*, two other genera were present at each site, namely *Bul. africanus* (24 individuals) and *G. costulatus* (80 individuals) at site LLF12; and *Bul. africanus* (one individual) and *Pseu. columella* (six individuals) at site LLF13.

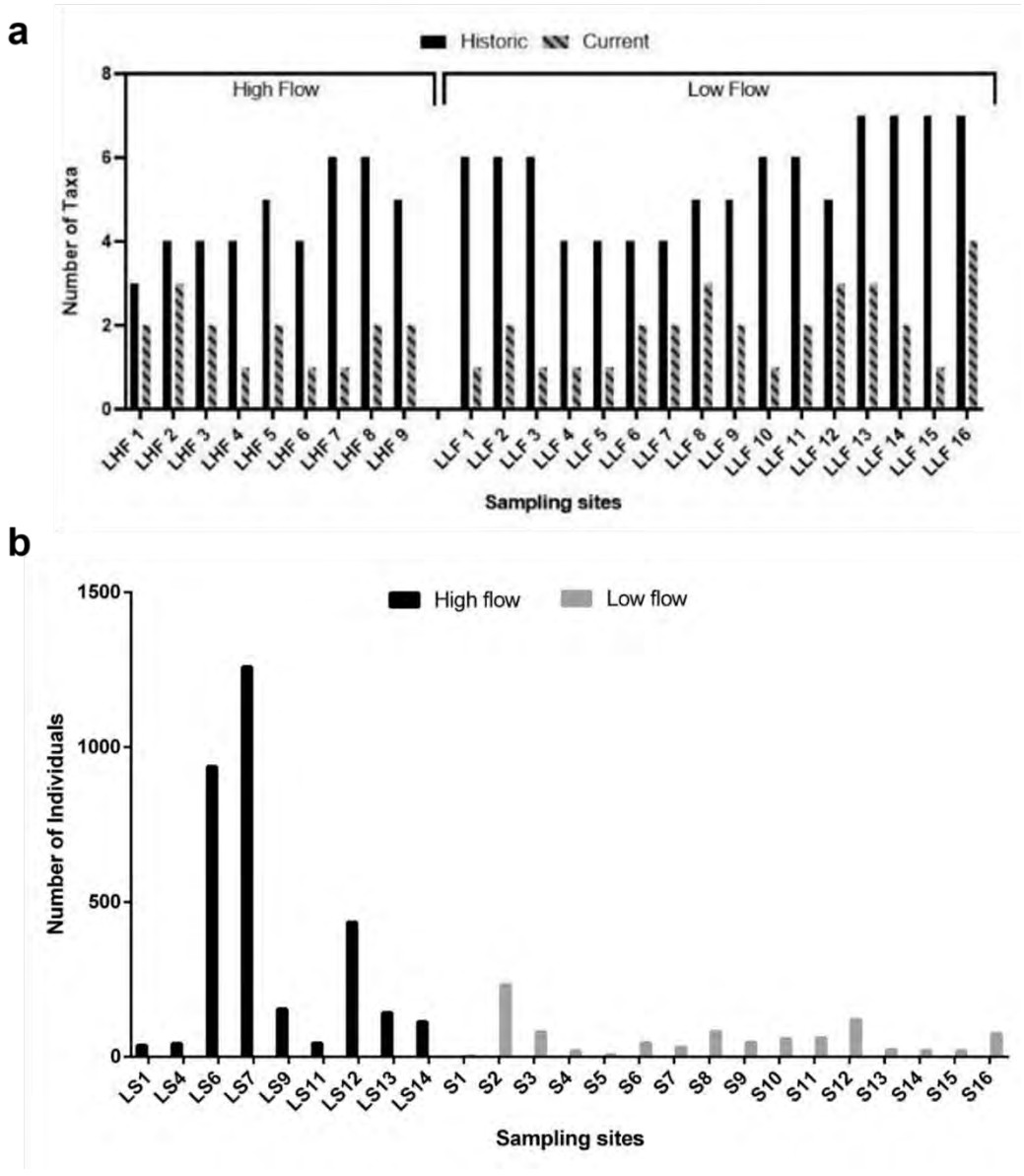

Figure 5.2: Graph indicating (a) the total number of genera historically collected at each site (retrieved from the NFSC), compared to the current number of genera collected during this study; and (b) the number of individuals collected during the high-flow sampling (LHF) season (April-May 2021) and low-flow sampling (LLF) season (August 2021), respectively, at the 25 selected sites within the north-eastern Provinces, South Africa.

5.3.3 Comparison of high-flow and low-flow sampling sites

The non-metric multidimensional scaling (nMDS) plot indicated a separation between the high-flow sites and the sites sampled during the low-flow period (Figure 5.3). The analysis of similarities (ANOSIM) further determined that there was a substantial difference between these groups based on the a priori factor “Flow” (R-value = 0.625) and that these differences were significant (significance level percentage = 0.1%).

The canonical correspondence analysis (CCA) indicated a clear separation between the sites sampled during the high-flow season compared to those sampled during the low-flow season (Figure 5.4). The CCA explained 44.06% of data variation, with 26.91% explained on the first axis and 17.15% explained on the second axis. The graph revealed that sites sampled during the high-flow period grouped together, and apart from sites sampled in the low-flow period with separation between the sampling seasons resulting from differences in mollusc diversity, water chemistry and physical habitat. Water temperature and NH_4^+ were positively associated with sites sampled in the high-flow season, with these values being much higher in the high-flow, compared to the low-flow period (see section 5.3.1), while PO_4^{3-} was much higher in the low-flow period and positively associated with sites sampled in this season along with oxygen saturation, that was much lower in the low-flow sampling season than in the high-flow sampling season (see section 5.3.1).

Furthermore, the qualitative assessment of substrate and aquatic vegetation indicated substantial differences between the high-flow and low-flow sampling sites (refer to Table S5.3). The sites sampled during the high-flow period had less aquatic vegetation, with the substrate primarily consisting of sand and gravel. No floating or submerged vegetation was observed at these high-flow sites, and algae were only present at sites LHF1 and LHF7. Conversely, the sites sampled during the low-flow period displayed a higher presence of aquatic vegetation. Specifically, floating vegetation, mainly water hyacinth, and water lilies were present at the majority of the sampling sites along with algae.

An additional factor causing separation between the sampling sites was the overwhelming presence of the invasive species *T. granifera* and the absence of native species during the high-flow period. Species diversity during the high-flow sampling season was lower, with only three genera collected, namely *T. granifera, M. tuberculata* and *Phy. acuta,* of which two, namely *T. granifera* and *Phy. acuta*, are considered invasive to South Africa, and *M. tuberculata* is regarded as native to South Africa. The invasive *T. granifera* was also extremely dominant during the high-flow sampling period, reaching an average of up to 241 individuals per site during this period. The native *M. tuberculata* was only collected during the high-flow season at one site (LHF3) in high abundance (932 individuals), the only site sampled during the high-flow sampling period where the lowest abundance of *T. granifera* was observed (five individuals collected). At the sites

sampled during the low-flow sampling period, six different genera were collected, ranging from an average of 58 individuals at each of the sites sampled during the low-flow sampling period. There was no clear dominance of one species with the four invasive species (*Phy. acuta, Pseu. columella, G. costulatus* and *T. granifera)* and three native species (*Bulinus* spp*., G. costulatus, R. natalensis* and *Biom. pfeifferi*) collected during the low-flow sampling period. The highest number of individuals collected at one site was 121 individuals of *Phy. acuta* at site LLF2. *Radix natalensis* was the most commonly found among the sites sampled during this period (LLF1, LLF3, LLF4, LLF5, LLF6, LLF7, LLF8, LLF9, LLF10, LLF12, LLF14).

There was a distinct separation between the sampling sites based on the presence or absence of the invasive snail species *T. granifera,* as observed in the nMDS plot, with two distinct groups forming (Figure 5.5). The analysis of similarities (ANOSIM) further confirmed a substantial difference between these two (R-value = 0.764) and the groupings were significant (significance level percentage = 0.1%) when considering the a priori factor "*T. granifera*".

Sites where *T. granifera* was present formed a distinct cluster separate from sites where *T. granifera* was absent, regardless of the sampling season. Notably, during the low-flow period, *T. granifera* was present at only two sites (LLF12 and LLF13). These two sites exhibited a distinct separation from all other low-flow sites, which lacked *T. granifera*, and instead displayed greater similarity to all the high-flow sampling sites where *T. granifera* was present.

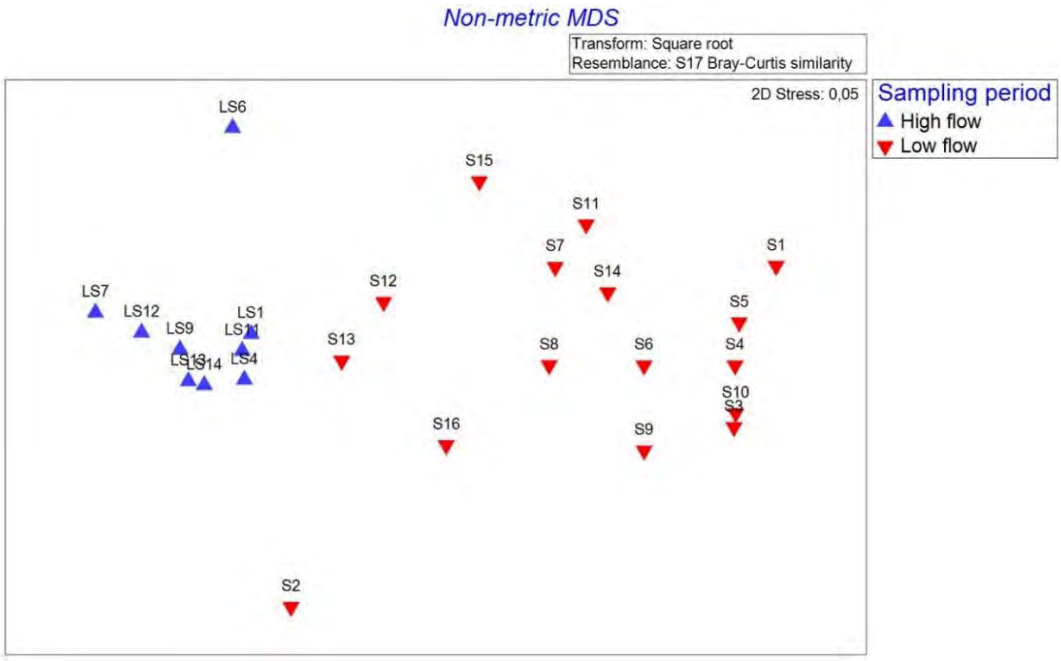

Figure 5.3: Non-metric multidimensional scaling (nMDS) indicating differences in mollusc species diversity and abundance during the high-flow sampling (LHF) season (April-May 2021) and low-flow sampling (LLF) season (August 2021), respectively, within the north-eastern Provinces.

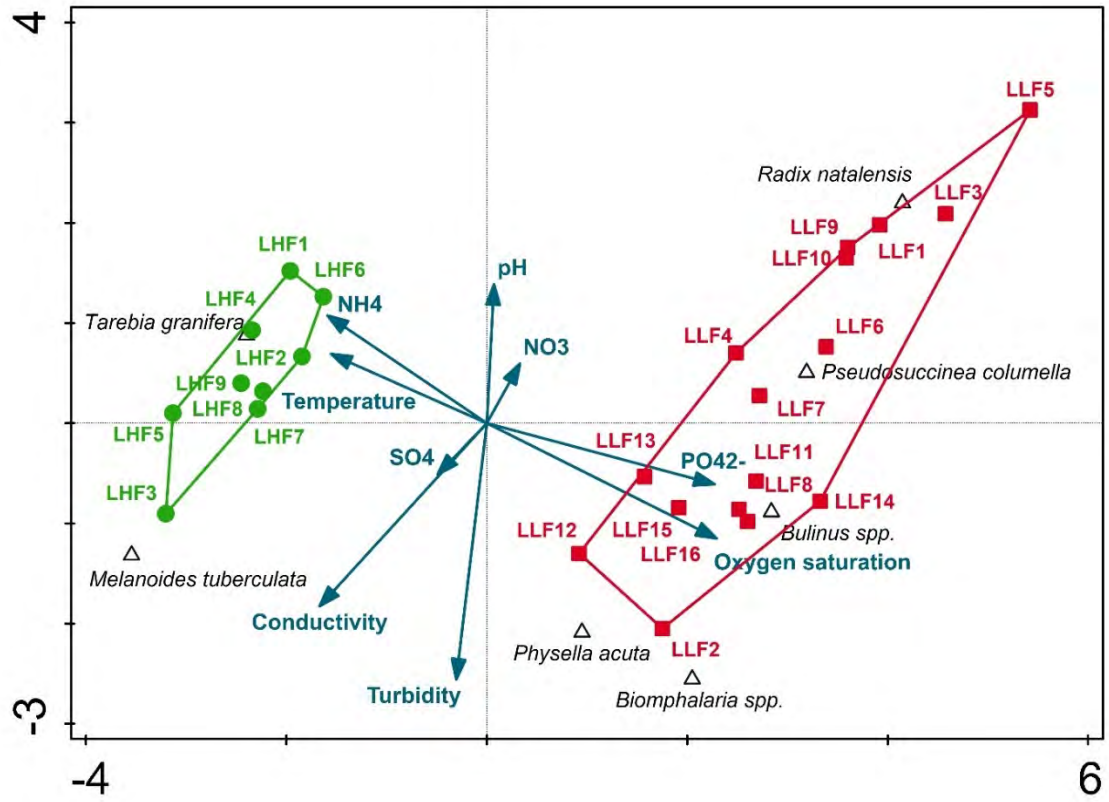

Figure 5.4: Canonical correspondence analysis (CCA) indicating differences between sites sampled during the high-flow sampling (LHF) season (April-May 2021) and low-flow sampling (LLF) season (August 2021), respectively. The CCA explains 44.06% of data variation, with axis 1 explaining 26.91% and axis 2 explaining 17.15%. Green circles and lines = sites sampled during the high-flow season; red squares and lines = sites sampled during the low-flow season; blue arrows = water quality parameters; black triangles = mollusc species collected.

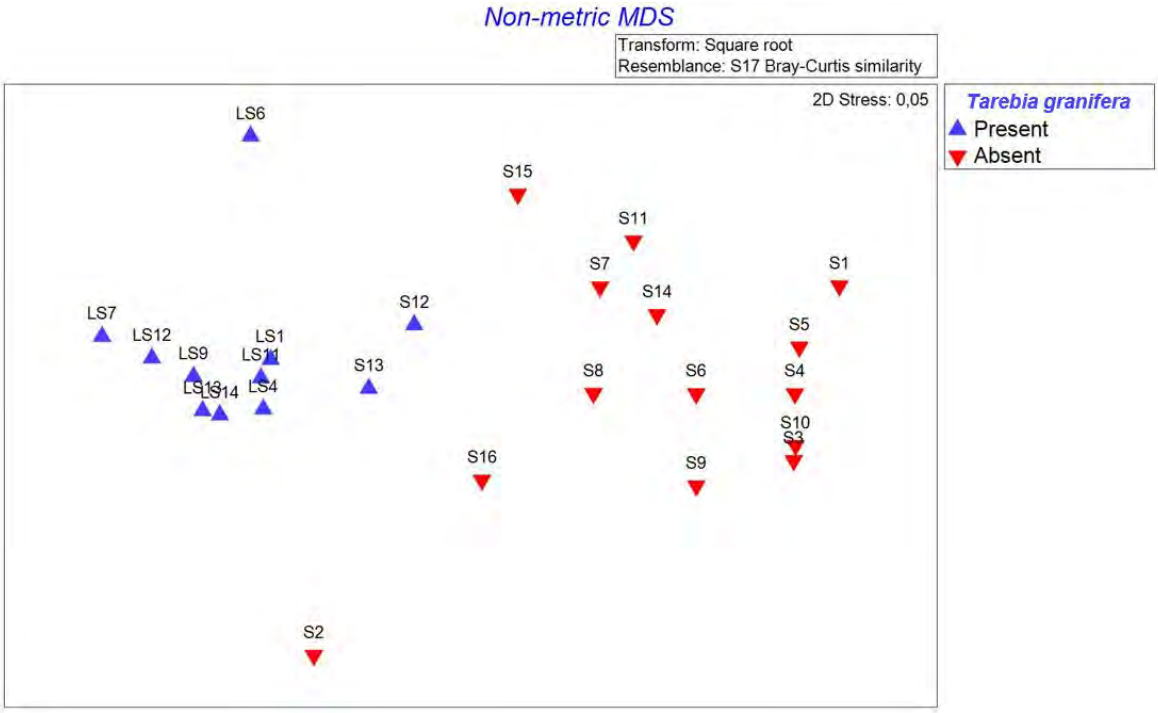

Figure 5.5: Non-metric multidimensional scaling (nMDS) indicating the presence of the invasive *T. granifera* and their effect on species distribution between sites sampled during the high-flow sampling (LHF) season (April-May 2021) and low-flow sampling (LLF) season (August 2021), respectively, at the 25 selected sites within the north-eastern Provinces.

5.3.4 Tarebia granifera *population density*

Of the 19 selected sites sampled specifically for *T. granifera*, aquatic molluscs were found to be present at 14 sites. *Tarebia granifera* were the only species that were present at all 14 of these sites. No molluscs were found at the CROC, MATL, LEPH, SAND, and LIMP-3, sites during the study. A total of four mollusc species were collected during the study; two invasives, namely *T. granifera* and *Physella acuta,* and two natives, namely *M. tuberculata* and *Corbicula fluminalis*. In total 29 378 aquatic molluscs were sampled, counted, and measured and *T. granifera* were the dominant species making up 26 178 individuals (89.11% of all sampled molluscs), followed by *C. fluminalis* (1 596 individuals; 5.43% of all sampled molluscs), *M. tuberculata* (1 456 individuals; 4.96% of all sampled molluscs) and *Phy. acuta* (148 individuals; 0.5% of all sampled molluscs).

Tarebia granifera was present at all four sites sampled on the Phongolo River (Figure 5.6), with an average density of 699 individuals/m^2 between sites. The PHON-2 and PHON-3 sites had the highest densities of *T. granifera* (1 648 and 610 individuals/m^2 respectively) while the PHON-1 and PHON-4 sites had the lowest densities (319 and 217 individuals/m^2 respectively). The highest snail densities recorded during the entire study (all 14 sites) were of *T. granifera* from the OLIF-1 and OLIF-2 sites during the 2020 survey on the Olifants River with 3 290 and 3 183 individuals/m^2, respectively. The same two sites (OLIF-1 and OLIF-2) sampled in 2021 had much lower densities of *T. granifera* (48 and 564 individuals/m^2, respectively). The OLIF-3 site (surveyed in 2020) had a density of 519 individuals/m^2. The OLIF-3 site was not revisited during the second survey in 2021 due to safety reasons following high flow events and the difficulty of the terrain. The three sites sampled on the Limpopo River had low densities of the invasive *T. granifera*. The highest density of the snail was recorded at the second site on the Limpopo River (LIMP-2) with 15 individuals/m^2, followed by the first site (LIMP-1) with three individuals/m^2, while the LIMP-3 site did not have any snails present. The sites on the Mogalakwena (MOGA) and Great Letaba (G-LETA) rivers had higher densities of *T. granifera* (136 and 214 individuals/m^2 respectively), while the sites on the Shingwedzi River (SHIN; 67 individuals/m^2), Letaba River (LETA; 56 individuals/m^2) and the Luvuvhu River (LUVU; 42 individuals/m^2) had lower densities of the invasive snail. Results from Pearson's correlation tests (Table 5.5) indicated no significant correlations between *T. granifera* population density and any of the water quality variables ($p>0.05$; $R^2<0.06$).

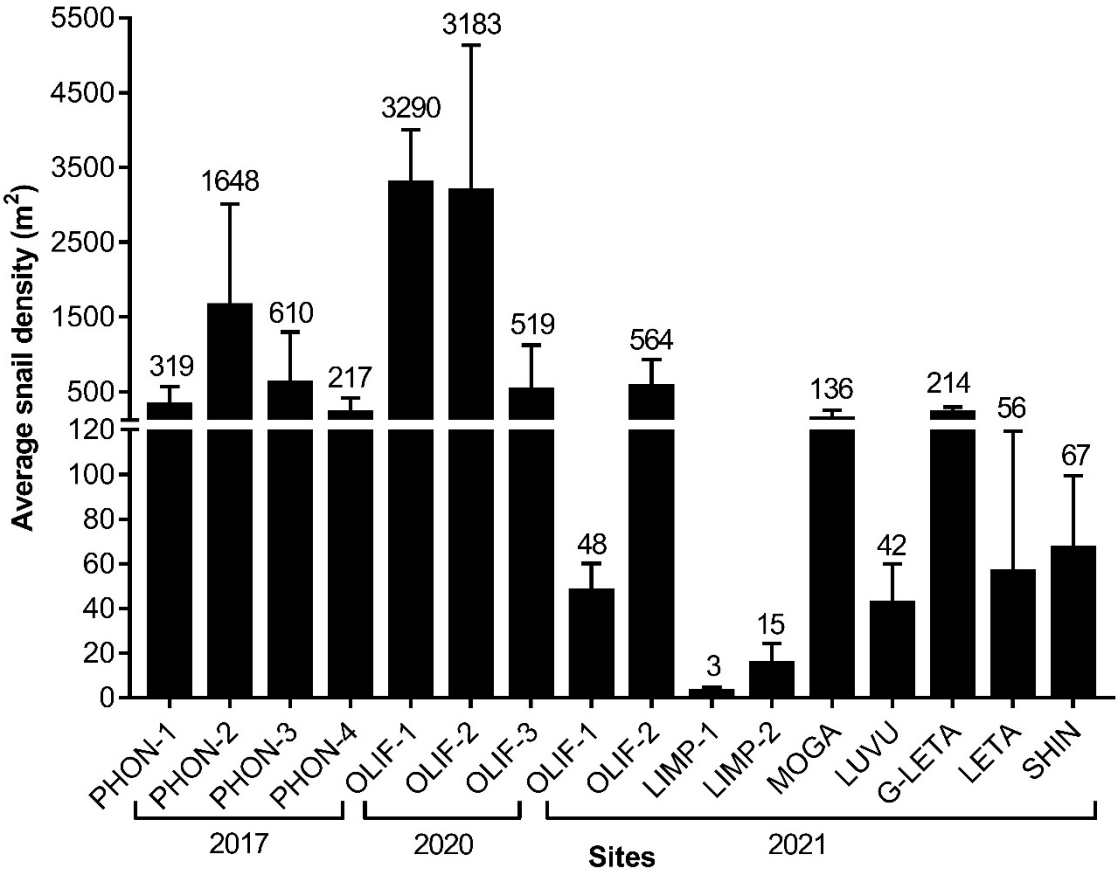

Figure 5.6: Average *Tarebia granifera* population density (±SD) sampled from the selected sites on the Phongolo River and Limpopo River system from 2017 to 2021.

Table 5.5: Pearson correlations between *Tarebia granifera* population density and water quality variables of all sites sampled with *Tarebia granifera* on the Phongolo River and Limpopo River system.

	Water quality variables							
	Temperature (°C)	Conductivity (µS/cm)	pH	Cl^- (mg/L)	SO_4 (mg/L)	NO_3 (mg/L)	NH_4 (mg/L)	PO_4 (mg/L)
T. granifera population density (m^2)	$R^2 = 0.034$ p = 0.50	$R^2 = 0.021$ p = 0.59	$R^2 = 0.0001$ p = 0.95	$R^2 = 0.026$ p = 0.55	$R^2 = 0.056$ p = 0.38	$R^2 = 0.046$ p = 0.42	$R^2 = 0.0054$ p = 0.79	$R^2 = 0.009$ p = 0.73

5.3.5 Tarebia granifera *population size structures*

The average birth rate of *T. granifera* was 1.89 new-born snails per day. The percentage contribution of *T. granifera* population size structures is illustrated in Figure 5.7 and described below. The *T. granifera* population from the Phongolo River (PHON) mainly consisted of adults (42.51%) and mature adults (41.30%) from the 5.28 - 10.39 and >10.39 mm size classes respectively, while 15.38 and 0.81% of the population consisted of juveniles (1.92 - 5.27 mm) and new-borns (<1.9 mm), respectively. During the 2020 survey of the Olifants River, the OLIF-1 population consisted mainly of adults (79%), followed by juveniles (14%) while only a few mature adults (5.89%) and new-borns (0.66%) were present. A few mature adults (5.82%) were also detected among the OLIF-2 population, while juveniles and new-born *T. granifera* were more abundant (48 and 27.82%, respectively). The contribution of new-borns was the highest recorded across all populations from all sites sampled. Adults (37.59%) dominated the population of the OLIF-3 population, followed by juveniles (31%), new-born *T. granifera* (18.62%) and mature adults (11.84%). The *T. granifera* population structure at OLIF-1 (2021) did not differ much temporally and the population consisted mainly of adults (55.81%) and juveniles (30%); between 2020 and 2021, an increase of 16% in the number of juveniles was observed. Mature adults again formed the smallest part of the population (13.95%) even though it increased by 8.06% from 2020 to 2021; no new-born *T. granifera* were sampled during the 2021 survey. The population of the OLIF-2 site (2021) indicated an increase in juveniles from 48 to 61%, adults from 17.76 to 21.72%, and mature adults from 5.82 to 16.80% while the relative contribution of new-borns declined from 27.82 to 0.33%. During the 2021 survey, the juvenile population sampled from the OLIF-2 site was the highest recorded contribution of juveniles from all populations across all sites sampled.

For both the LIMP-1 and LIMP-2 sites on the Limpopo River, no new-born *T. granifera* were sampled and adult snails formed the largest part of the populations, 57.1 and 82.93%, respectively. The adult population of the LIMP-2 site made the highest relative contribution to all population ages across all sites. Juvenile and mature adults contributed to 28 and 14.29% of the LIMP-1 snail population, while they contributed to 7 and 9.76% of the LIMP-2 population,

respectively. The *T. granifera* population of the Mogalakwena River (MOGA), had the largest proportion of mature adults (64.95%) of all the populations from the selected sites. Adults and new-borns made similar contributions (16.58 and 14.95%, respectively) while juveniles formed the smallest proportion of the population (3%). This was also the lowest contribution of juveniles and adults recorded for all *T. granifera* populations. The population from the Luvuvhu River site (LUVU) made the highest contributions to mature adults (55.26%) followed by adults (31.58%), juveniles (7%) and new-borns (5.26%). On the Great Letaba River, G-LETA populations mainly consisted of juveniles (56%), followed by adults (27.51%), mature adults (15.05%) and new-borns (1.38%). The lowest contribution of mature adults (5.26%) to all *T. granifera* populations was recorded for the LETA population on the Letaba River. Adults (75.66%) made the highest contribution to the LETA population, followed by juveniles (18%) and new-borns (0.66%). The last population sampled, on the Shingwedzi River site (SHIN), followed the same trend as the LETA population. Adults (61.33%) contributed the most, followed by juveniles (26%), mature adults (7.73%), and new-borns (4.42%).

Shell length (mean± SD) and length range (mm) of mature adult *T. granifera* sampled from the selected sites are summarised in Table 5.6. From all sampled sites, the shell length of mature adult *T. granifera* ranged from 10.40 to 25.21 mm, and they had an overall average length of 13.39 mm (± 1.12).

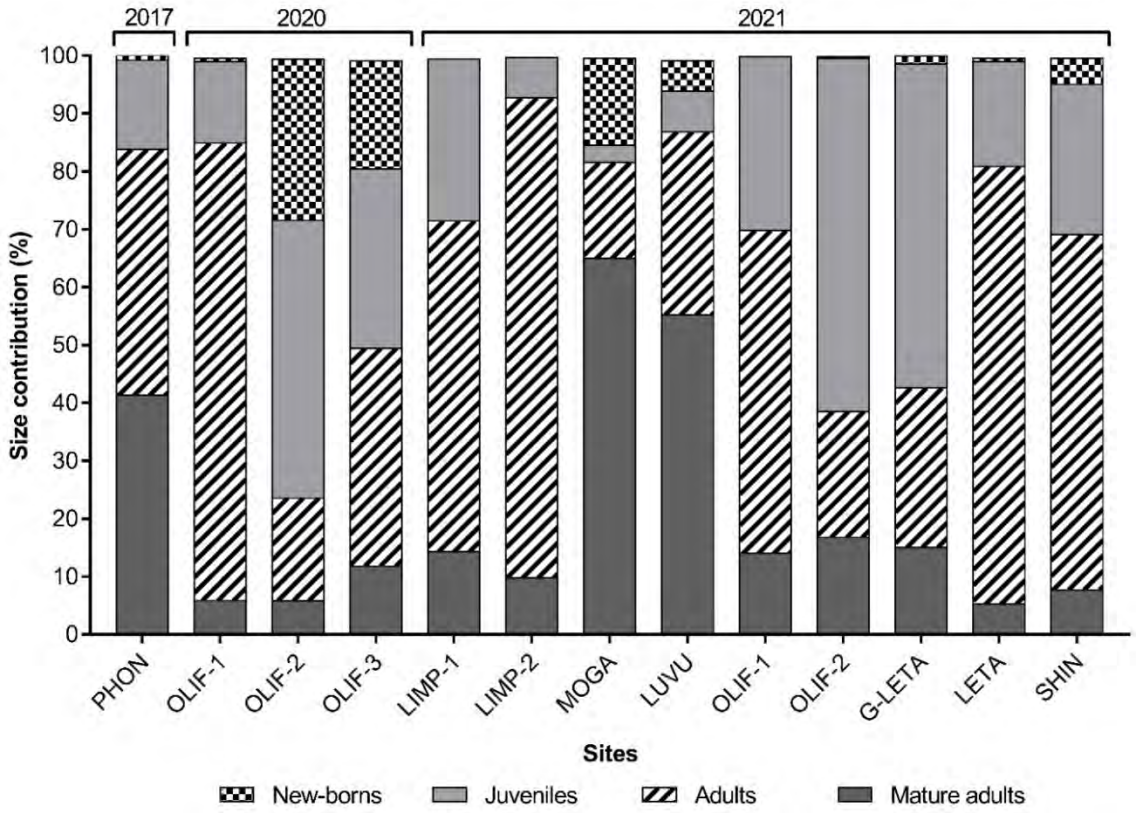

Figure 5.7: Percentage size contributions of *Tarebia granifera* populations from all selected sites sampled from 2017 to 2021. Snails were divided into mature adults (>10.39 mm), adults (5.28 - 10.39 mm), juveniles (1.92 - 5.27 mm), and new-borns (<1.9 mm).

Table 5.6: Average density (m^2), shell length (average ± SD), and length range (mm) of mature adult *Tarebia granifera* (>10.38 mm) sampled at the selected sites. Average densities were rounded up to the closest whole number. *Indicates Olifants River sites sampled in 2021.

Site	Average density (m^2)	Length in mm (average ± SD) *Tarebia granifera* > 10.39 mm	Length range (mm)
PHON	113	13.12 ± 2.63	10.43 - 25.21
OLIF-1	194	13.09 ± 2.31	10.40 - 20.63
OLIF-2	185	15.16 ± 1.85	10.41 - 20.34
OLIF-3	61	13.44 ± 2.23	10.42 - 19.87
LIMP-1	2	15.56 ± 3.15	10.45 - 15.56
LIMP-2	1	11.23 ± 0.68	10.57 - 12.27
MOGA	88	13.12 ± 1.88	10.45 - 20.54
LUVU	23	13.22 ± 1.54	10.80 - 17.04
OLIF-1*	7	12.46 ± 1.62	10.48 - 16.87
OLIF-2*	95	14.38 ± 1.78	10.45 - 20.52
G-LETA	32	12.18 ± 1.65	10.40 - 18.57
LETA	3	13.62 ± 1.63	11.26 - 15.88
SHIN	5	13.55 ± 3.50	10.40 - 20.54

5.4 Discussion

The aims of this study were to investigate the current distribution of freshwater mollusc species within the north-eastern region of South Africa, as well as the role of environmental variables and the invasive freshwater snail species *T. granifera* on the distribution of native molluscs and the population size and structure of this invasive species. Findings indicated that there has been a decrease in mollusc diversity since historical sampling took place. Results further indicated that differences in habitat and water chemistry affected the native mollusc species diversity between sampling seasons. However, an additional critical driver affecting mollusc species diversity and abundance during the current study was the invasive species *T. granifera.* The study further determined that sites with a dominance of *T. granifera* also correlated to a low diversity and density of other mollusc species, suggesting that this invasive mollusc likely has a negative effect on native snail distribution and density.

5.4.1 Current distribution of molluscs and the threat of invasive mollusc species

Nine mollusc species were collected during the current study, which is much reduced from the expected 16 that were historically present in the study area. One of the invasive species collected in the present study not historically recorded was *T. granifera.* This mollusc species was only recently discovered in South Africa with the first records occurring in 1999 from a reservoir in KwaZulu-Natal, South Africa. However, the actual date of introduction is conjectured to be earlier than 1996 (Appleton et al. 2009). The impact of this invasive mollusc is believed to be detrimental to native species diversity and abundance of not only molluscs but other biota as well, with additional environmental impacts also predicted including bottom-up effects on the natural food web and alterations in water quality (Jolly et al. 2008; Appleton et al. 2009; Hill et al. 2015). By using *T. granifera* as a key indicator for the distribution of freshwater snails in the present study, it was determined that all sampling sites where *T. granifera* was detected, grouped together and separately from those without this invasive species present. This suggests that the presence or absence of this invasive snail is likely associated with adverse ecological impacts on the diversity and abundance of native species. The presence of *T. granifera* has been found to diminish native species populations, and in particular instances, even lead to their complete elimination (Appleton 2003; De Kock and Wolmarans 2008; Appleton et al. 2009; Appleton et al. 2015a). At each of the sampling sites where *T. granifera* was present, the abundance of this invasive species was much higher than any other mollusc species. Similarly, Hill et al. (2015) observed that in the Nseleni River, South Africa, where both *T. granifera* and native species coexisted, the invasive species displayed a notably higher abundance compared to the native species. A paper by Pointier (1999) further reported that the presence of the invasive mollusc *T. granifera* resulted in decreased

populations and, in some cases, the disappearance of the native mollusc species *Bul. glabrata* in habitats affected by *T. granifera* in Puerto Rico and Venezuela. The effect of *T. granifera* numerically dominating native taxa, along with the absence of natural predators, could have a bottom-up influence on the food web within systems where they are present and affect resource competition between native and invasive mollusc species (Appleton et al. 2009; Hill et al. 2015).

5.4.2 Environmental factors affecting the distribution of native molluscs

The qualitative habitat assessment indicated that aquatic vegetation, a food source for grazing aquatic mollusc species, was largely absent during the high-flow sampling period, possibly contributing to the reduced species diversity observed during this season. Freshwater snails are commonly found in association with aquatic vegetation and their epiphyton as these biota use aquatic vegetation for attachment, food, shelter from predators and areas to lay eggs (Brown 1994; Oloyede et al. 2016; Davis et al. 2018; Olkeba et al. 2020). Our study results were in agreement, as there was an observed association between aquatic plants such as water lilies (family Nymphaeaceae) and the abundance of the mollusc species, particularly *Bulinus* sp. and *R. natalensis.* This phenomenon could be attributed to the fact that water lilies, among other floating and submerged vegetation, offer various advantages such as shelter, suitable sites for egg deposition (evidenced by the presence of egg deposits on the plant surfaces), access to the air-water interface since the snails are pulmonates, and surfaces for the development of epiphyton, which serves as an important food source (el-Dafrawy 2002). Low habitat diversity and/or absence of aquatic vegetation therefore negatively affects the diversity and abundance of aquatic biota, including molluscs.

Water quality parameters likely played a smaller role in the differences in snail diversity between sites than other variables as most did not differ greatly between the sampling seasons and were within acceptable ranges for the snail species according to De Kock and Wolmarans (2005). Water temperature ranges across all sites in the study area were within the optimal range for freshwater snail growth and survival, and study sites were relatively well buffered with pH ranges between neutral and slightly alkaline (6-8). These values were within the normal pH range for most freshwater snails (De Kock and Wolmarans 2005; Tchakonte et al. 2014; Oloyede et al. 2016) and thus, most likely had little effect on their abundance. However, salinity in particular likely played an important role as this was generally higher in the high-flow period compared to the low-flow period. High salinity levels have a negative impact on most snail species, either directly, due to their inability to tolerate elevated salinity, or indirectly, as increased salinity can affect the availability of their food sources (Pretorius et al. 1982; De Kock and Wolmarans 2005; Adekiya et al. 2020; Maes et al. 2021)

The lower abundance of many native snail species in the high-flow compared to the low-flow sampling period was more likely as a result of a combination of differences in water flow, snail habitat preference and the presence of the invasive snail *T. granifera.* The habitat and water flow in the high-flow sampling period was quite different from the low-flow period, with less aquatic vegetation available and many sites consisting of lotic systems thus likely contributing to the lower diversity and abundance of snail species. The absence of aquatic vegetation, along with the presence of fast-flowing water, creates unstable environments that impede the accumulation of sediment and the growth of aquatic vegetation. This is crucial as these factors serve as substrate and food sources necessary for the growth and development of freshwater snails (Brown 1994; Dida et al. 2014). Many native aquatic species also exhibit a preference for perennial habitats characterised by muddy, stony, and sandy substrates with high organic content (De Kock and Wolmarans 2006a; 2006b). These factors, in addition to the reported tolerance to high salinity of the invasive snail *T. granifera* and its streamlined shell shape, likely contributed to its higher abundance in the high-flow sampling period as it is known to easily survive in faster-flowing water (Appleton et al. 2009; Miranda et al. 2010; de Necker et al. 2021).

5.4.3 Tarebia granifera *population density*

Studies on the changes in *T. granifera* population densities have been contradictory as several studies reported maximum densities sampled at different times of the year (Appleton et al. 2009; López-López et al. 2009; Miranda et al. 2011a). Miranda et al. (2011b) found that *T. granifera* population density did not seem to undergo seasonal patterns in an estuarine environment of Catalina Bay, South Africa, but that differences in *T. granifera* population density were significant between locations during the wet season. In contrast, Makherana et al. (2022) reported significant differences in *T. granifera* population densities across seasons from a freshwater environment in Nandoni Dam, Limpopo Province, South Africa, but no significant differences were found between their sites. The results of the present study also found differences in *T. granifera* population density between seasons, where maximum densities were recorded on the Limpopo River system during spring (September and October), while densities were much lower during autumn (April and May) after the summer season. Similarly, López-López et al. (2009) found that *T. granifera* populations from the Tuxpam and Tecolutla rivers (USA) had pronounced depletions in snail density during the heavy rainfall season (summer), whereafter their populations increased rapidly during the dry season (winter) with increased food availability. Ecosystems sampled by De Kock and Wolmarans (2009) indicated that water velocity and rainfall played a significant role in the presence of the native thiarid species, *M. tuberculata.* It is thus not only important to note the time of the year, but also to consider the rainfall patterns prior to and during a survey (De Kock and Wolmarans

1998), as rainfall plays an important role in the densities and diversity of snail species (De Kock et al. 2002; Wolmarans and De Kock 2006).

The high densities of *T. granifera* recorded at selected sites during the present study correspond with those of previous studies conducted in South Africa with reported densities of over 1 000 individuals/m^2 (Appleton and Nadasan 2002; Appleton et al. 2009; Miranda et al. 2011a; 2011b). The lower densities found during the present study at selected sites correspond with the lower densities reported by Makherana et al. (2022) in the Nandoni Reservoir, South Africa. Nevertheless, even the low densities recorded during the present study were usually much higher than the densities reported for *T. granifera* (18 to 193 individuals/m^2) in its native ranges (Dudgeon 1980; Appleton and Nadasan 2002). The lower densities found during the present study may be explained by population crashes that have been observed to occur in *T. granifera* populations due to environmental disturbances such as drought, flooding, food availability and increased salinity (Appleton et al. 2009; López-López et al. 2009; Miranda et al. 2011a; Perissinotto et al. 2014). The low population densities on the Limpopo River may indicate an early invasion period or a low carrying capacity of the system at the time of sampling (Makherana et al. 2022). However, conclusions regarding such population density changes cannot be drawn in the present study due to a lack of regular sampling over a longer period of time.

It has also been proposed that the high densities reached by *T. granifera* populations potentially minimise the probability of eradication due to environmental disturbances such as drought or increased salinity (Ben-Ami and Heller 2005; Miranda et al. 2011a). Miranda et al. (2011b) reported that one adult *T. granifera* (18 to 20 mm) can have an average of up to 48.6 (~49) unborn juveniles in its brood pouch and larger adults (20 to 30 mm) were found with an average of 158 unborn juveniles in their brood pouch. This suggests that only a single snail is needed to start a new population, and also gives some insight into how rapidly these invaders can reach high population densities even after population crashes due to environmental disturbances (Miranda et al. 2011a).

Previous studies reported differences in *T. granifera* population density due to differences in water quality variables, such as pH, conductivity, and TDS (total dissolved solids) (Jones et al. 2017; Makherana et al. 2022). Miranda et al. (2011b) suggested that the invasive snail demonstrated increased abundance as a response to heightened environmental stress. Jones et al. (2017) found a strong association between conductivity and abundance of *T. granifera* in the Nseleni River (South Africa) and Makherana et al. (2022) reported significant differences in *T. granifera* population density in the Nandoni Reservoir (South Africa) attributed to increased pH, conductivity, and TDS. However, this does not seem to be the case in the present study as no significant correlations were found between *T. granifera* population

density and water quality variables in any of the sampled river systems. Miranda et al. (2011b) also found that, in comparison with other snails, *T. granifera* was the least associated with any of the water quality variables and was the most dominant and widely distributed species in all study areas. Alternatively, the higher conductivity and TDS levels could be a consequence of the ecological and biological characteristics of *T. granifera*, potentially stemming from their movement patterns within the sediment of an environment (Appleton et al. 2009) leading to sediment resuspension, for instance, salts and nutrients, and inducing the release of these substances back into the water column (Jolly et al. 2008). High densities of benthic gastropods are known to cause sediment and microphytobenthos resuspension (Jensen and Siegismund 1980; Hunt et al. 1987; Morrisey 1988; Andersen 2001; Kelaher et al. 2003; Orvain et al. 2004).

Appleton et al. (2009) also found that some *T. granifera* populations from KwaZulu-Natal declined in density by as much as 95% every few years. This decline was attributed to low birth rates during the spring/summer season. However, densities quickly started to increase again in only a few months (Appleton et al. 2009). Makherana et al. (2022) reported higher densities (368 individuals/m^2) of *T. granifera* during summer and lower densities (104 individuals/m^2) during autumn. The differences in density were attributed to low conductivity during autumn and winter and high conductivity and TDS during spring and summer. Results of the present study also indicated differences in *T. granifera* population density between seasons; the Olifants River was sampled during spring (September and October) and autumn (April and May). The highest snail densities of *T. granifera* were recorded during spring while the densities decreased by 86.87% during autumn. This reduction in snail density during autumn could be ascribed to heavy rain/seasonal change and increased water velocity during the wet season (summer), as no significant correlations were found between *T. granifera* population density and water quality variables. *Tarebia granifera* population densities may quickly increase again during the dry season (winter-spring) when availability of nutrients, and thus food, increases (Appleton et al. 2009; López-López et al. 2009). This may also explain why higher densities of *T. granifera* were present in the Phongolo River during winter-spring (August and September) compared to sites sampled on the Limpopo River system during autumn (April and May).

5.4.4 Tarebia granifera *population size structures*

At all the sites sampled during the present study, most *T. granifera* populations seemed to follow a trend of higher new-born recruitment during spring and summer, while fewer new-born snails were recorded during the autumn and winter months. Appleton and Nadasan (2002) also reported a peak in *T. granifera* births during the warmer summer months. *Tarebia granifera* have adapted to minimise mortality of early life stages during adverse events. Different sizes of unborn snails have been found in the brood pouches of adult snails,

suggesting that these unborn snails may be retained during unfavourable conditions (Miranda et al. 2011a). Larger individuals of *T. granifera* have also been found to exhibit greater resilience to desiccation and high salinity during drier periods, enabling them to survive until more favourable conditions occur (Chaniotis et al. 1980c; Facon et al. 2004; Miranda et al. 2011a). Interestingly, Miranda et al. (2011b) reported that some *T. granifera* populations seem to give birth all year round. This suggests that the birth rate may not be dependent on season but rather on environmental variables such as water depth, wet or dry conditions, salinity, temperature and the availability of food sources (López-López et al. 2009; Miranda et al. 2011a; 2011b).

A higher proportion of adult and mature adult *T. granifera* was found in the Phongolo River, indicative of a decreased birth rate. The lower proportion of new-born snails from the Phongolo population might be explained by seasonal environmental changes as sampling occurred during winter-spring (dry season). The population will most likely continue to grow due to the higher number of adult snails that can reproduce during spring/summer when environmental conditions become more favourable (Appleton et al. 2009). Miranda et al. (2011b) reported that *T. granifera* populations from Catalina Bay rapidly re-established when favourable conditions returned after a period of desiccation in 2009. The adult population gave birth to large numbers of new-born snails, and by the end of 2010 the population had recovered in structure and density. The number of unborn snails in the brood pouch increased during times of environmental stress (increased salinity or desiccation) as well as during times of population recovery when environmental conditions became more favourable (Miranda et al. 2011a). This suggests that *T. granifera* responds to environmental variability by increasing reproductivity and consequently speeding up population recovery (Miranda et al. 2011a).

In the Limpopo River system, the highest contributions of new-born *T. granifera* were recorded in the OLIF-2 and OLIF-3 populations on the Olifants River in 2020 during spring (September to October). In contrast to these populations, the OLIF-1 population was found to have low contributions of new-born snails during spring, while the highest contribution to the population was from adults. During the 2021 survey (autumn), the new-born age group contributed the least to the OLIF-2 population, indicating a reduction in birth during the survey, while the increase in the juvenile age group corresponded with the high contribution of new-borns from the previous season. The much lower average population density recorded during the 2021 survey and the decline in the new-born age group may indicate that the population is in a state of recovery. Although no new-born snails made a contribution to the populations of the Limpopo River, adults made up the largest part of the sampled populations. A similar trend in population size structure was found in the *T. granifera* populations from the Letaba River (LETA) and Shingwedzi River (SHIN). These sites were also sampled during autumn and the

low contributions of new-born snails may be due to environmental changes (dry periods and lower temperatures) causing low reproduction in adults during autumn and winter months. Even though sampling occurred during autumn, higher contributions of new-born snails compared to other sites sampled during autumn were recorded at the Mogalakwena, Luvuvhu, Shingwedzi and Great Letaba sites, while lower new-born contributions during autumn were recorded for all other sites. This finding is in line with the statements of Miranda et al. (2011b) that *T. granifera* reproduction corresponds to environmental factors rather than the season and it seems that if environmental factors are favourable, the snail will reproduce regardless of the season. The present study found similar results to those reported by Miranda et al. (2011a), with a higher abundance of smaller size classes (<10 mm). This finding is particularly concerning, since previous studies have indicated that juvenile snails may have higher impacts on food resources than larger adult snails, since juvenile snails consume more food by mass than adult snails (Boland et al. 2008; Tamburi and Martín 2009; Miranda et al. 2011b).

The average birth rate of 1.89 (~2) individuals per day recorded for *T. granifera* in the present study corresponds with reports that adults can birth one offspring every 12 hours (Abbott 1952; Chaniotis et al. 1980a; Sodeman 1991; Appleton et al. 2009). This ability to rapidly reproduce and the high environmental tolerance of mature adults play a key role in their success as an invader and ensure the persistence of *T. granifera* within invaded habitats as repeated introductions are not essential for them to successfully colonise new areas (Appleton et al. 2009; Miranda et al. 2011a). In the present study, *T. granifera* samples from only three sites (LIMP-1, LIMP-2 and OLIF-1 during 2021) had no new-born snails, although mature adults were recorded at all three of these sites. The reason for no new-born snails being recorded at these sites is unclear and further information on the reproductive biology and population dynamics of *T. granifera* in South Africa is needed (Appleton et al. 2009; Miranda et al. 2011a). Juvenile *T. granifera* have also been reported to move over smaller spatial ranges than larger individuals (de la Vega et al. 2003; Miranda et al. 2011a) and it is possible that they occurred in areas that were not sampled during the survey.

5.5 Conclusion

The purpose of this section of the study was to investigate the current distribution of freshwater snail species in north-eastern areas of South Africa, potential environmental factors affecting their distribution and densities, and population size structures of *T. granifera* in order to gain more information on how their populations persist and grow within these invaded habitats. Key findings included that native snails have decreased in spatial distribution and abundance since historic collections took place. This is possibly due to anthropogenic and natural alterations to the physical habitat, including increased rural development, and agricultural use. This has also

likely resulted in increased water use, causing changes in water quality and the presence of invasive mollusc species competing for the same ecological niche. Results further indicated that areas where *T. granifera* were the dominant species, few other mollusc species were present and, when present, they occurred at much lower densities than *T. granifera*. The densities reached by *T. granifera* were much higher than those of native molluscs and exceeded the densities reported from its native range. Higher population densities and higher new-born recruitment were observed during the sampling events in spring compared to autumn, likely as a result of more favourable environmental conditions during this period. Contrary to previous findings, no significant correlations were found between *T. granifera* population density and water quality variables in any of the sampled river systems. The ability of *T. granifera* to rapidly spread through the aquatic ecosystem, resist unfavourable conditions that are detrimental to native mollusc species, and the lack of natural predators is of concern, because of the negative consequences for the native species. At high population densities, the feeding dynamics of *T. granifera* may have further ecosystem-wide implications on the food web dynamics of invaded ecosystems. Without intervention, this invasive species has the potential to completely displace many of the native freshwater snail species in the Lowveld Ecoregion of southern Africa, including those that transmit schistosomiasis and other water-borne diseases, and further investigation is required to determine the effects of *T. granifera* on native aquatic biota and, potentially, human health.

CHAPTER 6: MODELLING THE HISTORIC AND CURRENT DISTRIBUTION OF MOLLUSCS IN THE NORTHERN PROVINCES OF SOUTH AFRICA

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6.1 Introduction

Studies in South Africa on schistosomiasis have provided useful insights into the schistosomiasis prevalence in rural communities, mainly in the Eastern Cape, KwaZulu-Natal, and Limpopo Provinces (De Kock et al. 2004). However, there seems to be an unclear understanding of how changes in climate-related factors such as temperature and rainfall will affect the distribution of the specific intermediate snail hosts, along with the development and the distribution of the parasite (Lafferty 2009; Stensgaard et al. 2019; Adekiya et al. 2020; Maes et al. 2021). Moodley (2003) and Kalinda et al. (2017b) researched the effects of changing temperatures on the growth, fecundity and survival of the intermediate snail host of schistosomiasis in South Africa and Africa, respectively, and found that *Bulinus* snails have a greater tolerance to high and low temperatures than *Biomphalaria* species. Additionally, it was reported by Appleton and Madsen (2012) that *Bulinus africanus* can survive in areas where there is frost and temperatures drop below zero at night. On the other hand, the optimum temperatures for the development of *Biom. pfeifferi* range from 20-25 ˚C (Appleton and Madsen 2012). Another study by Adekiya (2018) demonstrated that this species can also thrive in areas with temperatures ranging from 15-31 ˚C. Thus, if the temperatures vary outside of these intervals due to climate variability, this can alter the geographic distribution of these species.

