Nematodes diversity in *Mastomys* rodents (Rodentia: Muridae) and molecular characterization of *Trichuris* species in the Mnisi Community, South Africa

By

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DECLARATION

I Jesse Mukisa Mutesasira, declare that this dissertation hereby presented for the award of Master of Science in Global One Health to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria is my own work and has not been submitted previously to any other academic tertiary institution for the award of a degree.

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Approved by;

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Prof.Sonja Matthee ………………………………………

Dr. Charles Byaruhanga ………………………………………………………

LIST OF ABBREVIATIONS

AEC:	Animal Ethics Committee
AMO:	anterior end of muscular oesophagus
At:	part of anterior portion
bp:	base pair
Ch:	cervical head
CO1:	cytochrome C oxidase 1
DNA:	deoxyribonucleic acid
DRC	Democratic Republic of Congo
DVTD:	Department of Veterinary Tropical Diseases
Eg:	Eggs
HHRWS:	Hans Hoheisen Wildlife Research Station
ITS:	internal transcribed spacer
MAFFT:	Multiple Alignment using Fas Fourier Transform
MCC:	maximum clade credibility
MEGA:	Molecular Evolutionary Genetics Analysis
mt:	mitochondrial
Neg:	negative control
PCR:	polymerase chain reaction
Pf:	posterior end of female
Pl:	pseudo labia
RNA:	ribonucleic acid
rRNA:	ribosomal RNA
sp.	species (singular)
spp.	species (plural)
Ss:	spicule sheath
Swb:	spicule width at base
T:	teeth
Ue:	uterine portions
Ut:	uterus

Vu: vulva

ABSTRACT

Nematodes diversity in *Mastomys* rodents (Rodentia: Muridae) and molecular characterization of *Trichuris* species in the Mnisi Community, South Africa.

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Nematodes comprise of many species with diverse life histories and zoonotic potential. Understanding the distribution and diversity of nematodes in the commensal rodent genus *Mastomys* is crucial for assessing their impact on wildlife and livestock, and potential of zoonotic disease transmission. The current study investigated the nematode diversity in *Mastomys* species rodents in three habitats and characterized the recovered *Trichuris* sp. using morphometric and molecular techniques at a wildlife-human/domestic animal interface in the Savanna biome in Mnisi communal area, Mpumalanga, South Africa. Nematodes were recovered and identified in the gastrointestinal tracts of 68 *M. natalensis* and 27 *M. coucha* rodents which were trapped in crop, village and natural habitats in the Mnisi communal area in October 2020. Nematodes were microscopically identified using morphometric measurements. Molecular characterization of *Trichuris* sp. was achieved through deoxyribonucleic acid (DNA) extraction, polymerase chain reaction (PCR), Sanger sequencing and phylogenetic analyses of three genes: internal transcribed spacers (ITS) 1, ITS 2 and cytochrome B (CytB). Data were analyzed using descriptive statistics, univariate models, a zero-inflated negative binomial generalized linear model, and a binomial generalised linear model, to establish the frequency, measures of central tendency and the relationships between nematode counts or occurrence and predictor variables using R statistical software. Nematodes were recovered in 20% of the examined rodents, with a total of 46 nematodes recovered, representing two species: *Trichuris* sp. (mean abundance of 0.31± 0.22) primarily from the caecum and *Abbreviata* sp. (mean abundance of 0.15±0.14) primarily from the stomach. Almost all the rodents were infected with only one nematode species, while one rodent exhibited mixed infection of both nematode species. No significant differences ($p>0.05$) in nematode prevalence were observed between male and female *Mastomys* spp. Univariate and multivariable analysis confirmed a lack of significant differences ($p>0.05$) in nematode abundance concerning habitat type, rodent species, and sex. The obtained novel *Trichuris* sp. ITS1, ITS2 and CytB

sequences, clustered in a distinct clade from published sequences, but showing genetic relationships with known *Trichuris* spp. The current study emphasizes the importance of integrating morphometric identification and molecular analysis to accurately categorize *Trichuris* spp. and suggests a need for a larger sample size per habitat type in future research on nematode diversity.

Key words: *Abbreviata*, habitat, *Mastomys*, Mnisi communal area, Nematodes, Rodents, South Africa, taxonomy, *Trichuris*

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1. INTRODUCTION

1.1 Background

Nematodes (Phylum Nematoda) are round worms that comprise of a large number of species that include free-living and parasitic taxa (Morand et al., 2001). Parasitic species commonly infect vertebrate hosts, especially small mammals (Behnke et al., 2001). Vertebrate hosts become infected with nematodes through their diet (plants or invertebrates) (Anderson, 2000). When present in low infections, nematodes have no clinical effect on the host, but high-level infections can lead to enteritis, diarrhea and weight loss (Soulsby, 1968).