Changes in rainfall patterns are also linked to the formation of a breeding space for the snail (McCreesh and Booth 2013; Adekiya et al. 2020). Increased rainfall can increase the distribution of schistosomiasis in areas that previously provided unfavourable conditions, but can also reduce schistosomiasis in endemic areas through an increase in water flow, which will ultimately become unfavourable for the intermediate snail hosts of *Schistosoma* spp. (McCreesh and Booth 2013). Likewise, Manyangadze et al. (2021) argue that rainfall affects the snail habitats in several ways: (i) it can lead to the establishment of temporary snail habitats; and (ii) increases the creation of new habitats and reduces snail populations in endemic areas. Schistosomiasis is collectively propagated through the transportation of the infected snails by heavy rainfall events. Therefore, temperature and rainfall become crucial factors governing the distribution of the intermediate snail hosts of *Schistosoma* spp.

Alarmingly, it is observed that climate change will result in increased temperatures and other relative climate extremes (Kalinda et al. 2017b). These changes will undoubtedly alter the ecological interactions between the host and the parasite. Furthermore, it is argued that most studies have focused on the influence of climate change on malaria while neglecting the effects of climate change on schistosomiasis (Kalinda et al. 2017b). As a result, only a handful of studies have examined the influence of climate change on schistosomiasis and it is therefore essential to determine the impacts of climate variability on schistosomiasis-transmitting snails in South Africa.

There is a general lack of understanding of how climate factors affect the ecology and biology of schistosomiasis snail vectors, and there is therefore a need for models that incorporate climate factors along with the species occurrences to determine the influence of climate variability on the species distribution habitats. This will assist in identifying areas that may be currently at risk of increased schistosomiasis and/or may become suitable for schistosomiasis transmissions in the future. Ideally, the models will only be effective if parameterised separately for each snail species in relation to changes in the patterns of rainfall and temperature and other climate extremes. Also, equally important is the geographical scale in which the model is fitted (McCreesh and Booth 2013). Comparatively, species distribution models have been widely used in several African countries such as Madagascar and Zimbabwe (Deka 2022; Pedersen et al. 2014a). However, the modelling of the geographic distribution of suitable habitats for schistosomiasis-transmitting snails has not received similar attention in South Africa. To date, a similar modelling approach has only been adopted to model the spatial and seasonal distribution of suitable habitats for schistosomiasis-transmitting snails in KwaZulu-Natal, South Africa (Manyangadze et al. 2016b). Consequently, micro-geographical studies of the spatial and temporal distribution of the suitable habitats of the intermediate snail hosts of *Schistosoma* spp. are necessary in other areas of South Africa.

According to Manyangadze et al. (2016b), the MaxEnt model is the most recently developed technique which effectively models habitat suitability using species occurrences against a set of environmental constraints and it has been praised for its high predictive performance, most particularly when working with a small sample size (Miller 2010). Similarly, Lissovsky and Dudov (2021) defined MaxEnt as a machine learning algorithm that models the presence or the occurrence of species in a geographic space using presence-only points species without considering the location of absences. However, in order to carry out the analysis on MaxEnt, it is important to select the number of background points reflecting the entire environmental space being modelled. Moreover, the accuracy of the model can be assessed with the AUC (Scholte et al. 2012; Manyangadze et al. 2016b; Yang et al. 2018). Alternatively, the ROC can be used to measure the quality of the model (Lissovsky and Dudov 2021). A second model,

the RF model, is an algorithm derived from classification and regression trees, that combines numerous classification trees to produce more accurate classifications and is predominantly used in a range of fields such as remote sensing, ecology forestry, and climate change (Cutler et al. 2007; Evans et al. 2010). Random Forest models have been widely used in ecology for carrying out species distribution modelling and have been shown to encompass a wide range of advantages such as the ability to characterise the complex relationships among the variables and delineate a highly accurate classification of trees; most importantly, this algorithm is easy to interpret (Cutler et al. 2007). Comparatively, it is argued that the RF model forms part of the best available methods in machine learning and can outperform other modelling techniques (Cutler et al. 2007). The measures of variable importance and graphical representations are some of the outputs of the model and variable importance can be assessed or estimated by using AIC criterion or the statistical significance (Cutler et al. 2007). Apart from these approaches, there are other ways in which variable importance can be measured; for instance, jackknife analysis was used in this chapter, with the final results illustrating the relative importance of each variable in the model prediction. In other regression techniques, the use of a large set of predictor variables can result in overfitting of the data; in other words, the accuracy of the model can be hindered by using a large set of predictor variables, as this can propel the model to overfit the data, whereas the advantage of the RF model is that it is seemingly not sensitive to the tuning of parameters and overfitting the data (Fox et al. 2017).

The central aim of this chapter was to address Aim 2 of the larger study by modelling the historical distribution of the three schistosomiasis snail vectors across three Provinces of South Africa, Mpumalanga, Limpopo and Gauteng, in order to reconstruct the environmental requirements and conditions for the host snails in the past. This was to assist in determining how the pattern and habitat distribution of these snails and their parasites have changed over the years in relation to changes in climate and to work towards paving the way for studies that seek to understand how the snail vectors, along with the respective schistosome parasite, respond to changes in future climates. The first objective was to determine the historical distribution of schistosomiasis-transmitting vectors, *Biomphalaria pfeifferi* and *Bulinus globosus* in Mbombela and Nkomazi local municipalities, Mpumalanga. The second objective was to determine the distribution of *Biom. pfeifferi* and *Bul. globosus* within the Vhembe District Municipality, Limpopo Province and the third objective was to determine the historical distribution of *Biom. pfeifferi and Bul. africanus* in the Tshwane and Johannesburg metropolitan municipalities, Gauteng.

6.2 Methods

All analyses were done using the methodology explained in Chapter 2, sections 2.3.7 to 2.3.9.

6.3 Results

6.3.1 Historical distribution of Biomphalaria pfeifferi *and* Bulinus globosus *in Mbombela and Nkomazi local municipalities*

Model performance

The MaxEnt model provided high performance results for both snail species. The mean AUC score for *Biomphalaria pfeifferi* using MaxEnt was 0.939 (Figure 6.1 a). The resulting AUC score using GLM for *Biom. pfeifferi* was 0.892 (Figure 6.1b). Among the environmental variables used in MaxEnt and GLM, the precipitation of the wettest month (BIO 13) and precipitation of the driest month (BIO 14) had the highest relative variable importance as they had the highest contributing value that affected the distribution of *Biom. pfeifferi*. The jackknife test results for *Biom. pfeifferi* (Figure S6.1 a) showed that this variable provided high gains for both MaxEnt and GLM when used independently. Precipitation of the wettest month (BIO 13) had the highest relative importance (>0.8) when modelling for *Biomphalaria* using the GLM (Figure S6.1 b).

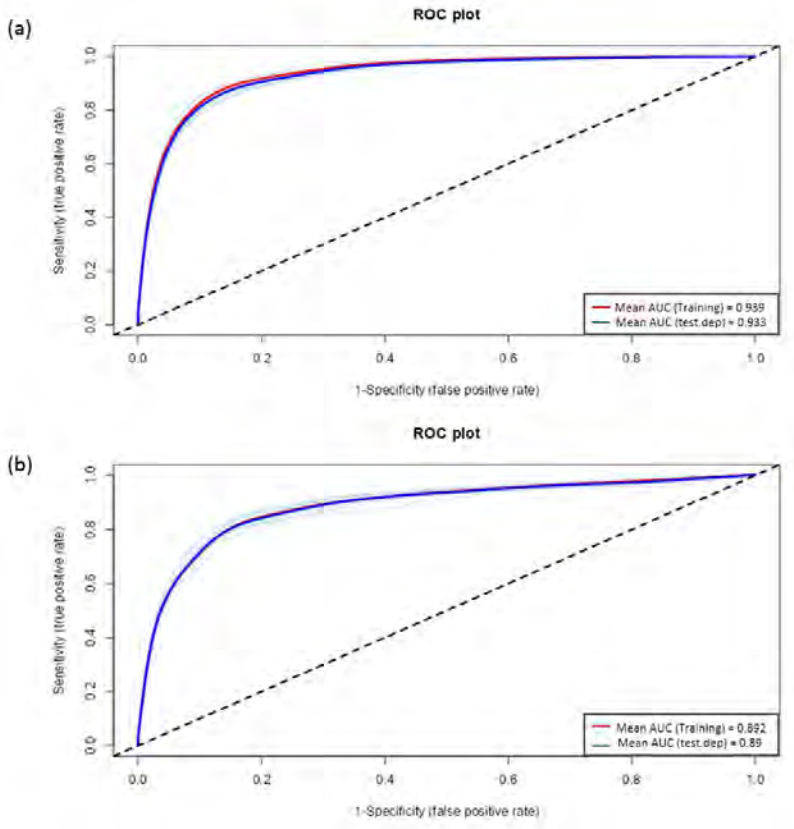

Figure 6.1: The ROC curves for both training and test data are shown for *Biomphalaria pfeifferi* in the Mbombela and Nkomazi local municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) maximum entropy; and (b) generalised linear model.

The AUC score for *Bul. globosus* was 0.942 using MaxEnt suggesting that the model predicted the distribution of *Bul. globosus* with high accuracy (Figure 6.2 a). The mean AUC score for the combined model output in GLM for *Bul. globosus* was 0.856 (Figure 6.2 b). Generally, the most important variables for *Bul. globosus* prediction were the mean diurnal range (BIO 2), precipitation of the wettest month (BIO 13), annual mean temperature (BIO 1), and mean temperature of wettest quarter (BIO 8), and elevation. The jackknife test results for *Bul. globosus* showed which variables made a high contribution to the species distribution. Surface runoff was found to be the least contributing variable to the historical distribution (Figure S6.2 a).

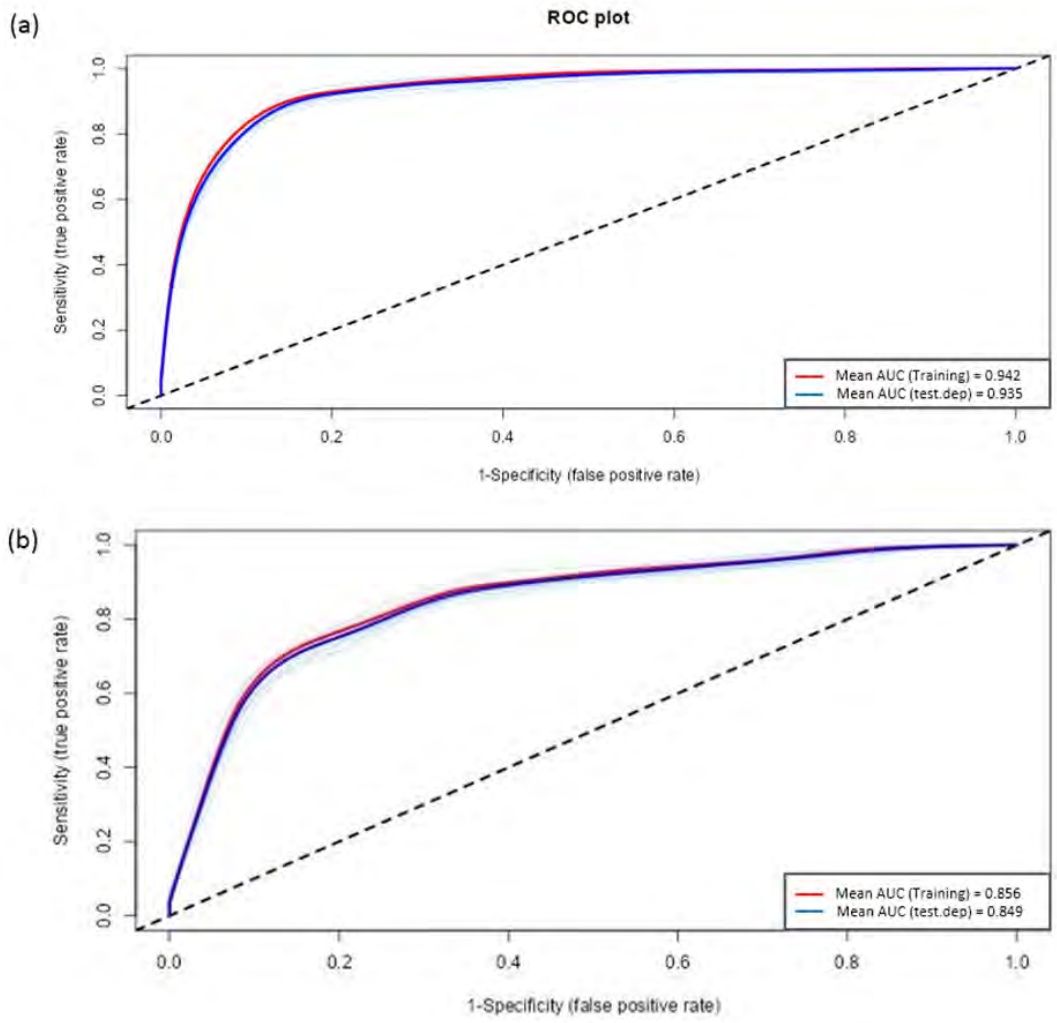

Figure 6.2: The ROC curves for both training and test data are shown for *Bulinus globosus* in the Mbombela and Nkomazi local municipalities. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) maximum Entropy and (b) generalised linear model

Maximum entropy and generalised linear model maps

Maximum entropy and GLM maps were used to model the distribution of intermediate host snails within the Mbombela and Nkomazi local municipalities, and the results were visualised using ArcMap 10.8.1. The results for the historical distribution of *Biom. pfeifferi* and *Bul. globosus* indicated that the species react to climate variables differently due to the difference in favourable conditions for each host snail species. The Mbombela local municipality was found to provide suitable conditions for the historical distribution of *Biom. pfeifferi and Bul. globosus* in both MaxEnt and GLM.

The high-suitability areas for the species were almost similar but GLM predicted that the *Biom. pfeifferi* distribution was high around the Crocodile River, Kaap River, Komati River and along the border of the local municipalities for both models (Figure 6.3). Although the historical

distribution of *Biom. pfeifferi was* found along permanent water sources, GLM showed a high distribution on the southern end of Nkomazi, and MaxEnt showed a small part on the northern parts of Mbombela to have moderate to high distribution. The MaxEnt model predicted that the high historical distribution of *Biom. pfeifferi* extended to the northern parts of the Nkomazi area, but overall, the municipality was largely predicted to have a moderate to low distribution of the snail species.

For both models, suitable conditions for *Bul. globosus* were highly probable and scattered in the Mbombela Local Municipality and along the border of the two municipalities. The species was found to be distributed on the eastern areas of the municipalities extending to the northern parts of Mbombela. The Mbombela Local Municipality presented conditions where the host snails would typically be found. Maximum entropy predicted the historical distribution of *Bul. globosus* to be almost similar to that of *Biom. pfeifferi,* but a lower distribution was noted in northern parts of the Nkomazi area.

The GLM results for the historical distribution of *Bul. globosus* showed that some south-western areas in Nkomazi had a moderate distribution of the snails. The model also indicated that a small area in the southern parts of Mbombela had a moderate to high distribution of *Bul. globosus*. Compared to *Biom. pfeifferi*, *Bul. globosus* was found by the GLM to be historically distributed beyond the main river systems as it can survive shallower waters (Figure 6.4). For both snail species, *Biom. pfeifferi* and *Bul. globosus,* MaxEnt was found to produce satisfactory results for the historical distribution within the Mbombela and Nkomazi local municipalities.

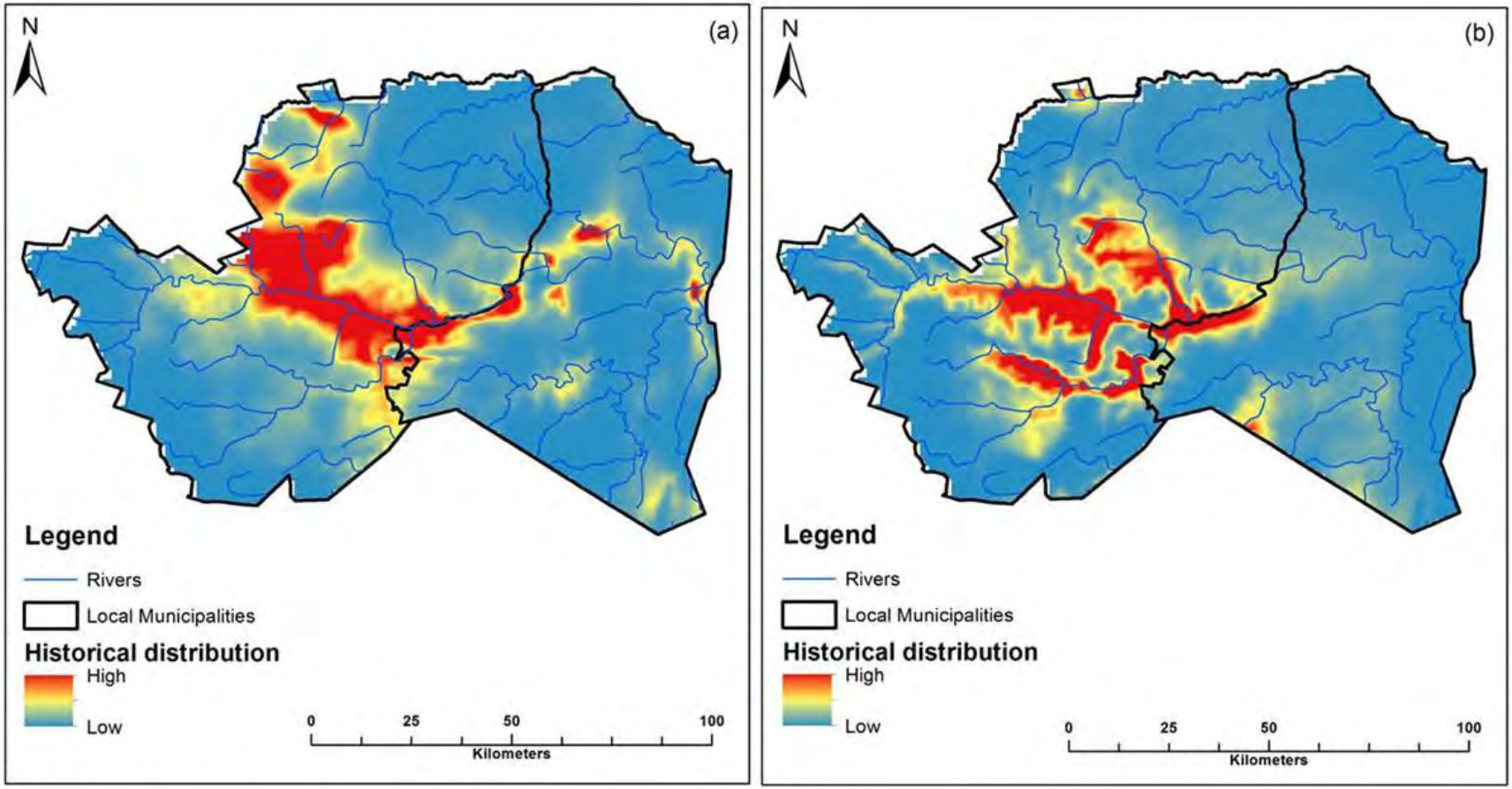

Figure 6.3: Predicted historical distribution of *Biomphalaria pfeifferi* within Mbombela and Nkomazi local municipalities: (a) maximum entropy and (b) generalised linear model predicted historical distribution.

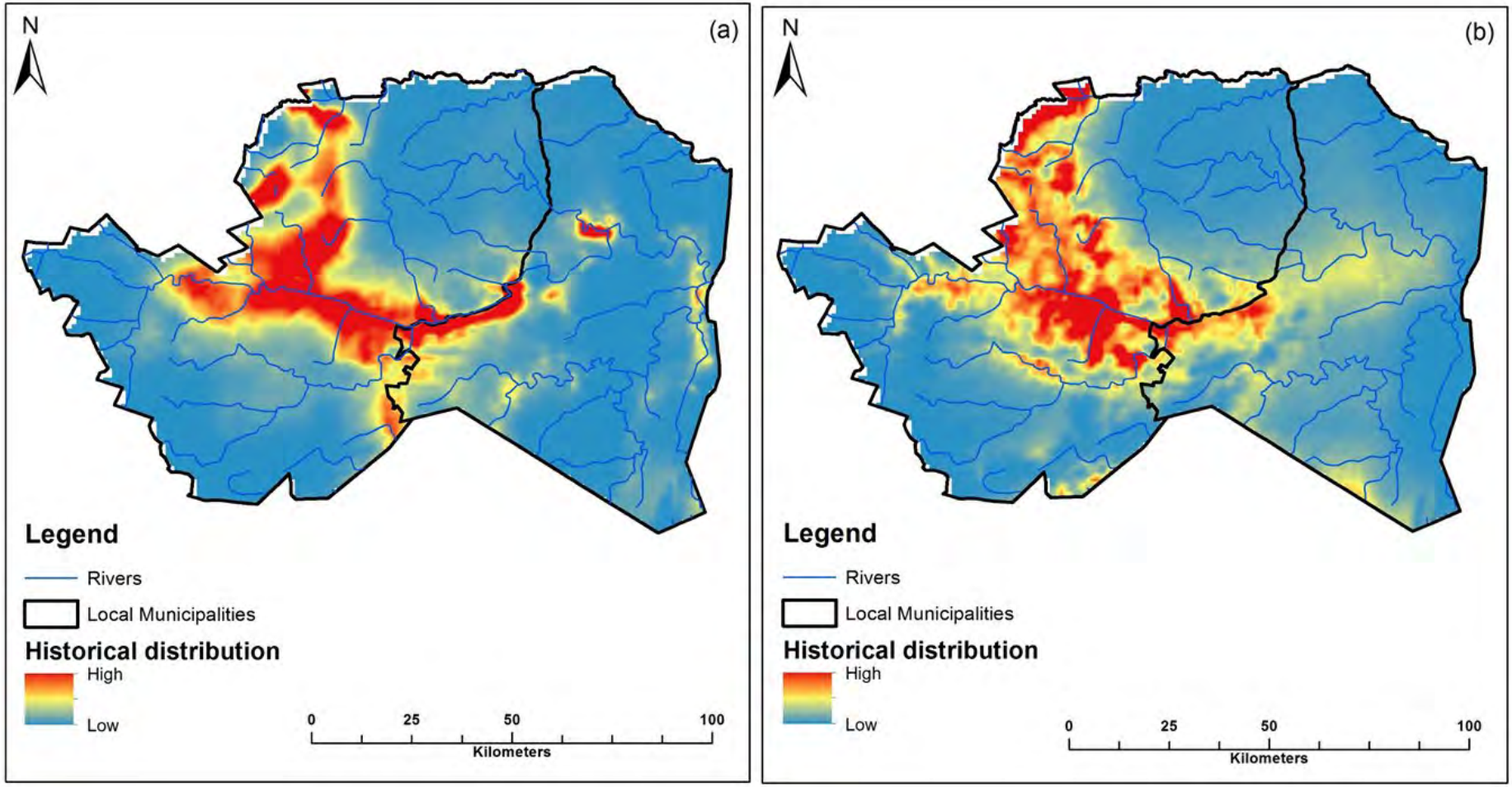

Figure 6.4: Predicted historical distribution of *Bulinus globosus* within Mbombela and Nkomazi local municipalities: (a) maximum entropy; and (b) generalised linear model.

Seasonal distribution

The results were obtained by making use of Kriging interpolation in ArcMap 10.8. Seasonal variation was observed for both species (Figure 6.5). Rainfall was negatively associated with both *Biom. pfeifferi* and *Bul. globosus* snails. The summer and winter seasons showed a high distribution of the *Biom. pfeifferi* snail within the Nkomazi Local Municipality (Figure 6.5 a and c). During the autumn season (Figure 6.5- b), a high distribution of the *Biom. pfeifferi* snail was predicted in the northern and eastern areas of Mbombela with Nkomazi having a low distribution. The results predicted that in spring there was a high distribution along the border of the local municipalities and in the north-eastern parts of Mbombela (Figure 6.5 d). During the summer season, *Biom. pfeifferi* was found to be highly distributed in the northern parts of the local municipalities (including Kruger National Park).

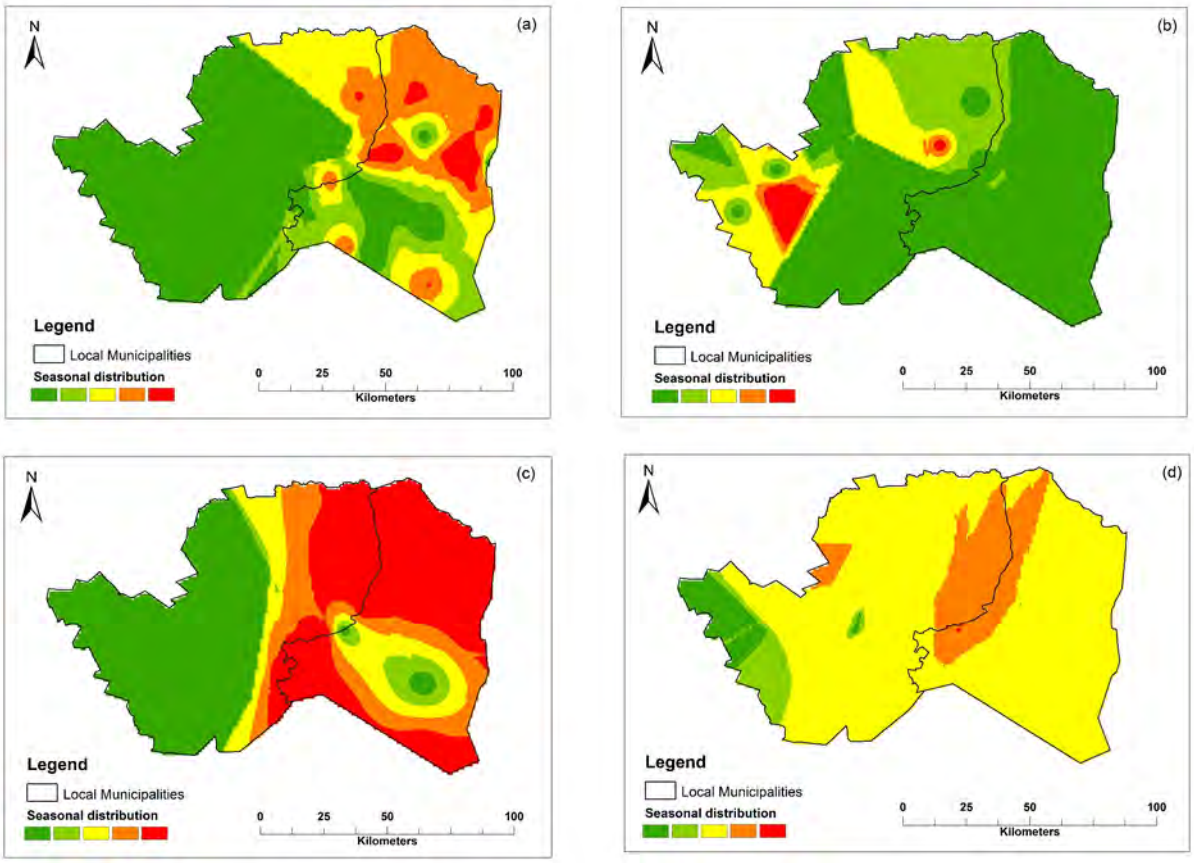

Figure 6.5: Predicted seasonal distribution of *Biomphalaria pfeifferi* within Mbombela and Nkomazi local municipalities for (a) summer, (b) autumn, (c) winter, and (d) spring. Dark green represents a very low distribution and red represents a very high distribution.

The *Bul. globosus* populations were relatively variable in inland locations, mostly occurring during and shortly after the wet season. The seasonal map results predicted that the distribution of *Bul. globosus* during the hot season, summer, was moderate and high within the Nkomazi Local Municipality and high in communities in the centre of Mbombela (Figure

6.6a). A few areas indicated a high distribution of *Bul. globosus* during the autumn season within the Mbombela Local Municipality and Nkomazi having mainly a low distribution (Figure 6.6 b). A low distribution of *Bulinus globosus* was indicated in the Mbombela region during the cold and dry winter season (Figure 6.6 c), while the opposite was true for the southern reaches of Nkomazi. Most of Mbombela and Nkomazi showed low and very low distribution of *Bul. globosus* in the spring except for some parts of Mbombela which showed moderate to very high distribution of the snail (Figure 6.6 d).

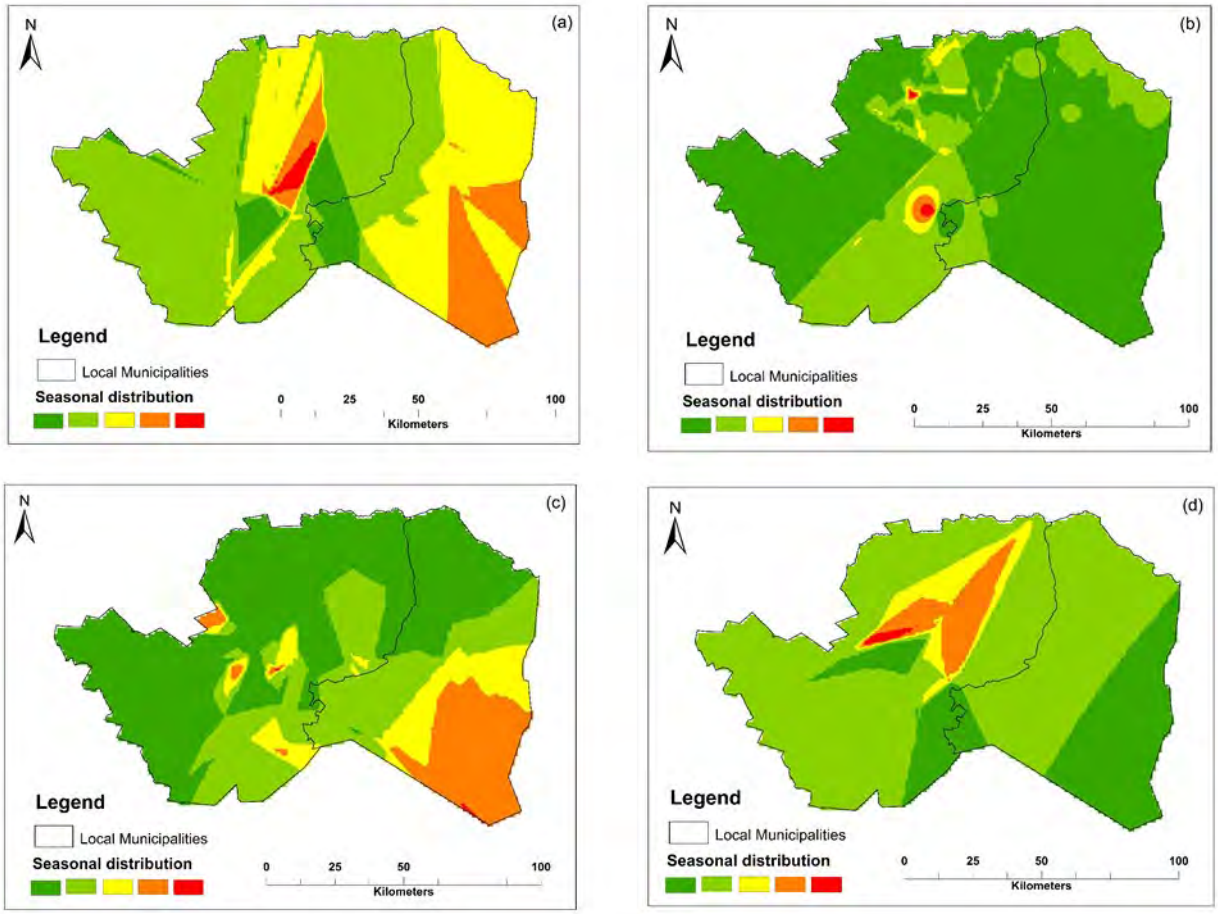

Figure 6.6: Predicted seasonal distribution of *Bulinus globosus* within Mbombela and Nkomazi local municipalities for (a) summer, (b) autumn, (c) winter, and (d) spring. Dark green represents a very low distribution and red represents a very high distribution.

6.3.2 Historical distribution of Biomphalaria pfeifferi *and* Bulinus africanus *in the Vhembe District Municipality*

Model performance

The AUC values of the species distribution model for *Biom. pfeifferi* are presented in Table 6.1. The RF had an AUC of 0.87 and MaxEnt had an AUC of 0.81. The models both performed well with excellent discrimination. The true skill statistic (TSS) for the RF model was 0.62 and that of MaxEnt was 0.49 (Table 6.1). The RF model performed well while MaxEnt performed moderately. The ROC curves for the RF and MaxEnt models are presented in Figure 6.7. The AUC for RF was 0.874, indicating a good measure of separability as the AUC was close to 1, and the ROC curve is above the 45-degree diagonal of the ROC space and close to the upper left corner (Figure 6.7 a). The AUC for the MaxEnt model was 0.808, and the ROC curve is above the 45-degree diagonal of the ROC space (Figure 6.7 b).

Table 6.1: Model performance of the Random Forest (RF) and maximum entropy (MaxEnt) in predicting the distribution of *Biomphalaria pfeifferi* habitats in the Vhembe District Municipality evaluated using area under the curve (AUC) and true skill statistic (TSS)

Model used	Methods	AUC	TSS
Random Forest	RF	0.87	0.62
Maximum entropy	MaxEnt	0.81	0.49

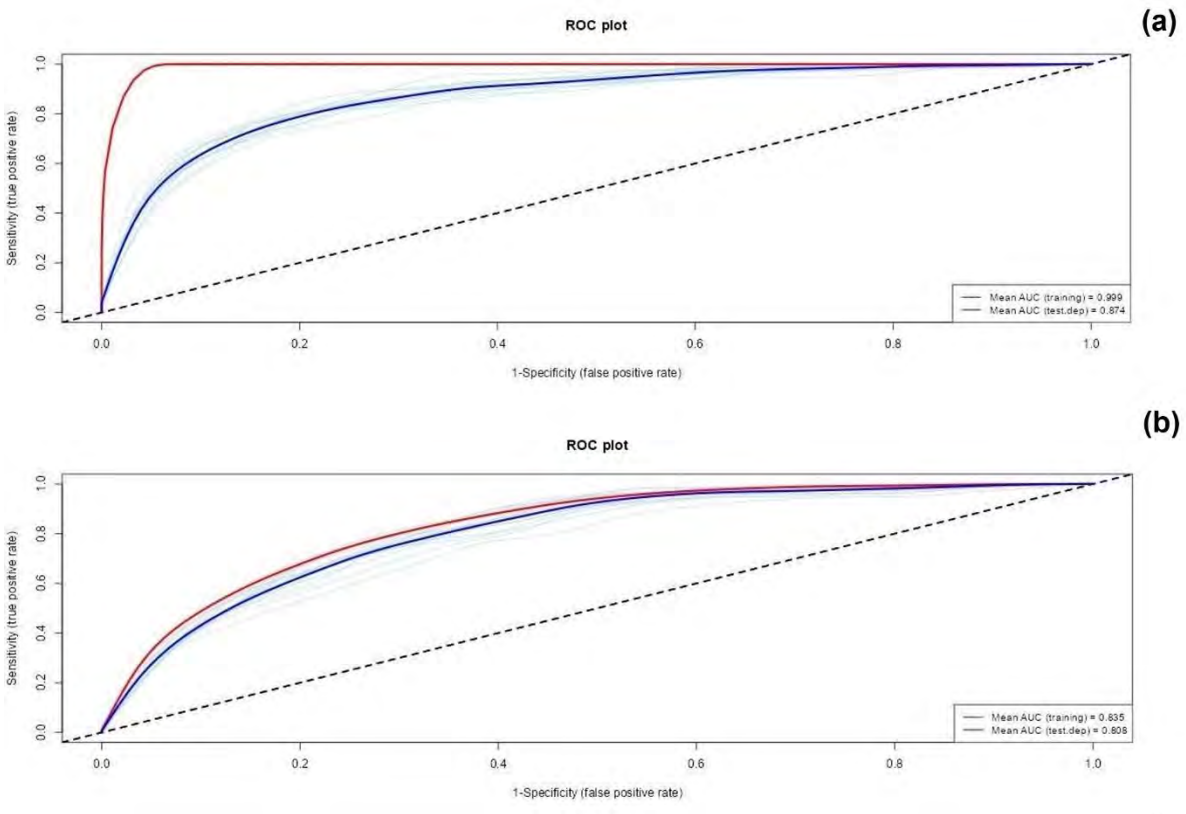

Figure 6.7: The ROC curves for both training and test data are shown for *Biomphalaria pfeifferi* in the Vhembe District Municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) Random Forest; and (b) maximum entropy.

The AUC of the species distribution model for *Bul. globosus* species is presented in Table 6.2. The RF had an AUC of 0.9 and MaxEnt had an AUC of 0.82. This is similar to the results obtained for *Biom. pfeifferi,* shown in Table 6.1. The models both performed well with excellent discrimination. Random Forest scored the highest TSS of 0.67 and MaxEnt scored 0.57. The RF performed well while MaxEnt performed moderately. The AUC for the RF model of *Bul. globus* in the Vhembe District Municipality was 0.9 and the AUC for MaxEnt was 0.82 (Figure 6.8 a and b, respectively). The ROC curve for RF is above the 45-degree diagonal of the ROC space and close to the upper left corner, while the ROC curve for MaxEnt is above the diagonal but not as close to the left-hand corner. Comparing RF and MaxEnt models in terms of AUC and TSS, the predictive performance of RF was better as the AUC for RF was larger than that for the MaxEnt.

Table 6.2: Model performance of the Random Forest (RF) and maximum entropy (MaxEnt) in predicting the distribution of *Bulinus globosus* habitats in the Vhembe District Municipality evaluated using area under the curve (AUC) and true skill statistic (TSS).

Model used	AUC	TSS
Random Forest	0.9	0.67
Maximum entropy	0.82	0.57

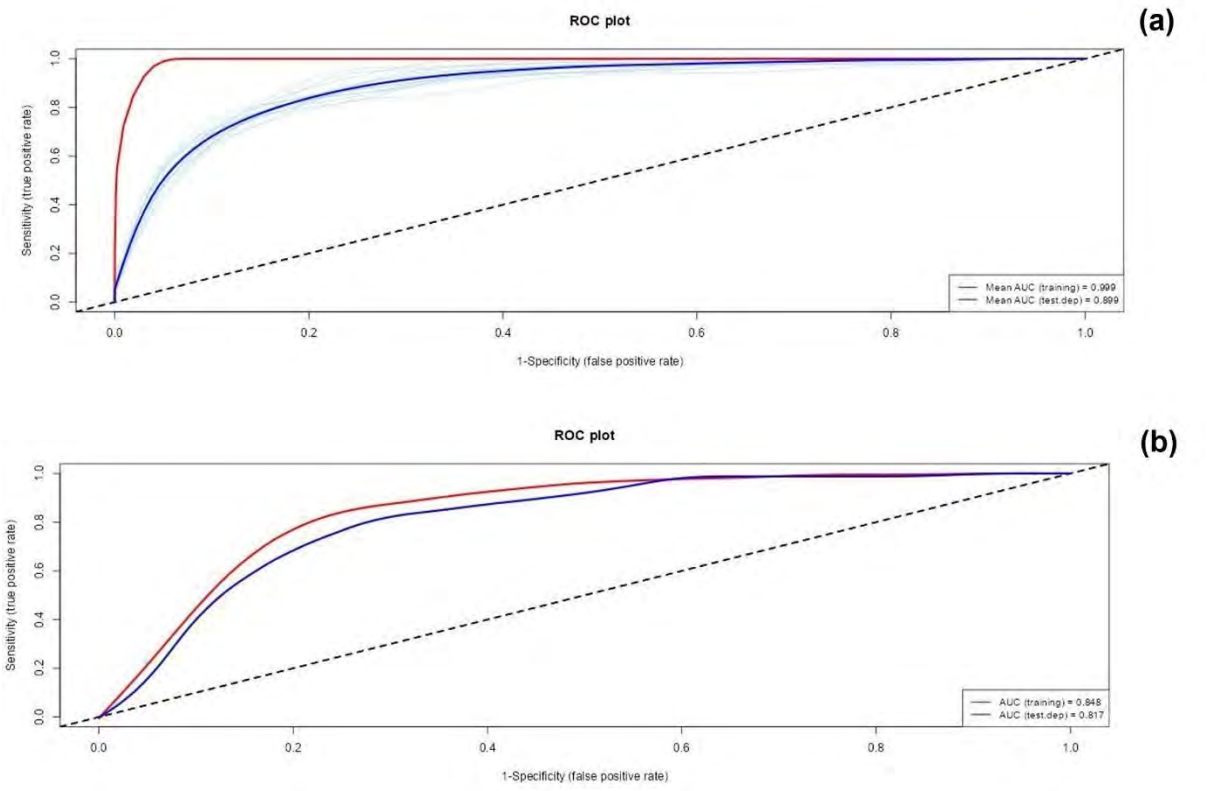

Figure 6.8: The ROC curves for both training and test data are shown for *Bulinus globosus* in the Vhembe District Municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) Random Forest; and (b) maximum entropy.

Variable importance for *Biomphalaria pfeifferi*

The relative importance of selected variables for *Biomphalaria pfeifferi* snail species was evaluated using jackknife tests. Minimum temperature of the coldest month (BIO 6) was shown to have a relatively higher variable importance than the other bioclimatic variables, followed by BIO 13, precipitation of the wettest month (Figure S6.3). Overall, the RF model showed a high relative importance, with all variables being more than 0.1 and peaking at 0.3 (Figure S6.3 a). The results of the jackknife analysis indicated that the variable with the least relative importance for RF was BIO 3, isothermality, with a value of 0.12. This was followed by the

variable BIO 2, mean diurnal range, with a value of 0.13, and BIO 15, precipitation seasonality, with a value of 0.14. The RF showed a higher variable importance than the MaxEnt.

The results of the jackknife analysis for the MaxEnt model showed a high relative variable importance for BIO 2, mean diurnal range, and BIO 6, minimum temperature of the coldest month, at a value higher than 0.2, and a low relative variable importance of less than 0.05 for BIO 3 and BIO 2 (Figure S6.3 b). Only the variables BIO 6 and BIO 2 provided high gains to the model, while BIO 3, BIO 15 and BIO 13 made a low contribution to the model, peaking at 0.03, 0.035 and 0.06, respectively. The AUC for the RF model was larger than that for MaxEnt model.

Variable importance for *Bulinus globosus*

The relative importance of selected variables for *Bul. globosus* snail species was evaluated using jackknife tests. The relative importance of the bioclimatic variables for *Bul. globosus* in the Vhembe District Municipality is presented in Figure S6.4 (a) (RF) and Figure S6.4 (b) (MaxEnt). The BIO 6 showed a relatively higher variable importance than the other bioclimatic variables with a value of 0.20 for the RF model (Figure S6.4 a). The least important variable was BIO 3 with a value of 0.09 and this was followed by BIO 2, BIO 13 and BIO 15 with values of 0.12, 0.16, and 0.162, respectively. Overall, the RF model showed high relative importance, with the contribution made by the variables BIO 6, BIO 2, BIO 15 and BIO 13 being more than 0.1.

The MaxEnt model showed a high relative variable importance for BIO 6, BIO 2 and BIO 13 at 0.29, 0.19, and 0.25, respectively, and a low relative variable importance for BIO 3 and BIO 15 at 0.03 and 0.06, respectively (Figure S6.4 b). In comparison to the RF model, which utilised all five bioclimatic variables very effectively, MaxEnt did not utilise the variables as well. The AUC translates well when the relative variable relevance of the model is high, and in this instance, the AUC for RF was larger than that for MaxEnt. The RF model utilised all relevant bioclimatic variables more effectively than MaxEnt, which only utilised a few and poorly utilised the other variables.

Random Forest

The response curves show the output predictions of the RF model for the relationship between the snails in the Vhembe District Municipality and the selected bioclimatic variables (Figure S6.6 a). The curves illustrate how the snail species respond to each environment. The RF model results for *Biom. pfeifferi* are presented in Figure S6.6 (a) and include BIO 1, BIO 15, BIO 2, BIO 3, and BIO 6. The variable BIO 13, the response to rainfall (100 mm), was low, and as rainfall gradually increased so did the response of *Biom. pfeifferi*. The response of BIO 15, the precipitation seasonality, was low in the 0 to 90% coefficient of variation and had a

sharp increase between 90 and 95%. This indicated that *Biom. pfeifferi* responded well to the precipitation around the mean. The variable BIO 2, the mean diurnal range, showed a low response with *Biom. pfeifferi* between 12 °C and 13.5 °C. At 13.5 °C, the response increased in an almost linear pattern, peaking at 14 °C and dropping at 15 °C. Overall, this showed that *Biom. pfeifferi* is suited to higher temperatures, with a minimum of 14 °C. There was also a sudden increase at 11.7 °C that immediately dropped at 12 °C. The variable BIO 3, isothermality, indicated a response at 55% to 57% with a sudden increase at 56% and dropping almost immediately thereafter. At 59%, the response increased in an almost linear relationship and dropped at 60%. This indicated that a high isothermality is associated with a high environmental suitable to *Biom. pfeifferi*. The variable BIO 6, the minimum temperature of the coldest month, indicated an almost linear increase in the relationship between the snail species response and the temperature of the coldest month. The peak was at temperatures higher than 10 °C, indicating that the presence of this species is associated with places that have higher temperatures. When the temperature rose to more than 8 °C, there was an increase in suitability for *Biom. pfeifferi*. The minimum temperature for *Biom. pfeifferi* to survive was at 9 °C.

The RF response curves for *Bul. globosus* are presented in Figure S6.5 (a). The variables BIO 13, BIO 15, BIO 2, BIO 3 and BIO 6. BIO 13 responded poorly at 100 mm and increased to a point of maximum suitability at 140 mm. From 200 mm, the suitability remained constant at 0.125. This indicated that *Bul. globosus* preferred precipitation levels of 140 mm to 200 mm and indicates that the snail prefers moderate rainfall with a minimum of 120 mm. The variable BIO 15, precipitation seasonality, showed a high coefficient of variation value for *Bul. globosus* at 78% and decreased to between 80% and 85%. At 85%, the suitability increased, peaking at 87.5%, 89% and 90%, respectively. This indicted that precipitation seasonality for *Bul. globosus* ranged from 85% to 95%. The variable BIO 2 showed no suitability at temperatures of 10 to 12 °C whereafter a linear increase occurred, peaking at 13.3 °C and dropping to a minimum point of 0.050 response at 13.6 °C. This indicated that *Bul. globosus* preferred warmer temperatures than colder temperatures, evident from the high values of 12 °C. The variable BIO 3 showed a maximum suitability at 56%, with a maximum point of 0.17 response, making it the most suitable point in the response curve. The sharp drop to low suitability at 56.1% was evident but the curve rose again at 57.5% and continued in an almost linear manner. Minimum temperature of the coldest month (BIO 6) showed that suitability for the snails was favoured at higher temperatures than lower temperatures, with the curve showing a decrease in suitability at 4 °C up to 6 °C, and then a sudden increase that peaked at 6.5 °C. Thereafter, an almost linear relationship was evident between *Bul. globosus* and the

minimum temperature of the coldest month between 8 °C and 8.5 °C, which was also the maximum point of suitability.

Maximum entropy (MaxEnt)

The MaxEnt model results for *Biom. pfeifferi* are presented in Figure S6.6 (b) and are compared to the bioclimatic variables BIO 13, BIO 15, BIO 2, BIO 3 and BIO 6. BIO 13 showed a high response at 100 to 150 mm and a sharp decrease at precipitation levels higher than 150 mm. The linear decrease dropped to zero by 300 mm, indicating no suitability for *Biom. pfeifferi*. The variable BIO 15 is the precipitation seasonality, which is the irregular distribution of rainfall during a normal year and a higher coefficient means the more scattered the precipitation is to the mean. The response curve showed a sharp increase in suitability to an increase in precipitation seasonality, peaking at 81% and remaining high at 0.75 response. This means that *Biom. pfeifferi* is better suited to precipitation levels that are close to the mean. The BIO 2 response curve showed a suitability increase from 10 °C to 12 °C and a decrease from 12 °C. The decrease became sharp at 13.5 °C and lasted up to 15 °C where there was no suitability. This indicated that *Biom. pfeifferi* survives well at temperatures of between 10 and 12 °C. The variable BIO 3 showed an increase when the coefficient of variation was at a value of 55% and 58%, and then decreased sharply when the coefficient of variation was more than 60%. This indicated that the *Biom. pfeifferi* is suited to areas that have a value of between 55% and 60%. The BIO 6, minimum temperature of the coldest month, showed a decrease in suitability at 4 °C up to 6 °C whereafter it started increasing at 6.5 °C and peaked at 8.5 °C where it started to decrease again until there was no suitability for *Biom. pfeifferi*. This means that *Biom. pfeifferi* favours temperatures warmer than 6 °C.

The MaxEnt response curves for *Bulinus globosus* against BIO 13, BIO 15, BIO 2, BIO 3 and BIO 6 are presented in Figure S6.5 (b). The BIO 13 response curve showed an increase in precipitation of 100 mm. The suitability in this variable remained high, meaning that the snail survives in higher rainfall areas. The BIO 15 response curve had 2 maximum points, which were 75% and 93%. This means the scattering around the mean is high in this species. For BIO 2, the response curve showed an increase at between 10 and 12.5 °C and only peaked at 13.7 °C. This means that the species favour a high mean diurnal range compared to a lower diurnal range. The variable BIO 3 showed a decrease in response with an increase in the coefficient of variation value. The response curve for BIO 6 showed an increase at temperatures between 5 °C and 9 °C, with a maximum point at 8.9 °C, whereafter the suitability decreased, indicating that the species prefer temperatures between 5 and 9 °C.

Ecological niche models

The study created maps for each species, *Bul. globosus* and *Biom. pfeifferi*, as well as each model. The RF model exhibited a better resolution compared to MaxEnt (Figures 6.9-6.12). The RF model indicated specific areas while MaxEnt showed a greater number of areas. The maps are presented in Figure 6.9 and Figure 6.10 for *Bul. globosus* as well as in Figure 6.11 and Figure 6.12 for *Biom. pfeifferi*.

Figure 6.9 shows the historical distribution for the *Bul. globosus* species using MaxEnt. The areas in green show a low distribution of the *Bul. globosus* species while areas in red show high distribution. The areas that show the highest historical distribution are the Green Farm, Bevula and Malamulele towns in the Collins Chabane municipality. Valdezia, Elim, Tshikuwi and Dzanani in the Makhado Local Municipality were also some of the areas that had a high distribution, as indicated in red. Tshipise, Bokmakierie, Musina and parts of Lost City are some of the towns in the Musina Local Municipality that showed the highest distribution for *Bul. globosus*. The Thulamela area had the lowest distribution, with only parts of Thengwe and Pile having some distribution. There were also low distribution areas in the Kruger National Park (KNP) and west of the Musina Local Municipality. A high distribution of *Bul. globosus* was recorded in all areas of the Nzhelele River that passes through Tshipise and Dzanani.

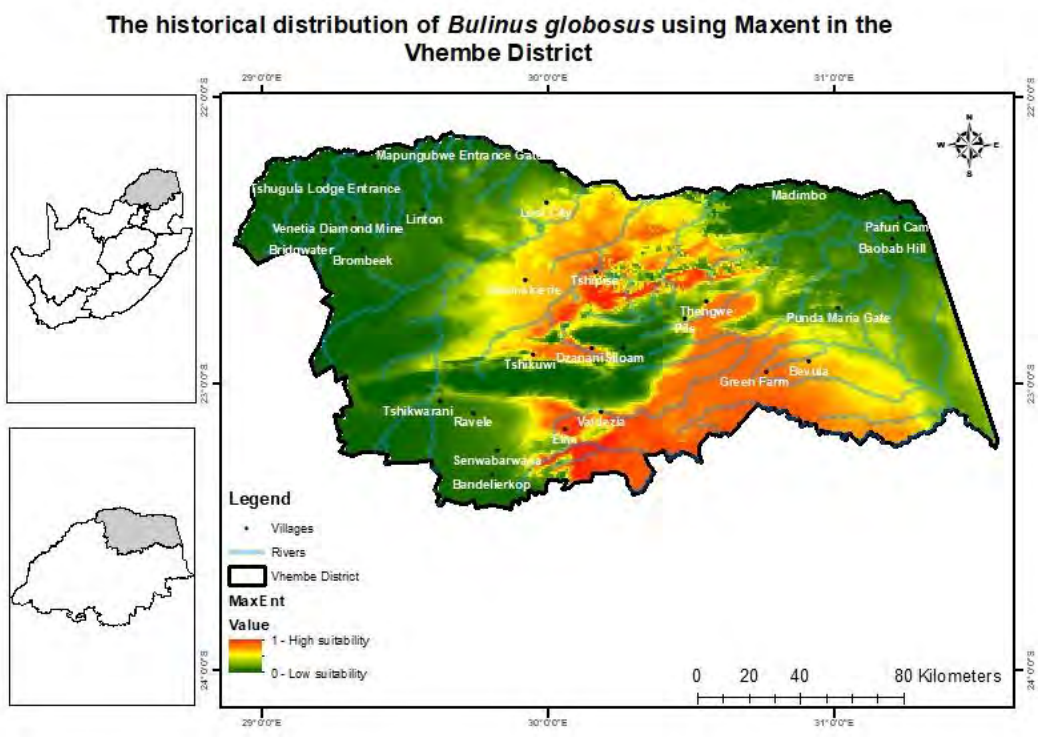

Figure 6.9: Historical distribution of *Bulinus globosus* in the Vhembe District Municipality using the maximum entropy model.

Compared to the MaxEnt model, the RF model showed more low distribution areas for *Bulinus globosus* in the Vhembe District Municipality with more green coverage than red coverage (Figure 6.10). The areas with low coverage were Tshipise, Green, Bevula, Elim, Valdezia, and Dzanani. All areas with high distributions were found near a water source such as the Luvuvhu River, which is near Elim and Valdezia, and the Nzhelele River, which is near the Tshipise and Dzanani areas, and the Green River which passes Green Farm. Overall, the RF had higher resolution and showed more details about which areas had a higher distribution of *Bulinus globosus* than the others.

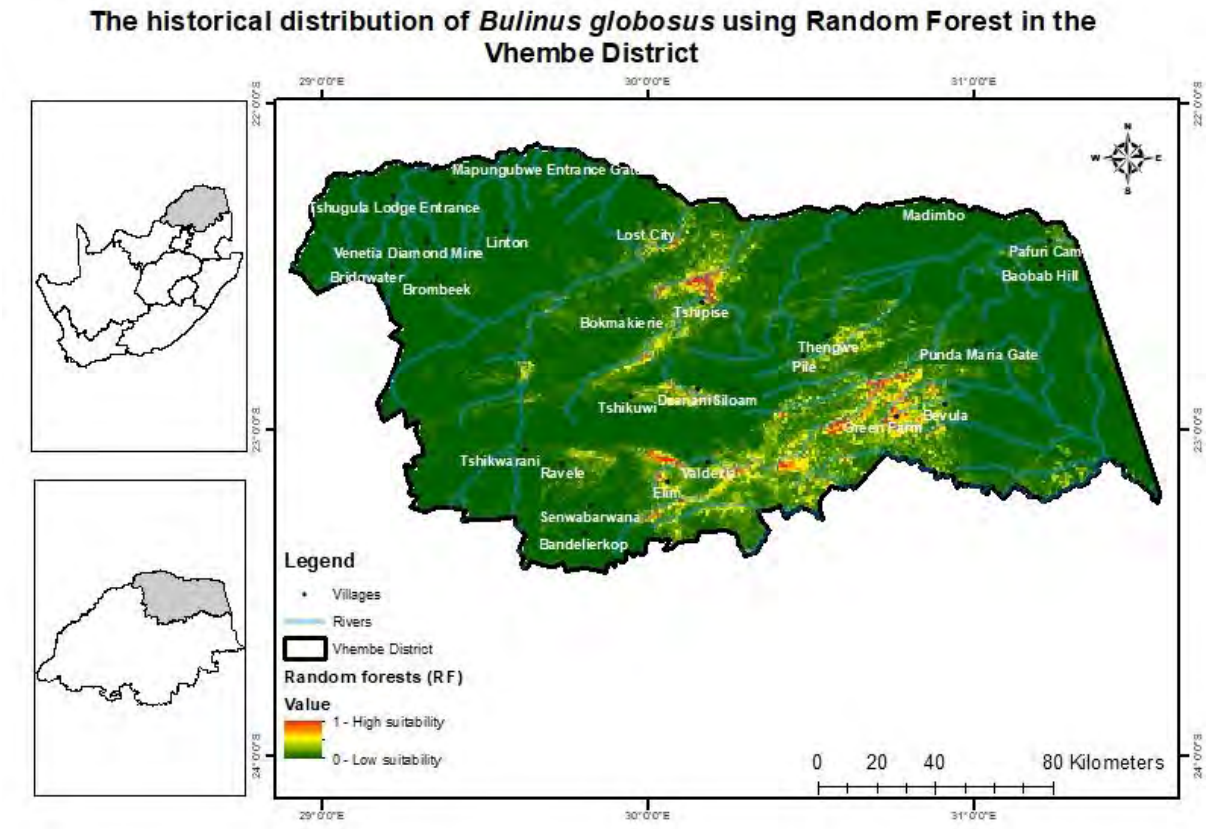

Figure 6.10: Historical distribution of *Bulinus globosus* in the Vhembe District Municipality using the Random Forest model.

Similar to the predictions for *Bul. globosus*, the MaxEnt model for the historical distribution of the *Biom. pfeifferi* indicated that areas such as Tshipise, Green Farm, Bevula, Elim, Valdezia, Dzanani, Tshikuwi, Bokmakierie, Musina and the Malamulele town were associated with a high distribution of the *Biom. pfeifferi* (shown in the colour red and yellow; Figure 6.11). However, the MaxEnt map generated for *Biom. pfeifferi* indicated additional areas such as Ravele, Tshikwarani and higher distribution in the Makhado municipality. The eastern parts of the Musina Local Municipality remained green, showing low distribution in this area, while the KNP indicated some areas of distribution. The RF modelled map for the historical distribution

of the *Biom. pfeifferi* was similar to the *Bul. globosus* map (Figure 6.12). The RF map generated for *Bulinus globosus* showed distribution in the northern regions of Tshipise, while *Biom. pfeifferi* showed a distribution in the southern areas of Tshipise towards the Dzanani area along the Nzhelele River. The distribution for this species was also low in the Green Farm, Bevula, Valdezia and Elim areas. A low distribution of *Biom. pfeifferi* was recorded in the eastern parts of the Musina Local Municipality and KNP.

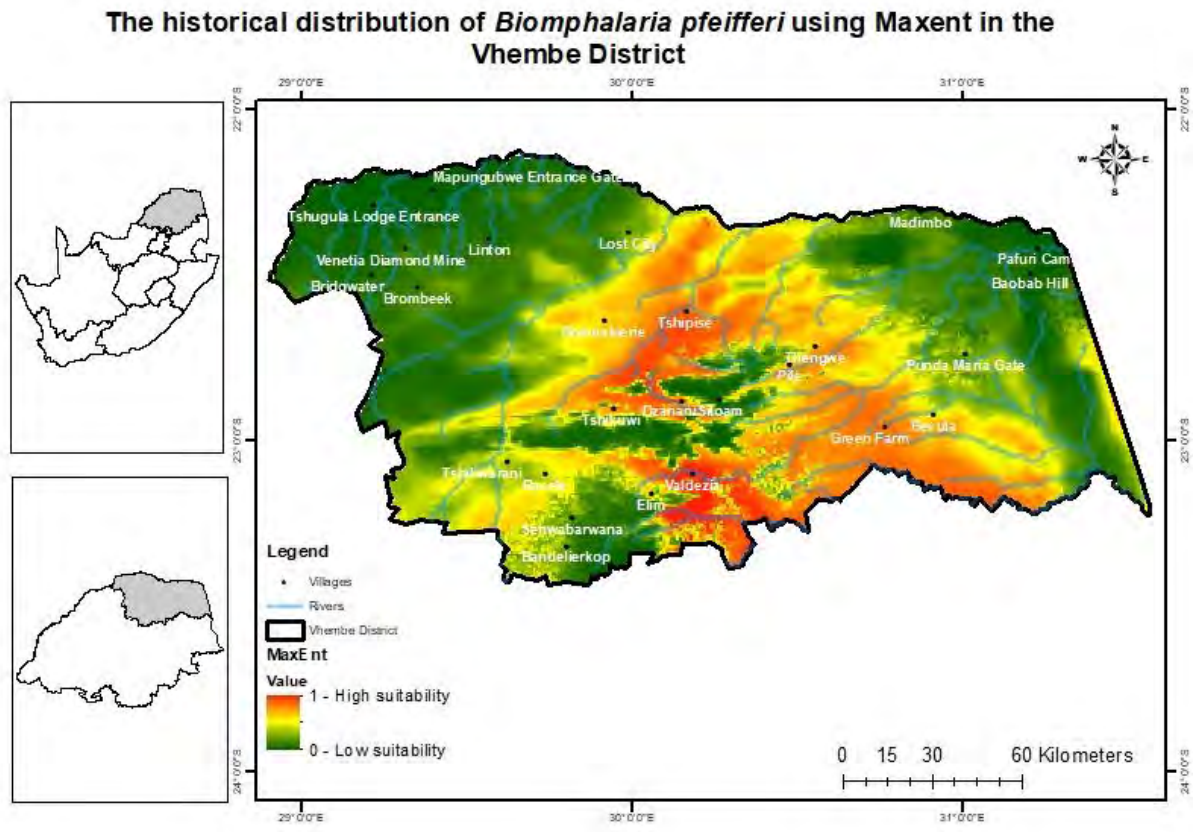

Figure 6.11: Historical distribution of *Biomphalaria pfeifferi* in the Vhembe District Municipality using the maximum entropy model.

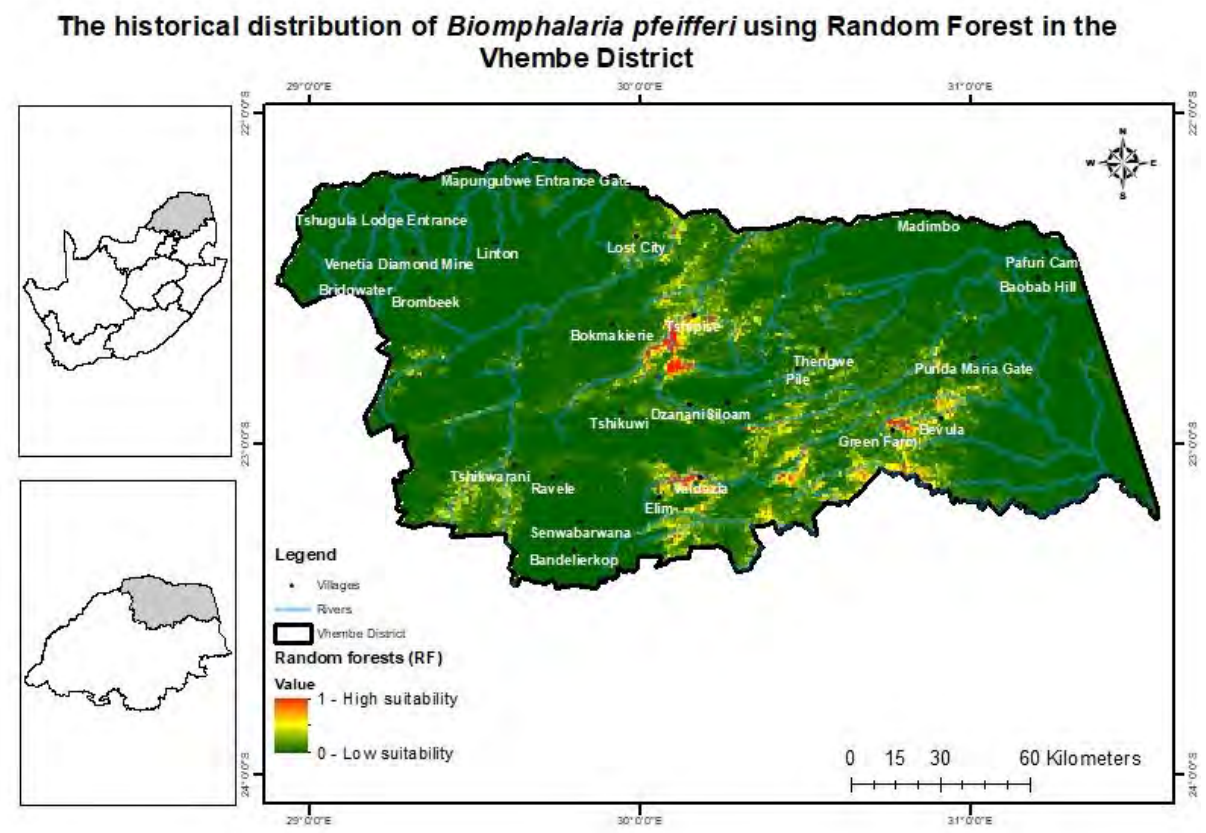

Figure 6.12: Historical distribution of *Biomphalaria pfeifferi* in the Vhembe District Municipality using the Random Forest model.

6.3.3 Historical distribution of Biomphalaria pfeifferi *and* Bulinus africanus *in the Tshwane and Johannesburg metropolitan municipalities*

The response curves shown in Figure S6.7 (a) and (b) were generated from both MaxEnt and Random Forest models. These curves showed the predicted habitat suitability for *Biom. pfeifferi* based on a range of factors governing the species distribution. The following bioclimatic variables were selected to run the MaxEnt and RF models: mean diurnal range (BIO 2), isothermality (BIO 3), min temperature of the coldest month (BIO 6), precipitation of the driest month (BIO 14), precipitation seasonality (BIO 15), precipitation of the driest quarter (BIO 17), and precipitation of coldest quarter (BIO 19). The response curve for BIO 2 showed a decline in the species' habitat suitability at the temperature interval 0 -14 °C. However, an increase in habitat suitability was present within the 14-16 °C temperature range. This relationship implies that the species responded positively to increasing temperatures. The following indicators of precipitation BIO 14, BIO 15, and BIO 19 illustrated a negative correlation between habitat suitability and rainfall. Likewise, the same observations were observed for BIO 14, and BIO 15 as generated by the RF model. The response curve for BIO 2 of the RF model (Figure S6.7 b) showed that habitat suitability fluctuated between the

temperature ranges 0-15 °C, followed by a slight increase in habitat suitability at 15.2 °C while habitat suitability for *Biom. pfeifferi* peaked at 16 °C.

The response curves generated using MaxEnt and Random Forest clearly showed that most of the variables retained after running the variance inflation factor were mainly rainfall parameters, implying that the temperature parameters were the main reason for multicollinearity issues on the original dataset (Figure S6.8). The variables used to predict habitat suitability for *Bul. africanus* In Tshwane Metropolitan Municipality included BIO 1, BIO 2, BIO 14, BIO 15, BIO 17, and BIO 19. These variables are represented on the x-axis of each response curve, and habitat suitability was measured against these climatic factors on the y-axis. For BIO 1, the predicted habitat suitability for *Bul. africanus* increased simultaneously with temperature specifically from the temperature range 17-18 °C and then subsequently declined at temperatures above 18 °C. According to the results obtained for the predicted habitat suitability against precipitation of the driest month (BIO 14), *Bul. africanus* favoured environments receiving precipitation between 100 mm-123 mm, and immediately after this range, habitat suitability declined greatly, which then affected the geographic dynamics of the snails. Similarly, BIO 15 showed that the species habitat suitability was negatively correlated with rainfall. Essentially, an increase in precipitation causes a decline in habitat suitability. Similar results were obtained for the remaining variables BIO 17 and BIO 19.

Correspondingly, the results generated through RF modelling showed that *Bul. africanus* reproduced well in cold conditions and showed a negative correlation with increased rainfall levels (i.e., BIO 14, BIO 15 and BIO 19; Figure S6.8 b). For example, BIO 1 indicated that species habitat suitability remained constant from 16 to 17 ˚C, however, this was followed by a slight increase extending up to 0.08 which is a high habitat suitability ratio. The probability of a predicted habitat suitability tremendously declined sharply at temperatures above 17.5 °C and this was followed by consecutive fluctuations from 17.5 to 18.5 °C. Above these temperature ranges, a constant trend from 19 to 20 °C was observed.

The response curves shown in Figure S6.9 indicated the predicted habitat suitability for *Bul. africanus* in Johannesburg Metropolitan Municipality. It was evident that the species responded differently to each respective climate parameter. The species habitat responded positively to BIO 3 up to 54%, where the predicted suitability ratio shifted from 0.08 to 0.06. At 55%, the species habitat suitability increased to 0.7 and a slight decline was experienced afterwards. On the other hand, the maximum temperature of the warmest quarter (BIO 5), showed that the habitat suitability increased from the temperature interval of 0-26.8 °C. A decline in habitat suitability was observed at 28.5 °C. The variables BIO 13 (precipitation of wettest month) and BIO 14 showed a negative correlation between habitat suitability and

precipitation. As rainfall increased, the habitat became unfavourable for the species to thrive. Therefore, it is unlikely to find *Bul. africanus* in areas where there are high levels of rainfall.

Model performance

The results for the models (MaxEnt and RF) that were used in this chapter study to predict the habitats of *Biom. pfeifferi* in the Tshwane Metropolitan Municipality are presented in Table 6.3. The AUC and TSS are the two metrics used in this analysis to assess how well each model performed in predicting the species habitat distribution (*Biom. pfeifferi*) in Tshwane Metropolitan Municipality. The RF model attained an AUC of 0.73, and scored a TSS of 0.35. Similarly, both the AUC and TSS values obtained on MaxEnt corresponded with the values obtained for the RF model in which the same metrics were used for evaluating model performance. Overall, the models performed satisfactorily in predicting the distribution of *Biom pfeifferi* habitats in Tshwane Metropolitan Municipality.

The performance of the respective species distribution models in terms of predicting the distribution of *Bul. africanus* habitats in the Tshwane Metropolitan Municipality is summarised in Table 6.4. The RF model attained an AUC value of 0.93 and scored a TSS value of 0.79. On the other hand, MaxEnt generated an AUC of 0.92, and scored a TSS value of 0.73. Collectively, both models performed well in predicting the historical distribution of *Bul. africanus* in Tshwane Metropolitan Municipality.

Likewise, the results of the distribution of *Bul. africanus* habitats in the Johannesburg Metropolitan Municipality were produced from RF and MaxEnt models (Table 6.5). For this species, an AUC value of 0.84 was attained by the RF model, which was accompanied by a TSS of 0.55. Conversely, MaxEnt showed different results for this species. According to MaxEnt, the model generated an AUC value 0.79, followed by a TSS of 0.48. Both models performed relatively well in predicting the distribution of *Bul. africanus* habitats in the Johannesburg Metropolitan Municipality.

Table 6.3: Model performance of maximum entropy (MaxEnt) and Random Forest (RF) in predicting the distribution of *Biomphalaria pfeifferi* habitats in Tshwane Metropolitan Municipality evaluated using area under the curve (AUC) and true skill statistic (TSS).

Model used	AUC	TSS
Maximum entropy	0.73	0.35
Random Forest	0.73	0.35

Table 6.4: Model performance of maximum entropy (MaxEnt) and Random Forest (RF) in predicting the distribution of *Bulinus africanus* habitats in Tshwane Metropolitan Municipality evaluated using area under the curve (AUC) and true skill statistic (TSS).

Model used	AUC	TSS
Maximum entropy	0.92	0.73
Random Forest	0.93	0.79

Table 6.5: Model performance of maximum entropy (MaxEnt) and Random Forest (RF) in predicting the distribution of *Bulinus africanus* habitats in Johannesburg Metropolitan Municipality evaluated using area under the curve (AUC) and true skill statistic (TSS).

SDM models	AUC	TSS
MaxEnt	0.79	0.48
Random Forest	0.84	0.55

The receiver operating characteristic (ROC) curve was generated for both models to determine the accuracy of each model in modelling the distribution of *Biom pfeifferi* habitats. The black dotted line (the 45-degree diagonal of the ROC space) indicates that the model performs no better than random. The x-axis represents the false positive rates which are measured against the true positive rates on the y-axis. The y-axis indicates a true model prediction whilst on the x-axis the model may have falsely predicted the probability of the species' presence (Figure 6.13 a and b). The AUC classifiers were skewed towards the true positives and closer to 1 which indicated accurate model performance. Similarly, MaxEnt showed that the AUC classifiers had a similar trend as on Random Forest model (Figure 6.13 b). Therefore, both these models performed well.

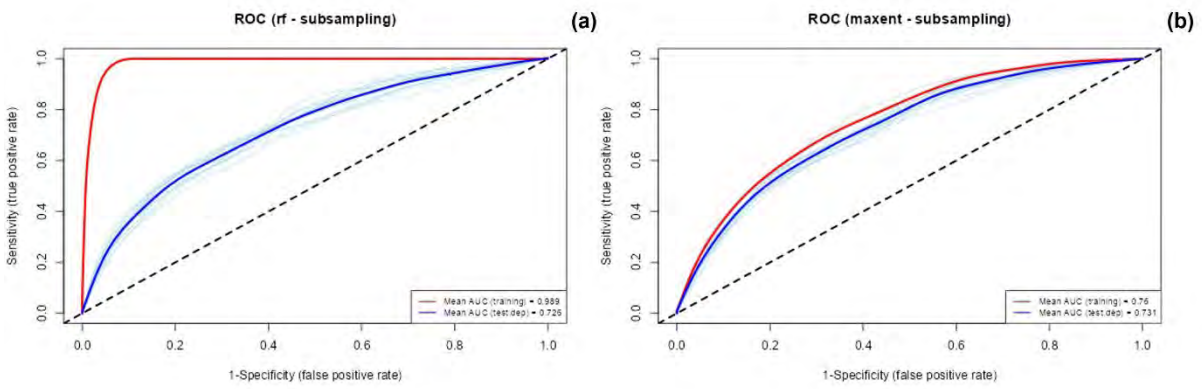

Figure 6.13: The ROC curves for both training and test data are presented for *Biomphalaria pfeifferi* in the Tshwane Metropolitan Municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) Random Forest; and (b) maximum entropy.

The ROC curves for *Bulinus africanus* generated by the RF and MaxEnt models depicted that the model performed well in predicting the distribution of *Bul. africanus* in Tshwane Metropolitan Municipality (Figure 6.14). The ROC curve for the RF model diverged from the black dotted line (the diagonal) and shifted closer to the true positive rate (1.0) which is indicative of excellent model performance. The ROC curve generated by MaxEnt showed a similar trend; however, the red line does not reach 1.0 which explains why this model has a slightly different AUC value than that of RF model. Overall, the models performed accurately in predicting the distribution of *Bul. africanus* in Tshwane.

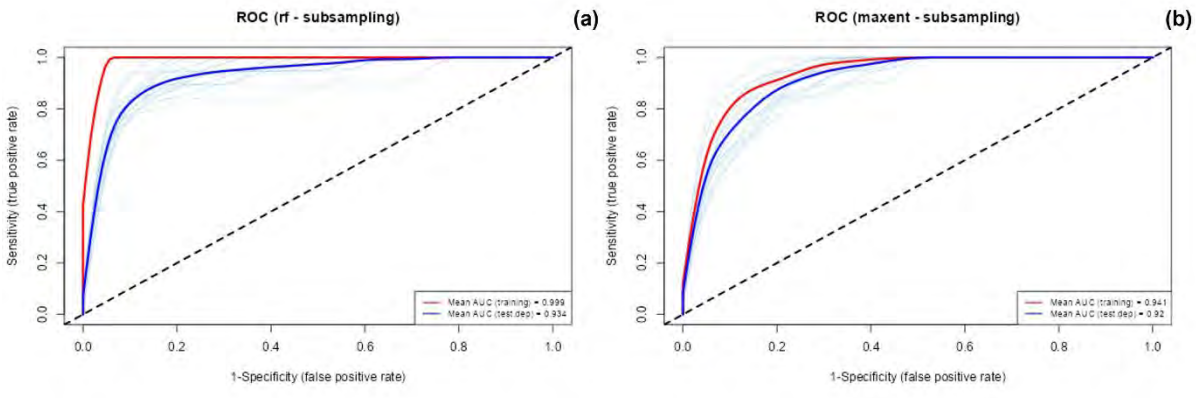

Figure 6.14: The ROC curves for both training and test data are presented for *Bulinus africanus* in the Tshwane Metropolitan Municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) Random Forest; and (b) maximum entropy.

The sensitivity of the model predictive ability was measured against the specificity see (Figure 6.15 a and b). The ROC curve for the RF model showed that the AUC was closer to the sensitivity ratio of 1.0 and extended far above the threshold line, thus representing good model performance. However, the ROC curve for the MaxEnt model showed that both AUC classifiers were not very far above the threshold line; however, the ROC did not touch the threshold implying that the model had an acceptable model performance.

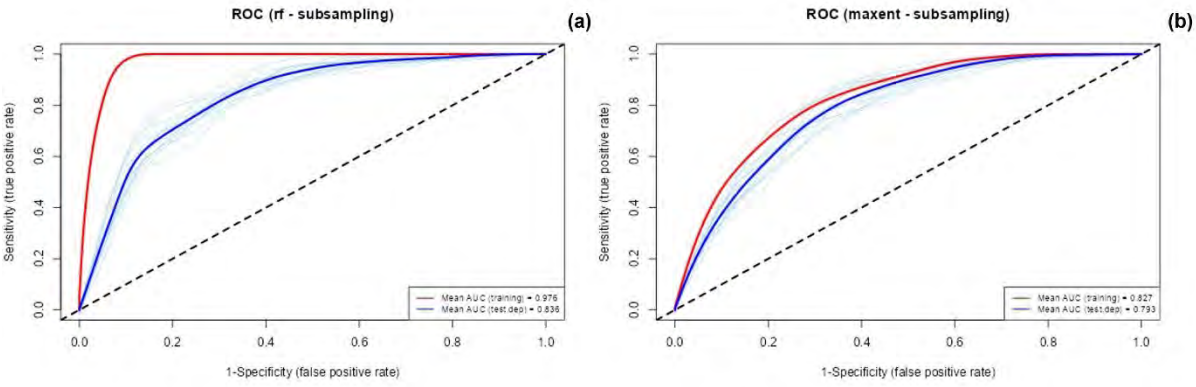

Figure 6.15: The ROC curves for both training and test data are presented for *Bulinus africanus* in the Johannesburg Metropolitan Municipality. The blue line indicates the test of the model's predictive power. The red line shows the fit of the model to the training data: (a) Random Forest; and (b) maximum entropy.

Jackknife test results

A jackknife test was used to measure the relative importance of each variable included in the models (RF and MaxEnt). Six bioclimatic variables were observed in both models (Figure S6.10). The relative importance of BIO 14 and BIO 6 was >0.10. However, BIO 15, BIO 17, and BIO 19 made a contribution that exceeds 0.05 and is closer to 0.10, which seemingly differed greatly from the remaining variable BIO 2, showing low relative importance in predicting the species distribution. In contrast, the results obtained on MaxEnt (Figure S6.10 b) varied greatly from those observed in the RF model. The contribution of BIO 6 was observed to be slightly above 0.15, followed by BIO 15, which had a relative importance of just below 0.15, and BIO 2 showed a relative importance >0.10. Comparatively, BIO 14 made a high contribution compared to BIO 17 and BIO 19.

The jackknife test results generated for the RF model illustrated that BIO 15 made the highest contribution in predicting *Bul. africanus* habitats in the Tshwane Metropolitan Municipality (see Figure S6.11 a) with an observed relative importance >0.25, followed by BIO 2. However, BIO 1 and BIO 19 made a higher contribution compared to the remaining bioclimatic variables (BIO 14, BIO 17) which showed a low relative importance (<0.10). However, the jackknife test results for the MaxEnt model (Figure S6.11 b) differed greatly. In this case, BIO 1 had a relative importance >0.25, and therefore contradicts what is illustrated in the RF results. Thus, this variable made the highest contribution to the model prediction compared to other variables, followed by BIO 14 with a relative importance >0.20. A major difference was observed between the variables with high importance and those with low importance, with BIO 2, BIO 15, BIO 17 and BIO 19 all remaining below 0.05, thus indicating that these variables made a low contribution to the distribution of *Bul. africanus* in the Tshwane Metropolitan Municipality.

The jackknife test results for the RF model measured six bioclimatic variables (BIO 1, BIO 2, BIO 14, BIO 15, BIO 17 and BIO 19) for both models in predicting *Bul. africanus* habitats in the Johannesburg Metropolitan Municipality (Figure S6.12 a). The RF model showed that BIO 8 and BIO 13 had an exceptionally high relative importance which exceeded 0.10. These variables were then followed by BIO 15 with an observed relative importance of 0.08, while BIO 3, BIO 5, and BIO 19 had a relative importance extending towards 0.08, and BIO 14 remained below 0.06. The jackknife test results for the MaxEnt model (Figure S6.12 b) showed that BIO 15 made the highest contribution to the model prediction compared to the other variables as it exceeded 0.25 (Figure S6.12 b). This was then followed by BIO 8 and BIO 13 which obtained a relative importance >0.15. However, BIO 5 contributed >0.10 in the model prediction, while BIO 3 and BIO 19 had made a much lower contribution to the model prediction compared to the other variables such as BIO 14, thus indicating that these variables made a

low contribution to the distribution of *Bul. africanus* in the Johannesburg Metropolitan Municipality.

Random Forest and maximum entropy model maps

Figure 6.16 (a) depicts the predicted habitat suitability that corresponds with the historical distribution of *Biom. pfeifferi* in Tshwane Metropolitan Municipality as generated from the MaxEnt model. The model predicted a possible high habitat suitability mostly in the northern and the southern regions of the Tshwane Metropolitan Municipality. The western and eastern parts of Tshwane were characterised by low to relatively moderate habitat suitability. Roodeplaat had favourable conditions for *Biom. pfeifferi*, characterised with high suitability. Similarly, the model predicted high habitat suitability in most parts of Bronkhorstspruit. Likewise, *Biom. pfeifferi* habitats were prevalent in Soshanguve, and in selected parts of Irene (Figure 6.16 (a). Conversely, the model showed a difference in terms of habitat suitability in areas such as Refilwe and Winterveldt. Refilwe had relatively moderate habitat suitability compared to the other parts of Tshwane. Similarly, moderate suitability was also evident in Winterveldt. However, the results showed some patches of high habitat suitability in selected parts of Winterveldt. High habitat suitability increases towards the central parts (Roodeplaat), north of Tshwane. Low habitat suitability was predicted in Atteridgeville, Centurion, and in selected parts of Bronkhorstspruit.

Habitat suitability for the historical distribution of *Biom. pfeifferi* predicted using the RF model are depicted in Figure 6.16 (b). There were some areas of both high and moderate habitat suitability in the northern, central, and southern regions of Tshwane Metropolitan Municipality. The Roodeplaat area had high habitat suitability, along with patches of moderate habitat suitability. Similarly, some parts of Bronkhorstspruit were characterised by high habitat suitability. Winterveldt and Soshanguve showed some areas of moderate habitat suitability along with a strong concentration of low habitat suitability. Similarly, this was present in other parts such as Centurion and Irene, where the model predicted a relatively moderate density of suitable habitats for *Biom. pfeifferi*. In areas such as Atteridgeville, Refilwe, and most parts of Centurion, low habitat suitability was present for *Biom. pfeifferi*. Overall, the eastern and the western parts of the Tshwane Metropolitan Municipality had considerably lower habitat suitability when compared to the northern, central, and southern regions.

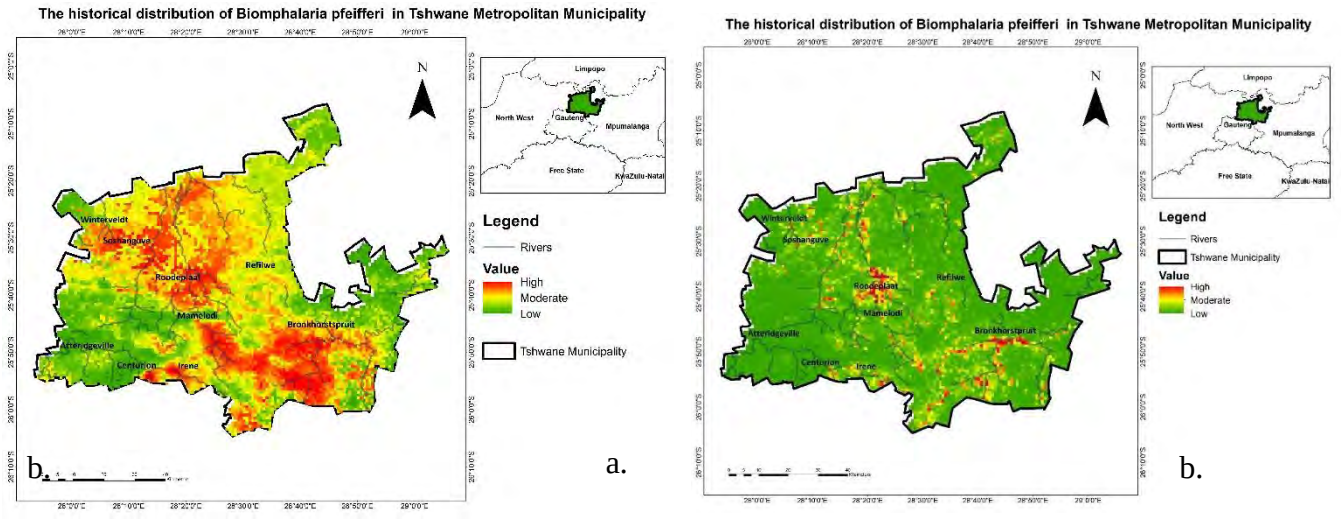

Figure 6.16: Predicted habitat suitability for the historical distribution of *Biomphalaria pfeifferi* in the Tshwane Metropolitan Municipality: (a) maximum entropy; and (b) Random Forest.

Figure 6.17 (a) represents the predicted probability of the historical distribution of the habitat suitability for *Bul. africanus* in Tshwane Metropolitan Municipality using the MaxEnt model. The robustness of the model was evident as there was a clear distinction between areas of high suitability and those of moderate to low habitat suitability. Low habitat suitability was present in the northern and the southern regions of Tshwane Metropolitan Municipality. The central parts of the Tshwane Metropolitan Municipality were characterised by relatively high and moderate habitat suitability. Bronkhorstspruit was shown to have both moderate and low habitat suitability while neighbouring locations such as Irene and Centurion showed low-suitability habitats. Similarly, a low-suitability habitat for this particular species was predicted in Winterveldt. Some parts of Soshanguve were moderately suitable for *Bul. africanus*. Likewise, Roodeplaat was shown to have moderate suitability with a high proportion of low-suitability habitats as well. Areas such as Refilwe were predicted to have some patches of high habitat suitability, and moderate suitability with low suitability in some parts. Mamelodi was highly suitable for *Bul. africanus*. Similarly, high habitat suitability was predicted in Atteridgeville with some patches of moderate suitability.

The predicted habitat suitability for the historical distribution of *Bul. africanus* in Tshwane Metropolitan Municipality generated from the RF model is represented in Figure 6.17 (b). These results differed from those predicted using MaxEnt. Firstly, low-suitability habitats were widely distributed across the Tshwane Metropolitan Municipality, with Bronkhorstspruit presenting low habitat suitability. Similarly, low-suitability habitats were observed in Winterveldt, Soshanguve, Irene, Centurion and in Refilwe. In contrast, Mamelodi and Roodeplaat were shown to be highly suitable areas for the distribution of *Bul. africanus*. However, Atteridgeville showed some patches of high habitat suitability, whereas other parts

of Atteridgeville had moderate suitability. Low-suitability habitats were also observed across some parts of Atteridgeville.

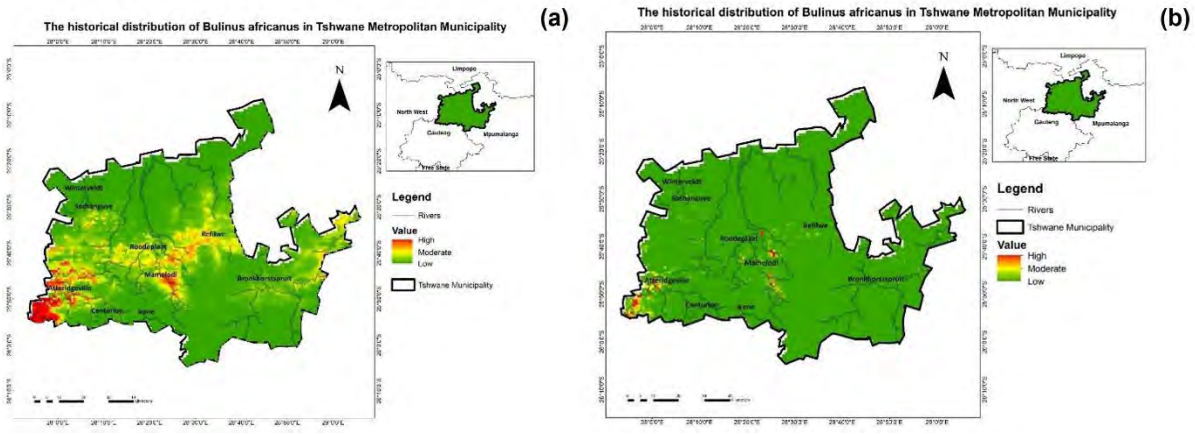

Figure 6.17: Predicted habitat suitability for the historical distribution of *Bulinus africanus* in the Tshwane Metropolitan Municipality: (a) maximum entropy; and (b) Random Forest.

Figure 6.18 (a) shows the predicted habitat suitability for the historical distribution of *Bul. africanus* in Johannesburg Metropolitan Municipality based on the MaxEnt model predictions. The model showed that habitat suitability for *Bul. africanus* was unevenly distributed across Johannesburg. As a result, high habitat suitability was mostly visible in the northern regions of Johannesburg and it decreased in small patches and towards the central parts of Johannesburg. The Randburg, Diepsloot, and Soweto areas showed high-suitability habitats for *Bul. africanus*, while low-suitability habitats dominated in the northern regions and in some eastern parts of Johannesburg. Although not highly distributed, there were some areas of high habitat suitability in some parts of Roodepoort, south of Johannesburg, and Midrand. Orange Farm, unlike the other locations, showed a low-suitability habitat. This was followed by areas south of Johannesburg, and in Midrand. Moderate suitability was sparsely distributed across the municipality. Moderate suitability could be observed in some parts south of Johannesburg, Roodepoort, Johannesburg, Midrand, Diepsloot, Randburg and just above Orange Farm.

The Random Forest model was another model used to determine the distribution of *Bul. africanus* in Johannesburg Metropolitan Municipality; this model generated significantly different results see (Figure 6.18 b). The model predicted an uneven distribution of habitat suitability across Johannesburg. Low-suitability habitats were widely distributed in the Johannesburg Metropolitan Municipality. Consequently, only some small patches were indicated to have moderate to high habitat suitability in this municipality. The Soweto, Randburg, Midrand, and Diepsloot areas and some parts of Roodepoort were characterised by both moderate and high suitability. On the other hand, areas such as Orange Farm and most parts of the south of Johannesburg had a considerably low-suitability habitat for the

distribution of *Bul. africanus*. High habitat suitability was observed in Randburg along some rivers. Moreover, the south of Johannesburg was shown to be a predominantly moderate-suitability habitat for *Bul. africanus.*

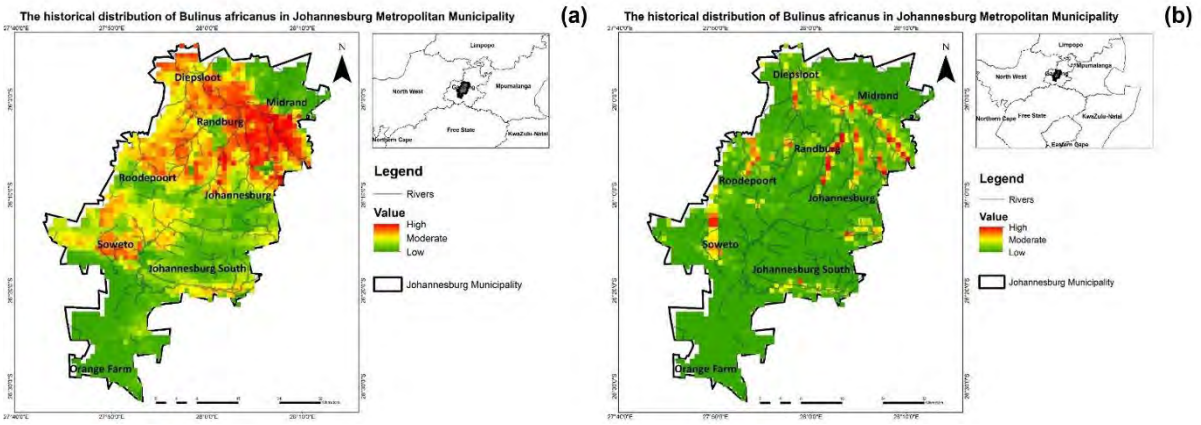

Figure 6.18: Predicted habitat suitability for the historical distribution of *Bulinus africanus* in the Johannesburg Metropolitan Municipality: (a) maximum entropy; and (b) Random Forest.

6.4 Discussion

6.4.1 Historical distribution of Biomphalaria pfeifferi *and* Bulinus globosus *in Mbombela and Nkomazi local municipalities*

The models used in this study indicated a good prediction of the occurrence probability for the historical distribution of suitable habitats for *Bulinus globosus* and *Biomphalaria pfeifferi* in Mbombela and Nkomazi local municipalities based on historic bioclimatic and climatic variables. The AUC scores for both species indicated that the GLM utilised in this study performed satisfactorily. The MaxEnt model generated high AUC scores which showed that the model predicted the best occurrence probability for the historical distribution. There are many factors that determine the ability of a model to successfully run, such as the spatial extent of study area and sample size. For many species, the sample field data are insufficient to characterise the geographic distribution of species in the study area and, often, must be supplemented by digital species information. However, the distribution records of some species must be confirmed by Google Earth due to the lack of detailed latitude and longitude information. Some distribution sites have been collected over long periods and their environment has changed over time.

Compared to the actual distribution, it was found that the range predicted by the model was in general agreement with the actual distribution. Although there are minor deviations, the core distribution area was consistent with the historical distribution. Based on the results of the MaxEnt model, the key factors affecting species' geographical distribution are not completely

consistent as it was seen that the variables contributed differently for each species. This is also true of the GLM.

The model predictions indicated that precipitation was the main environmental variable affecting species distribution. This was also supported by Manyangadze et al. (2021) who found similar results in a study investigating the spatial and seasonal distribution of *Bul. globosus* and *Biom. pfeifferi* snail species in KwaZulu-Natal. In summer, the seasonal distribution predicted *Biom. pfeifferi* snails have low to moderate historical distribution within Mbombela local municipality, which may be due to the high rainfall the municipality receives during the rainy season. This was also observed in a study conducted by Utzinger and Tanner (2000) in Tanzania. High distribution in the cold and dry season in the Nkomazi Local Municipality may be because of the dams and pools along the streams which provide suitable habitats for host snails. Seasonal fluctuations in rainfall affect temporary and permanent water bodies in which intermediate host snails are found. Seasonal precipitation can also be used to measure the availability of suitable temporary water bodies that host snails are known to inhabit (Dai et al. 2022).

The tolerance of the snail species *Bul. globosus* is highly dependent on the variations in climatic and environmental factors (Joubert et al. 1990, Manyangadze et al. 2016b, Kalinda et al. 2017b; Manyangadze et al. 2021). The model results for MaxEnt and GLM showed that under historical conditions, suitability of *Bul. globosus* was concentrated mainly in the Mbombela area, around Matsulu. In Nkomazi, the distribution was along the Malelane area and the southern parts of the municipality such as Thambokhulu. Similar findings were found in other studies assessing the distribution and habitats of schistosomiasis-transmitting snails (Brown 1966; Van Eeden and Combrinck 1966; De Kock and Wolmarans 2005). These were suitable areas for the snail to reside in due to the preferred conditions such as elevation, rainfall and higher temperatures found in these parts of the municipalities which represented the best conditions for the viability of the snail (Appleton and Miranda 2015a; 2015b; Adekiya et al. 2020).

The models indicated a decrease in the historical distribution and suitable habitats of *Biom. pfeifferi* with the species limited close to water sources such as the Crocodile River. These findings are in line with other studies on *Bul. globosus* and *Biom. pfeifferi* that have been conducted in southern Africa (Zimbabwe and Madagascar), showing a decrease in the geographic distribution of schistosomiasis host snails connected to changes in precipitation and temperature due to climate change (Pedersen et al. 2014b; Kalinda et al. 2017b; Kalinda et al. 2018; Adekiya et al. 2020). These studies found that areas that were considered suitable for intermediate host snails will either become too dry or too hot under future climate change scenarios to sustain schistosomiasis and its intermediate host snails (Pedersen et al. 2017).

A study by Kalinda et al. (2018) used data from field experiments and laboratories to create a deterministic compartmental simulation model based on difference equations using a weekly time step that represented the life cycle of *Bul. globosus* to simulate snail population dynamics of the species. The study concluded that the increase in temperature due to climate change may alter the prevalence of *Bul. globosus* and lead to a decrease in the species population. Pedersen et al. (2011b) modelled the impact of climate change on the spatial distribution of schistosomiasis host snails in Zimbabwe and concluded that climate change may cause a decrease in the spatial distribution of suitable habitats of host snails such as *Biom. pfeifferi.* This means that the historical distribution patterns within Mbombela and Nkomazi local municipalities may have changed, and will possibly continue to change, due to shifts in the environmental variables. Climate change will more likely shift rather than expand the geographic ranges of the snail species within Mbombela and Nkomazi, which will likely create new areas vulnerable to schistosomiasis and this is something that has also been reported in other studies (McCreesh and Booth 2013; Stensgaard et al. 2013; Pedersen et al. 2014b; Stensgaard et al. 2019).

6.4.2 Historical distribution of Biomphalaria pfeifferi *and* Bulinus globosus *in the Vhembe District Municipality*

In this study, the models performed well, with RF performing better than MaxEnt. Similar findings were found in a study done in China comparing MaxEnt and RF models and found that RF had a higher AUC than MaxEnt (Zhao et al. 2022). A study conducted by Mi et al. (2017) found that RF had the highest AUC value of 0.830 with MaxEnt at 0.805. The present study determined that RF performed the best for the majority of evaluation techniques, offered a better model fit to the testing data, and produced better species range maps. The TSS for both models was higher for RF than for MaxEnt, and this finding is in agreement with a study done by Abdulwahab et al. (2020) which showed higher TSS for RF compared to MaxEnt.

Random Forest

According to RF modelling results, BIO 6 exhibited the highest variable importance for *Biom. pfeifferi* species. Furthermore, the response curve showed a linear increase in the suitability of *Biom. pfeifferi* with the minimum temperature of the coldest month. The RF findings showed that when the temperature rose to more than 8 °C, there was an increase in habitat suitability for the *Biom. pfeifferi* species. This means that *Biom. pfeifferi* is suitable for higher temperatures, minimum at 14 °C, as observed in the RF response curve. A study done by De Kock et al. (2004), showed similar results with *Biom. pfeifferi* having a peak at 15-20 °C. The BIO 2 findings for *Biom. pfeifferi* species showed the moderate relative importance of this bioclimatic variable at a value of 0.13. The response curves showed a linear relationship

between the *Biom. pfeifferi* species and the mean diurnal temperature, peaking at 14 °C. This means that the *Biom. pfeifferi* prefers temperatures close to the mean. A study done by McCreesh and Booth (2014) showed a reduced survival rate for *Biom. pfeifferi* at temperatures outside the 15-31 °C range. This shows that temperature plays an important role in the suitability of the *Biom. pfeifferi* because temperatures outside the preferred temperature range lead to an increased mortality rate of the snails. For *Biom. pfeifferi*, BIO 3 was indicated to be of least relative importance of all the bioclimatic variables at a value of 0.12. Multiple previous studies have reported that isothermality has a moderate to high relative importance to the species distribution of the *Biomphalaria* species (Scholte et al. 2012, Habib et al. 2016, Yang et al. 2018).

The response curves showed that high habitat suitability is found in areas that have a mean diurnal temperature of more than half the annual temperature. *Biomphalaria pfeifferi* is better suited to habitats with greater temperature variability within an average month. Using the RF model, BIO 13 was shown to have the second highest variable importance for *Biom. pfeifferi*, followed by BIO 15. The BIO 13 response curves showed an increase in suitability with an increase in the rainfall, peaking at 150 mm. In terms of RF, this means that the *Biom. pfeifferi* favours wetter seasons over drier seasons. According to a study by De Kock et al. (2004), the *Biom. pfeifferi* had a higher preference for rainfall between 300-900 mm, although 2.7% of the species were also present in habitats with 0-300 mm of rainfall per year. However, based on a study by Manyangadze et al. (2021), the species thrives better in low rainfall areas because increased rainfall increases the mortality rate of the snails (Pedersen et al. 2014b). Similarly, a study in the Ingwavuma, uMkhanyakude district, KwaZulu-Natal, found that *Biom. pfeifferi* species were found between June and August 2014 in the cold and dry season; however, from September 2014 to May 2015, during increased rainfall, a decreased presence was observed (Manyangadze et al. 2021)

The RF findings for *Bul. globosus* species indicated a high relative importance for BIO 6, at a value of 0.2. Furthermore, the response curve showed a linear increase in the habitat suitability for *Bul. globosus* with the minimum temperature of the coldest month. The RF findings showed that when the temperature rose to more than 8 °C, there was an increase in suitability for the *Bul. globosus* species. However, at 10 °C a sudden drop in suitability can be seen in the response curve. It has been shown in studies such as that conducted by Adekiya et al. (2020) that constantly high temperatures are preferred by *Bul. globosus.* The temperature range for the survival of *Bul. globosus* is between 15-36 °C as reported by Kalinda et al. (2017b). A study done in China reported that the *Bul. globosus* survival temperature range is between 26-29 °C and the survival rate is reduced outside this range (Wang et al. 2022). This shows how temperature influences the growth, development, and survival of the

snails and how important it is to understand their suitability to changing conditions. Using the RF model, BIO 15 was indicated to be of second highest relative variable importance for *Bul. globosus*, followed by BIO 13, which showed the highest suitability for *Bul. globosus* at 140 mm and 200 mm rainfall. eThe precipitation is essential in the post-rainy season because it provides the species with an aquatic environment to survive and grow (Pedersen et al. 2014b; Manyangadze et al. 2021).