Parasitic nematodes either have a direct life cycle, involving only a definitive host, or an indirect life cycle, requiring both intermediate and definitive hosts. In the direct life cycle, hosts are infected by ingestion of infective stages of the parasite in contaminated food, through coprophagy or during grooming activities (Anderson, 1988, Carlberg and Lang, 2004, Taffs, 1976b). Eggs are then excreted in faeces, contaminating the environment (Khatoon et al., 2004). Nematodes that follow an indirect life cycle require an intermediate host(s) that facilitate the nematode's development or reproduction, before emerging to infect the definitive host to complete their life cycle (Anderson, 2000, Morand and Krasnov, 2006).

Nematodes also vary in their level of host-specificity with most of the taxa adapted to parasitize a broad range of host species, while others are more host specific (Baylis, 1922). The type of life cycle may influence the spatial distribution of the nematode species, as directly transmitted species with free-living life stages tend to be more localized, while species with indirect life cycles can disperse through the movement of intermediate hosts (Spickett et al., 2019a). In addition, host and environmental factors, such as paratenic hosts and hypobiosis, may cause variations in nematode developmental biology (Morand and Krasnov, 2006). Host size, sex and reproductive status have been found to influence nematode diversity and infections (Arneberg, 2002, Folstad and Karter, 1992, Moore and Wilson, 2002, Wirsing et al., 2007). For example, male rodents tend to harbour high-level infections and more nematode species than females mainly due to their larger body size (Pakdel et al., 2013, Rungwe, 2022). In addition, reproductively active rodents often exhibit higher nematode infections compared to non-reproductive individuals (Spickett et al., 2017a).

Environmental factors such as climate and vegetation structure can directly affect free-living parasitic stages, indirectly affecting host susceptibility, and ultimately influencing nematode diversity and distribution (Spickett et al., 2017a). This is mainly due to the susceptibility of free-living nematode stages and intermediate hosts to desiccation under higher temperatures, thereby reducing larval sizes and reproductivity (Andersen et al., 1966, Pandey, 1972, Ricci and Pagani, 1997) thus limiting food availability for invertebrate intermediate hosts (Froeschke et al., 2010, Stromberg, 1997).

South Africa boasts a large diversity of wildlife, and rodents are no exception. Nonetheless, nematode diversity associated with naturally occurring rodents remains poorly understood (Julius et al., 2018, Spickett et al., 2019a). While some studies have focused on rodents, they mainly centred on commensal species (e.g. *Rattus* and *Mastomys*) in urban areas (Archer et al., 2017, Julius et al., 2018) or on specific species like the four-striped mouse (*Rhabdomys pumilio*) in the Western Cape (Froeschke et al., 2010, Froeschke and Matthee, 2014). A baseline study in South Africa identified a large diversity of nematodes in 13 murid rodent species captured from 26 different localities (Spickett et al., 2019a). The study by Spickett et al. (2019a) is the first broad scale study on multiple rodent species and highlights the large diversity of nematodes associated with rodents, providing evidence that a large proportion of the nematodes are currently undescribed in South Africa.

One nematode genus in South Africa, *Trichuris* (Trichuridae), commonly called whipworms, requires taxonomic revision and phylogenetic assessment. To date, three morphologically described *Trichuris* species, *T. contorta* Rudolphi, 1819, *T. vondwei* Ortlepp, 1938 and *T. parvispicularis* Clapham, 1945 (Verster, 1960) are recognized as infecting rodents in South Africa. However, recent studies have documented an undescribed *Trichuris* sp. in *R. dilectus* in the northern parts of South Africa (Spickett et al. (2019a) as well as an unidentified *Trichuris* sp. in the rodent genus *Mastomys* in the north-eastern summer rainfall region in South Africa (Rungwe, 2022). These discoveries, based on limited morphometric data points, raise concerns about accurate species classification. The morphometric keys used in these studies have been criticized for their potential to produce overlaps in the measurements between *Trichuris* spp. (Feliu et al.,

2000a) making this method of species identification erroneous. Given the above, it is quite possible that the *Trichuris* diversity in South Africa is currently underestimated.

1.2 Problem Statement

Sub-Saharan Africa has a rich diversity of wildlife, including about 463 known rodent species (Van Wilgen, 2018). Nonetheless, studies focusing on helminth and especially nematode diversity associated with rodents in South Africa are scarce. Archer et al. (2017) recorded two species of acanthocephalans, two species of cestodes and three nematode species in three rodent species (*Rattus rattus, R. norvegicus* and *M. natalensis*) at a single locality, in Durban, KwaZulu Natal Province. In this study two nematode sp., *Gongylonema* sp. (25.3%) followed by *Angiostrongylus cantonensis* (15%) were the most prevalent. Subsequently, in Gauteng Province, an overall helminth infection of 70% was recorded in four rodent species (*R. norvegicus*, *R. rattus*, *R. tanezumi* and *M. coucha*) and again three nematode species were the most prevalent *Heterakis spumoda* (29.1%), *Nippostrongylus brasiliensis* (16.7%) and *Heligmonellid* sp. (16.2%) (Julius et al., 2018). An unknown *Trichuris* sp. was also recorded but in low prevalence (0.8%) (Julius et al., 2018).