MaxEnt

The MaxEnt findings indicated that BIO 6 exhibited the second highest variable importance for *Biom. pfeifferi*. The response curve showed a linear increase in the suitability of the *Biom. pfeifferi* between 6 and 8.9 °C, and a linear decrease at 9 °C. A study done in Ethiopia reported that *Biom. pfeifferi* can survive in minimum temperatures as low as 10 °C (Kristensen et al. 2001), and another study observed how survival can be seen at temperatures as low as 5 °C for a short time (De Kock and van Eeden 1986). This shows that although *Biom. pfeifferi* thrives better at higher temperatures, they can also be found at lower temperatures with fewer individuals. The lowest variable importance for *Biom. pfeifferi* habitat suitability was for BIO 3 *Biomphalaria pfeifferi* does not do well with temperature variability as presented by a greater habitat suitability when the mean diurnal range was less than the temperature annual range. *Biomphalaria pfeifferi* is better suited to low temperatures than higher temperatures. The highest relative variable importance was indicated for BIO 2, further supporting the BIO 6 results, and shows that more habitat suitability can be seen in low temperatures. Although the study done by De Kock et al. (2004) showed 53.7% of the species surviving at temperatures of between 15 and 20 °C, 0.3% of the species are found at temperatures of between 10 and 15 °C. A linear decrease in BIO 13 was shown for the *Biom. pfeifferi* results which can be because the species thrives better during post-rainy periods (Nwoko et al. 2023).

According to the MaxEnt findings, BIO 6 exhibited high relative importance for *Bul. globosus* species, at a value of 0.3. The findings also showed that peak suitability is at a temperature of 9 °C. A study done in China showed that the effective temperature range for the *Bul. globosus* was 7-40.2 °C (Wang et al. 2022). This species thrives at a mean temperature of less than 14 °C. Moderate variable importance was indicated for BIO 2, meaning that this species thrives better in low-temperature variation because it shows high suitability when the mean diurnal range is lower than the annual temperature. The second most variable importance was indicated for BIO 13, and BIO 15 had the second lowest variable importance, and thus providing further support that *Bul. globosus* does not favour high rainfall areas (Manyangadze et al. 2021, Pedersen et al. 2014b).

Ecological niche model

The final maps were also a good indication of the better performance of RF compared to MaxEnt, with MaxEnt showing a distribution of a large area, and RF showing which areas had a strong distribution for each species. For *Biom. pfeifferi* and *Bul. globosus* species, temperature and precipitation are important factors that determine the suitability of an area (Adekiya et al. 2020). The findings also indicated that the high distribution areas were near a water source, such as the Luvuvhu River and Nzhelele River. This is because water sources play a major role in the habitat of the species. The temperature range of the Vhembe District Municipality is suitable for both species. However, the precipitation may often be too high for the snails to survive. This is because high precipitation areas increase water velocity and this increases mortality (Pedersen et al. 2014b).

The MaxEnt model findings for *Biom. pfeifferi* showed that the Tshipise, Dzanani, Elim, Green Farm and Thengwe areas have high suitability for *Biom. pfeifferi* and aligned with the snail observation data inserted into the model. Tshipise is a holiday resort in the Vhembe District Municipality that is well known for having a spring of naturally scalding hot water at approximately 58 °C (Olivier et al. 2011). It is an attraction site visited by multiple tourists and a holiday spot. Other areas that have thermal springs are Sagole (located 57 km to the east of the Tshipise resort) with water at approximately 45 °C, and Mphephu (located near Siloam) at approximately 43 °C. *Biomphalaria pfeifferi* has been shown to favour temperatures that are between 15 and 20 °C, and the number of times the *Biom. pfeifferi* was collected in spring water was 0.5% (De Kock et al. 2004). The high temperature of the spring water is not favoured by these species and they cannot survive in this area. However, the surrounding water bodies can be supported due to lower water temperatures in the rivers. Furthermore, the number of times the species was collected in the rivers was 27.2%, and 27.5% in dams (De Kock et al. 2004). The Sagole spring water was noted to flow northwards into the Tshipise River (Olivier et al. 2011). The findings also show high suitability in areas near a water source such as the Luvuvhu River and Nzhelele River. The findings of the RF model for *Biom. pfeifferi* show that high suitability is seen in Elim, Green Farm, and south of Tshipise. The dams near these highly suitable areas in the Vhembe District Municipality are the Nandoni Dam situated near Thohoyandou, the Albasini Dam situated near Elim, and the Nzhelele Dam situated south of the Tshipise resort. These dams are likely highly suited to the snails as they are standing waters with a great deal of nutrients that provide food for the molluscs which are important to ensure their survival (Sidy et al. 2021)

The observation (snail points) of the *Bul. globosus* aligns well with the maps created with MaxEnt and RF with extremely high suitability found near Tshipise resort and surrounding water sources. Several studies have shown the relationship between temperature and

precipitation with the *Bul. globosus* distribution. Studies have shown that *Bul. globosus* thrives better in warmer temperatures (Adekiya et al. 2020, Kalinda et al. 2017b, Wang et al. 2022). This would explain the high suitability seen in areas that are near thermal water springs such as Tshipise and Sagole. A study done in the Vhembe District Municipality showed that between the years 1960 to 1970, there was more rainfall in the Thohoyandou area (approximately 1 198.2 mm) than in the Musina area (approximately 302.5 mm) (Shikwambana et al. 2021). *Bulinus globosus* is abundant in areas that receive less rainfall due to the increased mortality rate associated with the high rainfall (Pedersen et al. 2014b; Manyangadze et al. 2021). This would explain the higher suitability in the Musina and surrounding areas than in the Thohoyandou area.

6.4.3 Historical distribution of Biomphalaria pfeifferi *and* Bulinus africanus *in the Tshwane and Johannesburg metropolitan municipalities*

The results shown on MaxEnt and Random Forest models explicitly demonstrated that the distribution of *Biom. pfeifferi* habitat changes with a given bioclimatic factor. The models showed that *Biom. pfeifferi* thrives well in relatively warmer temperatures, in contrast to the cooler temperatures favoured by *Bul. africanus*. The bioclimatic variable BIO 2 illustrated that the species habitat suitability declined at the temperature range of 0-14 °C. This trend is expected as studies have shown that *Biom. pfeifferi* inhabits environments with warm temperatures, therefore these environmental conditions were undoubtedly unsuitable for *Biom. pfeifferi* species. Previous studies reported a decline in snail populations at temperatures fluctuating below 14 °C and above 31 °C for the same species (Adekiya 2018). This explains why an increase in habitat suitability was evident in the temperature interval 14-16 °C. Correspondingly, Moodley et al. (2003) argued that the optimum temperatures for *Biom. pfeifferi* range from 16-35 °C. In contrast, Manyangazde et al. (2021) reported that *Biom. pfeifferi* only tolerates temperature ranges of 20-27 °C; any temperature below or above this range reduces snail breeding, thus providing an explanation for the observations made in the present study. Likewise, similar observations were made for *Biom. pfeifferi* in terms of the relationship between precipitation and the species habitat suitability. A negative correlation between BIO 14, BIO 15 and BIO 19 as collective measures of precipitation was observed against habitat suitability. It is clear that changes in precipitation have an impact on the species habitat suitability, thus *Biom. pfeifferi* thrives well in environments where there are no high levels of rainfall.

Similarly, these observations were also derived from the RF model for BIO 14 and BIO 15. Therefore, it can be argued that varying levels of rainfall can change the spatial distribution of the infected snails. On the other hand, the response of the species habitat to temperatures

was noted at 15.2 °C, where the species habitat increased at this temperature measurement supporting the supposition that *Biom. pfeifferi* prefers relatively warm conditions.

The response curves generated by RF models for *Bul. africanus* in Tshwane Metropolitan Municipality demonstrated how habitat suitability varies across different environmental conditions which essentially depends on the species response to a given environmental parameter. The mean annual temperature variable had an influence on habitat suitability, arising from a simultaneous increase in habitat suitability and mean annual temperature between the temperature intervals 17-18 °C. However, a subsequent decrease in that trend was observed immediately after the temperatures increased above 18 °C. Therefore, it can be deduced that *Bul. africanus* is threatened by increasing temperatures, thus warmer temperatures are mostly likely to cause a shift in the spatial distribution of *Bul. africanus* habitats in Tshwane Metropolitan Municipality. Appleton and Madsen (2012) also agreed that this species is commonly found in areas where frost occurs and temperatures drop below zero. These observations correspond to previous findings that *Bul. africanus* tolerates cold temperatures (Moodley 2003). Moreover, it was observed that this species can withstand environments that experience 100 mm-123 mm of rainfall. Therefore, areas characterised by excessive rainfall pose devastating effects on the infected snails. This can potentially result in a shift in dynamics and distribution of the intermediate snail host, for instance areas that did not previously harbour snails can ultimately become new breeding sites. These observations complement what other studies have reported.

Similarly, the results generated from the RF model are not dissimilar from the trends portrayed in the MaxEnt. It is clear that this species reproduces well in cold temperature conditions, and a negative correlation is noticeable between the species habitat suitability and enhanced rainfall levels. All the rainfall indicators (BIO 15, BIO 17 and BIO 19) showed a similar relationship to species habitat suitability as habitat suitability decreased with increased rainfall. A decline in habitat suitability was observed when the temperatures increased above 17. 5 °C, thus showing how sensitive *Bul. africanus* is to relatively warmer optimums.

Temperature and precipitation variables were selected in this study as they have been reflected by many studies to have a major influence on the distribution of schistosomiasis (Manyangadze et al. 2021). In the Tshwane Metropolitan Municipality, an increase in habitat suitability was observed at 0-26.8 °C for *Bulinus africanus* when the species habitat suitability was measured against the temperature of the warmest quarter. Consequently, habitat suitability dropped greatly at 28.5 °C. A study by De Kock and Wolmarans (2005) reported the presence of *Bul. africanus* in the following temperature categories, namely 15-20 °C and 20-25 °C. Essentially, it can be argued that *Bul. africanus* is sensitive to increasing temperatures. The species habitat suitability dropped significantly at 57% isothermality. Therefore, as

isothermality decreases, the habitat suitability becomes threatened. There was also a negative correlation between precipitation and habitat suitability, which therefore implies that habitat suitability decreases as the rainfall increases. Hence, it is impossible to find *Bul. africanus* in areas characterised by increased levels of rainfall.

In the present study the AUC for the RF and MaxEnt models was >0.80. Therefore, these models produced accurate results in determining the distribution of *Bul. africanus* in Tshwane. In the context of this study, particularly in modelling the distribution of *Bul. africanus* habitats in Johannesburg, it must be noted that the RF model achieved the highest AUC value (0.84) compared to MaxEnt (0.79). The models differed greatly from one another; the RF model had a good predictive ability in determining the probability of the historical distribution of *Bul. africanus* in Johannesburg. On the other hand, MaxEnt had an acceptable performance, as also observed by Yang et al. (2018).

Distribution of *Biomphalaria pfeifferi* habitats in Tshwane Metropolitan Municipality

Two models were used to determine the historical distribution of *Biom. pfeifferi* in Tshwane. Interestingly, MaxEnt had a seemingly accurate visualisation of habitat suitability. However, both models were satisfactory in predicting habitat suitability in Tshwane. This has also been drawn from comparisons based on the AUCs obtained. As a result, the north and the south east of the municipality showed a high habitat suitability. Thus, it is most likely to find *Biom. pfeifferi* species in areas that are located in these regions. These include Bronkhorstspruit, Roodeplaat and areas north of Tshwane such as Soshanguve, and in some parts of Irene. All these have a relatively high species occurrence which can be attributed to the favourable environmental conditions for *Biom. pfeifferi* to survive.

Distribution of *Bulinus africanus* habitats in Tshwane Metropolitan Municipality

The MaxEnt model showed a seemingly better display of the results; however, the robustness of the Random Forest model was clearly demonstrated in a way that specific areas with low suitability are discernible from those with high and moderate suitability. On the other hand, MaxEnt produced more generalised approximations of habitat suitability in the Municipality. Nevertheless, both models were excellent in predicting habitat suitability in Tshwane. High suitability was found along the central parts of the Tshwane Metropolitan Municipality. This could mean that environmental requirements for *Bul. africanus* are mostly met in the central parts of Tshwane. In simple terms, the central part of the municipality provides suitable conditions which are not detrimental on the species. The Atteridgeville and Mamelodi areas were reported to have a high habitat suitability. On the other hand, low suitability was shown in the northern and the southern parts of Tshwane in areas such as Winterveldt, Irene and

Centurion, while moderate suitability was indicated for the southeastern areas of the municipality and some parts of Bronkhorstspruit.

Distribution of *Bulinus africanus* habitats in Johannesburg Metropolitan Municipality

The results showed high-suitability habitats for *Bul. africanus* in Randburg, Diepsloot and Soweto; therefore, these areas provide suitable environmental conditions for this species to thrive. Essentially, the results presented on the maps correspond with trends shown on the response curves. Thus, suitable habitats for *Bul. africanus* have cold temperatures and do not receive high levels of rainfall. Orange Farm reported a low habitat suitability, which therefore implies there are unfavourable conditions for the species to thrive.

6.5 Conclusion

The aim of this study was to identify historic areas of distribution for schistosomiasis-transmitting snails in three Provinces of South Africa using various modelling methods. The impact of different bioclimatic and climatic variables on the distribution of *Biomphalaria pfeifferi* and *Bulinus globosus* in Mbombela and Nkomazi local municipalities was evaluated with the use of the MaxEnt model and GLM. Both the MaxEnt model and GLM results showed that within the two municipalities, Mbombela provided the most suitable distribution area with some parts of Nkomazi, which corresponded with studies conducted previously. It is therefore evident that these factors, such as temperature, precipitation and elevation, play an important role in the distribution dynamics of these intermediate host snails, and that climate change can alter the known distribution of schistosomiasis host snails. The predictions generated by MaxEnt were highly accurate compared to the model produced by GLM. However, it was also shown that MaxEnt could correctly operate with a higher similarity with models of presence/absence when the review coverage conducted was uniform and extensive. Unsuitable areas might become new risk areas for the distribution and transmission of schistosomiasis due to changes in the climatic and bioclimatic variables. It is vital to predict the distribution of the species using a sound ecological niche model so that an understanding may be gained of areas that might be vulnerable to infection and transmission, in case officials require information to implement schistosomiasis control and elimination measures currently and in the future. These historically suitable areas might have already shifted due to an increase in human population, increase in agricultural activities, and land development. The findings of this study regarding the historical distribution of schistosomiasis host snails, can be used to identify additional localities where the intermediate host snails may already exist but have not been detected yet, and where the disease is likely to spread, creating new vulnerable areas. Water (rainfall) was noted as one of the main factors that affect the

distribution of schistosomiasis vectors, hence it was important to assess how changes in water quality may influence the distribution.

In the Vhembe District Municipality, the RF model made use of more variables and achieved a higher AUC than MaxEnt. The *Biom. pfeifferi* and *Bul. globosus* preferred low precipitation and higher temperatures as seen on the response curves. The maps showed that areas near a water source had higher distributions of *Biom. pfeifferi* and *Bul. globosus* than any other area. However, a more thorough study needs to be conducted to determine the seasonal distribution for both species because the precipitation seasonality (BIO 15) showed a good relationship with *Bul. globosus* in the MaxEnt model.

Precipitation parameters were negatively correlated with habitat suitability for both *Biom. pfeifferi* and *Bul. africanus* in the Tshwane and Johannesburg metropolitan municipalities. Temperature was another determinant in which *Biom. pfeifferi* were found to prefer relatively warmer temperatures compared to *Bul. africanus.* Inarguably, a shift in climate factors due to climate variability will undoubtedly change the distribution of schistosomiasis snail vectors, as the environmental requirements and conditions in which these snails mainly occur have been highlighted in the study based on the reconstruction of historical habitats. Climate variability increases the spread of schistosome-infected snails to areas in which the disease may not have been recorded and areas providing fluctuations in climate factors may be sensitive to the snail vectors, thus changing the dynamics of the snail vectors. This informs future studies by showing how habitat suitability is influenced by climate variability and how schistosomiasis endemicity is linked with areas providing suitable climate conditions in which the snail vectors thrive.

CHAPTER 7: MODELLING THE POTENTIAL FUTURE DISTRIBUTION OF MOLLUSCS IN THE NORTHERN PROVINCES OF SOUTH AFRICA IN RELATION TO FUTURE PREDICTED CLIMATE CHANGE

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7.1 Introduction

Climate change can modify the geographical distribution of schistosomiasis by affecting or disturbing the population of the intermediate host snail species and freshwater bodies. In a study conducted in Zimbabwe, Pedersen et al. (2014b) agreed that the spatial distribution of appropriate or suitable habitats would change with the variation in the condition of the climate. Climate change has an impact on schistosomiasis at temperate latitudes. Sub-Saharan Africa and the Pacific and Indian Oceans are affected by temperature extremes (WHO 2003). The occurrence and distribution of schistosomiasis are closely related to fluctuations in average temperature because schistosome transmission depends on the reproduction and survival of their intermediate hosts (Mangal et al. 2008). As intermediate hosts have no thermoregulatory mechanisms, their survival and reproduction rates depend on the temperature change.

The Representative Concentration Pathway (RCP) includes four scenarios that describe GHG emissions and concentrations up to 2100, based on assumptions (Meinshausen et al. 2011; van Vuuren et al. 2011). As described by the IPCC, RCP 4.5 and RCP 6 are stabilisation scenarios. The RCP 2.6 is a low-emission scenario where greenhouse gas (GHG) emissions peak in 2020 and decline as populations peak and renewable energy increases (Meinshausen et al. 2011; van Vuuren et al. 2011; Hayhoe et al. 2017). In RCP 8.5, greenhouse gases are expected to increase, creating a “high emission scenario’’. Consequently, this indirectly affects the distribution of *Schistosoma*-transmitting snails (Meinshausen et al. 2011). These RCPs superseded the IPCC proposed set of emission scenarios in the Special Report on Emissions Scenarios (SRES) published in 2000, and were previously used for this purpose. Unlike simpler models that make mixing assumptions regarding processes internal to a presumed cell, while other functions govern the interface between such cells, the general circulation models (GCMs) describe four climate futures, all considered possible depending on how much GHGs are emitted in the years to come. The IPCC also relies on Coupled Model Intercomparison Project (CMIP) experiments, a collaborative series designed to improve our knowledge of the GCMs and their interactions. The most recently completed phase of the project is CMIP5, which includes more metadata describing model simulations than previous phases.

Temperature and precipitation are expected to vary in low- and high-emission scenarios, which could affect the geographical distribution of schistosomiasis (Khasnis and Nettleman 2005;

Weber et al. 2018). Under moderate climate scenarios, global average surface temperatures will increase by between 1.4 and 3.1 °C by 2081-2100 (IPCC 2013). The global hydrological cycle and precipitation patterns will be affected by this warming (IPCC 2013). These changes will likely impact vector-borne diseases with intermediate invertebrate hosts (Parham et al. 2015). As a result, the likelihood of transmission in any location is influenced partly by abiotic factors that affect free-living organisms and intermediate host organisms such as snails.

The average annual temperature in South Africa has increased 1.5 times the observed global average increase of 0.65 °C during the past 50 years (Ziervogel et al. 2014; Botai et al. 2018; He and Ding 2021). Increased temperatures are expected to reduce precipitation in some parts of South Africa (Longxing et al. 2014). By 2015–2035, temperatures are predicted to rise by between 1 °C and 2 °C in the interior of South Africa (DEA 2013). Temperatures along the coast could increase by 1 °C between 2015-2035 (DEA 2013). South Africa is likely to experience changes in rainfall and temperature due to climate change. Variations in these conditions could affect disease transmission (DEA 2013).

In response to ongoing climate change, precipitation patterns shift, temperatures rise, and extreme climate events (e.g., droughts and flooding) are more frequent and severe (IPCC 2013). Schistosomiasis-transmitting snails are likely to be affected by climate change. South Africa's eastern regions are experiencing severe storms, landslides and flash flooding caused by changing rainfall patterns. It has been shown that floods can cause schistosomiasis outbreaks (Zhang et al. 2004; Longxing et al. 2014). People are exposed to contaminated water during flooding, increasing their risk of infection with schistosomes. The distribution of *Biom. pfeifferi* increases significantly during post-rainy seasons compared to *Bul. globosus*, and therefore, the presence of *S. haematobium* reduces during dry periods as it is transmitted by *Bul. globosus* (Ernould et al. 1999). Temperature and rainfall are major climate factors contributing to the distribution of *Schistosoma* species (Mangal et al. 2008; Stensgaard et al. 2013; Monde et al. 2016). In eastern Africa, studies (McCreesh and Booth 2014; McCreesh et al. 2014; Adekiya et al. 2020) indicated that increasing temperatures could provide a favourable environment for the proliferation of *S. mansoni* infections. In contrast, it was reported that the incidence of schistosomiasis might decline in other areas due to temperature increases (Adekiya et al. 2020). This may result in adverse conditions for intermediate host snails (McCreesh and Booth 2014; McCreesh et al. 2015; Adekiya et al. 2020). As a result of a warmer and drier climate, it has been reported that the incidence of schistosomiasis in Zimbabwe has decreased over the past three decades (Midzi et al. 2011; Pedersen et al. 2017).

Modelling future distributions of schistosomiasis-transmitting snails is necessary for identifying mitigation and response strategies; however, only a few efforts have been made to predict how climate change would affect the spread of schistosomiasis, and the results have been

inconsistent (Stensgaard et al. 2013). In some studies (Martens et al. 1997; Zhou et al. 2008; McCreesh et al. 2015) transmission rates have been reported to increase in colder areas. In contrast, some studies have reported a contraction of transmission and geographical shift due to climate change (Martens et al. 1997; Stensgaard et al. 2013). This emphasises a complex relationship between temperature and schistosomiasis risk (Mangal et al. 2008). There are two widely used approaches for modelling the impact of climate change on the distribution of schistosomiasis: i) the use of statistical models to model the relationship between *Schistosoma* snails and environmental variables (Stensgaard et al. 2013; Pedersen et al. 2017); and ii) an experimental approach based on the relationship between parasite and temperature (McCreesh et al. 2015).

Climate change is already reducing the number of schistosomiasis-transmitting snails in other areas (McCreesh and Booth 2014; McCreesh et al. 2015; Adekiya et al. 2020). Although climate change is predicted to limit suitable areas for schistosomiasis transmission, future predictions of the distribution of schistosomiasis-transmitting snails in South Africa remain unclear. The aim of the current chapter is to predict the national spatial distribution of three intermediate snail host species, namely *Bulinus globosus*, *Bul. africanus* and *Biomphalaria pfeifferi* for the current climate, and to forecast the distribution in a future climate, using climate change projection. Three ecological models, three GCMs, under two emission scenarios (RCP 4.5 and RCP 8.5) for three time periods (baseline (1979-2018), (2040-2070) and (2070-2100)), were used. The ecological niche models (ENMs) were used to create high-resolution maps for South Africa for the first time in this field, indicating areas that will exhibit an increase or decrease in transmission and where new infections may occur.

7.2 Methods

All analyses were carried out using the methodology explained in section 2.3.10 (Chapter 2).

7.3 Results

7.3.1 Model performance

Tables S7.1 to 7.6 illustrate the AUC, correlation (COR) and TSS values for the different climate and ecological models and species (*Bul. africanus*, *Bul. globosus*, and *Biom. pfeifferi*). All three ecological models had significant AUC and TSS values, demonstrating excellent performance. The RF outperformed the other models regarding model performance across distinct species, followed by MaxEnt and GLM. Results showed that the ensemble models showed the best predictive performance among the three ENM models as they achieved the highest AUC scores (0.99-1). Evaluation of the model showed that it was based on competence rather than chance in modelling the historical distribution of schistosomiasis. It must be noted that the high AUC

value provides confidence that the ensemble model can be used to examine schistosomiasis-prone areas under current and future climates.

The species distribution predictions were analysed for three periods: baseline (1979-2018), 2040-2070, and 2070-2100. Across these periods, the models performed well in predicting the species distribution by having similar AUC and TSS scores, although slight variations are observed. This could be due to ecological model differences and species distribution pattern shifts.

All three climate models for the different periods and emission scenarios performed well. Access1-0 performed well in predicting the future spatial distribution (RCP 4.5 and RCP 8.5) of *Bulinus africanus, Bulinus globosus and Biomphalaria pfeifferi* by demonstrating AUC values of above 0.9 for all ecological models. The bcc-csm1-1-m model performed second-best to access1-0; the AUC values of the ensemble model for the 2040-2070 and 2070-2100 periods are 0.98 and 1.00, respectively. The high AUC in the latter period suggests that the ensemble model anticipates a substantial shift in species distribution, potentially due to the climate changes projected by bcc-csm1-1-m. The hadgem2cc model performed third-best to access1-0 due to slight variations in the climate models. Overall, the AUC values consistently remain high across different climate scenarios, indicating that the ensemble model predicts the distribution of *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi*. The variations in AUC values across periods and climate scenarios can reflect changes in the range of species due to projected climate changes. These statistics highlight the reliability of the ensemble model in predicting the potential impacts of climate change on the distribution.

7.3.2 Distribution curves

RCP 4.5 (2040-2070)

Two of three climate models agree that the suitable habitat for *Bul. africanus* in BIO 3 lies at a temperature of between 51 and 55 °C (Table 7.1). All three models agree that the optimum temperature for *Bul. africanus* in BIO 5 is between 20 and 27 °C. For all three climate models, *Bul. africanus* prefers temperatures of between 25 and 30 °C in BIO 8. The BIO 13 climate models are similar; preferred habitats for *Bul. africanus* are between 50 and 100 mm. In contrast to BIO 13, BIO 15 shows little variation among the models, with peaks at between 0.50 and 1.35. The climate models agree that *Bul. africanus* favours BIO 16 in areas with rainfall of between 500 and 1 000 mm. Across all climate models, *Bul. africanus* thrives at 0.00 to 0.40 for soil water in the wettest, warmest, and coldest quarters. The habitats suitable for *Bul. globosus* in BIO 4 differ between the three models, access1-0 (100), bcc-csm1-1m (80), and hadgem2 (55) (Table 7.1). According to BIO 7, *Bul. globosus* thrives at temperatures of between 13 and 17 °C. Similar to BIO 7, BIO 8 suggests that *Bul. globosus* prefers 24 and 26 °C, while BIO 15 shows that *Bul. globosus* peaks at between 0.80 and 1.2. *Bulinus globosus* prefers lower levels of precipitation (BIO 16) between 260 and 450 mm, with little variation across models. All three models show

that low BIO 17 (10 mm) levels are favourable for *Bul. globosus*. The optimum soil water temperature for *Bul. globosus* in the warmest quarter is 0.20-0.30 for all three models. For *Biom. pfeifferi*, different optimum values are observed in BIO 4, access-1-0 (120), bcc-csm1-1m (50), and hadgem2cc (50) (Table 7.1). There is agreement among all models that *Biom. pfeifferi* prefers habitats between 23 and 24 °C. For BIO 12, slight variations are shown in all three models: access1-0 (750 mm), bcc-csm1-1m (450-500 mm) and hadgem2cc (600-750 mm). All three models indicate that BIO 16 is optimal for *Biom. pfeifferi* at 350 mm. As with BIO 16, BIO 18 confirms that *Biom. pfeifferi* thrives in environments with low rainfall (250 mm).

RCP 4.5 (2070-2100)

Similar to the period 2040-2070, BIO 3 shows a variation for *Bul. africanus* among the three models, access1-0 (84), bcc-csm1-1m (50), and hadgem2cc (65) (Table 7.1). Specifically, *Bul. africanus* responds differently to BIO 5, with optimum temperatures being access1-0 (25 and 32 °C), bcc-csm1-1m (20-25 °C), and hadgem2cc (26-27 °C). All three models are in agreement that *Bul. africanus* prefers similar habitats in BIO 8 at a temperature of 28 to 30 °C. The optimal environments in BIO 13 vary across the three models, access1-0 (5-100 mm), bcc-csm1-1m (180 mm), and hadgem2cc (70 mm). Precipitation seasonality (coefficient of variation) (BIO 15) shows that *Bul. africanus* is suitable for rainfall habitats at 0-1.20, while BIO 16 indicates that *Bul. africanus* prefers rainy habitats with a rainfall of 500 mm to 750 mm. *Bulinus africanus* prefers soils between 0.30 and 0.4, where it has been found in the warmest, wettest, and coldest quarters. There is a variation in optimum for BIO 4 between access1-0 (100), bcc-csm1-1m (75), and hadgem2cc (-50) for *Bul. globosus*. All climate models favour BIO 7 at 14-17 °C (Table 7.1). Climate models show that *Bul. globosus* thrives at 24 and 27 °C. In all models, *Bul. globosus* prefers environmental conditions of BIO 15 between 0.80 and 1.25. Contrary to the results for the period 2040-2070, *Bul. globosus* highly responds to BIO 16 at 600-1 000 mm of rainfall. For BIO 17, *Bul. globosus* shows high optimum rates in low rainfall environments of 0 to 35 mm, and thrives in areas with soil and water temperatures of 0.20 to 0.30 in the warmer quarters. According to the different models, there is a variation in the response of *Biom. pfeifferi* to BIO 4 between access1-0 (-50), bcc-csm1m-1 (50), and hadgem2cc (-75). In BIO 8, *Biom. pfeifferi* exhibits optimal temperatures between 22 and 27 °C across all models. Based on the climate models, the optimum rainfall range for *Biom. pfeifferi* in BIO 12 is between 520 and 700 mm. There is a peak in *Biom. pfeifferi* in BIO 16 when all models have 350-500 mm of rainfall. According to BIO 18, *Biom. pfeifferi* prefers low rainfall environments with peak rainfalls of 180 to 350 mm (Table 7.1).

RCP 8.5 (2040-2070)

There is a slight variation in isothermality (BIO 3); however, the three climate models agree that *Bulinus africanus* thrives at 80 °C (Table 7.2). In BIO 5, all three climate models agree that the

optimal environment for *Bul. africanus* is between 22 and 26 °C. According to the three climate models, BIO 8 shows different optimum temperatures; access1-0 illustrates 27 °C, whereas bcc-bcsm1-1 indicates 10-16 °C, and hadgem2cc indicates 15-17 °C. The consensus among climate models is that the ideal rainfall amount for *Bul. africanus* in BIO 13 is 180-200 mm. Based on BIO 15, *Bul. africanus* prefers 0.80-1.2 across all models. In BIO 16, *Bul. africanus* has a rainfall preference of 400 mm for the access1-0 model, 500-750 mm for bcc-csm1-1, and 220 mm for hadgem2cc. The soil water for the wettest quarter indicates that *Bul. africanus* thrives at 0.10-0.30. Conversely, the soil water for the coldest quarter indicates that *Bul. africanus* thrives at 0.30-0.35 for access1-0 and bcc-csm1m-1, and at 0.4 for hadgem2cc. All the models show high suitability for soil water in the warmest quarter.

In all three climate models, BIO 4 shows different optimum climates for *Bul. globosus*; according to access1-0, *Bul. globosus* thrives at -50, whereas bcc-cm1-1 indicates 200, and hadgem2cc indicates -5. In access1-0 and bcc-csm1m-1, the optimum temperatures for BIO 7 are 17-19 °C and 17-18 °C, respectively, and 10-13 °C for the hadgem2cc climate model. For BIO 8, all three models are in agreement that *Bul. globosus* thrives at 25-30 °C. All three models indicate that *Bul. globosus* is suitable for BIO 15 at 1.00-1.50. The thresholds for *Bul. globosus* in BIO 16 differ across the three climate models. Access1-0 indicates that 600 mm is suitable, and bcc-csm1-1 shows 250-400 mm is optimum for *Bul. globosus* and hadgem2cc suggests suitability at between 0 and 250 mm. Similar to BIO 16, BIO 17 also displays a range of optimum values in the models. The access1-0 model depicts 25 mm, the bcc-csm1-1 model depicts 50 mm, and the hadgem2cc model displays 0 to 100 mm. The soil water for the warmest quarter indicates that *Bul. globosus* thrives at 0.10-0.40, consistent with all three climate models.

In BIO 4, *Biom. pfeifferi* flourishes at -10 for access1-0e, at 100 for bcc-csm1-1m, and at 50 for hadgem2cc. Across all three models, *Biom. pfeifferi* varies in terms of mean temperature of wettest quarter (BIO 8), with a temperature of between 23 and 28 °C. This indicates that *Biom*. pfeifferi thrives in these environments. All models agree that *Biom. pfeifferi* flourishes in BIO 12 when rainfall is between 500 and 800 mm. Climate models predict that *Biom. pfeifferi* thrives in BIO 16 when rainfall is between 350 and 400 mm. Comparable to BIO 16, BIO 18 also indicates that *Biom. pfeifferi* thrives well in environments with rainfall of between 200 and 450 mm.

RCP 8.5 (2070-2100)

Despite some differences among the climate models, they indicated that *Bul. africanus* can thrive in areas of BIO 3 at between 73 and 80 °C (Table 7.2). Climate models agree and indicate that *Bul. africanus* prefers BIO 5 at 22-28 °C, while BIO 8 should be 25-30 °C for *Bul. africanus* and models agree with this trend. The habitat for *Bul. africanus* is likely to be more suitable when BIO 13 is between 180 and 300 mm, and all models appear to agree to an extent. The results of BIO 15 demonstrate that there is a variation in the preferred habitat of *Bul. africanus* among the

models, with suitable habitat being determined by access1-0 (0.50), bcc-csm1-1m (0.80) and hadgem2cc (0.40). Precipitation of wettest quarter (BIO 16) varies according to the model, with access1-0 (700 mm), bcc-csm-1m (450 mm), and hadgem2cc (550-750 mm). This study shows that *Bul. africanus* prefers environments with 550 mm and 750 mm of rainfall. For the precipitation of wettest quarter of *Bul. africanus*, all models agree that suitable soil water environments are between 0.15 and 0.16. *Bulinus africanus* thrives in 0.25-0.45 for the coldest and warmest quarters.

There are different optimum models for *Bul. globosus* in BIO 4: Access1-0 shows (50-100), bcc-csm1-1m (150) and hadgem2cc (0). It is deduced that the species thrives at 50-150. Based on BIO 7, *Bul. globosus* prefers temperatures of between 10 and 15 °C. Based on all models, *Bul. globosus* thrives at temperatures of between 23 and 30 °C in BIO 8 (Table 7.2). According to BIO 15, the *Bul. globosus* prefers areas between 0.7 and 1.20 in South Africa. Climate models show a slight variation in BIO 16; access1-0 (0-100 mm), bcc-csm-1m (250-350 mm) and hadgem2cc (500 mm). As a result, *Bul. globosus* likely prefers rainy environments with over 500 mm. In the same way, climate models exhibit variation also in BIO 17, as follows: Access1-0 (0-20 mm), bcc-csm1-1m (50-100 mm) and hadgem2cc (25 mm). According to climate models, suitable areas for soil water during the warmest quarter (BIO 18) are between 0.25 and 0.35 for *Bul. globosus*.

Biomphalaria pfeifferi thrives in BIO 4 at -10 °C, but -50 °C is indicated by hadgem2cc. Based on the climate models, the optimum temperature for BIO 8 is 22 to 26 °C for *Biom. pfeifferi*. According to the models, there are three optimum rainfall environments for BIO 12: access1-0 (700-850 mm), bcc-csm1-1m (350-475 mm) and hadgem2cc (400 mm). The preferred environment for *Biom. pfeifferi* is similar in both bcc-csm1-1m and hadgem2cc. However, models show variation in terms of precipitation of wettest quarter, BIO 16, with access1-0 (600-850 mm), bcc-csm1-1m (350 mm), and hadgem2cc (400-500 mm). The optimum thresholds for *Biom. pfeifferi* in BIO 18 are similar for all models at between 200 and 450 mm (Table 7.2).

Table 7.1: Preferable environments for *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi* using different climate models for RCP 4.5 (periods 2040-2070 and 2070-2100).

Species			access1–0		bcc–csm1m–1		hadgem2cc	
Bulinus africanus		Period	2040–2070	2070–2100	2040–2070	2070–2100	2040–2070	2070–2100
	Bioclimatic variables	BIO 3 (°C)	55	84	51	50	75	65
		BIO 5 (°C)	20–25	25 and 32	20–27	20–25	20–25	26–27
		BIO 8 (°C)	25	28	25	26	26–30	27–30
		BIO 13 (mm)	100	5–100	0–80	180	50	70
		BIO 15 (coefficient of variation)	0.50	0–0.50	1.35	1.20	0.50	0.50
		BIO 16 (mm)	500	515–750	500–700	500–750	540–1000	500
		Soil water volume for the wettest quarter	0.30	0.50	0.20	0.20	0.00	0.30
		Soil water volume for the coldest quarter	0.40	0.40	0.35	0–0.1	0.30	0.30
		Soil water for the warmest quarter	0.40	0.30	0.30	0.30	0.35	0.25
Bulinus globosus		BIO 4 (standard deviation ×100)	100	100	80	75	55	–50
		BIO 7 (°C)	13–17	14–16	16–17	17	14–15	14
		BIO 8 (°C)	26	27	26	25	24–25	26–27
		BIO 15 (coefficient of variation)	1.00	0.80	1.35	1.2	0.80	1.25
		BIO 16 (mm)	260	600–1000	350–450	650	350	700–800
		BIO 17 (mm)	10	0	30	35	10	0
		Soil water for the warmest quarter	0.20	0.3	0.30	0.20	0.25	0.25

Table 7.1 continued

Species			access1–0		bcc–csm1m–1		hadgem2cc	
Biomphalaria pfeifferi		Period	2040–2070	2070–2100	2040–2070	2070–2100	2040–2070	2070–2100
	Bioclimatic variables	BIO 4 (standard deviation ×100)	120	–50	50	50	50	–75
		BIO 8 (°C)	24	22	24	25–27	23	25–26
		BIO 12 (mm)	750	520	450–500	550–700	600–750	600
		BIO 16 (mm)	350	470	350	350	350	500
		BIO 18 (mm)	250	350	250	180	250	350

Table 7.2: Preferable environments for *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi* using different climate models for RCP 8.5 (periods 2040-2070 and 2070-2100).

Species			access1-0		bcc-csm1-1		hadgem2cc	
Bulinus africanus		Period	2040-2070	2070-2100	2040-2070	2070-2100	2040-2070	2070-2100
	Bioclimatic variables	BIO 3 (°C)	80	80	60	75	75	73
		BIO 5 (°C)	22–26	22–26	20–26	25–28	20–27	24–27
		BIO 8 (°C)	27	28	10–16	25	15–17	28–30
		BIO 13 (mm)	180	250	180–200	200	180	250–300
		BIO 15 (coefficient of variation)	0.80	0–0.50	1.20	0.80	0.40	0.40
		BIO 16 (mm)	400	700	500–750	450	220	550–750
		Soil water volume for the wettest quarter	0.10	0.16	0.30	0.15	0.10	0.15
		Soil water volume for the coldest quarter	0.30	0.25	0.35	0–0.10	0.4	0.45
		Soil water for the warmest quarter	0.25	0.30	0.30	0.35	0.30	0.25
Bulinus globosus	Bioclimatic variables	BIO 4 (standard deviation ×100)	-50	50–100	200	150	-5	0
		BIO 7 (°C)	17–19	15	17–18	17–18	10–13	12
		BIO 8 (°C)	25–30	25–30	25	25	25 and 30	23 and 30
		BIO 15 (coefficient of variation)	1.00–120	1.00	0.90–1.00	0.7	1.50	1.20
		BIO 16 (mm)	600	0–100	250–400	250–350	0–250	500
		BIO 17 (mm)	25	0–20	50	50–100	0–100	25
		Soil water for the warmest quarter	0.10–0.20	0.3	0.25	0.35	0.4	0.25

Table 7.2 continued

Species			access1-0		bcc-csm1-1		hadgem2cc	
		Period	2040-2070	2070-2100	2040-2070	2070-2100	2040-2070	2070-2100
Biomphalaria pfeifferi	Bioclimatic variables	BIO 4 (standard deviation ×100)	-10	-10	100	-10	50	-50
		BIO 8 (°C)	28	22	26	25-27	23	25–26
		BIO 12 (mm)	500–750	700–850	700–800	350–475	500	400
		BIO 16 (mm)	400	600–850	400	350	350	400–500
		BIO 18 (mm)	200–350	200–350	300–450	350–450	250	400

7.3.3 Ecological modelling

The ecological models were found to be reasonably accurate at predicting the future distribution of intermediate hosts based on bioclimatic variables using climate models. According to the results, temperature and rainfall can influence the distribution of schistosomiasis in SA. Figures 7.1 to 7.3 illustrate the results for the three selected climate models and snail species. Grey areas show non-suitable habitat (0-0.33), green depicts regions that have low-suitability habitat (0.33-0.66), orange illustrates areas that have a moderate-suitability habitat (0.66-0.90), and red shows high-suitability habitat (0.90-1.00).

Access1-0

For the baseline period (1979-2018), the ecological models showed moderate-suitability habitat for *Bul. africanus* around the eastern part of Gauteng Province, parts of Mpumalanga and the North West Province (Figure 7.1 (a). *Bulinus africanus* was found to be widespread west of the Eastern Cape Province; this species can survive in areas with temperatures of between 15 and 30 °C and moderate rainfall, making it well-suited to its distribution range.

For the access1-0 climate model, RCP 4.5 and 8.5 (2040-2070), GLM, MaxEnt and RF showed that areas in the Eastern Cape could more likely become highly suitable (Figure 7.1 a), while GLM further showed that in RCP 8.5, newly suitable locations could be established around the Drakensberg and eastern Free State Province. Moderately suitable areas are declining, and more areas can be considered non-suitable habitats for *Bul. africanus*. From (Figure 7.1 b) it is evident that in RCP 8.5 (2070-2100), new highly suitable areas are established around the northeast coast of KwaZulu-Natal and the Eastern Cape. Notably, while new geographical locations are found, the overall contraction of the suitable regions is evident.

Bulinus globosus was highly distributed in the eastern and northeastern areas of SA, particularly in the Limpopo, Mpumalanga, Gauteng Provinces, and in the eastern regions of the North West Province. The distribution extends to the coastal areas in KwaZulu-Natal (Figure 7.1 b). For the access1-0 climate model, RCP 4.5 and 8.5 (2040-2070), GLM and RF show an increase in highly suitable habitats for *Bul. globosus* around the northeastern areas of Limpopo, Mpumalanga, and the northeastern regions of the North West Province (Figure 7.1 c). In RCP 8.5 (2040-2070), highly suitable areas are more defined in the eastern geographical regions of Limpopo; RF showed highly concentrated suitable locations around the North West Province. Overall, for RCP 4.5 and 8.5, there is a decrease in low-suitability areas that have become non-suitable for *Bul. globosus* (Figure 7.1 c), and Figure 7.1 d) shows a reduction in highly suitable areas and an increase in moderately suitable geographic locations. In GLM, highly suitable areas can be found over the eastern areas in the Limpopo

Province and on the north coast of KwaZulu-Natal. The ensemble models show a decline in high- and moderate-suitability habitats. Previously low-suitability areas are now non-suitable for *Bul. globosus*.

It was found that *Biom. pfeifferi* was more abundant and distributed spatially across SA than *Bul. africanus* and *Bul. globosus*. Hence, most *Biom. pfeifferi* observations were found across SA. The spatial distribution of *Biom. pfeifferi* is comparable to that of *Bul. globosus*, and *Biom. pfeifferi* can be seen distributed in the northern and coastal areas of KwaZulu-Natal and around the coast of the Eastern Cape (Figure 7.1 e). The distribution covers the majority of Gauteng, Limpopo, and Mpumalanga. For the access1-0 climate model, RCP 4.5 and 8.5 (2040-2070) GLM showed a decline in highly suitable habitats for *Biom. pfeifferi,* especially in the extreme eastern parts of Limpopo and Mpumalanga (Figure 7.1 e). The RF model showed slight variations between the different periods. However, highly suitable areas are similar to the baseline. In the baseline period, *Biom. pfeifferi* was found in parts of the Gauteng Province; by RCP 4.5 (2040 -2070), there was a contraction of highly suitable habitats. The new geographical areas extend from the KwaZulu-Natal north coast to the Eastern Cape coast. Overall, for RCP 4.5 (2040-2070), the ensemble model depicts a more moderately suitable habitat and less highly suited areas. Compared to RCP 4.5, RCP 8.5 showed more moderate-suitability areas and a decline in low suitability (Figure 7.1 e). In RCP 4.5 and 8.5 (2070-2100), GLM showed an increase in suitable areas for *Biom. pfeifferi* compared to RCP 4.5 (2040-2070), while MaxEnt showed a low suitability for most areas. The ensemble model showed a decline in moderate and highly suitable areas in South Africa (Figure 7.1 f).

Bcc-csm1-1m

According to the bcc-csm1-1m model, there is a slight variation in suitable habitats. However, the distribution showed a similar pattern to that given in access-1.0. For *Bul. africanus*, RCP 4.5 showed a decline in moderate suitability over the Northwest, Gauteng, and Eastern Cape Provinces. The GLM results showed new potentially suitable areas over the north coast of KwaZulu-Natal and Drakensberg. Overall, the ensemble model showed a decline and contraction of *Bul. africanus* in low- and moderate-suitability regions of South Africa (Figure 7.2 a). In RCP 8.5, GLM showed an increase in moderately suitable habitats for *Bul. africanus* extending from Gauteng to the northern parts of the Free State Province; similarly, newly suitable habitats can be expanded over the eastern coast. The ensemble model for RCP 8.5 showed similar patterns as in RCP4.5; Gauteng and parts of the North West Province depicted a decrease in low- and moderate-suitability habitats and an increase in non-suitable areas (Figure 7.2 b).

RCP 4.5 (2040-2070) showed an increase or expansion of suitable areas for *Bul. globosus* towards the eastern part of the country (Figure 7.2 c). The GLM results showed an increase

in moderately suitable areas towards the Free State Province and highly suitable locations along the east coast of South Africa. In the MaxEnt model, moderately suitable areas are concentrated over the northeastern and eastern parts of South Africa. Overall, there is a decline in low-suitability habitat in South Africa. The ensemble model shows that there will be an increase in moderately suitable areas, similar to that shown for RCP 8.5 in Figure 7.2 (c). In contrast to RCP 4.5 and 8.5 (2040-2070), RCP 4.5 and 8.5 (2070-2100) showed a decline in highly suitable areas in South Africa. According to the GLM and MaxEnt models, moderately suitable habitats for *Bul. globosus* are shown to be over the eastern regions of South Africa, such as Limpopo and Mpumalanga. Ensemble models showed moderate-suitability habitats over the North West and Gauteng Provinces (Figure 7.2 d).

In RCP 4.5, GLM and MaxEnt showed a decline in moderate-suitability habitats of *Biom. pfeifferi* over the Gauteng and North West Provinces. The GLM results showed an increase in suitable areas around the eastern parts of South Africa extending towards the north coast of KwaZulu-Natal. The ensemble model showed that modern, suitable regions would decline in South Africa, particularly in the Eastern Cape Province (Figure 7.2 e). Similar to RCP 4.5, GLM in RCP 8.5 also showed an increase in suitable areas around the north coast of KwaZulu-Natal and the eastern regions of Mpumalanga. Moreover, in the MaxEnt model, low-suitability areas are increasing and there is a decline in moderate-suitability areas. The ensemble model shows a widespread increase in moderate-suitability habitats across the eastern Provinces of South Africa, such as KwaZulu-Natal, and in the eastern regions in the North West and Gauteng Provinces, extending towards the eastern part of Mpumalanga Province (Figure 7.2 e).

According to GLM in RCP 4.5 (2070-2100), there is an expansion in highly suitable coastal areas and a decline of highly suitable areas over the eastern Mpumalanga and Limpopo Provinces (Figure 7.2 f). GLM also showed an increase in moderately suitable habitats of *Biom. pfeifferi* across north-eastern areas of the North West Province. The MaxEnt model showed an increase in moderately suitable areas in parts of the Mpumalanga and Limpopo Provinces. The ensemble model depicted a decrease in suitable habitats around the coast and an increase in moderate-suitability habitats in the eastern parts of South Africa, and an increase in low-suitability habitats in South Africa. In RCP 8.5, GLM showed newly suitable areas in the eastern part of South Africa along the borders of Limpopo and Mpumalanga. The MaxEnt model showed a decrease in suitable habitats and an increase in low-suitability areas. The ensemble model showed an increase in low-suitability habitat and a decrease in moderate-suitability habitats, with areas along the east coast of South Africa being high-suitability habitats for *Biom. pfeifferi* (Figure 7.2 f).

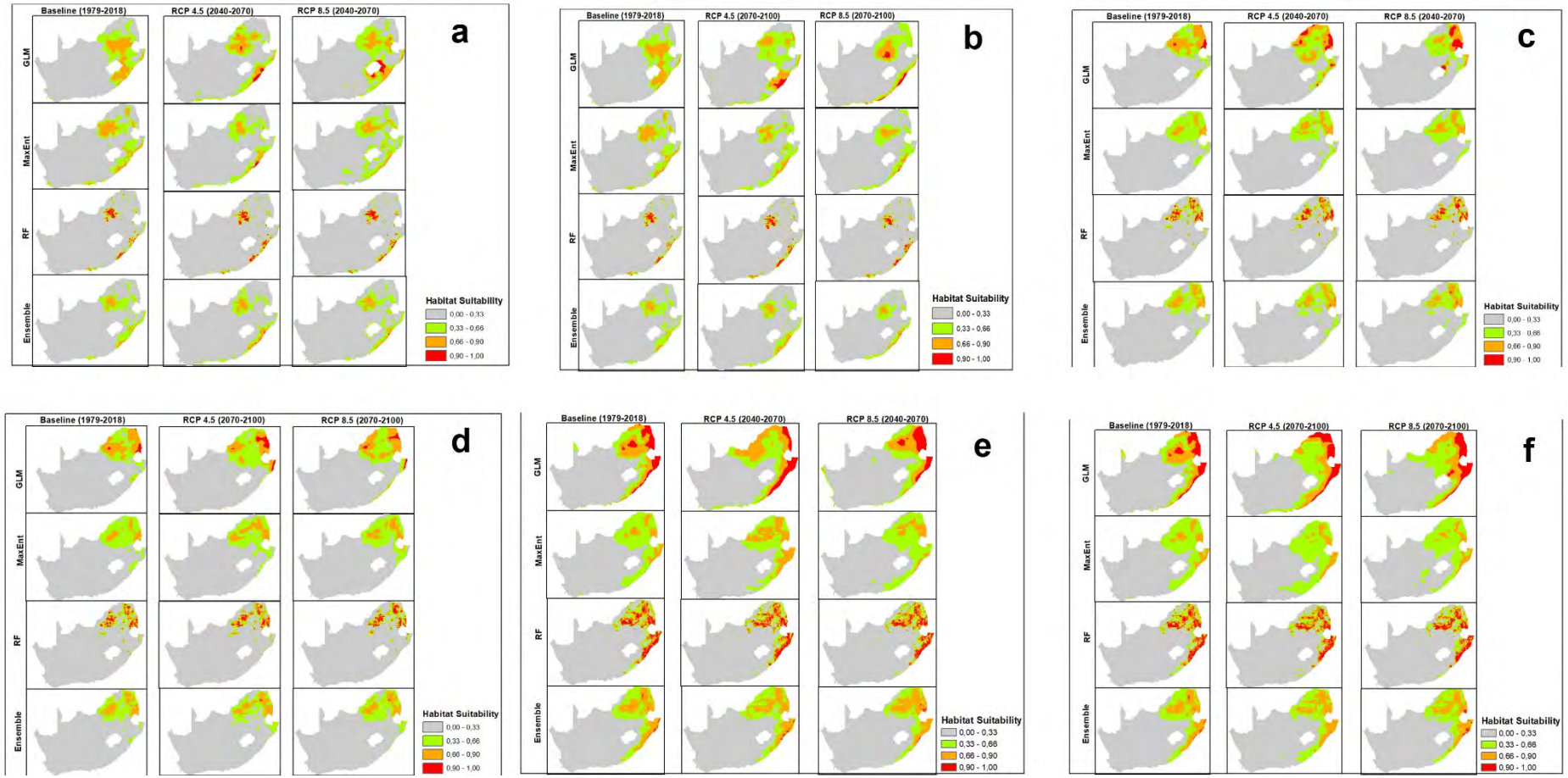

Figure 7.1: Ecological models using access1-0 for the three time periods: a-b) *Bulinus africanus*; c-d) *Bulinus globosus*; and e-f) *Biomphalaria pfeifferi.*

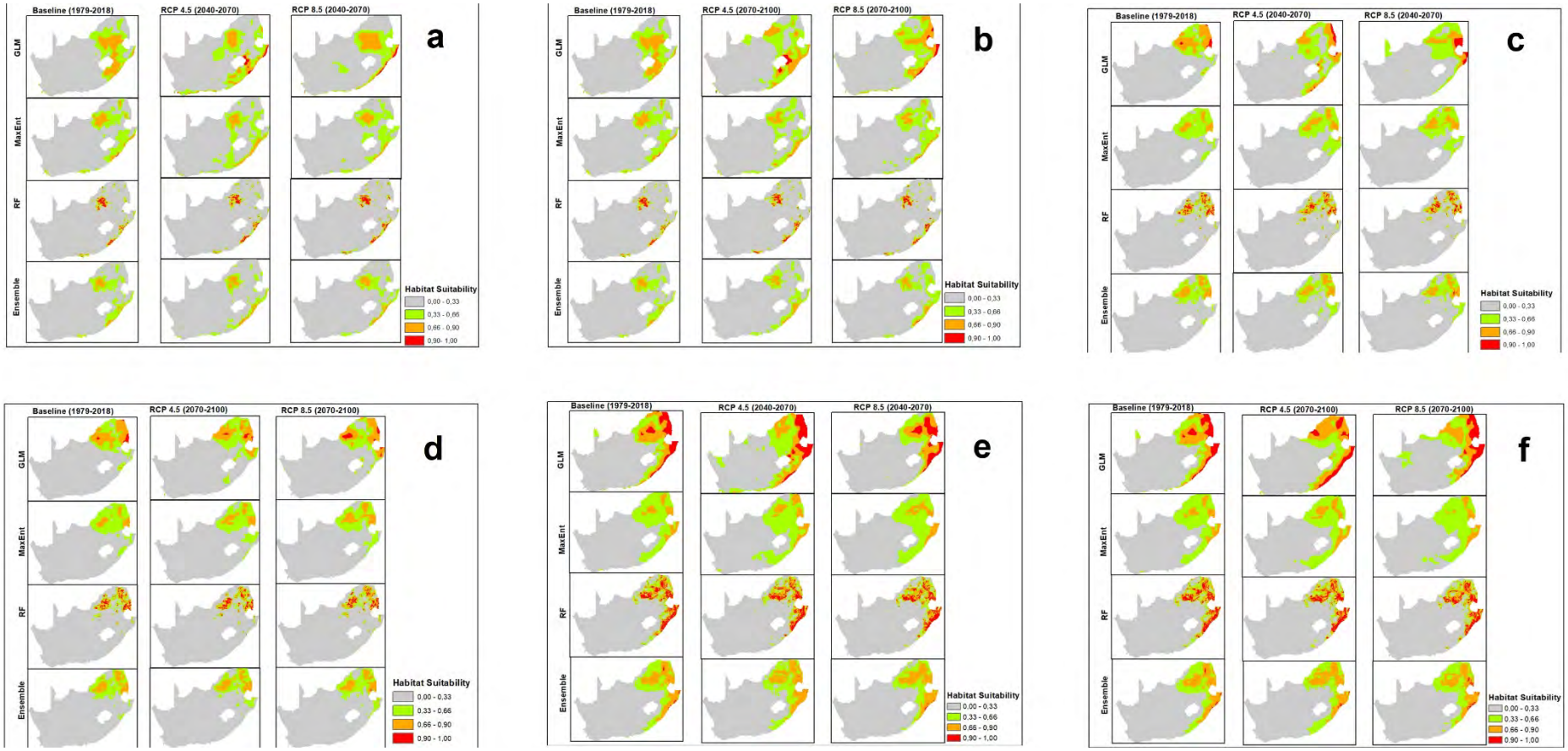

Figure 7.2: Ecological models using bcc-csm1-1m for the three time periods: a-b) *Bulinus africanus*; c-d) *Bulinus globosus;* and e-f) *Biomphalaria pfeifferi.*

Hadgem2cc

Based on the hadgem2cc model, there is a slight variation in suitable habitats. However, the distribution showed a similar pattern to those indicated by access-1.0 and bcc-csm1-1m. For *Bul. africanus*, RCP 4.5 (2040-2070) there is a contraction in moderate-suitability areas, with most of the suitable habitats distributed in the Gauteng Province and along the coastal regions of the Eastern Cape Province, while low-suitability habitats increased in the KwaZulu-Natal Province (Figure 7.3 a). In RCP 8.5 (2040-2070), similar distribution patterns for *Bul. africanus* were observed. moderate-suitability habitats declined over the Gauteng Province and increased over the coastal areas of KwaZulu-Natal. There was an increase in non-suitable habitats for *Bul. africanus* and a decrease in low-suitability habitat areas. Similar distribution patterns are observed in RCP 4.5 and 8.5 (2070-2100); there is a decline in habitat suitability in South Africa, with more concentrated moderate-suitability habitats in the Gauteng Province and high suitability along the Eastern Cape coast. There is a decline in low-suitability areas and an increase in non-suitable habitats of *Bul. africanus* in South Africa (Figure 7.3 b).

The *Bul. globosus* distribution patterns were found to be similar to those of the baseline period (1979-2018). Under RCP4.5 (2040-2070) suitable habitats are indicated along the border of Mpumalanga and Swaziland (Figure 7.3 c). There is an increase in the distribution of moderate-suitability habitats along the northeastern areas of Mpumalanga and Limpopo and parts of the North West Province. In RCP 4.5 and 8.5 (2070-2100), there is an increase in moderate-suitability habitats in the northeastern part of South Africa. A decline in low-suitability habitats and an increase in non-suitable areas for *Bul. globosus* were observed. (Figure 7.3 d).

For *Biom. pfeifferi*, highly suitable areas contracted along the eastern areas of South Africa, especially in KwaZulu-Natal. Widespread moderate-suitability habitats are indicated for *Biom. pfeifferi* along the northern areas of KwaZulu-Natal, northeastern areas of Limpopo and Mpumalanga, and eastern parts of the North West and Gauteng (Figure 7.3 e). There is an increase in low-suitability habitats for *Biom. pfeifferi* in the western areas of the Eastern Cape Province and a decrease in low-suitability areas around the Gauteng Province. In RCP 4.5 and 8.5 (2070-2100), a decline in high-suitability areas is shown for *Biom. pfeifferi* (Figure 7.3 f). As shown for RCP 4.5 and 8.5 (2040-2070), most moderate-suitability habitats for *Biom. pfeifferi* are widely distributed towards the northeastern and eastern regions of the country.

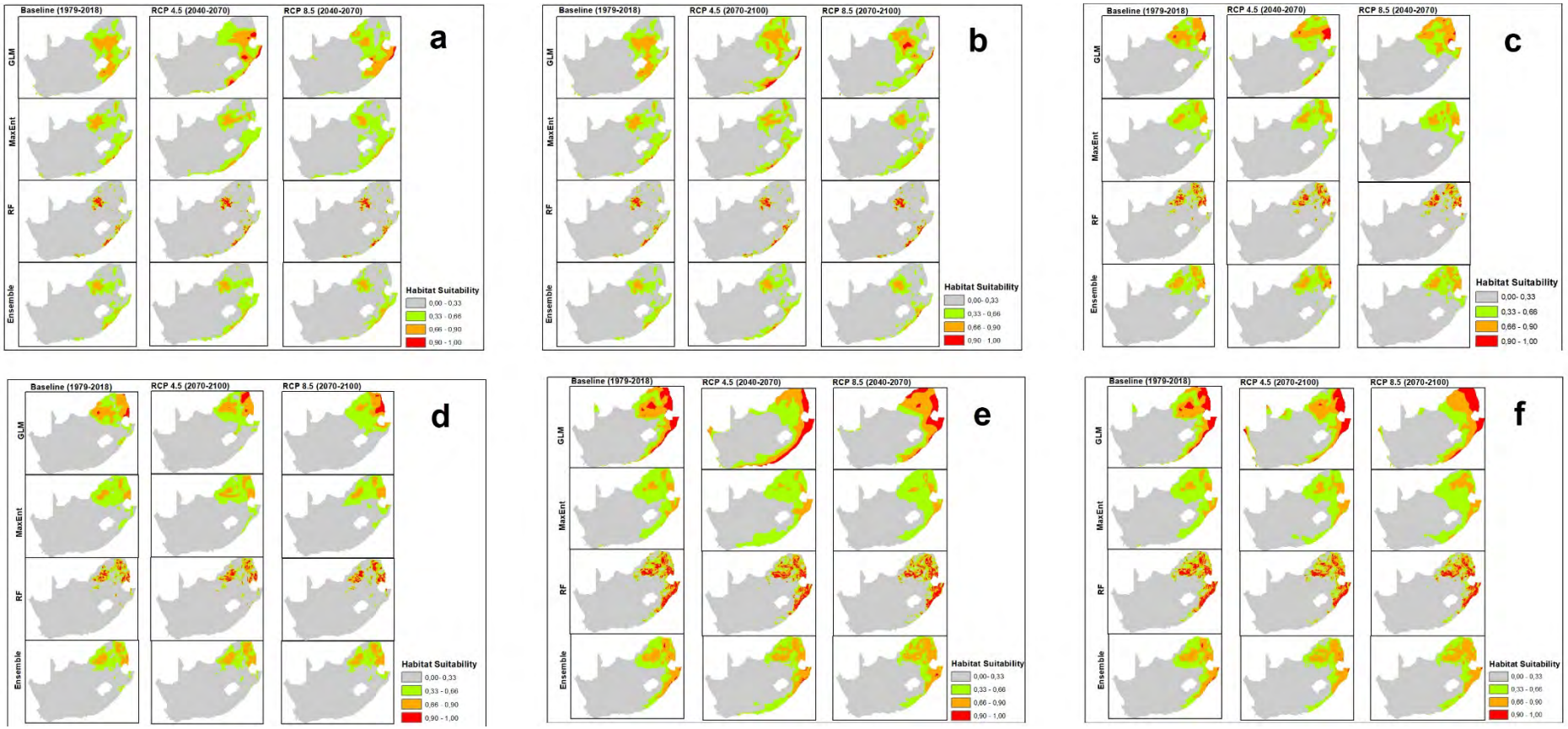

Figure 7.3: Ecological models using hadgem2-cc for the three time periods:e a-b) *Bulinus africanus*; c-d) *Bulinus globosus;* and e-f) *Biomphalaria pfeifferi.*

7.3.3 Change in suitability

Figures 7.4-7.6 present the effects of different climate scenarios on the suitability of three species: Bio*mphalaria pfeifferi*, *Bulinus globosus*, and *Bulinus africanus*. Suitability refers to the suitability of climate conditions for the survival and growth of these species. Figures 7.4-7.6 also showcase the impact of two emission scenarios, RCP 4.5 and RCP 8.5, over two time periods, 2040-2070 and 2070-2100. The suitability changes are represented as decline, no change, and increase.

For *Bul. africanus*, the access1-0 climate model for RCP 4.5 (2040-2070), eshows a 12.72% decline in suitability, a 74.89% chance of no change, and a 12.38% increase in suitability. Gains can be seen in *Bul. africanus* moving west of South Africa and over the central Free State. Parts of KwaZulu-Natal and the northern regions in the Eastern Cape can expect a decline in the distribution of *Bul. africanus* (Figure 7.4). Under RCP 4.5 (2070-2100), there is an 8.97% decline in suitability, a 76.05% chance of no change, and a 14.97% increase in suitability. Increases in habitat suitability of *Bul. africanus* are seen towards the west of South Africa and east of Mpumalanga. A contraction in the decline of distribution is indicated towards the northeastern parts of Limpopo and KwaZulu-Natal. Under RCP 8.5 (2040-2070), there is a 15.38% decline in suitability, a 64.49% chance of no change, and a 20.11% increase in suitability. Increases in suitability can be seen towards the inland of the Eastern Cape. Under RCP 8.5 (2070-2100), there is a 13.11% decline in suitability, a 72.69% chance of no change, and a 14.19% increase in suitability (Figure 7.4). A contraction in the increase in suitability is indicated towards the coastal areas of the Eastern Cape as well as the Gauteng and Free State Provinces. The decline in suitability is shown in parts of Mpumalanga and KwaZulu-Natal.

For *Bul. globosus*, the access1-0 climate model for RCP 4.5 (2040-2070), showed a 7.91% decline in suitability, a 77.16% chance of no change, and a 14.91% increase in suitability. An increase in suitability can be seen towards the inland of the Eastern Cape and parts of the Free State. The decline in suitability is observed in the eastern areas of South Africa. Under RCP 4.5 (2070-2100) a 6.01% decline in suitability is indicated, with a 92.6% chance of no change, and a minor increase in suitability (1.4%). Under RCP 8.5 (2040-2070), there is a 7.2% decline in suitability, an 80.9% chance of no change, and an 11.88% increase in suitability. The decrease in the suitability of *Bul. globosus* is seen in the extreme northeastern areas of Limpopo, KwaZulu-Natal coastal areas, and parts of Gauteng, North West, and eastern areas of the Free State. Under RCP 8.5 (2070-2100), there is a 10.71% decline in suitability, an 81.16% chance of no change, and an 8.13% increase in suitability. A sharp decrease in *Bul. globosus* species is indicated in eastern areas of South Africa (Figure 7.4).

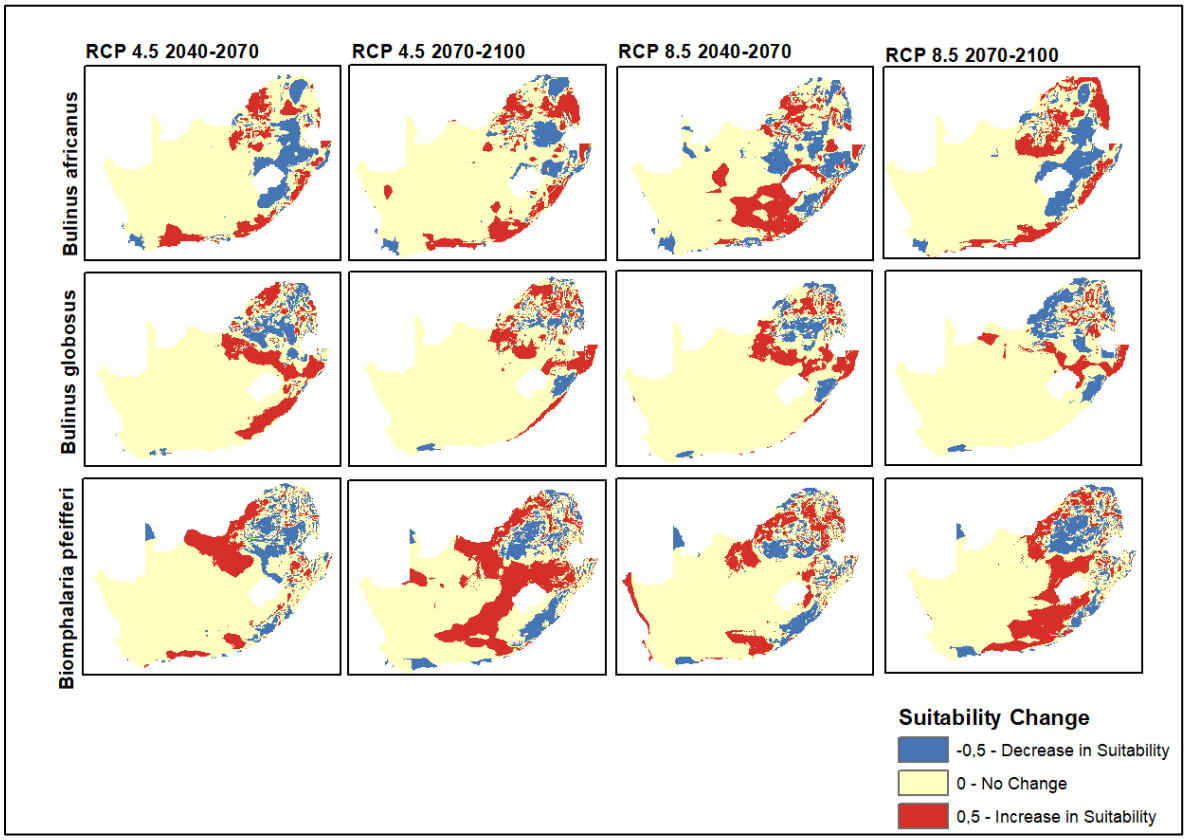

Figure 7.4: Change in suitability for *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi* using the access1-0 climate model for RCP 4.5 and 8.5.

For *Biomphalaria pfeifferi*, the access1-0 climate model for RCP 4.5 (2040-2070), showed an 11.5% decline in suitability, a 74.6% chance of no change, and a 13.9% increase in suitability. A significant decrease in suitability in the eastern areas of South Africa and in parts of the Eastern Cape is indicated. An increase in suitability is projected to shift to the west and south of the North West Province and towards the coastal areas of the Western Cape of South Africa. Under RCP 4.5 (2070-2100) an 11.4% decline in suitability was indicated, and a 60.45% chance of no change, and a significant 28.06% increase in suitability. The gains can be seen in the North West, Free State, parts of the Northern Cape and Eastern Cape. The access1-0 climate model for RCP 8.5 (2040-2070) showed a 10.95% decline in suitability, a 73.3% chance of no change, and a 15.71% increase in suitability (Figure 7.4). Under RCP 8.5 (2070-2100), a 12.01% decline in suitability is indicated, with a 65.39% chance of no change, and a 22.59% increase in suitability. Increases in suitability are seen towards the inner areas of the Eastern Cape, and parts of the North West and Free State Provinces. The decline in suitability areas includes the coast of the Eastern Cape, Gauteng, and parts of Limpopo.

For the bcc-csm1-1m model, *Bul. africanus*, RCP 4.5 (2040-2070), there is a 7.84% decline in suitability, a 72.1% chance of no change, and a substantial 20.1% increase in suitability (Figure 7.5). The increases are projected to shift to the west of South Africa, including parts of the Free State, Eastern Cape Province, and the southwest coast. The decline in suitability can be seen in Limpopo, Gauteng, Mpumalanga, and parts of the KwaZulu-Natal Province. Under RCP 4.5 (2070-2100) an 8.8% decline in suitability is indicated, with a 72% chance of no change, and a 19.2% increase in suitability. Under RCP 8.5 (2040-2070), a 7.8% decline in suitability is projected, with a 72.1% chance of no change, and a substantial 20.1% increase in suitability. There is a decline in the inland of the Eastern Cape and in the eastern parts of Limpopo Province. Under RCP 8.5 (2070-2100), there is a significant decline (23%) in suitability, a 64% chance of no change, and a 13% increase in suitability. Most of the areas that experience a decrease in suitability are the Free State and parts of the North West and Gauteng Provinces, respectively (Figure 7.5).

For the bcc-csm1-1m model, *Bul. globosus*, RCP 4.5 (2040-2070), an 11.9% decline in suitability is projected, an 86.6% chance of no change, and a minor 1.5% increase in suitability. Under RCP 4.5 (2070-2100), there is a 9.21% decline in suitability, an 80.89% chance of no change, and a 9.9% increase in suitability. Increases in suitability can be seen in the KwaZulu-Natal Province and in the southern parts of the Eastern Cape (Figure 7.5). Under RCP 8.5 (2040-2070), there is a 10.31% decline in suitability, a 75.5% chance of no change, and a 14.18% increase in suitability. The increase in suitability extends from the southern Free State to the northern areas of KwaZulu-Natal and the southwestern coastal areas of the Eastern Cape Province. Under RCP 8.5 (2070-2100), there is an 8.75% decline in suitability, an 82.77% chance of no change, and an 8.48% increase in suitability.

For *Biom. pfeifferi*, the bcc-csm1-1m model for RCP 4.5 (2040-2070) indicates that there is an 11.2% decline in suitability, a 65.54% chance of no change, and a significant increase (23.3%) in suitability. Increased suitability is shown to shift westwards in South Africa (Figure 7.5). The decline in habitat areas is focused on Gauteng, northern parts of Limpopo, eastern regions of the North West Province and the east coast. Under RCP 4.5 (2070-2100), there is a 12.29% decline in suitability, a 75.98% chance of no change, and a modest 11.73% increase in suitability. Under RCP 8.5 (2040-2070), there is a 10.25% decline in suitability, a 76.19% chance of no change, and a 13.56% increase in suitability. Under RCP 8.5 (2070-2100), there is a 10.46% decline in suitability, a 65.88% chance of no change, and a significant 23.66% increase in suitability. An increase in suitable areas is shown in the northern parts and Eastern Cape Provinces in South Africa. Parts of the northern North West and KwaZulu-Natal Provinces are also ideal for *Biom. pfeifferi*.

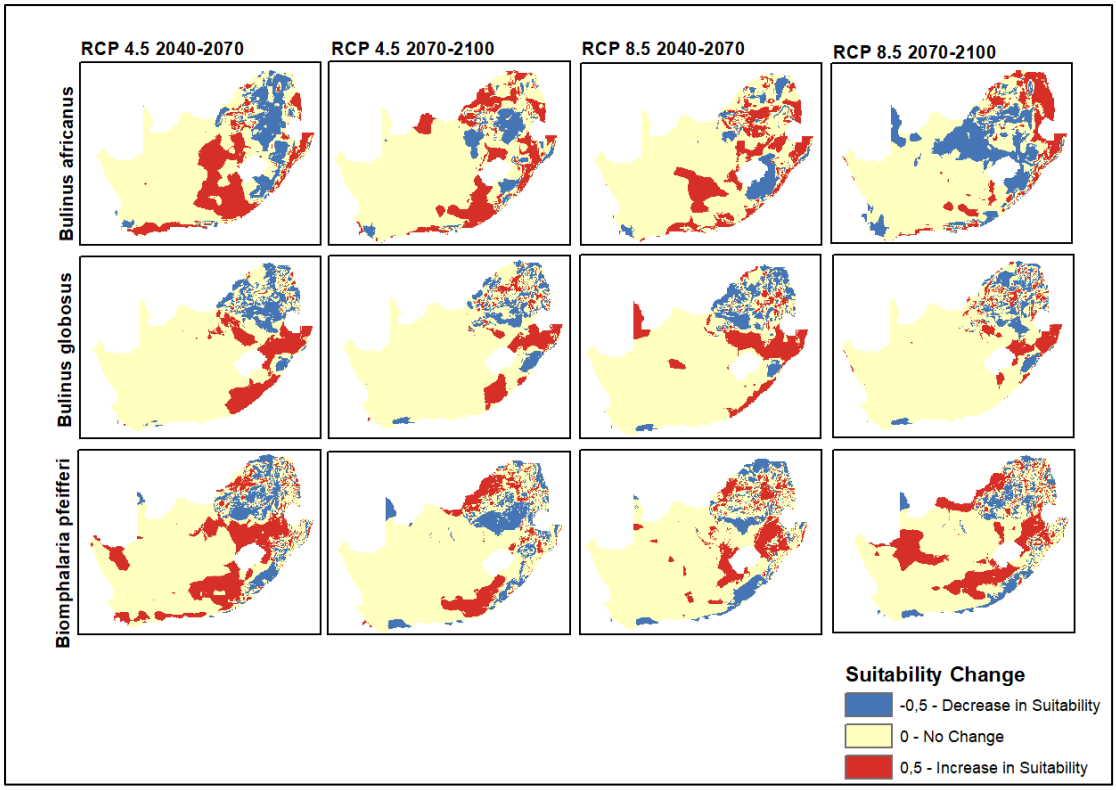

Figure 7.5: Change in suitability for *Bulinus africanus, Bulinus globosus,* and *Biomphalaria pfeifferi* using the bcc-csm1-1m climate model for RCP 4.5 and 8.5.

For *Bul. africanus*, the hadgem2cc model for RCP 4.5 (2040-2070) indicates that there is a 12.86% decline in suitability, a 71.57% chance of no change, and a 15.55% increase in suitability (Figure 7.6). Under RCP 4.5 (2070-2100), there is a substantial decline (44.57%) in suitability, a 45.37% chance of no change, and a 10.06% increase in suitability. Areas that exhibit a decrease in suitability are in the Eastern Cape, and parts of the North West Province. Under RCP 8.5 (2040-2070), there is a 17.37% decline in suitability, a 71.31% chance of no change, and an 11.32% increase in suitability. The decrease in suitability is projected across the eastern parts of South Africa, such as eastern areas of Mpumalanga, Gauteng, and areas in the North West Province. Increases in suitability are seen in northwestern areas of Limpopo and along the north coast in KwaZulu-Natal Province. Under RCP 8.5 (2070-2100), there is a 13.01% decline in suitability, a 73.35% chance of no change, and a 13.5% increase in suitability. There is a significant increase in suitability towards the west of the Eastern Cape Province and eastern areas of the North West Province and parts of Gauteng (Figure 7.6).

For *Bul. globosus*, the hadgem2cc model for RCP 4.5 (2040-2070) indicates an 8.16% decline in suitability, with an 80.51% chance of no change, and an 11.33% increase in suitability. An increase in suitable areas is found in the Eastern Cape, parts of the Drakensberg, and northern areas of KwaZulu-Natal. The decline can be seen in parts of the North West Province, eastern regions of Limpopo and Mpumalanga. Under RCP 4.5 (2070-2100), there is an 8.6% decline

in suitability, an 82.3% chance of no change, and a 9.14% increase in suitability. Most of the increase in suitability is found in northeastern areas of South Africa (Figure 7.6). Under RCP 8.5 (2040-2070), there is a 6.9% decline in suitability, an 81.3% chance of no change, and an 11.8% increase in suitability. Under RCP 8.5 (2070-2100), there is an 8.5% decline in suitability, a 78.46% chance of no change, and a 13.03% increase in suitability. Suitable areas expand from the eastern Free State into the northern and northeastern regions of KwaZulu-Natal.

For *Biom. pfeifferi*, the hadgem2cc model for RCP 4.5 (2040-2070) showed a 13.06% decline in suitability, a 75.04% chance of no change, and a 12% increase in suitability. Areas that exhibited a decrease are concentrated over Gauteng and North West Provinces. Other decline regions include parts of Limpopo, Mpumalanag and Eastern Cape (Figure 7.6). Under RCP 4.5 (2070-2100), there is a 9.2% decline in suitability, a 74.8% chance of no change, and a 16.01% increase in suitability. Under RCP 8.5 (2040-2070) a 10.06% decline in suitability is indicated, with a 78.24% chance of no change, and an 11.71% increase in suitability. Under RCP 8.5 (2070-2100), there is an 11.08% decline in suitability, a 72.3% chance of no change, and a 16.62% increase in suitability.

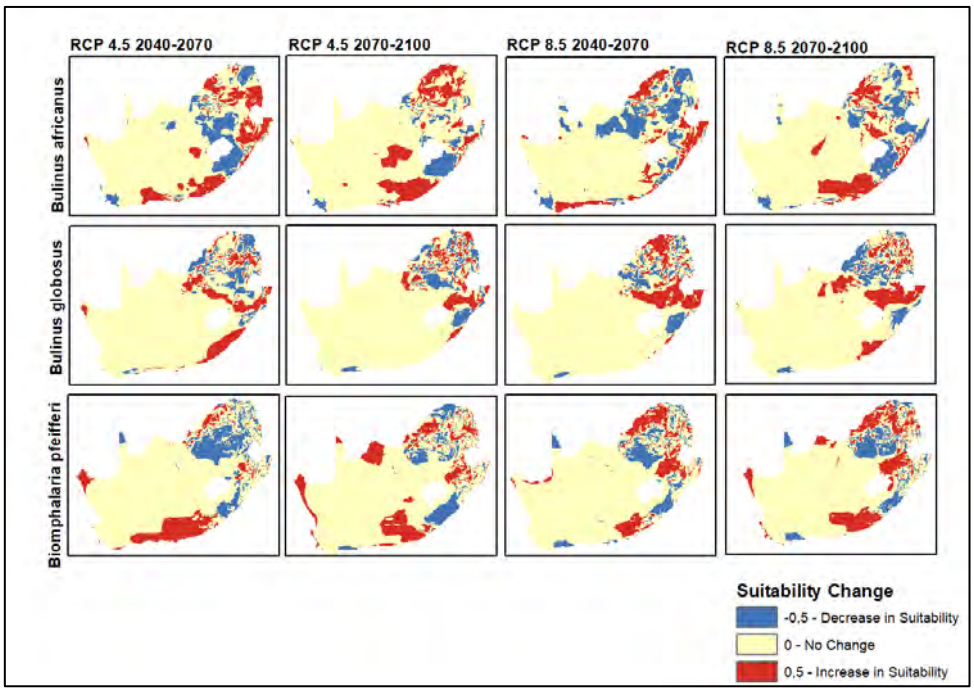

Figure 7.6: Change in suitability for *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi* using the hadgem2cc climate model for RCP 4.5 and 8.5.

7.4 Discussion

7.4.1 Suitable habitats for Bulinus africanus*,* Bulinus globosus *and* Biomphalaria pfeifferi

Bulinus africanus showed preferred habitats in BIO 5 and 8, reaching optimal temperatures of 20 to 30 °C. The results indicated that *Bul. africanus* survived in temperatures as high as 30 °C, higher than in its baseline range. In contrast, (Appleton, 1978) concluded that cooler temperatures contributed the most to suitable habitats for *Bul. africanus*. De Kock and Van Eeden (1986) found that the optimal temperature for *Bul. africanus* reproduction was between 23 °C and 26 °C. It must be noted that historically*, Bul. africanus* preferred cooler temperatures. However, this is further supported by the fact that the optimal temperature for reproduction of *Bul. africanus* is still within the cool range, indicating it is best equipped to reproduce in cooler temperatures. *Bulinus africanus* responds poorly to increased rainfall in the wettest month. They thrive in environments with less than 180 mm of rain. In contrast to precipitation in the wet quarter, *Bul. africanus* prefers a habitat that receives 500 to 750 mm of rainfall. A study (De Kock et al. 2004) concluded that most *Bul. africanus* species occur in regions with 300 mm - 700 mm/a of precipitation. This is likely because too much rain in the wettest quarter can cause flooding and create unsuitable conditions for the species. A study (Adekiya et al. 2020) found that rainfall patterns are associated with the prevalence of schistosomiasis. Moderate precipitation allows for the transportation of snails and supports the creation of new habitats and temporary snail habitats (Freitas-Galvão et al. 2020). *Bulinus africanus* thrives under conditions when the soil has more water in the wettest quarter. The optimal conditions for *Bul. africanus* are when there is enough rainfall throughout the wet quarter to keep the soil moist but not to the extent that flooding occurs. Under these conditions, the species can benefit while avoiding the negative impacts of flooding, such as desiccation. This indicates that *Bul. africanus* thrive in both cold and warm climates. Thus, *Bul. africanus* suitability depends are primarily on precipitation, temperature, and seasonal variations, rather than annual averages. This is because the environment in SA varies significantly from season to season, and the parasites that cause schistosomiasis require specific conditions to survive and thrive (Moodley 2003; Manyangadze et al. 2021).

Typically, *Bul. globosus* thrives at an annual temperature range of between 13 and 17 °C, and the mean temperature of the wettest quarter is between 25 and 27 °C. This is because *Bul. globosus* prefers warm to hot temperatures for survival. A study conducted by Joubert et al. (1984) concluded that *Bul. globosus* is more resistant to high temperatures than *Bul. africanus*. There has been an increase in temperatures in SA, especially in tropical areas like Limpopo and Mpumalanga. Consequently, it could lead to an increase in the abundance of *Bul. globosus* species in the future. Under RCP 4.5 (2040-2070), *Bul. globosus* thrives in the precipitation of the wettest quarter at (260 -350 mm). However, it is more tolerant to higher

rainfall between 2070 and 2100 (600 to 1 000 mm). This is because the species has adapted to the changing climate and can survive in more extreme weather conditions. In terms of precipitation of the wettest quarter (BIO 16), heavy rainfall can cause floods, resulting in snail species migrating to new habitats. Therefore, potential habitats are created, allowing schistosomiasis-transmitting snails to migrate actively to nearby favourable habitats (Guo et al. 2021). *Bulinus globosus* can also survive less rainfall in the driest quarter (winter). A study by Pennance et al. (2016) found that schistosomiasis-transmitting snails are more abundant during post-rainy seasons and more likely to transmit the disease. *Bulinus globosus* responds well to soil water in the warmest quarter at 0.2 to 0.30 m^3. This is because *Bul. globosus* is adapted to flourish in low rainfall conditions, meaning it can thrive in drier environments with less water. However, there is more rainfall in the warmer quarters of the year, and the soil water content is higher. This allows *Bul. globosus* to thrive and reproduce.

Biomphalaria pfeifferi thrives well in the mean temperature of the wettest quarter (summer), with an optimal temperature of 22 to 27 °C for 2040-2070 and 2070-2100. This is likely because this snail species is well adapted to tropical climates, and the warmer summer months provide the ideal conditions for its growth and development. In SA, *Biom. pfeifferi* is marginally suited to the environment because of variations in night and day temperatures and high daytime temperatures (30 °C). There was a notable difference in temperature tolerance for *Biom. pfeifferi* in comparison to *Bul. globosus*. It is pertinent to note that despite being widely distributed throughout the country, *Biom. pfeifferi* snails are not tolerant of high temperatures (Brown 1994). Furthermore, a study by Maes et al. (2021) showed that the mortality of these snails was high at 32 °C. Other studies (McCreesh et al. 2014; 2015) have shown that seasons are integral to schistosomiasis distribution and transmission. *Biomphalaria pfeifferi* requires annual rainfall of between 450 to 750 mm. This snail species thrives in warm, wet climates and can reproduce rapidly. As a result, an increase in populations when the conditions are favourable leads to a rise in the spread of the disease. *Biomphalaria. pfeifferi* prefers precipitation of the wettest quarter to be 350 to 470 mm; they respond well to low-moderate rainfall in summer. A study in Ethiopia (Xue et al. 2022) showed that moderate rainfall is instrumental in increasing schistosomiasis by accumulating sufficient surface water in ponds. During heavy rainfall events, water levels rise, causing water turbulence. As a result, flow rates may increase, disrupting snail habitats and reducing cercariae survival (Xue et al. 2022). In terms of precipitation of the warmest quarter, *Biom. pfeifferi* prefers moderate rainfall levels, less than 350 mm. Manyangadze et al. (2016a) studied schistosomiasis in the Ndumo area of the uMkhanyakude district and found that *Biom. pfeifferi* were suitable in the cold and dry seasons (winter) following the rainy season.

RCP 8.5 (2040-2100)

In both periods of RCP 8.5, it was illustrated that *Bul. africanus* responded to the maximum temperature of the warmest month ranging from 20 to 27 °C. For *Bulinus africanus,* the preferred mean temperature of the wettest quarter for the 2040-2070 period is 10 to 20 °C, and increases from 25 to 28 °C in the 2070-2100 period. Furthermore, the preferred temperature range of 10-20 °C indicates that the species is adapted to cooler climates and may not survive in hot environments. In contrast, under RCP 4.5 *Bul. africanus* preferred warmer temperatures. *Bulinus africanus* thrives at precipitation ranging from 180 to 300 mm. This shows that *Bul. africanus* do not prefer moderate to high rainfall environments. During heavy rainfall events, water levels rise, causing water turbulence. As a result, flow rates may increase, disrupting snail habitats and reducing cercariae survival (Xue et al. 2022). In terms of precipitation of wettest quarter (BIO 16)*, Bul. africanus* favours 400 to 750 mm, with low responses to high soil water volume in the wettest quarter. This suggests that the species thrives in areas with moderate moisture and cannot survive in areas with too much or too little precipitation.

Bulinus globosus prefers environments with an annual temperature range of 12 to 19 °C and an increase in temperature is projected from 2040-2070. This indicates that the species is adapted to survive in cooler climates, but as temperatures rise, they will benefit from the warmer environment rather than be negatively impacted by it. This could be seen as a form of evolution as the species adapts to its changing environment. An average of 23 to 30 °C in the wettest quarter suits *Bul. globosus*. This shows that *Bul. globosus* thrives during summer in South Africa. This is because this snail species is native to South Africa's tropical areas, where temperatures are warmest in summer. Joubert et al. (1984) showed that *Bul. globosus* can withstand higher temperatures (34 to 40 °C). Shiff et al. (1975) concluded that *Bul. globosus* could survive in thermally harsh environments as their numbers are relatively high in increasing temperatures. Additionally, the varying rainfall supports snail species, as they require moisture to survive and thrive. The optimal rainfall for *Bul. globosus* is between 250 and 400 mm in the wettest quarter and 50 mm in the driest quarter.

Due to its wide spatial distribution, *Biom. pfeifferi* has varying optimum temperatures and rainfall. *Biomphalaria pfeifferi* prefers two temperature ranges, 22-23 °C and 25-28 °C, respectively. Pedersen et al. (2017) found a positive correlation between temperature and the abundance of *Biom. pfeifferi* in Zimbabwe. This means that as temperatures increase, so does the species' abundance. They concluded that if temperatures continue to rise due to climate change, there could be a shift in the distribution of this snail species. Similarly, in terms of annual rainfall, *Biom. pfeifferi* prefers two rainfall estimates, 350 to 500 mm and 500 to 850 mm. *Biomphalaria pfeifferi* thrives in rainfall environments of precipitation of the wettest quarter

(BIO 16) at 350 to 500 mm and 600 to 850 mm. The species can tolerate various temperatures and rainfall due to its broad geographical range. For example, this snail species can survive in areas with lower temperatures, but its growth rate and reproductive success will be reduced. Similarly, this snail will survive in areas with higher rainfall, but its growth rate and reproductive success will increase. Unlike *Bulinus africanus* and *Bulinus globosus, Biomphalaria pfeifferi* prefers low to moderate rainfall 200 to 400 mm for precipitation of the driest quarter (BIO 17). This snail species prefers habitats with low to moderate rainfall because too much rain can cause flooding and disrupt their habitats. After a period of rain, *Biom. pfeifferi* are found in low-lying areas not prone to flooding.

7.4.2 The future distribution of Schistosoma*-transmitting snails in South Africa*

Bulinus africanus, *Bulinus globosus* and *Biomphalaria pfeifferi* have different habitats but coexist at certain locations. Similarly, Pedersen et al. (2017) found that species have different foci but overlap at most locations. Changes in spatial distribution over time were apparent, with a trend towards more unsuitable habitats, but pockets of suitable habitats were also found in areas previously unsuitable (Pedersen et al. 2014b).

During the baseline period (1979-2018), the ecological models demonstrated that moderate-suitability habitat existed for *Bul. africanus* in the eastern part of the Gauteng Province, portions of Mpumalanga, and the North West Province. The species thrived in areas with temperatures ranging from 15 °C to 30 °C and moderate rainfall, which aligned well with its distribution range. The study focused on examining the habitat suitability for *Bul. africanus* under various future climate scenarios (RCP 4.5 and RCP 8.5) from 2040 to 2100. The findings revealed that elevated suitability for *Bul. africanus* was probable in the Eastern Cape region under both scenarios. This was attributed to the emergence of new habitat areas around the Drakensberg and in the eastern parts of the Free State Province for RCP 8.5. In the later timeframe (2070-2100), RCP 8.5 indicated the emergence of highly favourable regions along the northeast coast of KwaZulu-Natal and the Eastern Cape. In contrast, areas with moderate suitability were diminishing, while non-suitable habitats were expanding. The study employed an ensemble model to uncover the declining and contracting nature of *Bul. africanus* in regions with low- and moderate-suitability habitats. These distribution patterns remained consistent across different timeframes within each scenario. They displayed a decrease in low-suitability habitats and an increase in non-suitable habitats for *Bul. africanus* in South Africa. Similarly, under RCP 8.5, moderately suitable habitats extended from Gauteng to the northern parts of the Free State Province. This was coupled with the potential for creating new habitats along the eastern coastal areas. The consistent distribution patterns for both RCP scenarios indicated that shifts in suitable habitats and non-suitable areas were ongoing. This highlighted the evolving suitability levels in specific Provinces such as Gauteng and regions like the

Eastern Cape. This finding underscored the dynamic response of *Bul. africanus* to changing climate conditions in South Africa.

In specific regions of South Africa, *Bulinus globosus* was distributed across various regions under the different climate scenarios. Under the baseline scenario, the species was primarily found in the country's east and northeast. This included Limpopo, Mpumalanga, Gauteng, and the eastern parts of the North West Province. It extended to coastal KwaZulu-Natal. Emission scenarios RCP 4.5 and RCP 8.5 (2040-2070) showed an increase in favourable *Bul. globosus* habitats in these regions, particularly in northeastern Limpopo, Mpumalanga, and the northeastern areas of North West Province. Eastern areas of Limpopo emerged as a significant hub of suitability under RCP 8.5. Overall trends indicated a reduction in less suitable areas and an increase in moderate-suitability habitats. However, under the RCP 4.5 and RCP 8.5 scenarios (2070-2100), the prevalence of highly suitable regions declined. Throughout these simulated situations, the models consistently emphasised the suitability of the eastern parts of South Africa, like Limpopo and Mpumalanga. The study also noted suitable habitat patterns near the border of Mpumalanga and Swaziland. The ensemble models illustrated moderate habitat suitability in the North West and Gauteng Provinces. Similar to the baseline, *Bul. globosus* distribution patterns remained unchanged in these scenarios. This highlights the intricate interplay between climate and habitat suitability over time. The results showed that the spatial distribution of *Bul. globosus* is influenced by environmental conditions, with alterations impacting the species' distribution. Additionally, the results suggested that the species displayed resilience to environmental changes over time.

The baseline scenario showed that *Biom. pfeifferi* exhibited a higher abundance and broader spatial distribution across South Africa than the other two species. It was prevalent in various Provinces, including Gauteng, Limpopo, and Mpumalanga. The distribution of *Biom. pfeifferi* mirrored that of *Bul. globosus* in certain aspects, particularly within KwaZulu-Natal and along the Eastern Cape coast. The results showed the impact of climate change, using different climate models and scenarios (RCP4.5 and RCP8.5) for various periods (2040-2070 and 2070-2100). These models indicated potential contractions in highly suitable habitats, especially in the eastern regions like Limpopo and Mpumalanga. While the results varied slightly between models and scenarios, a general trend of a decline in highly suitable areas and a shift in distributions was observed under RCP 4.5 (2040-2070). Notably, the territories were revealed to range from the KwaZulu-Natal north coast to the Eastern Cape coast, indicating a shift in distribution. In South Africa, for example, *Biom. pfeifferi* is likely to expand its geographical distribution with global warming; however, this expansion will occur only if the absence of snails and disease transmission reflects unfavourable climatic conditions rather than a lack of otherwise suitable freshwater habitats (Stensgaard et al. 2013). This is because

the species can tolerate a wide range of temperatures; therefore, if the current lack of snails and disease transmission is due to the climate, the species should be able to expand its range as the climate warms. However, if the lack of suitable freshwater habitats is the limiting factor, the species may be unable to expand its range. Overall, the ensemble model suggested a transition to moderately suitable habitats and a decrease in well-suited regions, particularly in RCP 8.5 rather than RCP 4.5. In the subsequent timeframes (2070-2100) under RCP 4.5 and RCP 8.5, the models indicated an expansion of suitable areas for *Biom. pfeifferi* compared to the earlier 2040-2070 scenario under RCP 4.5. However, the ensemble model showed an opposing trend of decreasing moderate-suitability and increasing low-suitability habitat. This contrasted with the eastern coastal regions demonstrating high suitability with fewer extreme temperatures. This enabled *Biom. pfeifferi* to thrive in those regions. This indicates that the species can adapt to different climates, making it more resilient to changing environmental conditions.

7.4.3 Change in habitat suitability

The analysis of habitat suitability for *Bul. africanus* across different climate scenarios reveals a consistent pattern of gains, losses, and no change in suitability. Under RCP 4.5 (2040-2070), suitability increases are notable, mainly seen in the western regions of South Africa and the central Free State. Even though ecological models predict suitable precipitation and temperatures for the Western Cape, no disease occurs under these conditions. Appleton and Stiles (1976) concluded that the natural acidity of water bodies such as rivers and dams in this Province could negatively affect intermediate hosts. However, this is counterbalanced by declines, evident in KwaZulu-Natal and the northern parts of the Eastern Cape. Similarly, under RCP 4.5 (2070-2100), while there are increased suitability areas towards the west of South Africa and east of Mpumalanga, losses are observed in the northeastern parts of Limpopo and KwaZulu-Natal, with a substantial chance of no change. Under RCP 8.5, gains are evident in the inland areas of the Eastern Cape, while losses appear in areas such as Mpumalanga and KwaZulu-Natal. Throughout these scenarios and models, the balance between gains, losses, and no change underscores the dynamic interplay of climate impacts on the habitat suitability for *Bul. africanus*. This poses challenges and opportunities for distribution and survival. For example, some scenarios may show areas more suitable for the snail due to increased precipitation, while others may become less suitable due to rising temperatures. This means that some areas may become more favourable for the snail while others may become less favourable.

For *Bulinus globosus,* suitability across various scenarios and timeframes shows fluctuations. Under RCP 4.5 (2040-2070), suitable areas are projected to increase in the Eastern Cape, Drakensberg, and northern KwaZulu-Natal. The decline can be seen in parts of the North West

Province, eastern regions of Limpopo and Mpumalanga. Scenario RCP 4.5 (2070-2100) indicates a reduction in suitable areas in the extreme northeast of Limpopo, the coastal areas of KwaZulu-Natal, and parts of Gauteng, the North West, and the eastern Free State. However, under RCP 8.5 (2070-2100), suitable locations will expand from the eastern Free State into northern and northeastern regions of KwaZulu-Natal.

The habitat suitability for *Biomphalaria pfeifferi* is examined across different Representative Concentration Pathway (RCP) scenarios and timeframes. Under RCP 4.5 (2040-2070), suitability decreases in eastern areas of South Africa and in the Eastern Cape. Stensgaard et al. (2013) found that warmer climates will minimise the distribution range for *Biom. pfeifferi.* However, it increases to the west and south of the North West Province and along the coastal areas of the Western Cape. Under RCP 4.5 (2070-2100), gains are seen in the North West, Free State, parts of the Northern Cape, and the Eastern Cape. This could be due to the ideal temperature and rainfall conditions for intermediate hosts in this region. However, no snail vectors were found or sampled in the Northern Cape. Moodley (2003) concluded that no disease occurred in the Northern Cape, but the temperature in the Province was suitable for schistosomiasis.

Similar patterns are observed under RCP 8.5 scenarios for 2040-2070 and 2070-2100. Gains in suitability occur in the inland areas of the Eastern Cape, the North West, and the Free State. In contrast, declines are noted along the Eastern Cape coast, Gauteng, and Limpopo. A study by Blum and Hotez (2018) suggests that climate change could lead to changes in schistosomiasis transmission due to alterations in temperature and precipitation. This could support the growth of *Biom. pfeifferi*. African regions such as West Africa and Central Africa are prone to contraction, while suitability in southern Africa tends to expand, highlighting the importance of disease shifts with climate changes. Our findings suggest a shift in habitat suitability patterns for *Biom. pfeifferi* in various regions of South Africa based on climate scenarios. This indicates that the climate scenarios in RCP 8.5 will cause the geographical distribution of *Biom. pfeifferi* to shift. This will result in some areas becoming more suitable for the species while others become less suitable. A study by McCreesh et al. (2015) suggested that all else being equal, *S. mansoni* infection risk may increase across much of eastern Africa as temperatures increase over the next few years.

It is difficult to say whether our climate models are conservative, or whether these additional effects might amplify the extent of changes predicted by the bioclimatic variables alone. However, this chapter provided novel insights into the predicted snail habitat suitability and the spatial distribution of snails in South Africa using climate models with machine learning as the preferred approach. This study is also subject to limitations. The predicted geographical distribution may be wider than the actual range of *Bulinus africanus, Bulinus globosus and*

Biomphalaria pfeifferi. due to the fact that this study did not consider the impact of human activities such as snail control (Stensgaard et al. 2013). Another limitation is niche conservatism, where it is assumed that the adaptability of *Bulinus africanus*, *Bulinus globosus* and *Biomphalaria pfeifferi* will not change with environmental variations.

7.5 Conclusion

Three climate models were used to predict schistosomiasis-transmitting snail distribution in South Africa. Among the models, slight variations in predicting future habitats and the distribution of snails were observed. The slight variations in the models were attributed to different models, RCP scenarios and periods. Despite these variations, the models still showed similar patterns of snail distribution under different climate scenarios. The slight differences in the models allowed for more precise predictions about geographical areas where snails may or may not survive due to climate change. As a result, climate models have common patterns in their predictions of newly established zones and contraction in other areas of endemicity. The results showed that the distribution patterns of *Bulinus africanus*, *Bulinus globosus*, and *Biomphalaria pfeifferi* are not significantly different from the baseline. There will likely be schistosomiasis-transmitting snails in geographical areas such as Limpopo, Mpumalanga, KwaZulu-Natal, North West, Gauteng and Eastern Cape. However, there is a shift predicted in species distribution into new regions or provinces that were previously unsuitable, and the once desirable areas will become unsuitable. The Free State and Western Cape are two geographical regions where schistosomiasis-transmitting snails are predicted to be found.

CHAPTER 8: COMMUNITY KNOWLEDGE, ATTITUDES, PERCEPTIONS AND PRACTICES RELATING TO SCHISTOSOMIASIS AND ENVIRONMENTAL CHANGE IN HANESENGANI, LIMPOPO PROVINCE

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8.1 Introduction

Schistosomiasis is an endemic parasitic disease in South Africa that has significant implications for public health and socioeconomic development (Mbereko et al. 2020). The annual incidence of the disease in the country is estimated at 4.5 million cases, with another 25.7 million people at risk of infection (Hambury 2021). The disease is caused by a parasite that inhabits freshwater snails and penetrates the human skin upon contact with infested water. Human exposure to contaminated water occurs during various activities, such as washing clothes, bathing, work-related contact, or recreational activities (Banhela et al. 2017). However, prevalence is not only affected by environmental factors. The high prevalence of schistosomiasis in many affected communities is aggravated by insufficient knowledge and awareness of transmission and treatment, which increases susceptibility to infection (Mbereko et al. 2020). Most schistosomiasis education programmes in sub-Saharan Africa target school going children because they are more accessible (Sacolo et al. 2018). However, the drawback is that older individuals, including caregivers of school going children, are excluded from these programmes. Studies centred on a holistic community understanding are inclusive of most community members, and this is likely to help produce results and recommendations that can help the wider community, including caregivers who may unintentionally expose children entrusted in their care to schistosomiasis (Macharia et al. 2016).

The transmission of schistosomiasis is also influenced by factors that are beyond the control of the communities, such as low socioeconomic status, lack of safe drinking water, poor environmental sanitation, and climate change/variability that affects water availability and quality (De Leo et al. 2020). These factors, in combination with a lack of knowledge and inappropriate attitudes and practices, can further enhance the infection rates in these communities. The success of community-centred control measures depends largely on community acceptance and should be developed based on their socio-economic conditions and cultural realities (Sady et al. 2015; Mwai et al. 2016; Person et al. 2016; Sacolo-Gwebu et al. 2019). While this is the case, only a few studies have been conducted to gain an understanding of community experiences in the Vhembe District Municipality of the Limpopo Province, an area known for the high prevalence of schistosomiasis (Dickens et al. 2020). The

aim of this study was to fill the gap by investigating the sociodemographic and cultural factors that influence schistosomiasis transmission in the HaNesengani community. These insights can be useful for the successful development of community-centred control measures.

The aim of this study was to assess the community knowledge, attitudes, perceptions, and practices regarding schistosomiasis in HaNesengani, Limpopo Province, and to investigate whether they associated environmental conditions with the occurrence of schistosomiasis.

The specific objectives of the study were to 1) assess the HaNesengani community's level of knowledge on the transmission, cause and symptoms of schistosomiasis; 2) determine the attitudes and perceptions of the HaNesengani community members towards the treatment and prevention of schistosomiasis; 3) identify the practices that expose the HaNesengani community to schistosomiasis transmission; and 4) determine the views of the HaNesengani community members on the relationship between environmental conditions and the occurrence of schistosomiasis.

8.2 Methods

8.2.1 Research design, study site and data collection

A sequential explanatory research approach was adopted to assess schistosomiasis knowledge, attitudes, perceptions and practices and community views on the relationship between environmental changes and the occurrence of schistosomiasis. To start with, quantitative data were collected using a structured closed-ended household questionnaire. Following the analyses of the quantitative data, an in-depth interview guide was developed to explore the results obtained from the household questionnaires in more depth. Structured closed-ended questionnaires were analysed using descriptive and non-parametric inferential statistics whilst in-depth interviews were analysed manually and added as a narrative analysis to enrich the discussion of the results of the household questionnaire. A description of the study site is provided in Chapter 2 (section 2.2.2) and a more detailed discussion of the research design, sampling methods and data collection instruments employed in the study can be found in Chapter 2 (section 2.3.9).

8.2.2. Data analysis

A detailed description of data analysis procedures can be found in Chapter 2 (section 2.3.9) of this report.

8.3 Results and discussion

The following section will integrate the findings from the quantitative and qualitative components of the study. Discussing results from the different data sources in one section is common in mixed-methods research. This approach can enhance the validity of the study through data triangulation by comparing and contrasting the results from different methods or data sources to examine the consistency or discrepancy of the findings (O'Cathain et al. 2010).

8.3.1 Demographic and socio-economic profile of respondents

The household questionnaire was administered to 342 community members, consisting of 60% females, 39% males, and 1% who preferred not to disclose their gender. The majority fell within the 35–64 year age group (45%). A significant proportion had attained a secondary school education (59%), while 4% had not received any formal schooling. The majority of respondents were unemployed (49%), while those employed were predominantly engaged in agricultural activities (27%) such as farming and livestock herding for both domestic and commercial purposes (see Table 8.1).

The in-depth interviews primarily involved females (67%) and individuals aged 35 to 64 years (73%), with secondary or tertiary education levels (40% each). Farming constituted the predominant occupation (33%), followed by healthcare roles (13%), that included registered nurses, community health workers, and traditional healers.

Table 8.1: Demographic and socio-economic characteristics of study participants

Variable	Category	Number of respondents (n)	Percentage (%)
Age	18 - 34 years	138	40
	35 - 64 years	153	45
	65 years or more	51	15
Gender	Female	205	60
	Male	134	39
	Prefer not to say	3	1
Level of education	Primary school	35	10
	Secondary school	201	59
	Tertiary qualification	83	24
	Postgraduate qualification	10	3
	None	13	4
Employment status	Working full-time (over 30 hours per week)	39	11
	Working part-time (8 to 29 hours per week)	42	12
	Contract worker	24	7
	Unemployed	166	49
	Student	47	14
	Retired	24	7
Occupation description	Agricultural sector	92	27
	Office and administrative support	36	11
	Student	29	8
	Social grant	16	5
	Self employed	4	1
	Construction building and maintenance	36	11
	Retail and hospitality	19	6
	Unemployed	98	29
	Domestic worker	6	2
	Health care provider	6	2

8.3.2 Access to water and sanitation infrastructure

Inadequate sanitation and a lack of access to clean, safe sources of water are two risk factors associated with contracting schistosomiasis (Kabuyaya et al. 2018). According to the study's findings, the majority of respondents obtained their drinking water from piped systems, which

included communal taps (40%), yard pipes (21%), borehole sources (15%), and in-home piped systems (11%). This pattern was similar in water access for household needs, where respondents predominantly used piped water from communal taps (38%), yard pipes (21%), and boreholes (18%). Similarly, for gardening and crop irrigation, piped water from communal taps (37%), yard pipes (20%), and boreholes (18%) were the primary sources. A small fraction of respondents mentioned using harvested rainwater (2%) or greywater (0.3%) for gardening and crop production.

While the household questionnaires revealed a preference for using piped water sources (communal taps, yard pipes, or boreholes) for drinking, domestic use, and irrigation, the in-depth interviews uncovered that community members often resorted to bathing or doing laundry in the river. This was due to frequent water supply interruptions rendering communal pipes unreliable. This does not only increase the risk of infection, but also puts the community at risk of crocodile attacks. According to a male resident, *"last year it swallowed a woman who was doing laundry, and while the community was out there in a search party for her, it swallowed another man."* This is also a concern in other areas in the region, as other incidents of crocodile attacks during water collection, bathing, or laundry were reported near the Nandoni Dam on the Luvuvhu River (Sadike 2022).

The majority of respondents (87%) utilised pit latrines, while 11% used flush toilets, and 1% had no toilet facilities. However, improved water and sanitation infrastructure does not necessarily translate to its consistent use or reduced infection rates (Exum et al. 2019). Two interviewees admitted to urinating or defecating in the river during laundry activities, highlighting that access to home toilets might not deter open defecation and urination during water supply shortages in HaNesengani, as people do not want to leave their clothes unguarded. Access to a safe, reliable water supply could reduce risk of infection and other environmental health hazards in the community.

8.3.3 Community knowledge on the cause, transmission and symptoms of schistosomiasis

As indicated in Figure 8.1 below, the local name for the disease, *mutambotambo*, was known to 93% of the 342 participants. Familiarity with the common name, bilharzia, was reported by 70% of respondents, while only 29% were familiar with the scientific term, schistosomiasis. Those who were aware of *mutambotambo* named schools as their primary source of information (41%). These findings concur with those of Sady et al. (2015), who identified schools as pivotal in disseminating information within affected communities.

Interview responses confirmed the findings of the questionnaire. Interviewees confirmed that most adults in the community were knowledgeable about *mutambotambo* due to past school

awareness programmes. They were of the opinion, however, that many children were unaware of schistosomiasis, as these programmes were no longer integrated into local schools. All those interviewed unanimously agreed that schools were the most effective platforms for children to learn about the disease. They noted that children often disregard parental instructions at home, highlighting the importance of imparting information within a school environment. Efforts should be made to reintroduce awareness campaigns in local schools, and to include local terminology in any awareness materials.

Participants that had no knowledge of the disease were excluded from subsequent questionnaire sections.

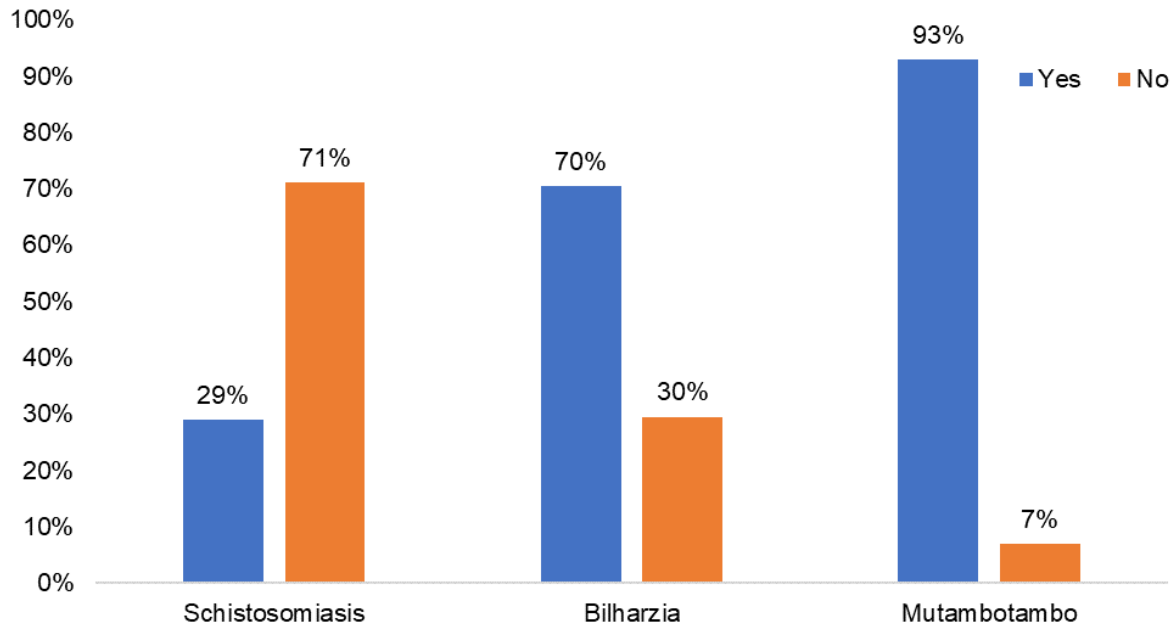

Figure 8.1: Respondent awareness of different terms for schistosomiasis (n = 342)

Knowledge of the cause of schistosomiasis

The 318 respondents who had knowledge of the disease were asked further questions aimed at gaining an understanding of their knowledge concerning the causes of the disease, transmission, and symptoms. This question employed a multiple-choice format that allowed respondents to select multiple applicable options. The majority (73%) of those aware of *mutambotambo* correctly attributed its origin to infected snails in freshwater sources. However, a significant number, 84%, also inaccurately believed the disease to be caused by a virus present in the water, while 83% also associated it with waterborne bacteria (refer to Figure 8.2).

Further probing during the in-depth interviews revealed that everyone understood contact with river water to be the cause of infection. As with the household questionnaire, there was a

misconception regarding the cause of infection. Respondents specifically held the belief that schistosomiasis develops due to the presence of discarded nappies and feminine products in the river, creating *"dirt that cannot be seen with the naked eye*". Only one farmer interviewed accurately associated the disease with the presence of snails in the river.

While it is encouraging that most participants understood contact with water to be the mode of transmission, it is evident that there is a lack of understanding of the disease vector. Awareness campaigns should include information about the life cycle of schistosomiasis, to empower community members to protect themselves against infection.

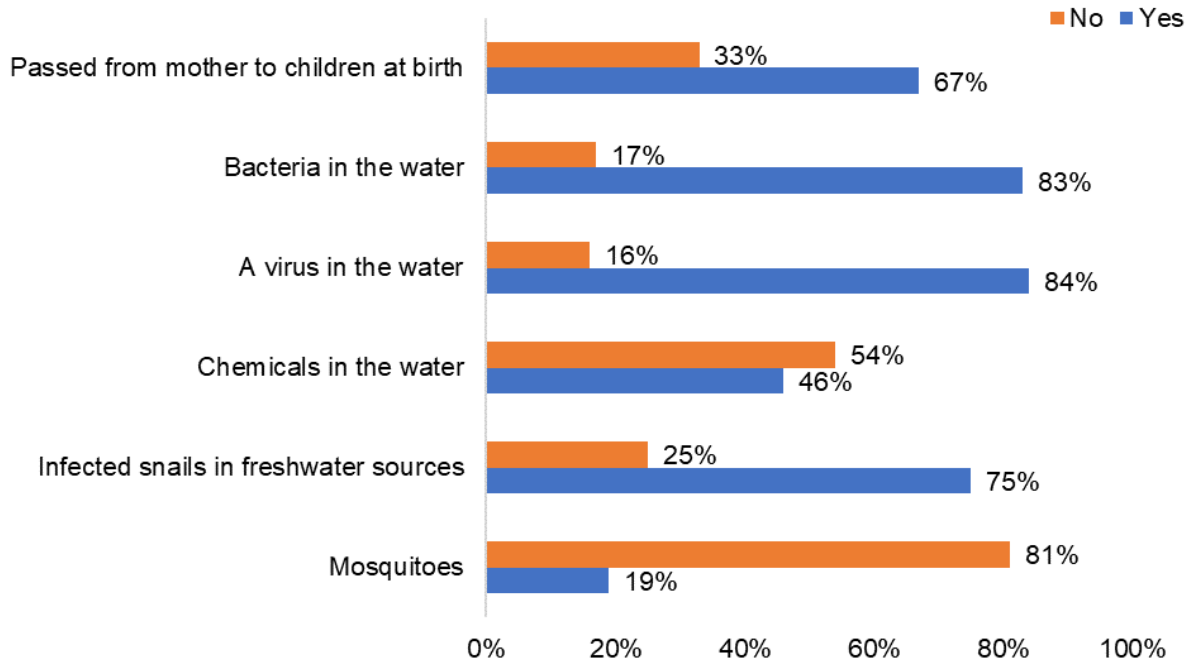

Figure 8.2: Community knowledge on the cause of schistosomiasis (n = 318).

Although not provided as an option in the questionnaire, many respondents indicated a belief that excessive consumption of salt, particularly coarse salt, could lead to *"getting mutambotambo".* This was mentioned frequently enough to warrant further exploration during the subsequent in-depth interviews.

Interviewees explained that the overconsumption of salt prompts kidney swelling and contraction during urination, which, in turn, causes the discharge of blood in the urine—a manifestation akin to the symptoms of schistosomiasis. Other in-depth interviewees recounted that during their younger years, elders would caution them that overindulgence in salt could result in contracting bilharzia. However, a male traditional healer expressed that he had never treated anyone infected due to salt consumption. He said that the notion of salt-induced bilharzia infection might be an unsubstantiated "old wives' tale," employed to discourage

excessive salt intake. Interestingly, this belief that excessive salt consumption contributes to schistosomiasis has surfaced in studies conducted in North Senegal and the Philippines as well (Frigerio et al. 2016; Lorenzo et al. 2019). Hence, this misperception is not confined solely to this particular community.

Knowledge about the mode of transmission of schistosomiasis

Schistosomiasis distinguishes itself from most waterborne diseases by infecting an individual through skin contact with infested freshwater, as opposed to contamination through water ingestion (Secor 2014). Regarding disease transmission, participants familiar with *mutambotambo* had reasonable knowledge levels. They identified the following transmission routes: contact with water containing human faeces (89%), swimming or wading in water inhabited by snails (84%) and using river water for washing or bathing (83%) (refer to Figure 8.3). Participants also incorrectly associated transmission with drinking unfiltered river water (86%) and consuming water shared with cattle (72%).

In-depth interviews revealed that interviewees understood infection to occur through skin exposure to contaminated water, but also believed transmission could occur by swallowing water while swimming in pools tainted by urine. This misconception reveals a lack of understanding of the schistosomiasis life cycle, as the parasite requires an intermediate snail host for its development (Colley et al. 2014). This finding once again emphasises the need for educational campaigns focusing on accurate information about the disease life cycle and routes of transmission.

The majority of questionnaire respondents (76%) recognised that unprotected sex would not lead to infection. However, further probing during interviews uncovered varied perceptions about the sexual transmission of schistosomiasis. For example, one traditional healer interviewed said the disease was not contagious and could not be transmitted sexually, while another believed the disease was contagious like herpes and could be transmitted sexually.

Other interviewees held the belief that transmission occurs through genital contact during swimming, with symptoms manifesting in the genitals.

This association with genital contact and sexual transmission is not isolated to this community. Participants in a similar study in Mozambique believed sexual conduct to be a route of transmission (Rassi et al. 2019). Another study by Lothe et al. (2018) found similar misconceptions in a South African community. Rassi et al. (2019) concluded that the misconception that schistosomiasis is a sexually transmitted disease is understandable, as most urogenital schistosomiasis symptoms affect the reproductive organs. These misconceptions are often responsible for reluctance to seek treatment, and awareness campaigns should highlight that transmission does not occur through sexual contact.

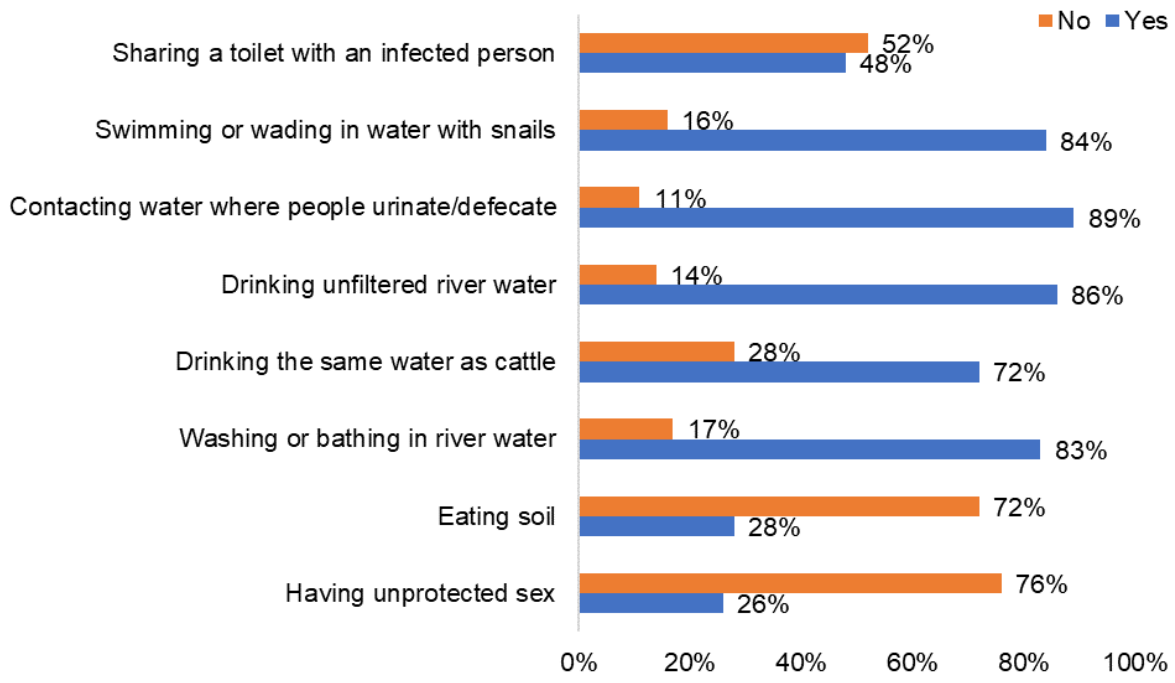

Figure 8.3: Community knowledge about the mode of transmission for schistosomiasis (n = 318).

Knowledge about the symptoms of schistosomiasis

Questionnaire respondents demonstrated a high level of knowledge regarding the signs and symptoms of schistosomiasis. They accurately identified the most prevalent symptoms as blood in the urine (97%) and pain during urination (85%) (see Figure 8.4). These findings were in line with those from prior research studies conducted in South Africa (Ndumo) and Zimbabwe (Ntale), where 82.5% and 77.0% of respondents, respectively, were aware of schistosomiasis symptoms, particularly blood in the urine (Mbereko et al. 2020). Respondents also correctly identified additional disease symptoms: abdominal pain and swelling (72%), blood in stool/faeces (73%), and fatigue or body aches (67%).

In-depth interviewees also identified many symptoms correctly, describing symptoms such as blood in urine, a burning sensation while urinating, passing small amounts of urine, headaches, pneumonia, and abdominal pain. These results show much higher levels of knowledge in the community when compared with a study in Kenya where participants lacked accurate knowledge of the disease's symptoms, even citing cracked foot soles as a symptom (Odhiambo et al. 2014).

Beyond the common symptoms acknowledged by the majority of respondents, healthcare providers (registered nurses and traditional healers) further elaborated on other symptoms. A nurse noted that the disease could manifest as painless gross haematuria (urinating blood

without discomfort), while a traditional healer incorrectly suggested that *mutambotambo* encompasses any form of abnormal bleeding, including nosebleeds in children and menstrual blood occurring thrice a month (adding up to 15 days if her typical cycle is five days).

Schistosoma haematobium infection can lead to female genital schistosomiasis (FGS), potentially resulting in uterine enlargement, menstrual irregularities, and infertility in women (Nour 2010); this could possibly be the reason for the traditional healer's perception. Our findings show that healthcare providers in the formal sector have adequate knowledge about the symptoms of the disease to diagnose and assist community members. Traditional healers, however, may not always understand the routes of transmission or diagnose the disease accurately. This points to a need to include traditional healers in awareness campaigns, and to increase collaboration between formal and traditional healthcare sectors for the effective management of neglected tropical diseases like schistosomiasis.

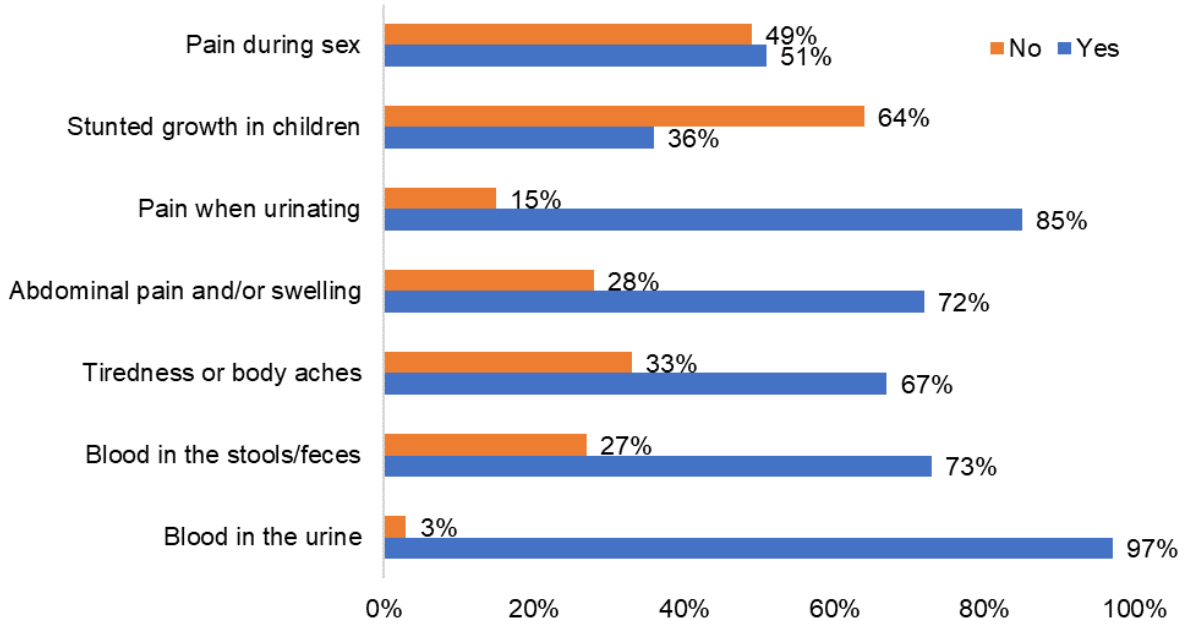

Figure 8.4: Community knowledge of the symptoms of schistosomiasis (n = 318).

8.3.4 Community attitudes and perceptions regarding the severity, treatment and prevention of schistosomiasis

Schistosomiasis was perceived to be a serious disease by 75% of questionnaire respondents (Table 8.2), a perception that was also predominant among those interviewed. Questionnaire respondents strongly agreed that hospitalisation (76%) and adherence to prescribed medication (79%) were necessary for treating bilharzia, especially in cases of bloody urine or stool. The majority (89%) also believed that only doctors (72%) and nurses (56%) could provide effective treatment.

While the clinic in HaNesengani does not provide medication or treatment for people who test positive for schistosomiasis, primary health care providers indicated their willingness to offer advice and support to patients who have been diagnosed. Some of the interviewees also reported visiting traditional healers for diagnosis and treatment. A female farmer said, *"Traditional healers can treat it because they can give you medication that can stop the blood from coming out. They know how to treat mutambotambo because it is a disease that has existed for a long time; it was there even before modern medicine."* This response once again highlights the need to include traditional healers in awareness campaigns and community-based interventions, as they are trusted practitioners in rural communities. The respondents knew that contact with freshwater sources caused infection, but they lacked knowledge about proper prevention measures. Perceptions of the effectiveness of protective gear varied depending on the understanding of the routes of infection and transmission. Those who knew that infection occurred through the skin believed that protective gear could prevent infection, but others that believed transmission to occur through ingestion wondered how *"people can swim in those things".* Study participants did not view avoiding contact with river water (46%) as a feasible preventive measure, probably because, as the in-depth interviewees explained, communal taps often run dry, so going to the river was a necessity rather than a choice. Table 8.2 summarises respondent attitudes towards the seriousness, treatment and prevention of schistosomiasis.

Table 8.2: Respondent attitudes towards the seriousness, treatment and prevention of schistosomiasis.

Items	Strongly agree		Agree		Unsure		Disagree		Strongly disagree	
	n	%	n	%	n	%	n	%	n	%
Bilharzia is a serious disease	238	75	61	19	12	4	2	1	5	2
Bilharzia is part of growing up	60	19	54	17	75	24	71	22	58	18
One outgrows bilharzia	51	16	50	16	87	27	83	26	47	15
Having unprotected sex can lead to infection with bilharzia	68	21	53	17	89	28	58	18	50	16
Defecating in the toilet is very important	214	67	61	19	33	10	9	3	1	0
When someone passes bloody urine/stool they should go to the hospital	243	76	49	15	16	5	5	2	5	2
A person can be infected with bilharzia many times	163	51	70	22	46	15	19	6	20	6
People can swim, fish or collect water from the river without wearing protective gear	94	30	69	22	56	18	44	14	55	17
It's important to learn about bilharzia	232	73	68	21	12	4	3	1	3	1
If infected, it is important to take bilharzia medication as prescribed	251	79	48	15	15	5	0	0	4	1

8.3.5 Responses from caregivers of children under 15 years of age

Caregivers (parents and guardians) of children under 15 years old were asked to answer some additional questions about their children's water-related practices in the community. Caregivers reported that children under their supervision never swam or played in river water (73%), collected river water for domestic use (72%), or crossed the river to go to school or visit others (75%).

Most of those interviewed agreed that children should be made aware of schistosomiasis and its risks, transmission and symptoms in a school setting. Nurses interviewed at the local clinic in HaNesengani stated that they used to host the children on weekends to keep them safe and prevent them from swimming in the river out of boredom. The children would participate in various activities, *"but they had to bring their own lunch, which was not affordable for the families"*. The initiative was discontinued due to a lack of funds. School awareness campaigns are not conducted due to staff shortages at the local clinic. These factors have increased the risk of schistosomiasis among the school-aged children in the community, who are the most vulnerable group. Equipping teachers at local schools with knowledge and materials about schistosomiasis should be made a priority in any future community-based awareness campaigns and interventions.

8.3.6 Participant perceptions of the relationship between environmental conditions and the occurrence of schistosomiasis

The questionnaire was administered to 342 respondents, of whom 235 (69%) met the inclusion criteria for this part of the questionnaire (lived in HaNesengani for more than 15 years at the time of the study). The respondents were more familiar with *tshanduko ya mupo* (the local term for climate change) (79%) than with the English term climate change (64%). Those who knew about *tshanduko ya mupo* correctly identified its effects: heavy rainfall (floods) (95%), drought (94%), hot weather (91%), and tropical storms (84%). However, those who claimed to know about climate change performed poorly in determining its effects. Only 60% cited drought as an example of climate change, followed by heat waves (57%), heavy rainfall (60%), and tropical storms (56%).

In-depth interviews revealed that many respondents in the study community confuse the concept of climate change with normal weather processes, indicating a need for further engagement on the topic. When asked whether temperature influences the occurrence of bilharzia, some in-depth interviewees indicated that there is a link because *"when it is sunny and hot, more people may go to the river to cool off"*. Osakunor et al. (2018) found that climate changes have a significant impact on human exposure patterns, with exposure increasing

during hotter seasons when more people are exposed to infected water sources during recreational activities. These findings concur with those of this study.

When asked whether heavy rainfall (flooding) expands areas where snails are found, 35% of respondents were unsure whether heavy rainfall (flooding) expands areas where snails are found. In-depth interview respondents associated periods of high rainfall with a decrease in schistosomiasis because people can collect rainwater at their homes and do not need to go to the river to swim or collect water. The effect of droughts on the occurrence of schistosomiasis was also explored, and 32% of respondents strongly agreed that during periods of low rainfall and drought, more people rely on rivers for water, increasing their risk of exposure. Table 8.3 below summarises all respondent associations between environmental factors and the occurrence of schistosomiasis.

Table 8.3: Respondent associations between environmental factors and the occurrence of schistosomiasis.

Items	Strongly agree		Agree		Unsure		Disagree		Strongly disagree	
	n	%	n	%	n	%	n	%	n	%
Rise in temperature increase the survival and number of infected snails	80	34	62	26	72	31	13	6	8	3
A rise in temperatures reduces snail survival and numbers	38	16	45	19	96	41	26	11	30	13
A rise in temperature increases the intensity of bilharzia infection	78	33	57	24	87	37	6	3	7	3
Intensive rainfall (flooding) expands areas where snails are found	81	34	54	23	82	35	9	4	9	4
Intensive rainfall (flooding) destroys snails and their habitats due to fast-flowing water	57	24	47	20	85	36	22	9	24	10
During times of low rainfall and drought, more people rely on the rivers for water	76	32	58	25	72	31	9	4	20	9
Fewer people get infected with bilharzia during times of low rainfall	76	32	46	20	80	34	15	6	18	8
As a result of the construction of a dam, more people have access to water leading to a reduction in bilharzia infection	85	36	49	21	83	35	5	2	13	6

8.3.7 Relationships between respondent age, gender, level of education and knowledge and attitudes regarding schistosomiasis

Respondents aged 18 to 34 years (the youngest category) had the lowest knowledge levels of schistosomiasis in the current study (28%). These results confirm interview responses that indicated a low level of knowledge and awareness among younger generations in the community. In HaNesengani, respondent age and knowledge of the cause, transmission and symptoms of the disease were statistically significant at a significance level of 0.05 ($p = 0.049$). These results reiterate the need for educational campaigns aimed at the youth and local schools. There was no statistically significant difference between gender and knowledge in the current study ($p = 0.118$). Respondents with secondary education had the lowest level of knowledge (35%) about schistosomiasis and a statistically significant difference between education level and knowledge about the disease was found ($p = 0.004$).

The attitudes of respondents aged 35 to 64 years toward the disease (Table 8.4) were neutral (21% positive vs. 21% negative), while those aged 18 to 34 years were more likely to have positive attitudes towards the seriousness and prevention of the disease (20%) (see Table 8.4). However, the association between respondents' ages and their attitudes was not statistically significant ($p = 0.441$). Female respondents (29%) were found to have more positive attitudes than male respondents (20%). This finding is in line with another study in South Africa, which reported that male participants underestimated the severity of schistosomiasis and had poorer health-seeking behaviours than female participants (Sacolo-Gwebu et al. 2019). The relationship between respondents' gender and their attitudes was also not statistically significant ($p = 0.097$). Respondents with secondary education had balanced attitudes (27% positive vs. 28% negative), whereas those with tertiary education had more positive attitudes (16%). The level of education of the respondents was statistically significant ($p = 0.007$).

Table 8.4: Attitudes regarding the treatment and prevention of schistosomiasis by age, gender and educational level.

Variable		Positive attitudes		Neutral		Negative attitudes		Total	
		n	%	n	%	N	%	n	%
Age	18 – 34 years	65	20	8	3	53	17	126	40
	35 – 64 years	68	21	10	3	66	21	144	45
	65 years or older	20	6	1	0	27	9	48	15
Age/Attitude Pearson Chi-Square		0.441							
Gender	Female	91	29	12	4	88	28	191	60
	Male	62	20	6	2	57	18	125	39
	Prefer not to say	0	0	1	0	1	0	2	1
Gender/Attitude Pearson Chi-Square		0.097							
Level of education	Primary school	9	3	2	1	21	7	32	10
	Secondary school	86	27	13	4	88	28	187	59
	Tertiary school	51	16	3	1	22	7	76	24
	Postgraduate qualification	3	1	0	0	7	2	10	3
	None	4	1	1	7	8	3	13	4
Level of education/Attitudes Pearson Chi-Square		0.007							

8.4 Conclusion

This study used a mixed-methods approach to investigate community knowledge, attitudes, perceptions and practices regarding schistosomiasis in the community of HaNesengani, Limpopo Province. Findings showed that the community's lack of access to a safe and clean water supply was the main factor that led to risky water practices and the consequent persistence of infection in the community. The findings also indicated that most participants were familiar with the local term for the disease, *mutambotambo*, and could correctly identify its signs and symptoms, as well as recognise its severity and need for treatment.

However, they had limited knowledge about the disease's life cycle, which increased their susceptibility to schistosomiasis, especially when combined with the unreliable piped water supply. This once again highlights the importance of awareness campaigns and a reliable and clean water supply, especially in rural communities where the disease is endemic.

The study further revealed that the local clinic had stopped conducting awareness campaigns in schools and infection prevention activities with children in the community due to a shortage of staff and financial resources. This is unfortunate, as the study found that the youngest age group had the lowest levels of awareness of schistosomiasis, and children aged five to 17 years were the most vulnerable to infection.

Respondents had deeply rooted misconceptions about disease transmission, such as attributing it to excessive salt intake or genital contact with water, or considering it a sexually transmitted disease. Many respondents also did not see the value of wearing protective gear when in contact with water, as it was impractical. The findings suggested that community-wide awareness campaigns, particularly about the disease's life cycle and transmission through any skin contact, are needed to disseminate accurate information and dispel existing myths or beliefs.

Traditional healers play an important role as health care providers in this community; yet, responses in this study showed that they also have misconceptions about the transmission, symptoms and treatment of schistosomiasis. There does not seem to be much collaboration between the formal and traditional healthcare sectors; this should be addressed to ensure collaboration and knowledge sharing among health care providers in the community.

The findings also highlight the importance of creating awareness materials that include important terminology in local languages, as more residents are familiar with these.

CHAPTER 9: CONCLUSIONS AND RECOMMENDATIONS

Compiled by: *L. de Necker, N. Ayob and L.F. Lindeque*

9.1 Introduction

Freshwater molluscs are a diverse group of aquatic biota with specific environmental preferences and are essential to nutrient cycling and ecosystem function. Freshwater molluscs also play a role in medical and economic aspects, acting as vectors of parasitic diseases and, occasionally, being used as a food source, particularly in rural communities. Several factors influence the distribution of freshwater molluscs, including habitat structure, water quality, flow velocity, rainfall, temperature, oxygen content, invasive species and environmental changes caused by anthropogenic alterations and climate change. Climate change, particularly alterations in water temperature and precipitation, is predicted to modify the geographical range of freshwater molluscs rather than causing complete extinction. Given the potential impacts of climate change and other threats, an understanding of the distribution and response of freshwater molluscs is crucial for their conservation and for the maintenance of aquatic ecosystems in southern Africa.

Schistosomiasis is a neglected tropical disease prevalent across sub-Saharan Africa, including South Africa, predominantly in poor and rural communities without access to potable water and appropriate sanitation. The disease is primarily transmitted through contact with natural water bodies used for various activities like domestic use, agriculture, fishing, and recreational activities. Although the goal of the World Health Organization is to eliminate schistosomiasis by 2030, this disease persists due to limited diagnostic methods and reliance on the medication praziquantel. Additionally, schistosomiasis is not considered a reportable disease in South Africa, and there are currently no control programmes for the disease in the country.

The overarching aim of this study was to investigate the potential impacts of climate change on the transmission of schistosomiasis in South Africa to inform public health and adaptation and mitigation strategies. Specifically, the study sought to 1) collect data on freshwater snail species and their parasites in historic and new potentially suitable distribution sites with a focus on schistosomiasis vectors, 2) use historic water quality information and climatic modelling to investigate schistosomiasis areas in South Africa, 3) determine the new potentially suitable distribution ranges of schistosomiasis vectors in relation to future predicted climate change, and 4) assess community knowledge, attitudes, and perceptions of the past and present incidence of schistosomiasis in communities located in known schistosomiasis areas.

By achieving these aims, the study sought to provide insights into the current and potential future distribution of schistosomiasis and its vectors, as well as the perceptions and experiences of affected communities. The following sections provide concluding remarks on each of the original set aims of this study.

9.2 Conclusions

Aim 1: Conduct field collection surveys of freshwater snail species, particularly those known to be schistosomiasis vectors, and their parasites in historic and potentially new suitable distribution sites.

Native and invasive mollusc species were collected across various Provinces in South Africa and identified using a combination of morphological and molecular analyses. Fifteen different mollusc species were historically collected, with two being invasive species and the rest native. The results of the current study indicated that the diversity of mollusc species has declined as only 10 species were collected of which three were invasive species. Of these, *T. granifera* was previously not recorded in the study area. Cercarial shedding experiments were unsuccessful, indicating that the mollusc species that tested positive for the presence of digenean trematodes had immature or early-stage infections. The low prevalence of *Schistosoma* sp. in snails, along with declining survival and reproduction rates of native mollusc species during colder seasons, and the life cycle complexity of *Schistosoma* and *Fasciola* digenean trematodes might explain why these species were not detected in the collected vectors. Positive results of other trematode parasites, including *N. smiti* and *Pet. variospinosus* within specimens of *Bulinus*, and the detection of *O. batrachoides* within specimens of *R. natalensis* provide new insights into the life cycle of these trematodes. All *T. granifera* samples from the selected sites were genetically identical females, supporting the hypothesis that they originate from few or even a single invasion event or a small population of introduced snails. The lack of trematode parasites within *T. granifera* enables this species to reach higher densities and spread rapidly, potentially contributing to their advantage over native snails in the invaded ecosystems of South Africa and, potentially, the reduction in diseases such as schistosomiasis. Areas dominated by *T. granifera* showed few other mollusc species, and if present, they occurred in much lower densities compared to *T. granifera*. The ability of this invasive mollusc to spread rapidly, resist unfavourable conditions that affect native mollusc species, and the lack of natural predators and parasites raise concerns for native species, snail-transmitted diseases such as schistosomiasis, and ecosystem dynamics. The high densities of this invasive snail species may also have broader implications for food web dynamics of invaded ecosystems.

Aim 2: Determine whether schistosomiasis infection areas have changed in the past half-century in South Africa with the use of historic water quality information and historic climatic modelling.

Three modelling methods, namely MaxEnt, RF and GLM, were employed to identify historic distributions of schistosomiasis-transmitting snails in three South African Provinces (Mpumalanga, Limpopo and Gauteng). Various bioclimatic and climatic variables, such as temperature, precipitation, and elevation, played an important role in shaping the distribution of *Biomphalaria pfeifferi, Bulinus africanus* and *Bulinus globosus*. The MaxEnt model demonstrated higher accuracy and effectiveness under uniform and extensive review coverage than GLM in the Mbombela and Nkomazi local municipalities. Generally, the eastern parts of Mpumalanga had the most suitable habitats for both *Biom. pfeifferi* and *Bul. globosus,* although suitable habitats for *Bul. globosus* were mainly present in the warm seasons while suitable habitats were present for *Biom. pfeifferi* in all seasons. The RF and MaxEnt models were used to analyse the historical distribution of *Biom. pfeifferi* and *Bul. globosus* in the Vhembe District Municipality and *Biom. pfeifferi* and *Bul. africanus* in the Tshwane and Johannesburg metropolitan municipalities with the RF model generally faring better than the MaxEnt model in both the Limpopo and Gauteng Provinces. The central parts of the Vhembe District Municipality had the most suitable habitats for both *Bul. globosus* and *Biom. pfeifferi*. In the Tshwane Metropolitan Municipality, the central, upper western and lower eastern regions were the most suitable for both mollusc species although more habitats were suitable for *Biom. pfeifferi* than for *Bul. globosus,* while in the Johannesburg Metropolitan Municipality, the northern regions were more suitable for *Bul. globosus.*

It was revealed that, although each of the studied mollusc species had preferences for specific climatic conditions and proximity to water sources, generally the most important climatic variables affecting habitat suitability for all the snails were temperature, precipitation and elevation. Importantly, many of the regions considered suitable for schistosomiasis-transmitting snails were also more populated regions. Notably, unsuitable areas could potentially become new transmission risk zones in the future due to changing climatic and bioclimatic conditions. The findings emphasise the importance of robust ecological niche modelling to predict vulnerability to infection and transmission, particularly in the light of factors such as population growth, agricultural activities, and land development.

Aim 3: Determine potentially new suitable distribution ranges of schistosomiasis vectors in relation to future predicted climate change.

The three distinct climate models utilised for predicting the distribution of schistosomiasis-transmitting snails across South Africa indicated slight variations in their projections of future

habitats and snail dispersion. These variations emerged due to the divergent model types, various Representative Concentration Pathway (RCP) scenarios, and temporal spans considered. Notwithstanding these variations, a consistent pattern of snail distribution emerged across the models, delineating potential survival areas and vulnerable zones under different climate scenarios. The findings indicate that the established distribution patterns of *Bul. africanus*, *Bul. globosus*, and *Biom. pfeifferi* remain relatively stable compared to the baseline. Projected habitats for schistosomiasis-transmitting snails encompass regions within Limpopo, Mpumalanga, KwaZulu-Natal, North West, Gauteng, and Eastern Cape Provinces. However, these species are progressively expanding into previously inhospitable territories within certain Provinces, while historically favourable areas are becoming unsuitable. Provinces such as the Free State and Western Cape are anticipated to become schistosomiasis transmission regions in the future as a result of a greater presence of suitable habitats for the snails in the future, as indicated by the model projections.

Aim 4: Assess community knowledge, attitudes, and perceptions of the past and present incidence of schistosomiasis in communities located in known schistosomiasis infection areas.

A mixed methods approach was employed to explore the knowledge, attitudes, perceptions, and practices related to schistosomiasis within the HaNesengani community in Limpopo Province. Regular piped water supply interruptions emerged as a primary driver of risky water behaviours and the persistent prevalence of schistosomiasis in the community. While participants exhibited familiarity with the local name for the disease and its symptoms, a lack of understanding about the disease life cycle and its transmission was observed among participants. The research also found that there was a discontinuation of awareness campaigns and infection prevention activities for school children due to resource constraints. Results revealed that the youngest age group, aged five to 17 years, exhibited the lowest awareness levels and the highest vulnerability to schistosomiasis. These findings underscore the importance of awareness campaigns in local languages and a reliable piped water supply, especially in endemic rural areas. The role of traditional healers as essential healthcare providers also emerged from the study; yet their misconceptions about schistosomiasis transmission, symptoms, and treatment highlighted a lack of collaboration between formal and traditional healthcare sectors. To address this gap, fostering collaboration and knowledge exchange among community healthcare providers is imperative. These findings highlight the need for comprehensive community-wide awareness campaigns, dispelling myths about the mode of transmission, and emphasising how to prevent infection. Ultimately, addressing these multifaceted challenges can contribute to the mitigation of schistosomiasis within endemic communities like HaNesengani.

9.3 Recommendations

The following recommendations were established for future studies to build upon. By addressing these research areas, future studies can contribute to a comprehensive understanding of snail-transmitted diseases such as schistosomiasis, invasive snail dynamics, their ecological implications, and the potential risks they pose to both ecosystems and human health.

- Further research into the co-evolutionary history of parasites and snails may provide insight into schistosome flexibility to parasitise various species. This has implications for predicting schistosome range shifts in response to climate change since the parasites might be more resilient than their intermediate snail hosts.
- Long-term monitoring of the distribution and abundance of both native and invasive freshwater snail species would further provide valuable data on population dynamics, trends, and potential impacts on native species over time.
- Examine the impact of invasive snails on aquatic food webs and ecosystem dynamics and investigate how changes in snail populations may affect other aquatic organisms and ecosystem services.
- Future studies on the interactions between digenean trematodes and both invasive and native snail species and the prevalence and transmission dynamics of parasites, including schistosomes and other water-borne diseases, within these snail populations.
- Further investigation of the role of *T. granifera* as a potential host for trematodes in invaded habitats across southern Africa. This will further assist in gaining an understanding of the role of *T. granifera* in native aquatic biota distribution and, potentially, effects on human health by affecting the distribution of snail-transmitted diseases such as schistosomiasis.
- Laboratory experiments investigating ecological interactions between invasive snail species, such as *T. granifera*, and native snails, including competition for resources, and predator-prey relationships would provide insight into the potential impacts of invasive snails on local biodiversity and ecosystem functioning.
- To predict disease contractions or expansions, future studies should incorporate human activities into predictive models as these may impact or create snail habitats.
- Further research should focus on factors affecting the micro-geographical scale of *Schistosoma* host snail distribution. Regional climate models must capture localised climate changes that assign meteorological parameters on relatively small scales.
- Further studies employing participatory knowledge co-creation approaches should be conducted in communities where schistosomiasis is still endemic. Studies should focus

on approaches that bring together stakeholders from the formal and traditional healthcare, and educational sectors. These stakeholders have a good understanding of factors that influence schistosomiasis prevalence in their communities, and could provide valuable insights towards the design of community wide awareness programmes.

9.4 Project outputs

9.4.1 Conference presentations

- Thibedi, D.Z., Ayob, N., Nkosi, N.C. & L. de Necker. 2023. The historic spatial distribution of *Bulinus globosus* using bioclimate variables in the Vhembe District, Limpopo Province, South Africa. South African Society for Atmospheric Sciences. University of Western Cape, Bellville, Cape Town. 29 October – 2 November 2023.

- Thibedi, D.Z., Ayob, N., Nkosi, N.C. & L. de Necker. 2023. The historic distribution of *Bulinus globosus* in the Vhembe district, Limpopo Province. NRF-SAIAB Student Symposium 24 November 2023

- Khwela, T., Ayob, N., Nkosi, N.C. & **L. de Necker.** 2023. Mapping historic schistosomiasis risk areas in Johannesburg and Tshwane Municipalities. NRF-SAIAB Student Symposium 24 November 2023.

- Thibedi, D.Z., Ayob, N., Nkosi, N.C. Khwela, T. & L. de Necker. 2023. The historic distribution of *Biomphalaria pfeifferi* and *Bulinus globosus* in the Vhembe district, Limpopo Province. 31st International Cartographic Conference (ICC 2023). Cape Town, South Africa. 13-18 August 2023.

- Le Roux, M.H., Smit, N.J. & **L. de Necker.** 2023. The distribution of aquatic molluscs and associated trematodes within the Limpopo Lowveld ecoregion, South Africa. South African Society of Aquatic Scientists conference. Lord Charles Hotel, Somerset West. 25-29 June 2023.

- Ayob, N., Burger, R.P., Belelie, M.D., Nkosi, N.C., **de Necker, L.** & D.P. Cilliers. 2023. Using climate models to determine the future distributions of *Schistosoma* transmitting snails in South Africa. International Society for Ecological Modelling Global Conference 2023. University of Toronto, Scarborough, Canada. 2-6 May 2023.

- Le Roux, M.H., Smit, N.J. & **L. de Necker.** 2022. Digenean trematode diversity in vectors transmitting schistosomiasis within ecosystem of southern Africa. NRF-SAIAB Online Student Symposium 2022. 9 December 2022.

- Letlaila, F., Nkosi, N., Ayob, N. & **L. de Necker.** 2022. The historical and seasonal distribution of schistosomiasis transmitting vectors in the Mpumalanga Province, South Africa. NRF-SAIAB Online Student Symposium 2022. 9 December 2022.

- Thibedi, D. W., Ayob, N., Nkosi, N. & **L. de Necker.** 2022. The historic distribution of *Biomphalaria pfeifferi* in the Vhembe district, Limpopo Province. NRF-SAIAB Online Student Symposium 2022. 9 December 2022.

- Ayob, N., Burger, R.P., Nkosi, N.C., Havenga, H., **de Necker, L.** & D.P. Cilliers. 2022. Predicting the effects of climate variability on *Schistosoma* transmitting snails in the Mpumalanga Province, South Africa. South African Society for Atmospheric Sciences Conference. University of Witwatersrand, Gauteng, South Africa. 31 October – 1 November 2022.
- **de Necker, L.,** Le Roux, M.H., Mothapo, N., Lindeque, F., Smit, N.J. & V. Wepener. 2022. Schistosomiasis in South Africa: vector distribution and human perceptions and practices. WaterNet Conference. Sun City, Rustenburg, North West, South Africa. 19-21 October 2022.
- Letlaila, F., Ayob, N., Nkosi, N. & **L. de Necker.** 2022. The influence of water quality on the *Schistosoma* ecosystems within Mbombela and Nkomazi local municipalities. WaterNet Conference. Sun City, Rustenburg, North West, South Africa. 19-21 October 2022.
- Ayob, N., Burger, R.P., Nkosi, N.C., **de Necker, L.** & D.P. Cilliers. 2022. Modelling the suitable habitats for *Schistosoma* host snails in Limpopo, South Africa. Society of South African Geographers Biennial Conference. University of Pretoria, Gauteng, South Africa. 12-14 September 2022.
- Le Roux, M.H., **de Necker, L.,** Lawton, S. & N.J. Smit. 2022. Decline in the native freshwater snail diversity of the Limpopo River system, South Africa: a cause for concern. South African Society of Aquatic Scientists. Amanzi Private Game Reserve, Brandfort, Free State, South Africa. 26-30 June 2022.
- **de Necker, L.,** Brendonck, L., van Vuren, J., Wepener, V. & N.J. Smit. 2021. Aquatic invertebrate community resilience and recovery in response to a supra-seasonal drought in an ecologically important naturally saline lake. NRF-SAIAB Online Student Symposium 2021. 3 December 2021.
- Coetsee, A., **de Necker, L.,** & N.J. Smit. 2021. Freshwater snail diversity and abundance in historic schistosomiasis distribution areas of the Limpopo Province. NRF-SAIAB Online Student Symposium 2021. 3 December 2021.
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- Pearson, H., Gerber, R., Malherbe., W. & **L. de Necker**. 2021. The role of *Tarebia granifera* (Lamarck, 1822) as an invasive in freshwater ecosystems of southern Africa. NRF-SAIAB Online Student Symposium 2021. 3 December 2021.
- Letlaila, R.F., Ayob, N., Nkosi, N., & **L. de Necker**. 2021. The historical and seasonal distribution of schistosomiasis transmitting vectors in the Mpumalanga Province, South Africa. NRF-SAIAB Online Student Symposium 2021. 3 December 2021
- **de Necker, L.,** Lindeque, F., Mothapo. N., Cilliers, D., Burger, R., Smit, N.J. & V. Wepener. 2021. The current and future predicted status of schistosomiasis in South Africa: What role will climate change play? South African Society of Aquatic Scientists online conference. 2-4 November 2021.

- **de Necker, L.,** Cilliers, D., Burger, R., Smit, N.J. & V. Wepener. 2021. The current and future predicted status of schistosomiasis in South Africa under climate change. British Society for Parasitology Online Meeting 2021. 21-25 June 2021
- **de Necker, L.,** Wepener, V., Smit, N.J. & O.L.F. Weyl. 2020. Current status and future predicted distribution patterns of bilharzia transmitting snails under climate change and implications for vector-borne diseases in South Africa. NRF-SAIAB Online Student Symposium 2020. 8 December 2020.
- Pearson, H., Gerber, R., Malherbe, W. & **L. de Necker.** 2020. The role of *Tarebia granifera* (Lamark, 1822) as an invasive in freshwater ecosystems of southern Africa. NRF-SAIAB Online Student Symposium 2020. 8 December 2020.
- Le Roux, M.H., Smit, N.J. & **L. de Necker.** 2020. The effect of sediment and water quality on parasite diversity. NRF-SAIAB Online Student Symposium 2020. 8 December 2020.
- Sithole, N., Smit, N.J. & **L. de Necker.** 2020. The effect of select environmental variables on the diversity and abundance of freshwater snails in three sites of the North-West Province. NRF-SAIAB Online Student Symposium 2020. 8 December 2020.**Poster presentation**
- Le Roux, M.H., Smit, N.J. & **L. de Necker**. 2023. Unravelling the hidden world of freshwater molluscs: combining taxonomic & molecular techniques. South African Society of Aquatic Scientists conference. Lord Charles Hotel, Somerset West. 25-29 June 2023,
- Le Roux, M.H., Smit, N.J. & **L. de Necker**. 2023. Unveiling the hidden world: molecular insights into trematode infections in freshwater molluscs of southern Africa. 38th Icthyoparasitological Symposium, Oer-Erkenschwick, Germany. 21-22 June 2023.

9.4.2 Conference proceedings

- Thekiso, L., Khwela, T., Ayob, N., Nkosi, N.C. & L. de Necker. 2022. Climate variability impacts on the historical distribution of *B. africanus* and *B. pfeifferi* in the Tshwane and Johannesburg Municipalities. Society of South African Geographers Biennial Conference. University of Pretoria, Gauteng, South Africa. 12-14 September 2022.
- Khwela, T., Nkosi, N.C., Ayob, N. & L. de Necker. 2023. Characterising the historic suitable habitats for *Biomphalaria pfeifferi* in the Tshwane Metropolitan Municipality. South African Society for Atmospheric Sciences. University of Western Cape, Bellville, Cape Town. 29 October – 2 November 2023.
- Khwela, T., Ayob, N., Nkosi, N. & L. de Necker. 2023. The influence of environmental factors on the historical distribution of *Biomphalaria pfeifferi* in the Tshwane Metropolitan Municipality. In: Proceedings of the International Cartographic association, 5(8): 2023. 31st International Cartographic Conference (ICC 2023), Cape Town, South Africa. 13-18 August 2023.
- Letlaila, F., Ayob, N., Nkosi, N. & L. de Necker. 2023. The historic distribution of schistosomiasis transmitting snails in Mbombela and Nkomazi local municipalities. In: Proceedings of the International Cartographic association, 5(8): 2023. 31st

International Cartographic Conference (ICC 2023), Cape Town, South Africa. 13-18 August 2023.

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9.4.3 Popular articles

- **de Necker, L.** 2020. Bilharzia and its snail vectors under the spotlight in current study. The Water Wheel 19 (6), 20-23.

9.4.4 Journal articles

Under review:

- Gerber, R., Pearson, J.J., Wepener, V., Malherbe, W. & **L. de Necker.** *Under review.* Current distribution and population dynamics of *Tarebia granifera* in aquatic ecosystems of north-eastern South Africa: drivers of density and impact on native mollusca. Ecology and Evolution.
- Letlaila, F., Ayob, N., Nkosi, N. & **L. de Necker.** *Under review.* Historic water quality patterns in endemic schistosomiasis regions of southern Africa: implications for disease vector distributions. Physics and Chemistry of the Earth, Parts A/B/C.
- Pearson, J.J., Gerber, R., Malherbe, W., Smit, N.J. & **L. de Necker.** *Under review.* Stranger in a strange land: ecological impacts of the invasive mollusc *Tarebia granifera.* African Journal of Aquatic Sciences.

Accepted for publication:

- Ayob, N., Burger, R.P., Nkosi, N.C., Havenga, H., **de Necker, L.** & D.P. Cilliers. *Accepted.* Modelling the historical distribution of schistosomiasis transmitting snails in South Africa using ecological niche models: Paper I. PLOS One.
- **de Necker, L.,** van Rooyen, D., Gerber, R., Brendonck, L., Wepener, V. & N.J. Smit. *Accepted.* Effects of river regulation on aquatic invertebrate community composition: a comparative analysis. Ecology and Evolution.

9.4.5 Community outreach

- **Snail Day school activity, Vhembe, Limpopo**. Aimed to teach children about schistosomiasis and river health. 1 April 2023. Pentecostal Holiness Church, Tshino, HaNesengani, Venda, Limpopo Province. Attended by 68 school children from four different schools in the Tshino Village area (Mpheni Primary, Tshino Primary, Tshivhazwaulu Primary and Rasivhetshele Primary). Further attended by eight teachers (two from each school) and local tribal leaders, community members and members from the Well of Hope Non-profit Organisation in the region. A total of 29 researchers, 18 from North-West University (NWU) and 11 from the University of Limpopo (UL), assisted with the workshop.

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APPENDICES

Chapter 2

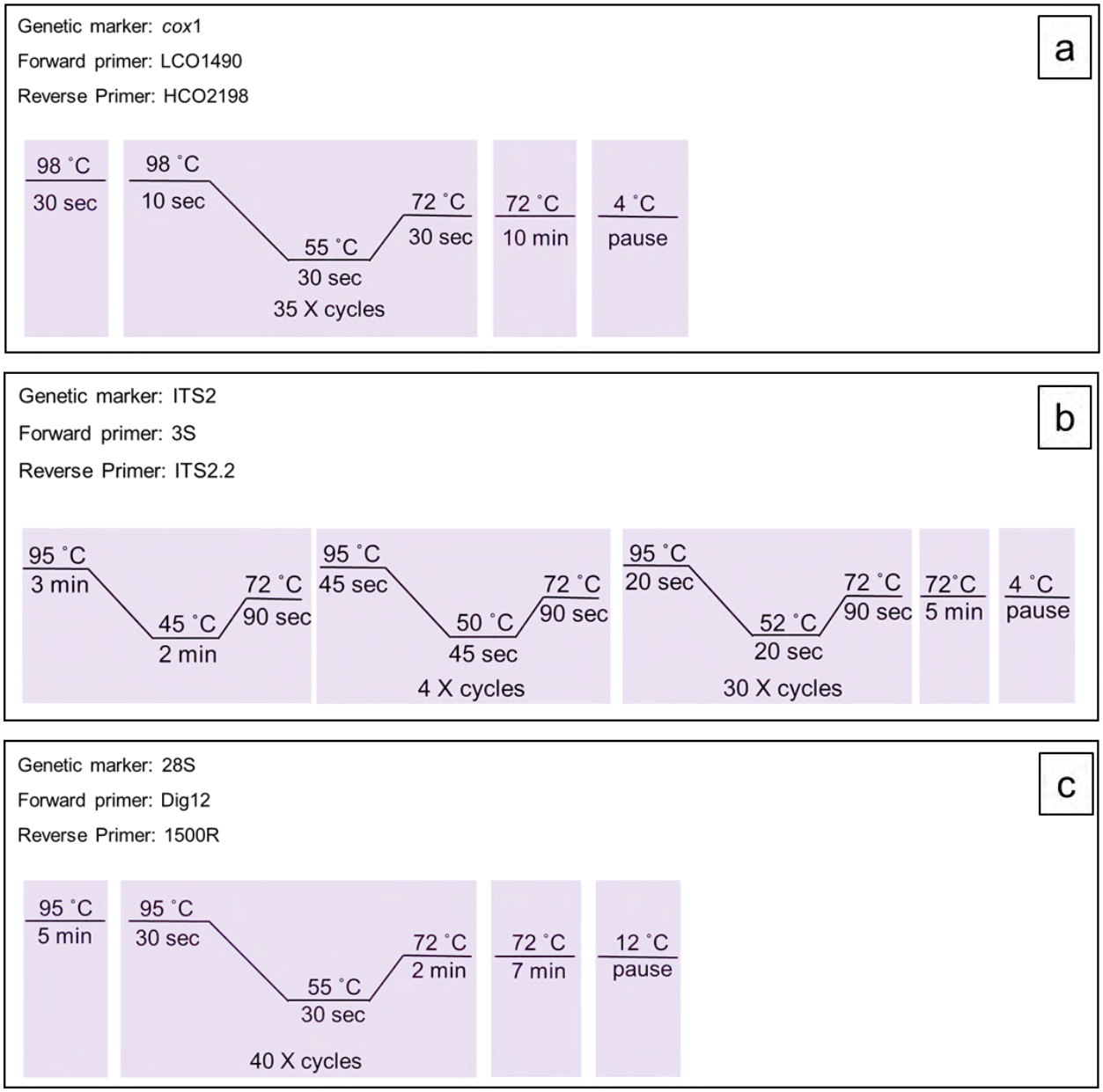

Figure S2.1: The PCR protocols used to amplify DNA of the three selected genetic markers: a) COI, b) ITS2, and c) 28S. Protocols were adapted from Vermaak (2021).

Table S2.1: The COI sequences used for the phylogenetic analysis during the present study. The GenBank accession numbers AY958764 and AY958760 were referred to as AF958764 and AF958760 in Genner et al. (2007). Percentage similarity is based on pairwise genetic distance calculations.

Snail species	Country	Site	Sample number	Similarity (%)	GenBank accession number	Reference
Tarebia granifera	South Africa	PHON	TP05	100	-	Present study
Tarebia granifera	South Africa	LIMP-2	M12	100	-	Present study
Tarebia granifera	South Africa	MOGA	M21a	100	-	Present study
Tarebia granifera	South Africa	LUVU	H39	100	-	Present study
Tarebia granifera	South Africa	OLIF-1	M61a	100	-	Present study
Tarebia granifera	South Africa	OLIF-2	M41	100	-	Present study
Tarebia granifera	South Africa	G-LETA	M71a	100	-	Present study
Tarebia granifera	South Africa	LETA	H59	100	-	Present study
Tarebia granifera	China	-	-	100	MZ662113	Yin et al. (2022)
Tarebia granifera	Thailand	-	-	100	MK000375	Veeravechsukij et al. (2018b)
Tarebia granifera	Thailand	-	-	98.2	MK000294	Veeravechsukij et al. (2018b)
Tarebia granifera	USA	-	-	98.2	MT671805	Tolley-Jordan and Triplett (2020)
Tarebia granifera	USA	-	-	98.1	AY958764	Genner et al. (2007)
Tarebia granifera	Singapore	-	-	96.9	AY958760	Genner et al. (2007)
Tarebia granifera	Singapore	-	-	96.4	AY958762	Genner et al. (2007)
Tarebia lineata (Gray, 1828)	India	-	-	83.9	KY511306	Jena and Srirama (2017)
Tarebia lineata	Malaysia	-	-	85.8	MW591171	Jamaluddin et al. (2022)
Melanoides tuberculata	Tanzania	-	-	85.6	AY791909	Von Gersdorff Sørensen et al. (2005)

Table S2.2: P-distance (pairwise genetic distance) given as the number of base pair differences between the sequences used for phylogenetic analysis during the present study.

	Sequence	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	M12_*Tarebia granifera* LIMP-2	-	0	0	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
B	M21a_*Tarebia granifera* MOGA	0	-	0	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
C	H39_*Tarebia granifera* LUVU	0	0	-	0	0	0	0	0	0	0	11	12	12	18	21	93	106	75
D	M41_*Tarebia granifera* OLIF-2	0	0	0	-	0	0	0	0	0	0	11	12	12	18	21	93	106	75
E	H59_*Tarebia granifera* LETA	0	0	0	0	-	0	0	0	0	0	11	12	12	18	21	93	106	75
F	M61a_*Tarebia granifera* OLIF-1	0	0	0	0	0	-	0	0	0	0	11	12	12	18	21	93	106	75
G	M71a_*Tarebia granifera* G-LETA	0	0	0	0	0	0	-	0	0	0	11	12	12	18	21	93	106	75
H	TP05_*Tarebia granifera* PHON	0	0	0	0	0	0	0	-	0	0	11	12	12	18	20	87	100	75
I	MZ662113_*Tarebia granifera*_China	0	0	0	0	0	0	0	0	-	0	11	12	12	18	21	93	106	75
J	MK000375_*Tarebia granifera*_Thailand	0	0	0	0	0	0	0	0	0	-	11	12	12	18	21	93	106	75
K	AY958764_*Tarebia granifera*_USA	11	11	11	11	11	11	11	11	11	11	-	0	12	19	22	77	87	79
L	MT671805_*Tarebia granifera*_USA	12	12	12	12	12	12	12	12	12	12	0	-	12	19	22	89	102	79
M	MK000294_*Tarebia granifera*_Thailand	12	12	12	12	12	12	12	12	12	12	12	12	-	17	20	87	100	71
N	AY958760_*Tarebia granifera*_Singapore	18	18	18	18	18	18	18	18	18	18	19	19	17	-	3	76	82	77
O	AY958762_*Tarebia granifera*_Singapore	21	21	21	21	21	21	21	20	21	21	22	22	20	3	-	79	85	77
P	MW591171_*Tarebia lineata*_Malaysia	93	93	93	93	93	93	93	87	93	93	77	89	87	76	79	-	13	76
Q	KY511306_*Tarebia lineata*_India	106	106	106	106	106	106	106	100	106	106	87	102	100	82	85	13	-	86
R	AY791909_*Melanoides tuberculata*_Tanzania OG	75	75	75	75	75	75	75	75	75	75	79	79	71	77	77	76	86	-

Chapter 3

Table S3.1: Base pair differences between members of the *Bulinus africanus/globosus* group. Above the diagonal are the percentage of identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured blue to match the phylogenetic tree generated (Figure 3.2).

	Species name	Sequence number	1	2	3	4	5	6	7	8
1	*Bulinus africanus*	BS01		100	100	100	100	100	100	97
2	*Bulinus africanus*	AM921970	0		100	100	100	100	100	97
3	*Bulinus africanus*	BS61	0	0		100	100	100	100	97
4	*Bulinus africanus*	BS04	0	0	0		100	100	100	97
5	*Bulinus africanus*	BS66	0	0	0	0		100	100	97
6	*Bulinus africanus*	BS37	0	0	0	0	0		100	97
7	*Bulinus africanus*	BS45	1	1	1	1	1	1		97
8	*Bulinus globosus*	AM921975	13	13	13	13	13	13	12	

Table S3.2: Base pair differences between members of the *Bulinus truncatus/tropicus* group. Above the diagonal are the percentage of identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured orange to match the phylogenetic tree generated (Figure 3.2).

	Species name	Sequence number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Bulinus truncatus	AM921965		100	100	100	100	100	100	100	100	100	100	99	86	86	86	82
2	Bulinus depressus	BS12	0		100	100	100	100	100	100	100	100	100	99	86	86	86	82
3	Bulinus depressus	BS15	0	0		100	100	100	100	100	100	100	100	99	86	86	86	82
4	Bulinus depressus	BS72	0	0	0		100	100	100	100	100	100	100	99	86	86	86	82
5	Bulinus depressus	BS29	0	0	0	0		100	100	100	100	100	100	99	86	86	86	82
6	Bulinus depressus	BS69	0	0	0	0	0		100	100	100	100	100	99	86	86	86	82
7	Bulinus depressus	BS73	0	0	0	0	0	0		100	100	100	100	99	86	86	86	82
8	Bulinus depressus	BS33	0	0	0	0	0	0	0		100	100	100	99	86	86	86	82
9	Bulinus depressus	BS50	0	0	0	0	0	0	0	0		100	100	99	86	86	86	82
10	Bulinus depressus	BS57	0	0	0	0	0	0	0	0	0		100	99	86	86	86	82
11	Bulinus depressus	BS54	0	0	0	0	0	0	0	0	0	0		99	86	86	86	82
12	Bulinus truncatus	AM921983	2	2	2	2	2	2	2	2	2	2	2		86	86	85	81
13	Bulinus natalensis	AM921976	60	60	60	60	60	60	60	60	60	60	60	62		99	98	88
14	Bulinus tropicus	MT707224	59	59	59	59	59	59	59	59	59	59	59	61	5		99	89
15	Bulinus tropicus	MT707227	61	61	61	61	61	61	61	61	61	61	61	63	8	3		88
16	Bulinus tropicus	MT707228	79	79	79	79	79	79	79	79	79	79	79	81	51	48	51	

Table S3.3: Base pair differences between members of the *Bulinus truncatus/tropicus* group and closest neighbouring branches of the phylogenetic tree (Figure 3.3). Above the diagonal is the percentage of identical base pairs, and below the diagonal are base pair differences of the COI gene. Gene sequences generated during this study are coloured orange to match the phylogenetic tree generated.

	Species name	Sequence number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*Bulinus natalensis*	AM286311		100	100	98	98	98	96	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97
2	*Bulinus natalensis*	AM921835	0		100	98	98	98	96	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97
3	*Bulinus natalensis*	AM921836	0	0		98	98	98	96	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97
4	*Bulinus tropicus*	AM921834	11	11	11		100	100	97	97	97	97	97	97	97	97	98	98	98	97	97	97	97	97
5	*Bulinus tropicus*	AM921837	11	11	11	0		100	97	97	97	97	97	97	97	97	98	98	98	97	97	97	97	97
6	*Bulinus tropicus*	AM921842	11	11	11	0	0		97	97	97	97	97	97	97	97	98	98	98	97	97	97	97	97
7	*Bulinus truncatus*	AM286312	19	19	19	15	15	15		99	99	99	99	99	99	99	98	98	98	98	98	98	98	98
8	*Bulinus truncatus*	AM286313	18	18	18	16	16	16	7		99	99	99	99	99	99	98	98	98	98	98	98	98	98
9	*Bulinus truncatus*	AM286314	14	14	14	14	14	14	5	4		100	100	100	100	99	99	99	99	99	99	99	99	98
10	*Bulinus truncatus*	AM921807	14	14	14	14	14	14	5	4	0		100	100	100	99	99	99	99	99	99	99	99	98
11	*Bulinus truncatus*	AM286316	15	15	15	14	14	14	6	5	1	1		100	100	99	99	99	99	99	99	99	99	98
12	*Bulinus truncatus*	AM286317	15	15	15	14	14	14	6	5	1	1	0		100	99	99	99	99	99	99	99	99	98
13	*Bulinus truncatus*	AM921806	15	15	15	15	15	15	4	5	1	1	2	2		99	99	99	99	99	99	99	99	99
14	*Bulinus truncatus*	AM286315	18	18	18	15	15	15	7	6	4	4	3	3	5		98	98	98	98	98	98	98	98
15	*Bulinus depressus*	BS15	14	14	14	13	13	13	10	9	7	7	8	8	8	9		100	100	99	99	99	99	99
16	*Bulinus depressus*	BS29	14	14	14	13	13	13	10	9	7	7	8	8	8	9	0		100	99	99	99	99	99
17	*Bulinus depressus*	BS33	14	14	14	13	13	13	10	9	7	7	8	8	8	9	0	0		99	99	99	99	99
18	*Bulinus depressus*	BS57	13	13	13	14	14	14	11	10	6	6	7	7	7	10	4	4	4		100	100	100	100
19	*Bulinus depressus*	BS72	13	13	13	14	14	14	11	10	6	6	7	7	7	10	4	4	4	0		100	100	100
20	*Bulinus depressus*	BS73	13	13	13	14	14	14	11	10	6	6	7	7	7	10	4	4	4	0	0		100	100
21	*Bulinus depressus*	BS69	14	14	14	15	15	15	12	11	7	7	8	8	8	11	4	4	4	0	0	0		100
22	*Bulinus depressus*	BS91	16	16	16	15	15	15	10	13	9	9	10	10	8	13	6	6	6	2	2	2	2	

Table S3.4: Base pair differences between members of the *Biomphalaria pfeifferi*. Above the diagonal are the percentage of identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured blue to match the phylogenetic tree generated (Figure 3.6).

	Species name	Sequence number	1	2	3	4	5	6	7
1	*Biomphalaria pfeifferi*	MG461588		100	100	100	100	100	93
2	*Biomphalaria pfeifferi*	BP08	1		100	100	100	100	93
3	*Biomphalaria pfeifferi*	BG12	1	0		100	100	100	93
4	*Biomphalaria pfeifferi*	BG11	1	0	0		100	100	93
5	*Biomphalaria pfeifferi*	BG05	0	1	1	1		100	93
6	*Biomphalaria pfeifferi*	BP01	0	1	1	1	0		93
7	*Biomphalaria glabrata*	AF198662	29	30	30	30	29	29	

Table S3.5: Base pair differences between members of the *Gyraulus* species. Above the diagonal are the percentage of identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured orange to match the phylogenetic tree generated (Figure 3.6).

	Species name	Sequence number	1	2	3
1	*Gyraulus costulatus*	BP11		99	98
2	*Gyraulus costulatus*	BP19	6		98
3	*Gyraulus convexiusculus*	KX060747	11	10	

Table S3.6: Base pair differences between members of the *Biomphalaria* above the diagonal are the percentage identical base pairs, and below the diagonal are base pair differences of the COI gene. Gene sequences generated during this study are coloured blue to match the phylogenetic tree generated (Figure 3.7).

	Species name	Sequence number	1	2	3	4
1	*Biomphalaria pfeifferi*	BG11		99	100	93
2	*Biomphalaria pfeifferi*	OL423116	6		99	93
3	*Biomphalaria pfeifferi*	MG780186	1	7		93
4	*Biomphalaria glabrata*	DQ084823	37	39	36	

Table S3.7: Base pair differences between members of the *Gyraulus* above the diagonal are the percentage identical base pairs, and below the diagonal are base pair differences of the COI gene. Gene sequences generated during this study are coloured orange to match the phylogenetic tree generated (Figure 3.7).

	Species name	Sequence number	1	2	3	4	5	6	7	8	9
1	*Gyraulus connollyi*	SX1		100	100	93	96	87	87	87	88
2	*Gyraulus connollyi*	SX15	1		100	93	96	88	88	88	88
3	*Gyraulus connollyi*	SX17	1	0		93	96	88	88	88	88
4	*Gyraulus connollyi*	JX12	37	36	36		93	87	87	87	87
5	*Gyraulus chinensis*	AF199086	20	19	19	36		88	88	88	88
6	*Gyraulus* sp.	KY697250	66	65	65	68	63		99	100	99
7	*Gyraulus* sp.	LC429485	66	65	65	68	64	3		99	99
8	*Gyraulus costulatus*	BP19	66	65	65	68	63	0	3		99
9	*Gyraulus* sp.	LC429526	65	64	64	67	62	4	3	4	

Table S3.8: Base pair differences between members of the *Radix.* Above the diagonal are the percentage of identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured grey to match the phylogenetic tree generated (Figure 3.9).

	Species name	Sequence number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Radix natalensis*	RN10		100	100	100	100	100	100	100	100	100	95	95	100	100
2	*Radix natalensis*	RN64	0		100	100	100	100	100	100	100	100	95	95	100	100
3	*Radix natalensis*	PC11	1	1		100	99	99	99	99	100	100	94	94	100	99
4	*Radix natalensis*	PC26	1	1	0		99	99	99	99	100	100	94	94	100	99
5	*Radix natalensis*	RN12	1	1	2	2		100	100	100	99	100	95	95	100	99
6	*Radix natalensis*	RN37	1	1	2	2	0		100	100	99	100	95	95	100	99
7	*Radix natalensis*	RN13	1	1	2	2	0	0		100	99	100	95	95	100	99
8	*Radix natalensis*	RN74	1	1	2	2	0	0	0		99	100	95	95	100	99
9	*Radix natalensis*	PC13	1	1	0	0	2	2	2	2		100	94	94	100	99
10	*Radix natalensis*	RN72	0	0	1	1	1	1	1	1	1		95	95	100	100
11	*Radix natalensis*	RN58	17	17	18	18	16	16	16	16	18	17		100	95	94
12	*Radix natalensis*	RN02	17	17	18	18	16	16	16	16	18	17	0		95	94
13	*Radix natalensis*	ON729288	0	0	1	1	1	1	1	1	1	0	17	17		100
14	*Radix natalensis*	ON729287	1	1	2	2	2	2	2	2	2	1	18	18	1	

Table S3.9: Base pair differences between members of the *Pseudosuccinea* above the diagonal are the percentage identical base pairs, and below the diagonal are base pair differences of the ITS2 region. Gene sequences generated during this study are coloured green to match the phylogenetic tree generated (Figure 3.9).

	Species name	Sequence number	1	2	3	4	5	6	7	8	9
1	*Pseudosuccinea columella*	FN598155		100	100	100	100	100	100	100	99
2	*Pseudosuccinea columella*	MN602683	0		100	100	100	100	100	100	99
3	*Pseudosuccinea columella*	PC04	0	0		100	100	100	100	100	99
4	*Pseudosuccinea columella*	PC17	0	0	0		100	100	100	100	99
5	*Pseudosuccinea columella*	PC07	0	0	0	0		100	100	100	99
6	*Pseudosuccinea columella*	RN26	0	0	0	0	0		100	100	99
7	*Pseudosuccinea columella*	RN51	0	0	0	0	0	0		100	99
8	*Pseudosuccinea columella*	RN28	0	0	0	0	0	0	0		99
9	*Pseudosuccinea columella*	JN614470	3	3	3	3	3	3	3	3	

Table S3.10: Base pair differences of *Radix natalensis* below the diagonal and percentage identical base pairs of the COI gene above the diagonal. Grouped and colour coded based on phylogenetic grouping as seen in Figure 3.11.

	Sample code	Site collected	1	2	3	4	5	6	7	8	9	10	11	12
1	RN64	LLF3		100	100	100	99	99	100	98	98	99%	99	99
2	RN72	LLF 3	0		100	100	100	100	100	98	99	99%	99	99
3	RN10	LLF 11	0	0		100	100	100	100	98	99	99%	99	99
4	PC11	LLF 13	2	2	2		100	100	100	99	99	99%	99	99
5	PC13	LLF 13	5	2	2	0		100	100	99	98	99%	99	99
6	RN39	LLF 6	5	2	2	0	0		100	99	98	99%	99	99
7	PC26	LLF 9	2	2	2	0	0	0		99	99	99	9	99
8	RN12	LLF 13	13	10	10	7	8	8	8		97	98	98	98
9	RN58	LLF 4	14	6	6	4	13	13	4	17		98	99	99
10	RN13	LLF 10	8	7	6	4	7	7	5	13	14		100	100
11	RN34	LLF 8	6	6	6	4	4	4	4	9	6	0		100
12	RN37	LLF 8	6	6	6	4	4	4	4	9	6	0	0	

Table S3.11: Base pair differences between members of the *Pseudosuccinea.* Above the diagonal are the percentage identical base pairs, and below the diagonal are base pair differences of the COI gene. Gene sequences generated during this study are coloured green to match the phylogenetic tree generated (Figure 3.10).

	Species name	Sequence number	1	2	3	4	5	6	7	8	9	10	11
1	*Pseudosuccinea columella*	KM594692		100	100	100	100	100	100	100	100	100	100
2	*Pseudosuccinea columella*	ON953198	0		100	100	100	100	100	100	100	100	100
3	*Pseudosuccinea columella*	PC04	0	0		100	100	100	100	100	100	100	100
4	*Pseudosuccinea columella*	RN51	0	0	0		100	100	100	100	100	100	100
5	*Pseudosuccinea columella*	LC015522	0	0	0	0		100	100	100	100	100	100
6	*Pseudosuccinea columella*	PC07	0	0	0	0	0		100	100	100	100	100
7	*Pseudosuccinea columella*	RN26	0	0	0	0	0	0		100	100	100	100
8	*Pseudosuccinea columella*	RN28	0	0	0	0	0	0	0		100	100	100
9	*Pseudosuccinea columella*	PC17	0	0	0	0	0	0	0	0		100	100
10	*Pseudosuccinea columella*	RN50	0	0	0	0	0	0	0	0	0		100
11	*Pseudosuccinea columella*	KP242797	0	0	0	0	0	0	0	0	0	0	

Table S3.12: Base pair differences between members of *Physella acuta* from the African clade 3. Above the diagonal are the percentage identical base pairs, and below the diagonal are base pair differences of the COI gene. Gene sequences generated during this study are coloured blue to match the phylogenetic tree generated (Figure 3.13).

	Species ID	**GenBank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*Physella acuta* (South Africa)	MH649323		99	100	100	98	98	98	98	98	98	98
2	*Physella acuta* (South Africa)	MH649329	3		99	99	97	98	98	98	98	97	97
3	*Physella acuta* (South Africa)	MH649330	2	3		100	98	98	98	98	98	98	98
4	*Physella acuta* (South Africa)	MH649327	2	5	2		98	98	98	98	99	98	98
5	*Physella acuta* (Egypt)	KF412768	11	14	11	9		100	100	100	100	99	99
6	*Physella acuta* (South Africa)	MH649324	9	12	9	9	2		100	100	100	99	99
7	*Physella acuta* (South Africa)	MH649333	10	13	10	8	1	1		100	100	99	99
8	*Physella acuta* (present study)	RN56	10	13	10	8	1	1	0		100	99	99
9	*Physella acuta* (South Africa)	MH649336	9	12	9	7	2	2	1	1		99	99
10	*Physella acuta* (Canada)	KM611969	12	15	12	10	5	5	4	4	5		100
11	*Physella acuta* (Canada)	EU038356	12	15	12	10	5	5	4	4	5	0	

Table S3.13: The COI sequences used for the phylogenetic analysis during the present study. The GenBank accession numbers AY958764 and AY958760 were referred to as AF958764 and AF958760 in Genner et al. (2007)e. Percentage similarity is based on pairwise genetic distance calculations.

Snail species	Country	Site	Sample number	Similarity (%)	GenBank accession number	Reference
Tarebia granifera	South Africa	PHON	TP05	100	-	Present study
Tarebia granifera	South Africa	LIMP-2	M12	100	-	Present study
Tarebia granifera	South Africa	MOGA	M21a	100	-	Present study
Tarebia granifera	South Africa	LUVU	H39	100	-	Present study
Tarebia granifera	South Africa	OLIF-1	M61a	100	-	Present study
Tarebia granifera	South Africa	OLIF-2	M41	100	-	Present study
Tarebia granifera	South Africa	G-LETA	M71a	100	-	Present study
Tarebia granifera	South Africa	LETA	H59	100	-	Present study
Tarebia granifera	China	-	-	100	MZ662113	Yin et al. (2022)
Tarebia granifera	Thailand	-	-	100	MK000375	Veeravechsukij et al. (2018b)
Tarebia granifera	Thailand	-	-	98.2	MK000294	Veeravechsukij et al. (2018b)
Tarebia granifera	USA	-	-	98.2	MT671805	Tolley-Jordan and Triplett (2020)
Tarebia granifera	USA	-	-	98.1	AY958764	Genner et al. (2007)
Tarebia granifera	Singapore	-	-	96.9	AY958760	Genner et al. (2007)
Tarebia granifera	Singapore	-	-	96.4	AY958762	Genner et al. (2007)
Tarebia lineata (Gray, 1828)	India	-	-	83.9	KY511306	Jena and Srirama (2017)
Tarebia lineata	Malaysia	-	-	85.8	MW591171	Jamaluddin et al. (2022)
Melanoides tuberculata	Tanzania	-	-	85.6	AY791909	Von Gersdorff Sørensen et al. (2005)

Chapter 4

Table S4.1: Summary data for trematodes transmitted by *Tarebia granifera*.

Trematodes species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Acanthatrium histaense (Koga, 1953)	Lecithodendriidae	*Tarebia granifera*	Insects, Amphibia	Thailand (Veeravechsukij et al. 2018a; Dechruksa et al. 2007)
Acanthotrema tridactyla (Martin and Kuntz, 1955)	Heterophyidae	*Tarebia granifera*	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij et al. 2018a)
Alaria mustelae (Bosma, 1931)	Diplostomidae	*Tarebia granifera*	Amphibia, Mammals	Thailand (Veeravechsukij et al. 2018a; Bosma, 1934)
Cardicola alseae (Meade and Pratt, 1965)	Sanguinicolidae	*Tarebia granifera*	Fish	Thailand (Veeravechsukij et al. 2018a; Ukong et al. 2007)
Centrocestus formosanus (Nishigori, 1924)	Heterophyidae	*Tarebia granifera*	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij et al. 2018a; Dechruksa et al. 2007)
Diorchitrema formosanum (Katsuta, 1932)	Heterophyidae	*Tarebia granifera*	Fish, Mammals	Taiwan (Abbott, 1952)
Haematoloechus similis (Looss, 1899)	Haematoloechidae	*Tarebia granifera*	Amphibia	Thailand (Dechruksa et al. 2007; Grabda, 1960)
Haplorchis pumilio (Looss, 1896)	Heterophyidae	*Tarebia granifera*	fish, Birds, Mammals, Humans	Thailand (Veeravechsukij et al. 2018a)
Haplorchis sp.	Heterophyidae	*Tarebia granifera*	Fish, Birds, Mammals, Humans	Laos (Giboda et al. 1991)
Haplorchis taichui (Nishigori, 1924)	Heterophyidae	*Tarebia granifera*	Fish, Birds, Mammals, Humans	Thailand (Veeravechsukij et al. 2018a; Le et al. 2017; Ditrich et al. 1992; Chontananarth and Wongsawad, 2010; Chontananarth and Wongsawad, 2013)

Table S4.1 continued

Trematodes species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Loxogenes liberum (Seno, 1907)	Lecithodendriidae	*Tarebia granifera*	Insects, amphibians	Thailand (Veeravechsukij et al. 2018a)
Loxogenoides bicolor (Kaw, 1945)	Lecithodendriidae	*Tarebia granifera*	Insects, amphibians	Thailand (Veeravechsukij et al. 2018a; Dechruksa et al. 2007)
Maritreminoides caridinae (Yamaguti and Nisimura, 1944)	Microphallidae	*Tarebia granifera*	Crustacea, birds	Thailand (Veeravechsukij et al. 2018a)
Maritreminoides obstipus (Van Cleave and Mueller, 1932)	Microphallidae	*Tarebia granifera*	Crustacea, birds	Thailand (Veeravechsukij et al. 2018a)
Metagonimus yokogawai (Katsurada, 1912)	Heterophyidae	*Tarebia granifera*	Fish, mammals, humans	Japan, Korea, Formosa, Taiwan, India (Abbott, 1952)
Philophthalmus gralli (Mathis and Léger, 1910)	Philophthalmidae	*Tarebia granifera*	Birds, mammals, humans	Thailand (Veeravechsukij et al. 2018a), United States (Heneberg et al. 2014; Tolley-Jordan and Owen 2008)
Philophthalmus sp.	Philophthalmidae	*Tarebia granifera*	Birds, mammals, humans	Jamaica (McKoy et al. 2011)
Transversotrema laruei (Velasquez, 1958)	Transversotrematidae	*Tarebia granifera*	Fish	Thailand (Veeravechsukij et al. 2018a)
Notocotylus sp. (Diesing, 1839)	Notocotylidae	*Tarebia granifera*	?	Jamaica (McKoy et al. 2011)

Table S4.2: Summary data for trematodes transmitted by *Melanoides tuberculata*. Data were adapted from Pinto and de Melo (2011).

Trematodes species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Acanthatrium histaense (Koga, 1953)	Lecithodendriidae	*Melanoides tuberculata*	Insecta, Amphibia	Thailand (Krailas et al. 2014)
Acanthatrium sp.	Lecithodendriidae	*Melanoides tuberculata*	Insecta, Amphibia	Vietnam (Besprozvannykh et al. 2013)
Acanthostomum burminis (Bhalerao, 1926)	Cryptogonimidae	*Melanoides tuberculata*	Fish, Amphibia	India (Madhavi et al. 1997; Roopa and Janardanan, 1998)
Acanthotrema tridactyla (Martin and Kuntz, 1955)	Heterophyidae	*Melanoides tuberculata*	Fish, birds, mammals	Thailand (Krailas et al. 2014)
Alaria mustelae (Bosma, 1931)	Diplostomidae	*Melanoides tuberculata*	Amphibia, mammals	Thailand (Krailas et al. 2014)
Apatemon gracilis (Szidat, 1928)	Strigeidae	*Melanoides tuberculata*	Fish	Thailand (Krailas et al. 2014)
Calicophoron microbothrium (Fischoeder, 1901)	Paramphistomidae	*Melanoides tuberculata*	Mammals	Zimbabwe (Chingwena et al. 2002)
Cardicola alseae (Meade and Pratt, 1965)	Sanguinicolidae	*Melanoides tuberculata*	Fish	Thailand (Krailas et al. 2014)
Centrocestus caninus (Leiper, 1913)	Heterophyidae	*Melanoides tuberculata*	Mammals	Taiwan (Waikagul et al. 1990)
Centrocestus formosanus (Nishigori, 1924)	Heterophyidae	*Melanoides tuberculata*	Fish, birds, mammals	China (Chen, 1948; Chao et al. 1993), Japan (Yanohara, 1985; Yanohara et al. 1987), Mexico (Arizmendi-Espinosa, 1992; Amaya-Huerta and Almeyda-Artigas, 1994; Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado, 2000; Scholz et al. 2000; Ortega et al. 2009), Malaysia (Bayssade-Dufour et al. 1982), Taiwan (Lo and Lee, 1996a; 1996b), India (Madhavi et al. 1997), United States (Mitchell et al. 2002; 2005; Tolley-Jordan and Owen 2008; Huston, 2014),

Table S4.2 continued

Trematodes species	Family	1st Intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
				Venezuela (Hernández et al. 2003), Iran (Farahnak et al. 2005), Colombia (Velásquez et al. 2006; Vergara and Velásquez, 2009), Brazil (Pinto and de Melo, 2010a; Pulido-Murillo et al. 2018; Ciccheto et al. 2021), Thailand (Krailas et al. 2014), Peru (Pulido-Murillo et al. 2018)
Centrocestus unequiorchalis (Saad, 1994)	Heterophyidae	*Melanoides tuberculata*	Birds, mammals	Egypt (Saad, 1994)
Cloacitrema philippinum (Velasquez, 1969)	Philophthalmidae	*Melanoides tuberculata*	Birds	Thailand (Krailas et al. 2014)
Clonorchis sinensis (Cobbold, 1875)	Opisthorchiidae	*Melanoides tuberculata*	Mammals	China (Galliard, 1938; Lun et al. 2005), Vietnam (Kino et al. 1998), unspecified Asian country (Chai et al. 2005)
Echinochasmus bagulai (Verma, 1935)	Echinostomatidae	*Melanoides tuberculata*	Birds	India (Dhanumkumari et al. 1991a)
Echinochasmus japonicus (Tanabe, 1926)	Echinostomatidae	*Melanoides tuberculata*	Fish, birds, mammals	China (Cheng and Fang, 1989)
Echinochasmus milvi (Yamaguti, 1939)	Echinostomatidae	*Melanoides tuberculata*	Birds and mammals	Iran (Farahnak et al. 2005)
Echinochasmus pelecani (Johnston and Simpson, 1944)	Echinostomatidae	*Melanoides tuberculata*	Birds	Thailand (Krailas et al. 2014)
Eumegacetes artamii (Mehra, 1935)	Eumegacetidae	*Melanoides tuberculata*	Birds	India (Kumari and Madhavi, 1994; Madhavi et al. 1997)
Eumegacetes spinosus (Fahmy, Khalifa and Abdel-Rahman, 1981)	Eumegacetidae	*Melanoides tuberculata*	Birds	Egypt (Sakla and Khalifa, 1983)
Gastrodiscus aegyptiacus (Cobbold, 1876)	Gastrodiscidae	*Melanoides tuberculata*	Mammals	Zimbabwe (Mukaratirwa et al. 2004)
Gastrothylax crumenifer (Creplin, 1847)	Paramphistomidae	*Melanoides tuberculata*	Mammals	Thailand (Krailas et al. 2014)

Table S4.2 continued

Trematodes species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Gigantobilharzia sp.	Schistosomatidae	*Melanoides tuberculata*	Birds	Egypt (Fahmy et al. 1976), United Arab Emirates (Schuster et al. 2014)
Grysoma indica (Umadevi and Madhavi, 1995)	Psilostomidae	*Melanoides tuberculata*	Birds	India (Umadevi and Madhavi, 1995; Madhavi et al. 1997)
Haematoloechus similis (Looss, 1899)	Haematoloechidae	*Melanoides tuberculata*	Amphibia	Thailand (Krailas et al. 2014)
Haplorchis pleurolophocerca (Sonsino, 1896)	Heterophyidae	*Melanoides tuberculata*	Birds, mammals	Egypt (Faust and Nishigori, 1926; Khalil, 1932)
Haplorchis pumilio (Looss, 1896)	Heterophyidae	*Melanoides tuberculata*	Fish, birds, mammals	China (Shen, 1959; Chao et al. 1993; Wang et al. 2002), Malaysia (Ow-Yang and Yen, 1975), Egypt (Khalil, 1932; Khalifa et al. 1977; Saad and Abed, 1995), Kenya (Sommerville, 1982b), India (Dhanumkumari et al. 1991b; Madhavi et al. 1997; Umadevi and Madhavi, 2006; Dechruksa et al. 2007), Taiwan (Lo and Lee, 1996a; 1996b), Mexico (Scholz et al. 2000; Scholz et al. 2001), Iran (Farahnak et al. 2005), Thailand (Dechruksa et al. 2007; Krailas et al. 2011; 2014), United States (Tolley-Jordan and Owen 2008; Huston, 2014), Venezuela (Díaz et al. 2008), Vietnam (Skov et al. 2009; Van et al. 2009), Brazil (Lopes et al. 2020), Peru (Pulido-Murillo et al. 2018)

Table S4.2 continued

Trematode species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Haplorchis sp.	Heterophyidae	*Melanoides tuberculata*	Fish	United States (Harvey et al. 2005), Zimbabwe (Schols, 2019), Vietnam (Besprozvannykh et al. 2013)
Haplorchis taichui (Nishigori, 1924)	Heterophyidae	*Melanoides tuberculata*	Fish, Birds, Mammals	India (Dhanumkumari et al. 1991b; Madhavi et al. 1997), Iran (Farahnak et al. 2005), Thailand (Chuboon and Wongsawad, 2009; Chontananarth and Wongsawad, 2010; Krailas et al. 2011; 2014)
Haplorchis yokogawai (Katsuta, 1932)	Heterophyidae	*Melanoides tuberculata*	Mammals	Thailand (Manning et al. 1971), (Chai et al. 2005)
Haplorchoides cahirinus (Looss, 1896)	Heterophyidae	*Melanoides tuberculata*	Fish	Egypt (El-Naffar, 1980)
Haplorchoides mehrai (Pande and Shunkla, 1977)	Heterophyidae	*Melanoides tuberculata*	Fish	India (Shameem and Madhavi, 1988; Madhavi et al. 1997)
Heterophyes heterophyes (Siebold, 1852)	Heterophyidae	*Melanoides tuberculata*	Mammals	(Malek, 1980; Plotnikov, 2003)
Heterophyidae sp.	Heterophyidae	*Melanoides tuberculata*	?	Brazil (Bogéa et al. 2005)
Lecithodendriidae sp.	Lecithodendriidae	*Melanoides tuberculata*	Amphibia, birds	Zimbabwe (Schols, 2019), Brazil (Lopes et al. 2021)
Loxogenoides bicolor (Kaw, 1945)	Lecithodendriidae	*Melanoides tuberculata*	Insecta, Amphibia	Iran (Farahnak et al. 2005), Thailand (Dechruksa et al. 2007; Ukong et al. 2007; Krailas et al. 2014)
Mesostephanus appendiculatus (Ciurea, 1916)	Cyathocotylidae	*Melanoides tuberculata*	Fish, mammals	Thailand (Krailas et al. 2014).
Mesostephanus haliasturis (Tubangui and Masilungan, 1941)	Cyathocotylidae	*Melanoides tuberculata*	Birds	Australia (Barker and Cribb, 1993)
Microparyphium sp.	Echinostomatidae	*Melanoides tuberculata*	Fish	Vietnam (Besprozvannykh et al. 2013)

Table S4.2 continued

Trematode species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
Neopronocephalus triangularis (Mehra, 1932)	Pronocephalidae	*Melanoides tuberculata*	Reptiles	India (Thapar, 1968)
Notocotylus mamii (Hsu, 1954)	Notocotylidae	*Melanoides tuberculata*	Mammals	China (Hsu, 1957)
Orthetrotrema monostomum (Macy and Basch, 1972)	Eumegacetidae	*Melanoides tuberculata*	Birds	India (Madhavi and Swamakumari, 1995; Madhavi et al. 1997)
Paragonimus westermani (Kerbert, 1878)	Paragonimidae	*Melanoides tuberculata*	Mammals	Taiwan (Nakagawa, 1917), China (Blair et al. 1999), unspecified Asian country (Davis et al. 1994)
Paralecithodendrium pyramidium (Looss, 1896)	Lecithodendriidae	*Melanoides tuberculata*	Mammals	Egypt (Azim, 1936)
Paralecithodendrium sp.	Lecithodendriidae	*Melanoides tuberculata*	Mammals	Vietnam (Besprozvannykh et al. 2013)
Paramonostomum aegyptiacus (Khalifa and El-Naffar, 1978)	Notocotylidae	*Melanoides tuberculata*	Mammals	Egypt (Khalifa and El-Naffar, 1978)
Philophthalmus palpebrarum (Looss, 1899)	Philophthalmidae	*Melanoides tuberculata*	Birds	Egypt (Ayoub et al. 2020)
Philophthalmus distomatosa (Looss, 1896)	Philophthalmidae	*Melanoides tuberculata*	Birds, mammals	Israel (Radev et al. 2000)
Philophthalmus gralli (Mathis and Leger, 1910)	Philophthalmidae	*Melanoides tuberculata*	Birds, mammals	United States (Murray and Haines, 1969; Nollen and Murray, 1978; Tolley-Jordan and Owen 2008; Church et al. 2013), China (Zhongzhang et al. 1980), Jordan (Ismail and Issa, 1987), India (Rao and Rao, 1981; Karim, 1982; Saxena, 1984), United Arab Emirates (Ismail and Arif, 1992), Saudi Arabia (Kalantan et al. 1997), Mexico (Scholz et al. 2000), Venezuela (Díaz et al. 2002), Zimbabwe (Mukaratirwa et al. 2005),

Table S4.2 continued

Trematode species	Family	1st intermediate hosts	2nd intermediate/ definitive host	Parasite distribution (reference)
				Brazil (Pinto and de Melo, 2010b), Costa Rica (Rojas et al. 2013)
Philophthalmus nocturnus (Looss, 1907)	Philophthalmidae	*Melanoides tuberculata*	Birds	India (Madhavi et al. 1997)
Philophthalmus sp.	Philophthalmidae	*Melanoides tuberculata*	Birds	Thailand (Krailas et al. 2014), United Arab Emirates (Schuster et al. 2014)
Podocotyle lepomis (Dobrovolny, 1939)	Opecoelidae	*Melanoides tuberculata*	Fish	Thailand (Krailas et al. 2014)
Procerovum cheni (Hsu, 1950)	Heterophyidae	*Melanoides tuberculata*	Birds	China (Hsu, 1951)
Procerovum varium (Onji and Nishio, 1916)	Heterophyidae	*Melanoides tuberculata*	Birds	China (Hsu, 1951), India (Madhavi et al. 1997; Umadevi and Madhavi, 2000; Arya et al. 2016)
Pygidiopsis genata (Looss, 1907)	Heterophyidae	*Melanoides tuberculata*	Mammals	Egypt (Youssef et al. 1987)
Stellantchasmus falcatus (Onji and Nishio, 1915)	Heterophyidae	*Melanoides tuberculata*	Birds, Mammals	Iran (Farahnak et al. 2005)
Transversotrema laruei (Velasguez, 1958)	Transversotrematidae	*Melanoides tuberculata*	Fish	Thailand (Krailas et al. 2014)
Transversotrema patialense (Soparkar, 1924)	Transversotrematidae	*Melanoides tuberculata*	Fish	India (Soparkar, 1924; Anantaraman, 1948; Rao and Ganapati, 1967; Nadakal et al. 1969; Pandey, 1971; Madhavi et al. 1997), Sri Lanka (Crusz, 1956), Israel (Ben-Ami et al. 2005), Thailand (Ukong et al. 2007)

Chapter 5

Table S5.1: Number of individuals collected at each of the sites in the Limpopo Lowveld Ecoregion sampled during the high-flow period.

Limpopo high-flow species collection				
Site description	Site code	*Tarebia granifera*	*Melanoides tuberculata*	*Physella acuta*
Limpopo River	LHF1	36		
Limpopo River	LHF2	37		
Limpopo River	LHF3	5	932	
Olifants River	LHF4	1 258		
Shingwedzi River	LHF5	43		
Luvuvhu River	LHF6	434		
Groot Letaba River	LHF7	139		2
Letaba River	LHF8	107		
Olifants River	LHF9	112		
Total number of individuals collected		2 171	932	2

Table S5.2: Number of individuals collected at each of the sites in the Limpopo Lowveld Ecoregion sampled during the low-flow period.

Limpopo low-flow species collection								
Site description	Site code	*Physella acuta*	*Bulinus* group	*Biomphalaria pfeifferi*	*Gyraulus costulatus*	*Radix natalensis*	*Pseudosuccinea columella*	*Tarebia granifera*
Hex River	LLF1					2		
Roodekopjes Dam	LLF2	121		111				
Crocodile River	LLF3					79		
Buffelspruit	LLF4					18		
Donkerpoort Dam	LLF5					6		
Klein Sand River	LLF6		28			17		
Mokolo River	LLF7		29			1		
Bloed River	LLF8		52		22	7		
Stanford Lake	LLF9					24	22	
Tzaneen Dam	LLF10					57		
Mothomeng Dam	LLF11		55			5		
Middle Letaba Dam	LLF12		24		80			16
Luvuvhu River	LLF13		1				6	16
Magic Dam	LLF14		14			5		
Dam, Valdezia	LLF15		18					
Luvuvhu River	LLF16	17	4		38		15	
Total number of individuals collected		138	225	111	140	221	43	32

Table S5.3: Qualitative habitat assessment observed at the Limpopo high- and low-flow sites collected in the high-flow (HF) and low-flow (LF) sampling seasons during the current study .

Limpopo high flow sites											
Date	Site code	GPS coordinates	Weather	Habitat Type	Habitat Assessment						
					Marginal	Floating	Emergent	Submerged	Algae	Stone	GSM
2021/04/22	LHF1	22°26'47.9"S 28°52'44.6"E	Sunny, Warm	Limpopo River	Yes	No	Yes	No	Yes	Yes	Yes
2021/04/25	LHF2	22°28'54.5"S 28°55'07.1"E	Sunny, Warm	Mogalokwena	Yes	No	No	No	No	Yes	Yes
2021/04/26	LHF3	23°56'40.9"S 26°55'50.9"E	Sunny, Warm	Limpopo River	No	No	No	No	No	No	Yes
2021/04/29	LHF4	24°03'14.7"S 31°43'35.1"E	Sunny, Warm	Olifants River	Yes	No	Yes	No	No	Yes	No
2021/05/03	LHF5	23°13'15.8"S 31°33'18.4"E	Sunny, Warm	Shingwedzi River	Yes	No	Yes	No	No	No	Yes
2021/05/04	LHF6	22°25'45.4"S 31°15'27.4"E	Sunny, Warm	Luvuvhu River	No	No	No	No	No	Yes	Yes
2021/05/05	LHF7	23°40'39.1"S 31°05'55.1"E	Sunny, Warm	Groot Letaba River	Yes	No	Yes	No	Yes	Yes	Yes
2021/05/06	LHF8	23°56'36.2"S 31°44'06.4"E	Sunny, Warm	Letaba River	Yes	No	Yes	No	No	Yes	Yes
2021/05/07	LHF9	24°04'00.2"S 31°14'33.0"E	Sunny, Warm	Olifants River	Yes	No	Yes	No	No	Yes	Yes
Limpopo low flow sites											
Date	Site code	GPS Coordinates	Weather	Habitat Type	Habitat Assessment						
					Marginal	Floating	Emergent	Submerged	Algae	Stone	GSM
2021/08/23	LLF1	25°31'18,85"S 27°22'55,3"E	Sunny, Warm	Hex River	Yes	No	No	Yes	No	Yes	Yes
2021/08/23	LLF2	25°23'0.6936"S 27°35'54.8052"E	Sunny, Warm	Roodekopjes Dam	Yes	Yes	Yes	Yes	Yes	No	Yes
2021/08/23	LLF3	25°22'25,3"S 27°33'59.6"E	Sunny with a light breeze	Crocodile River	Yes	Yes	No	No	No	No	Yes
2021/08/23	LLF4	24°45'43.848"S 28°13'3.0324"E	Sunny, Warm	Buffelspruit	Yes	Yes	Yes	Yes	Yes	No	Yes
2021/08/24	LLF5	24°40'14.2716"S 28°19'12.8604"E	Overcast & Cool	Donkerpoort Dam	Yes	No	Yes	Yes	Yes	Yes	Yes
2021/08/24	LLF6	24°24'31.4208"S 28°7'20.2224"E	Overcast & Cool	Klein sand River	Yes	Yes	No	Yes	Yes	Yes	Yes
Date	Site code	GPS Coordinates	Weather	Habitat Type	Habitat Assessment						
					Marginal	Floating	Emergent	Submerged	Algae	Stone	GSM
2021/08/24	LLF7	24°16'32.3724"S 28°5'0.9708"E	Sunny, Warm	Mokolo River	Yes	Yes	No	Yes	Yes	No	Yes
2021/08/24	LLF8	23°50'49.56"S 29°21'37.9008"E	Sunny, Warm	Bloed River	Yes	Yes	Yes	Yes	Yes	No	Yes
2021/08/25	LLF9	23°54'41.22"S 29°58'53.4216"E	Sunny, Windy (cool)	Stanford Lake	Yes	Yes	Yes	None	Yes	No	yes
2021/08/25	LLF10	23°45'47.6172"S 30°8'30.3612"E	Overcast & windy	Tzaneen Dam	No	No	Yes	Yes	Yes	No	Yes
2021/08/25	LLF11	23°41'57.2712"S 30°19'35.5296"E	Sunny with a light breeze	Mothomeng Dam	Yes	Yes	Yes	No	Yes	Yes	Yes
2021/08/25	LLF12	23°16'34.2084"S 30°23'51.1044"E	Sunny, Warm	Middle Letaba Dam	Yes	No	Yes	Yes	Yes	No	Yes
2021/08/26	LLF13	23°6'24.408"S 30°24'4.1184"E	Sunny, partly cloudy	Luvuvhu River	Yes	No	No	No	Yes	No	Yes
2021/08/26	LLF14	23°5'20.7132"S 30°23'1.6836"E	Sunny, Warm	Magic Dam	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2021/08/26	LLF15	23°7'22.0152"S 30°13'7.6116"E	Sunny, Warm	Dam, Valdezia	Yes	Yes	No	Yes	Yes	No	Yes
2021/08/26	LLF16	23°6'25.0416"S 30°7'32.8044"E	Sunny, Warm	Luvuvhu River	No	No	No	No	No	Yes	Yes

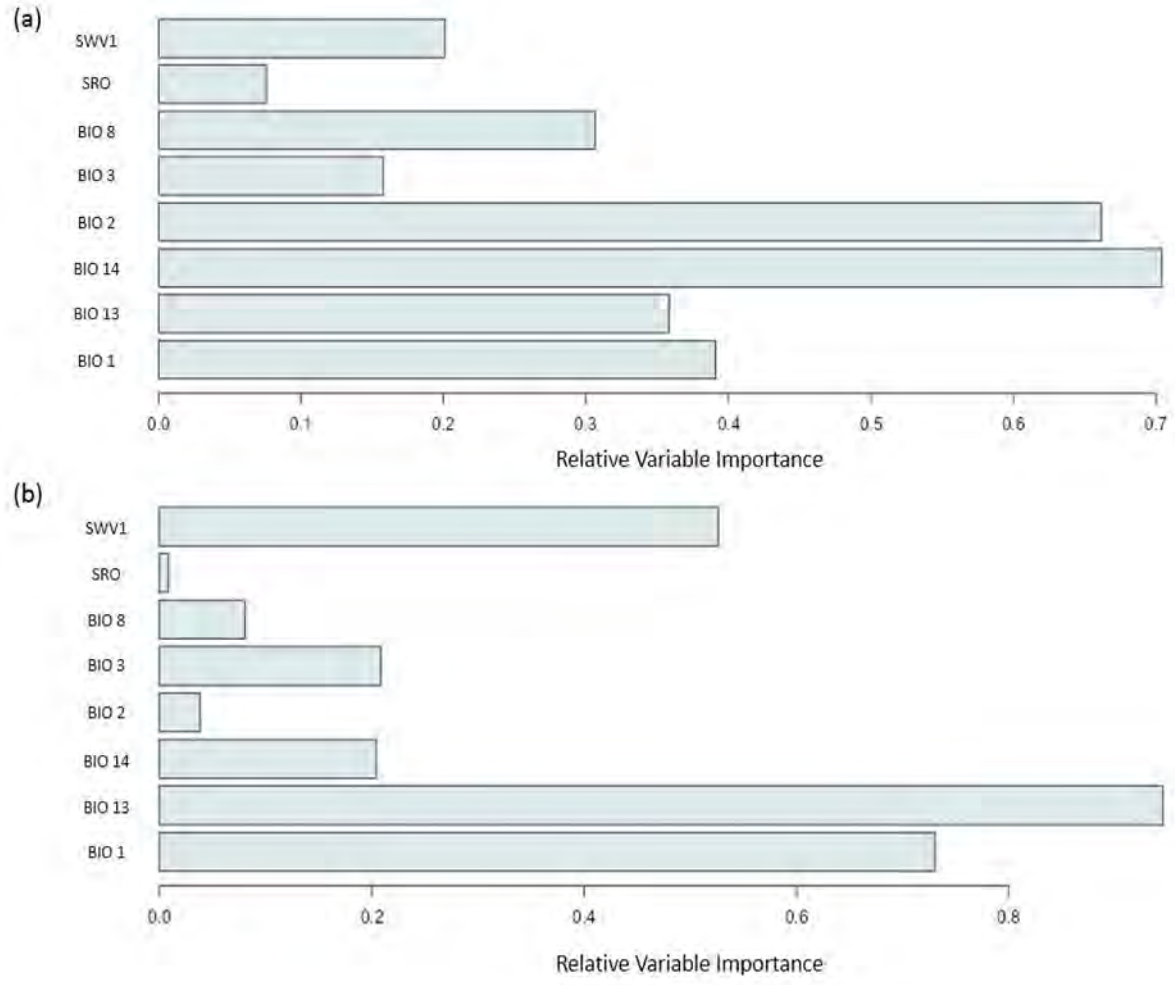

Figure S6.1: Jackknife test results for *Biomphalaria pfeifferi* snail species in the Mbombela and Nkomazi local municipalities with estimates of important variables when running (a) the maximum entropy model, and (b) the generalised linear model.

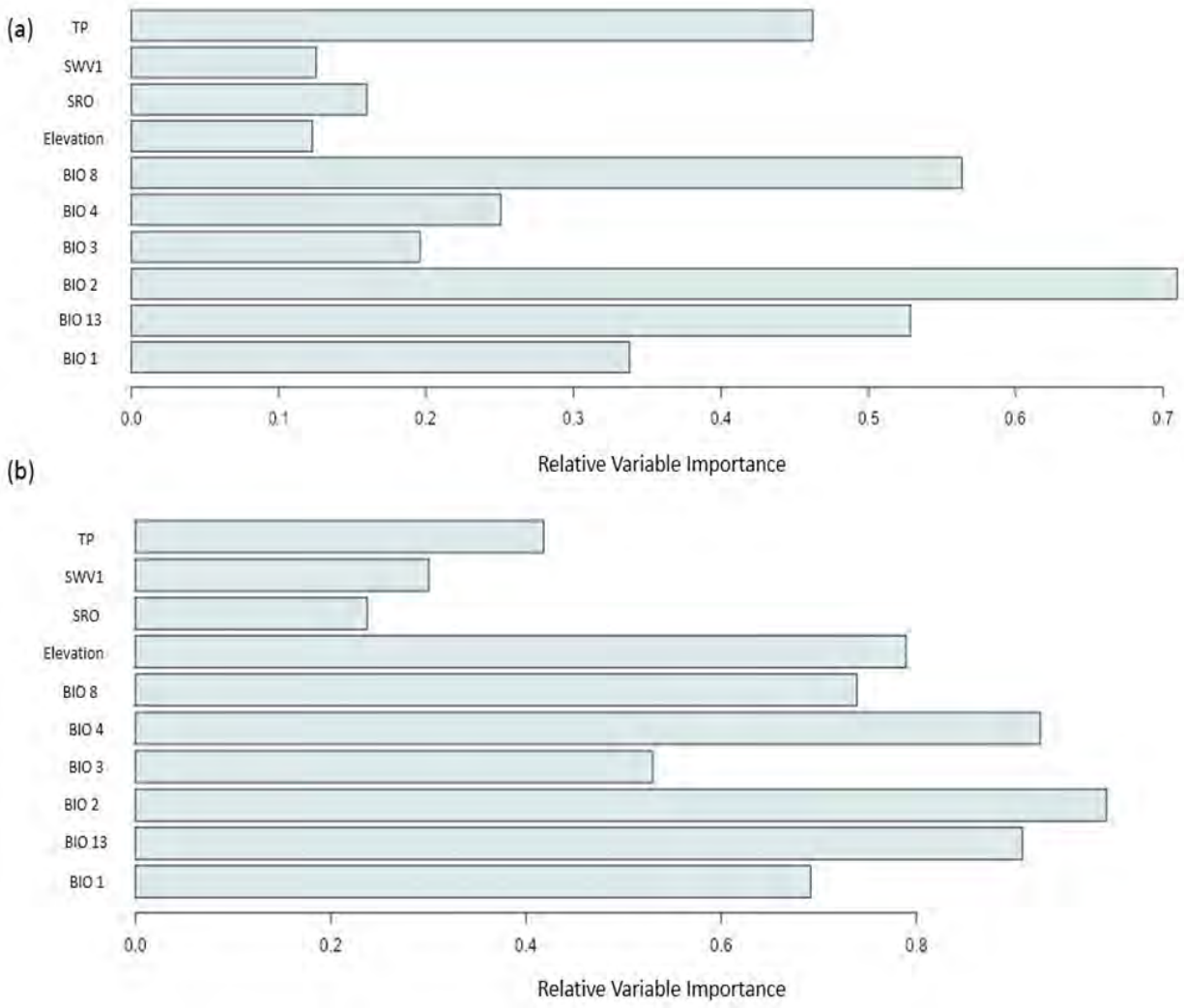

Figure S6.2: Jackknife test results for *Bulinus globosus* snail species in the Mbombela and Nkomazi local municipalities with estimates of important variables when running (a) the maximum entropy model, and (b) the generalised linear model.

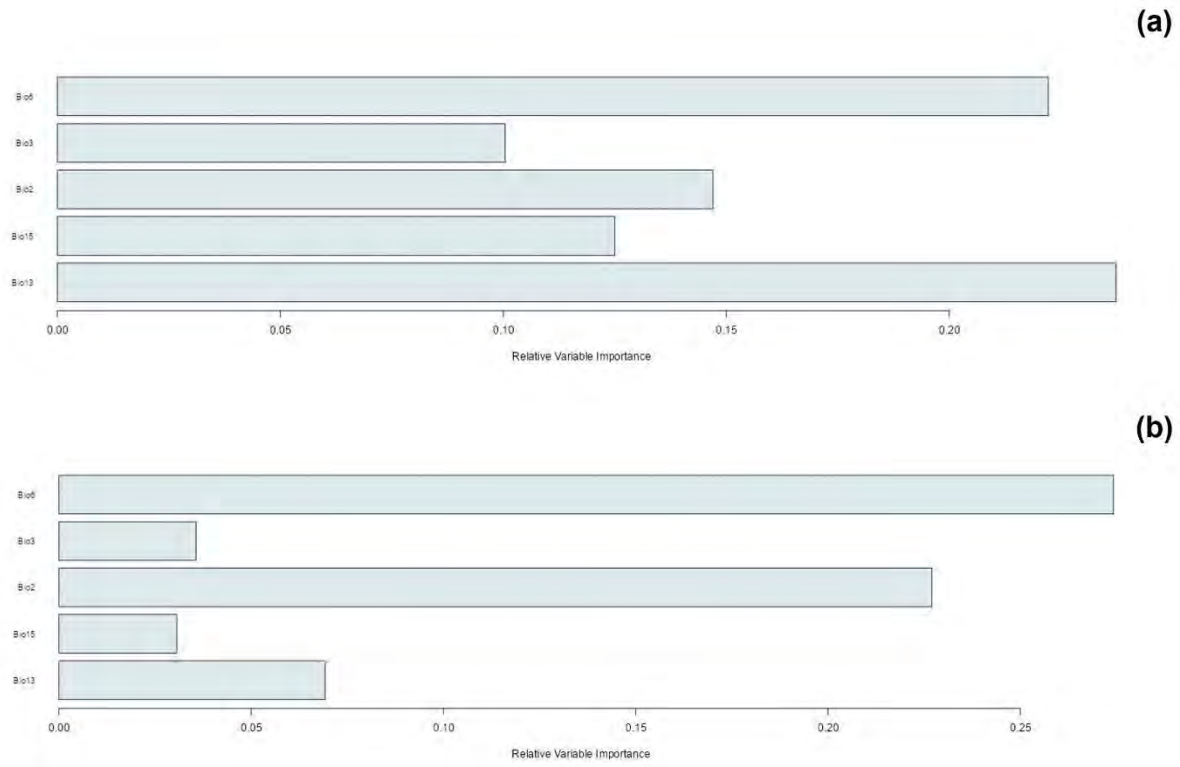

Figure S6.3: Relative variable importance of selected bioclimatic variables (BIO 6, BIO 3, BIO 2, BIO 15 and BIO 13) for *Biomphalaria pfeifferi* snail species using a) Random Forest, and b) maximum entropy in the Vhembe District Municipality.

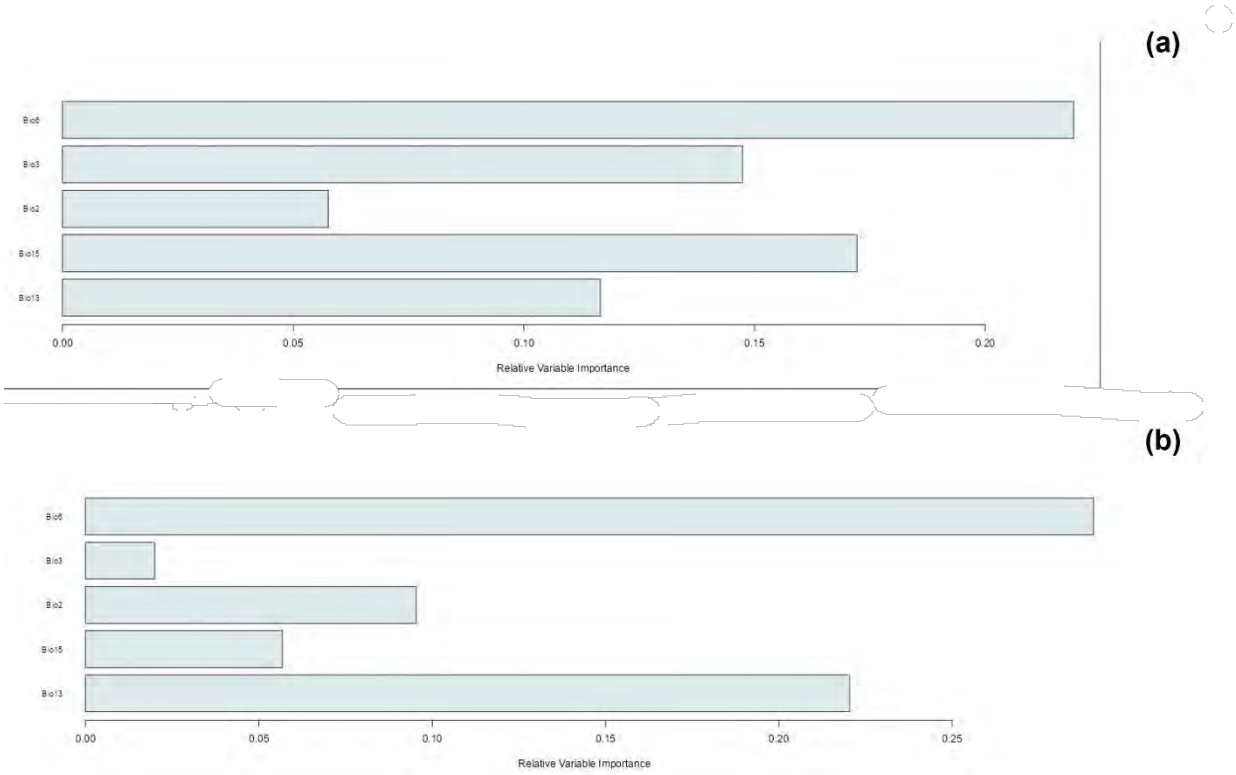

Figure S6.4: Relative variable importance of selected bioclimatic variables (BIO 6, BIO 3, BIO 2, BIO 15 and BIO 13) for *Bulinus globosus* snail species for (a) Random Forest; and (b) maximum entropy in the Vhembe District Municipality.

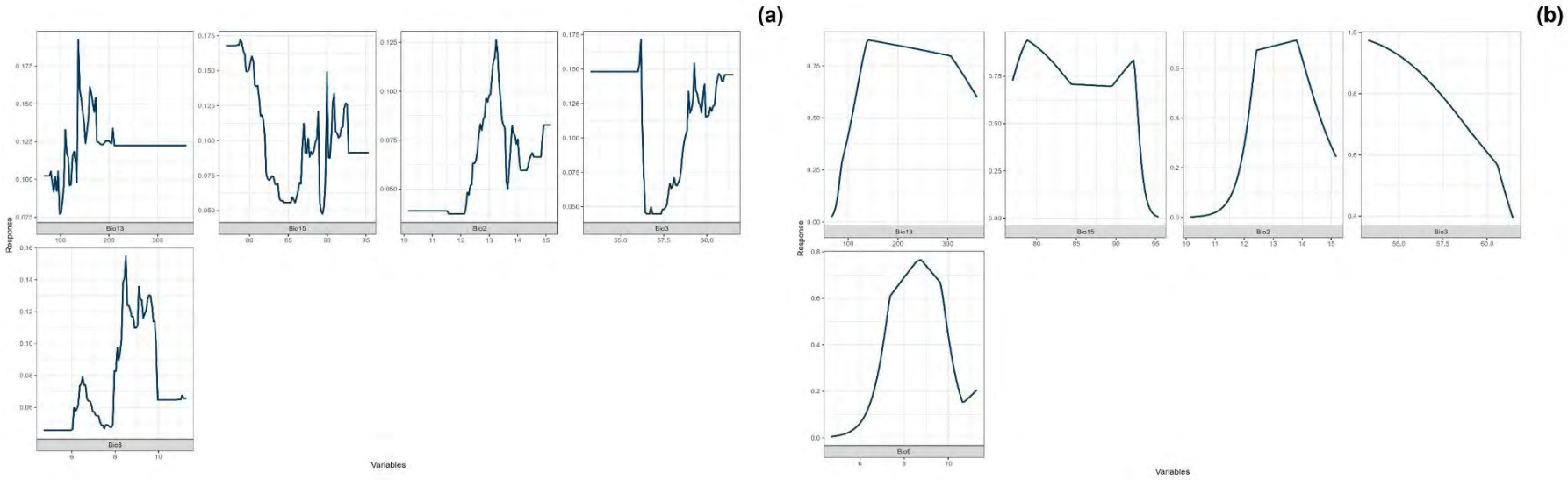

Figure S6.5: The response curves generated using (a) Random Forest; and (b) maximum entropy showing the relationship between *Bulinus globosus* and selected bioclimatic variables (BIO 13, BIO 15, BIO 3, BIO 2, BIO 6) in the Vhembe District Municipality.

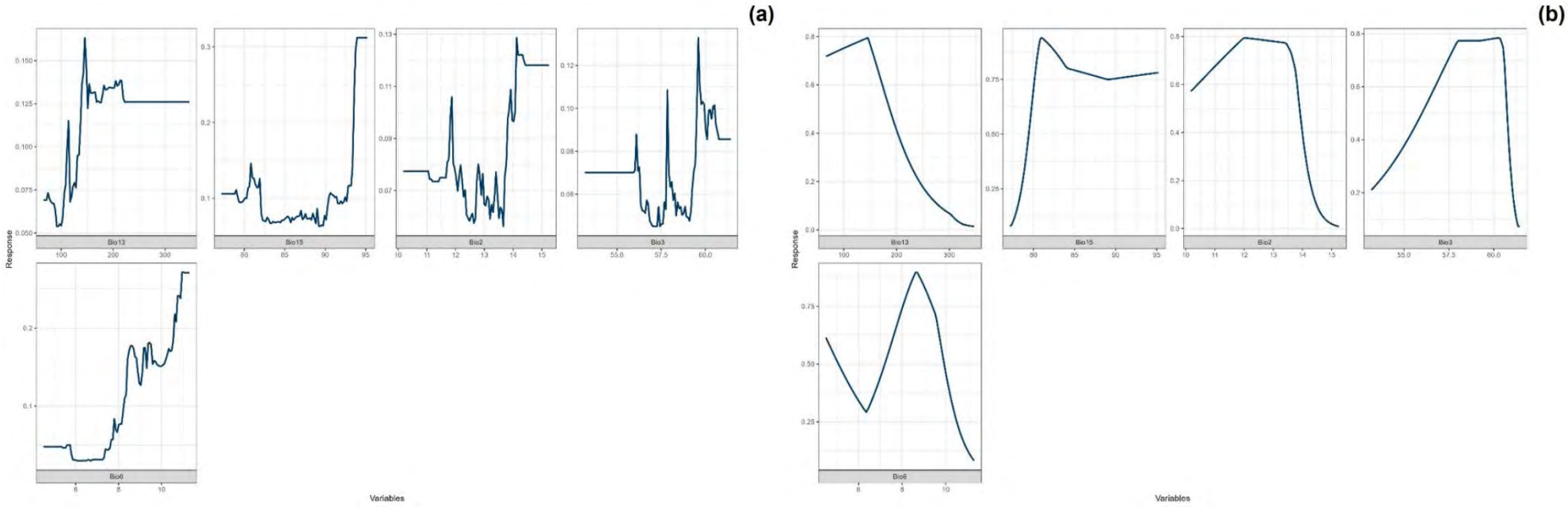

Figure S6.6: The response curves generated using (a) Random Forest; and (b) maximum entropy showing the relationship between *Biomphalaria pfeifferi* and selected bioclimatic variables (BIO 13, BIO 15, BIO 3, BIO 2, BIO 6) in the Vhembe District Municipality.

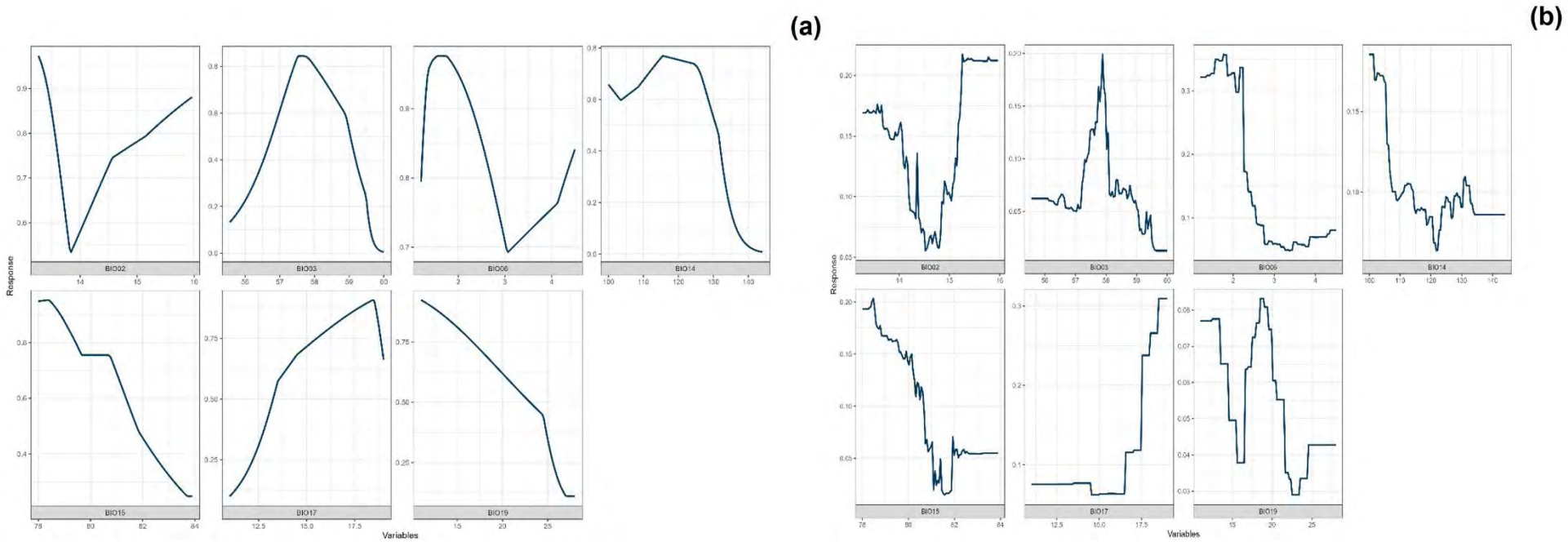

Figure S6.7: The response curves generated using (a) maximum entropy; and (b) Random Forest showing the relationship between *Biomphalaria pfeifferi* and selected bioclimatic variables (BIO 2, BIO 3, BIO 6, BIO 14, BIO 15, BIO 17, BIO 19) in the Tshwane Metropolitan Municipality.

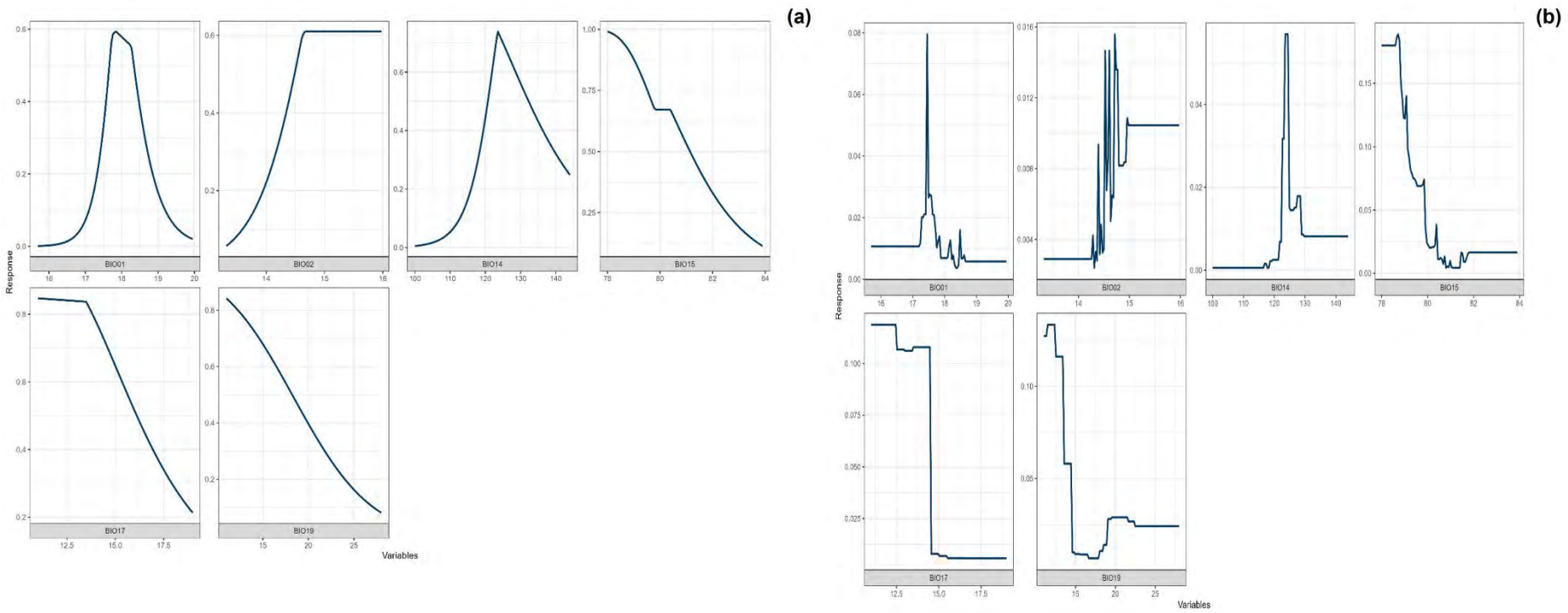

Figure S6.8: The response curves generated using (a) maximum entropy; and (b) Random Forest showing the relationship between *Bulinus africanus* and selected bioclimatic variables (BIO 1, BIO 2, BIO 14, BIO 15, BIO 17 and BIO 19) in the Tshwane Metropolitan Municipality.

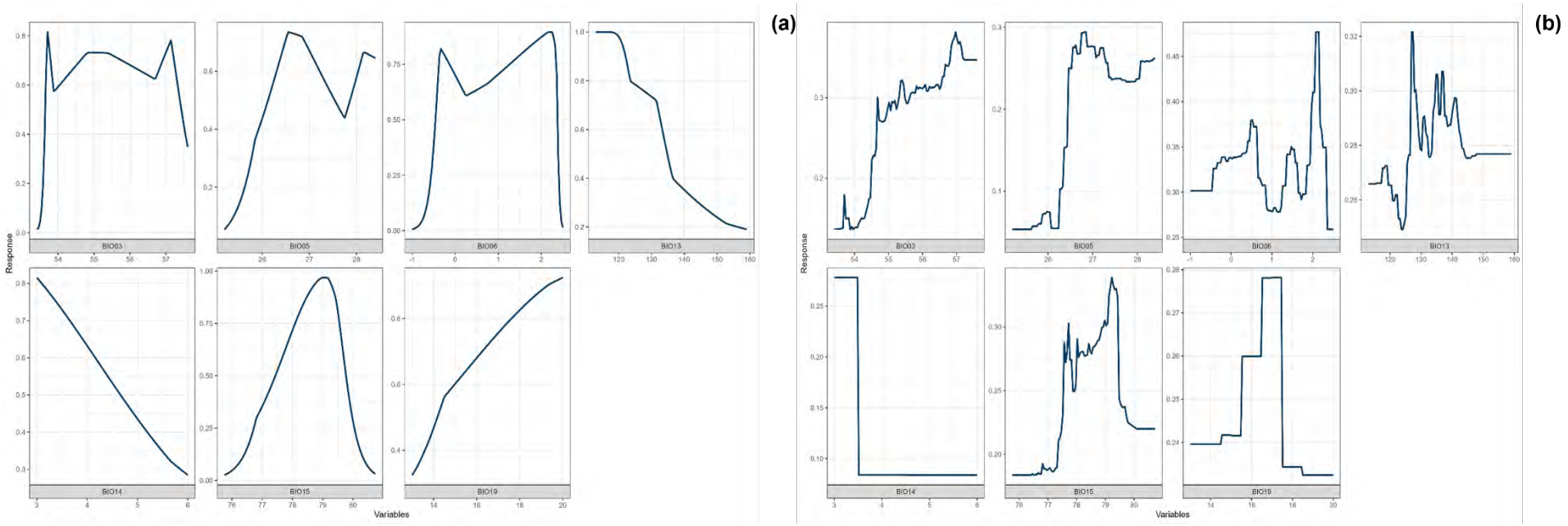

Figure S6.9: The response curves generated through (a) MaxEnt, and (b) Random Forest for predicting habitat suitability for *Bulinus africanus* based on a set of bioclimatic factors (BIO 3, BIO 5, BIO 6, BIO 13, BIO 14, BIO 15 and BIO 19) in the Johannesburg Metropolitan Municipality.

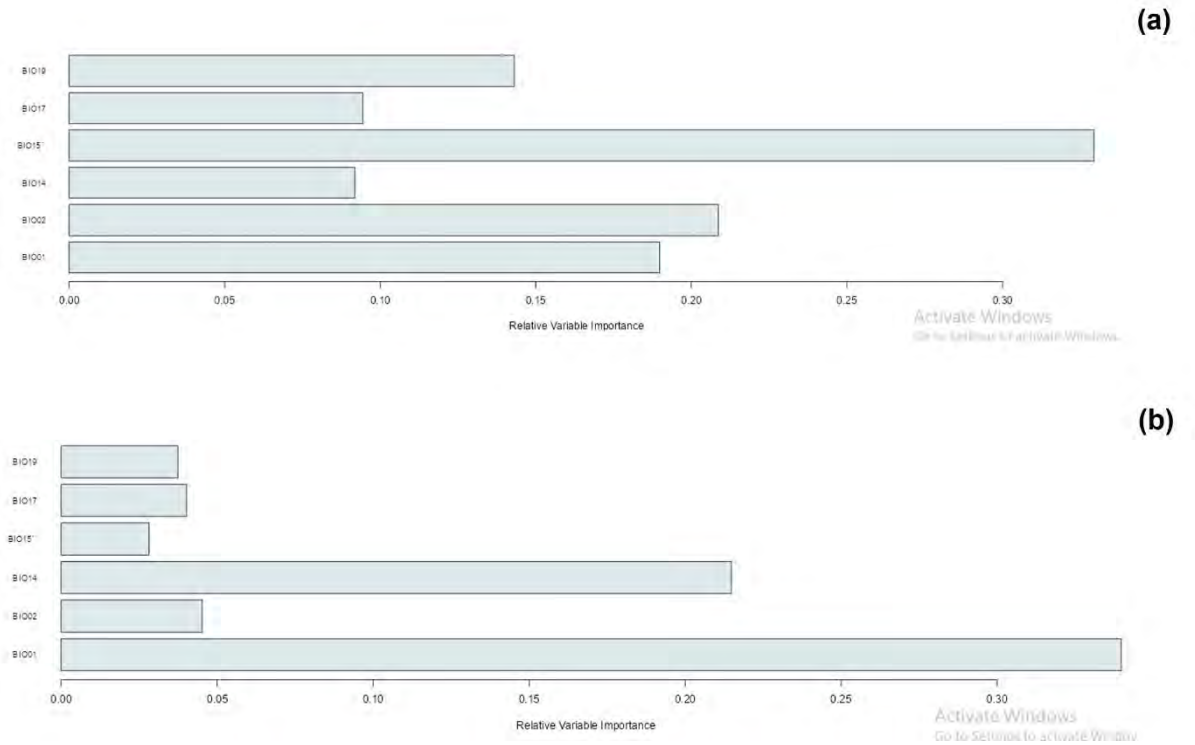

Figure S6.10: Relative variable importance of selected bioclimatic variables (BIO 1, BIO 2, BIO 14, BIO 15, BIO 17 and BIO 19) to *Biomphalaria pfeifferi* snail species using (a) Random Forest, and (b) maximum entropy models in the Tshwane Metropolitan Municipality.

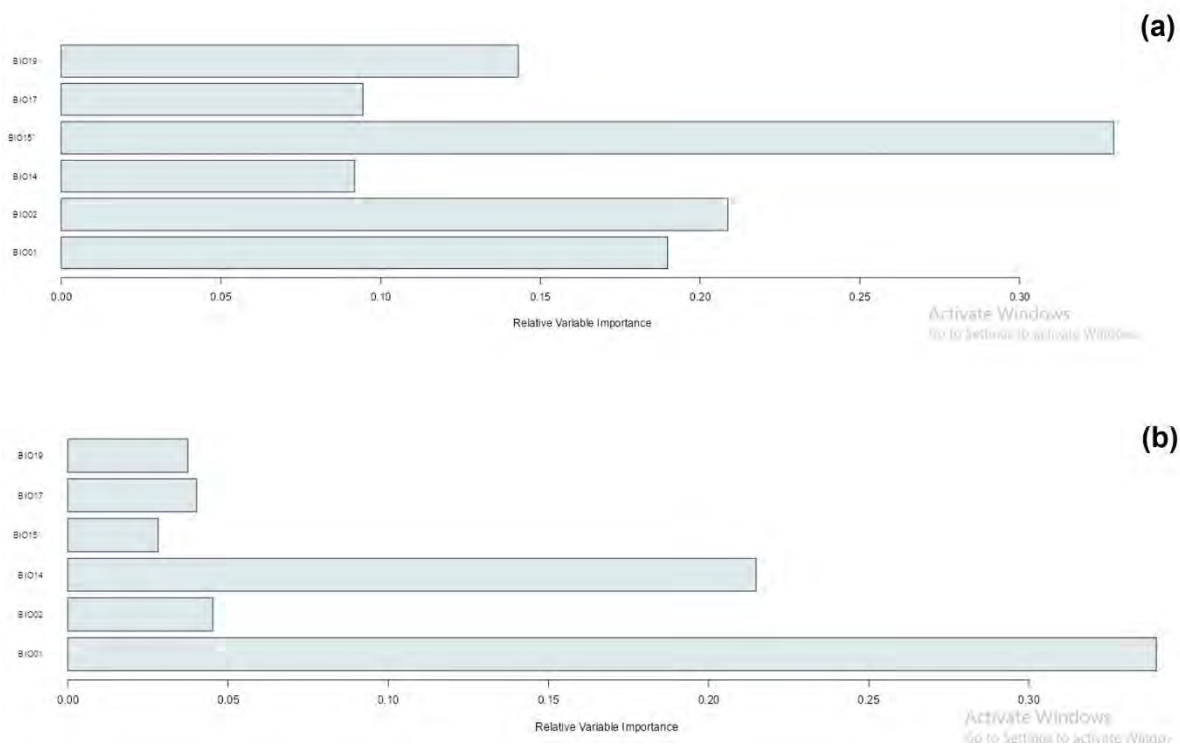

Figure S6.11: Relative variable importance of selected bioclimatic variables (BIO 1, BIO 2, BIO 14, BIO 15, BIO 17 and BIO 19) to *Bulinus africanus* snail species using (a) Random Forest, and (b) maximum entropy models in the Tshwane Metropolitan Municipality.

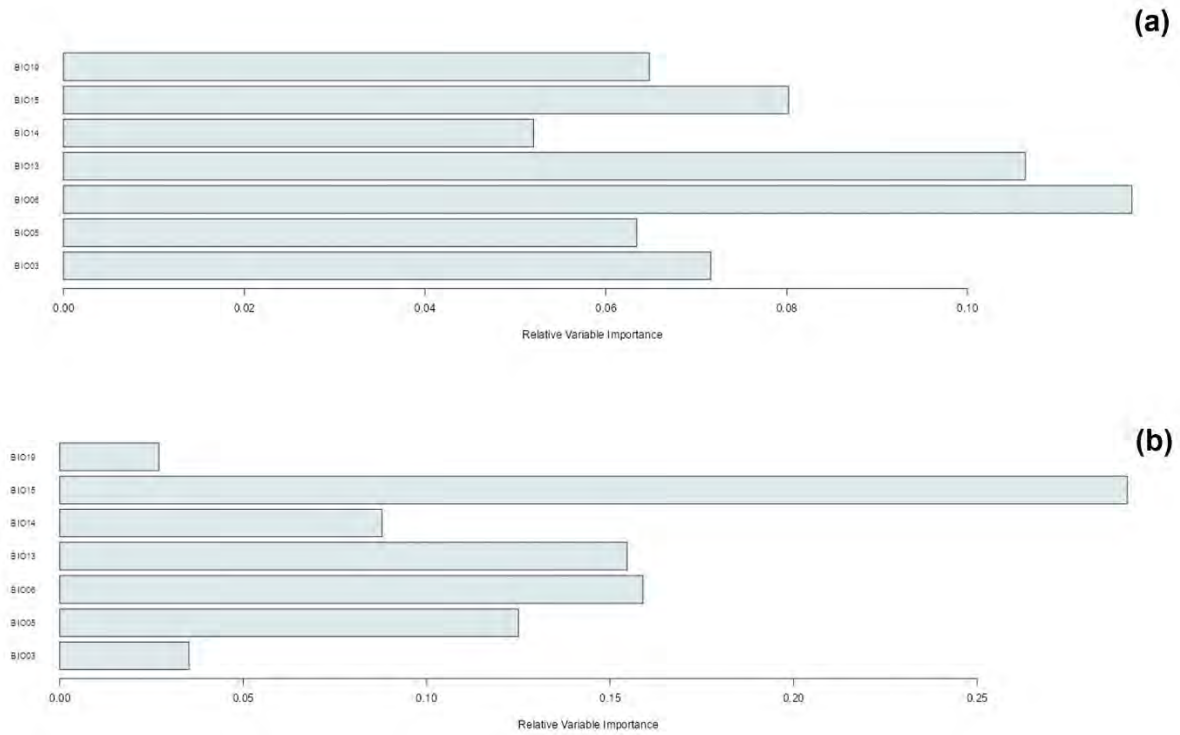

Figure S6.12: Relative variable importance of selected bioclimatic variables (BIO 3, BIO 5, BIO 6, BIO 13; BIO 14, BIO 15, and BIO 19) to *Bulinus africanus* snail species using (a) Random Forest; and (b) maximum entropy models in the Johannesburg.

Chapter 7

Table S7.1: The AUC and TSS and COR (correlation) for *Bul. africanus* for the different climate and ecological models using RCP 4.5 in this study.

Climate model	Ecological model	AUC	COR	TSS
Baseline	1. GLM 2. MaxEnt 3. RF 4. Ensemble	0.87 0.95 0.95 0.99	0.65 0.79 0.79 0.92	0.70 0.77 0.93 0.95
Access1-0	• GLM	0.92	0.74	0.71
(2040-2070)	• MaxEnt • RF • Ensemble	0.95 0.99 0.98	0.79 0.92 0.91	0.77 0.91 0.90
(2070-2100)	• GLM • MaxEnt • RF • Ensemble	0.88 0.95 0.99 0.98	0.65 0.80 0.92 0.91	0.66 0.80 0.91 0.93
bcc-csm1-1-m	• GLM	0.88	0.66	0.69
(2040-2070)	• MaxEnt • RF • Ensemble	0.94 0.99 0.98	0.78 0.91 0.91	0.77 0.92 0.92
(2070-2100)	• GLM • MaxEnt • RF • Ensemble	0.85 0.94 0.99 1	0.6 0.77 0.91 0.97	0.65 0.77 0.91 0.94
hadgem2-cc	• GLM	0.86	0.64	0.69
(2040-2070)	• MaxEnt • RF • Ensemble	0.94 0.99 0.99	0.77 0.91 0.91	0.77 0.91 0.91

Table S7.2: The AUC and TSS and COR (correlation) for *Bul. africanus* for the different climate and ecological models using RCP 8.5 in this study.

Climate model	Ecological model	AUC	COR	TSS
Access1-0 (2040-2070)	• GLM	0.87	0.64	0.66
	• MaxEnt	0.94	0.77	0.76
	• RF	0.99	0.91	0.91
	• Ensemble	1	0.95	0.93
(2070-2100)	• GLM	0.91	0.72	0.71
	• MaxEnt	0.95	0.80	0.79
	• RF	0.99	0.91	0.91
	• Ensemble	1	0.95	0.92
bcc-csm1-1-m (2040-2070)	• GLM	0.90	0.70	0.69
	• MaxEnt	0.96	0.81	0.81
	• RF	0.99	0.92	0.92
	• Ensemble	1	0.96	0.92
(2070-2100)	• GLM	0.86	0.61	0.65
	• MaxEnt	0.94	0.77	0.75
	• RF	0.99	0.91	0.91
	• Ensemble	1	0.94	0.92
hadgem2-cc (2040-2070)	• GLM	0.88	0.64	0.66
	• MaxEnt	0.95	0.79	0.77
	• RF	0.99	0.91	0.91
	• Ensemble	1	0.95	0.93
(2070-2100)	• GLM	0.88	0.65	0.63
	• MaxEnt	0.95	0.80	0.79
	• RF	0.99	0.91	0.92
	• Ensemble	1	0.93	0.91

Table S7.3: The AUC and TSS and COR (correlation) for *Bul. globosus* for the different climate and ecological models using RCP 4.5 in this study.

Climate model	Ecological model	AUC	COR	TSS
Baseline	• GLM	0.94	0.79	0.78
	• MaxEnt	0.95	0.80	0.79
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.97	0.94
Access1-0 (2040-2070)	• GLM	0.90	0.70	0.74
	• MaxEnt	0.95	0.80	0.79
	• RF	0.98	0.90	0.89
	• Ensemble	0.99	0.97	0.93
(2070-2100)	• GLM	0.92	0.74	0.74
	• MaxEnt	0.94	0.79	0.78
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.97	0.93
bcc-csm1-1-m (2040-2070)	• GLM	0.88	0.65	0.64
	• MaxEnt	0.94	0.78	0.77
	• RF	0.98	0.89	0.88
	• Ensemble	0.99	0.97	0.93
(2070-2100)	• GLM	0.93	0.75	0.73
	• MaxEnt	0.94	0.78	0.76
	• RF	0.98	0.89	0.88
	• Ensemble	0.99	0.97	0.93
hadgem2-cc (2040-2070)	• GLM	0.91	0.72	0.72
	• MaxEnt	0.95	0.80	0.78
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.95	0.90
(2070-2100)	• GLM	0.93	0.76	0.75
	• MaxEnt			
	• RF			
	• Ensemble			

Table S7.4: The AUC and TSS and COR (correlation) for *Bul. globosus* for the different climate and ecological models using RCP 8.5.

Climate model	Ecological model	AUC	COR	TSS
Access1-0	• GLM	0.93	0.75	0.76
(2040-2070)	• MaxEnt	0.95	0.80	0.79
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.96	0.94
(2070-2100)	• GLM	0.90	0.73	0.74
	• MaxEnt	0.94	0.78	0.78
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.96	0.94
bcc-csm1-1-m	• GLM	0.89	0.67	0.66
(2040-2070)	• MaxEnt	0.95	0.79	0.77
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.97	0.95
(2070-2100)	• GLM	0.92	0.75	0.74
	• MaxEnt	0.95	0.80	0.80
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.97	0.95
hadgem2-cc	• GLM	0.92	0.74	0.73
(2040-2070)	• MaxEnt	0.95	0.79	0.79
	• RF	0.98	0.89	0.89
	• Ensemble	0.99	0.96	0.94
(2070-2100)	• GLM	0.92	0.74	0.75
	• MaxEnt	0.95	0.80	0.78
	• RF	0.98	0.90	0.89
	• Ensemble	0.99	0.98	0.98

Table S7.5: The AUC and TSS and COR (correlation) for *Biom. pfeifferi* for the different climate and ecological models using RCP 4.5 in this study.

Climate model	Ecological model	AUC	COR	TSS
Baseline	• GLM	0.92	0.78	0.73
	• MaxEnt	0.93	0.77	0.75
	• RF	0.97	0.88	0.83
	• Ensemble	0.99	0.95	0.94
Access1-0 (2040-2070)	• GLM	0.89	0.72	0.66
	• MaxEnt	0.93	0.79	0.74
	• RF	0.96	0.87	0.84
	• Ensemble	0.98	0.94	0.90
(2070-2100)	• GLM	0.87	0.70	0.67
	• MaxEnt	0.92	0.78	0.75
	• RF	0.97	0.88	0.84
	• Ensemble	0.98	0.94	0.90
bcc-csm1-1-m (2040-2070)	• GLM	0.86	0.68	0.64
	• MaxEnt	0.91	0.77	0.73
	• RF	0.96	0.87	0.83
	• Ensemble	0.97	0.93	0.89
(2070-2100)	• GLM	0.89	0.75	0.69
	• MaxEnt	0.92	0.79	0.74
	• RF	0.97	0.88	0.84
	• Ensemble	0.98	0.92	0.88
hadgem2-cc	• GLM	0.88	0.68	0.66
	• MaxEnt			

Table S7.6: The AUC and TSS and COR (correlation) for *Biom. pfeifferi* for the different climate and ecological models using RCP 8.5 in this study.

Climate model	Ecological model	AUC	COR	TSS
Access1-0	• GLM	0.92	0.79	0.76
(2040-2070)	• MaxEnt	0.92	0.79	0.76
	• RF	0.97	0.88	0.84
	• Ensemble	0.99	0.96	0.92
(2070-2100)	• GLM	0.87	0.70	0.68
	• MaxEnt	0.92	0.78	0.73
	• RF	0.97	0.88	0.84
	• Ensemble	0.99	0.96	0.92
bcc-csm1-1-m	• GLM	0.92	0.79	0.74
(2040-2070)	• MaxEnt	0.92	0.79	0.74
	• RF	0.97	0.87	0.83
	• Ensemble	0.99	0.95	0.91
(2070-2100)	• GLM	0.89	0.74	0.69
	• MaxEnt	0.92	0.78	0.73
	• RF	0.97	0.88	0.84
	• Ensemble	0.99	0.96	0.92
hadgem2-cc	• GLM	0.88	0.75	0.70
(2040-2070)	• MaxEnt	0.93	0.79	0.74
	• RF	0.97	0.88	0.84
	• Ensemble	0.99	0.97	0.94
(2070-2100)	• GLM	0.88	0.73	0.70
	• MaxEnt	0.91	0.78	0.74
	• RF	0.97	0.88	0.84
	• Ensemble	0.99	0.97	0.95