More recently, Spickett et al. (2019a) recorded 15 nematode, nine cestode and one *Acanthocephala* sp. from four rodent species (*R. pumilio*, *R. dilectus, M. coucha* and *M. natalensis*) at multiple localities across South Africa. The nematode *Heligmonia spira* was the most prevalent (27.3%) followed by two other nematodes: *Neohelgmonella* sp. (24.1%) and *Syphacia* sp. (22.8%). In addition, 16 undescribed nematode species that included an unknown *Trichuris* sp. (present in one rodent species and at prevalence range of 0.1%) were also recorded (Spickett et al., 2019a). Lastly, Rungwe (2022) recorded two undescribed nematodes species (*Trichuris* and *Syphacia*) at an overall prevalence of 30% and cestodes in rodents from 73 *Mastomys* spp. individuals across a wildlife-human/domestic animal interface in Mpumalanga during September (spring) 2019. To date, nematode identification in South Africa has mostly been conducted based on morphological characteristics. Given the presence of unknown nematode species in rodents in South Africa there is a need for molecular studies to confirm the taxonomy and systematics of these nematodes in rodents.

Apart from the taxonomic uncertainties on the nematode diversity there is also an urgent need to explore the role of ecological factors (e.g. vegetation/habitat types) that shape nematode infections in rodents. Froeschke and Matthee (2014), found land-use type differences and in particular higher nematode infection in *R. pumilio* in fragmented natural vegetation, surrounded by an agricultural mosaic, compared to extensive nature reserves in the winter rainfall Western Cape Province. Spickett et al. (2017a) recorded biome-related difference and in particular higher nematode infection in the more mesic Fynbos compared to the more xeric Succulent Karoo biome. The recent study by Rungwe (2022) in the Savanna biome noted habitat differentiations in the two nematode species, infecting *Mastomys* spp. with *Trichuris* sp. occurring exclusively in the village habitat type, while *Syphacia* sp. was recorded from the village and agricultural habitats. These studies provide evidence that the incidence of nematodes may be influenced by environmental factors and there is a need for further research in this neglected field.

1.3 Justification

Rodents often co-exist in proximity with humans, domestic and wild animals in various habitats. This allows them to serve as bridges that link wildlife ecosystems and human settlements which increases exposure risks of humans to zoonotic diseases circulating in the natural ecosystems (Meerburg et al., 2009). The erosion of the boundaries between the pristine natural environment and human habitat caused by various human activities has created new ecological scenarios, facilitating parasitic exchange across wildlife-human-livestock interface (Thompson et al., 2009, Thompson et al., 2010). This phenomenon warrants thorough investigation.

In the Sub-Saharan Africa region species in the rodent genus *Mastomys* are among the most common agricultural pests (Mulungu, 2017). The species *M. natalensis* is a semi commensal rodent that survives well in human-occupied habitats and has been reported as a candidate for zoonotic bacteria, viruses, protozoa and helminths (Holt et al., 2006, Meerburg et al., 2009, Mariën et al., 2020). Rodents have been reported to be reservoirs or carriers of human trichinosis, capillariasis and angiostrogylosis (Meerburg et al., 2009). Despite the zoonotic potential of these nematodes, complete understanding of nematode diversity within *Mastomy*s species, particularly *M. natalensis*, within wildlife interfaces of Africa, remains limited and warrants investigation. The

Mnisi community, located at a wildlife-human/domestic animal interface provides an ideal site for studying the detection of such pathogens.

A foundational study by Rungwe (2022) in September 2019 provided baseline data on the nematode diversity in the gastrointestinal tract (GIT) of the rodent genus *Mastomys*, across a wildlife-human/domestic animal interface in the South African Savanna biome. However, the study lacked species-level identification and classified rodents as *Mastomys* spp. This, coupled with the identification of an unknown *Trichuris* sp. based on morphological characters, prompted the need for taxonomic verification in an expanded study employing molecular techniques.

Expanding on Rungwe (2022) work, the present study included additional *Mastomys* samples collected at the same study sites and utilised standardized methods in October 2020, aiming to achieve species-level assessment of nematode diversity through molecular typing of the hosts (Derycke et al., 2013, Derycke et al., 2008). Furthermore, the role of host factors and habitat type on nematode infections required evaluation, given that biotic and abiotic factors influence the survival of both the host and their nematode infections (Sousa and Grosholz, 1991).

The current study addressed the dearth of studies characterizing *Trichuris* sp. (using morphological measurements and molecular techniques) in *M. natalensis* in the Savanna biome. Morphometric measurements obtained in the present study were compared with published data (e.g. (Feliu et al., 2000a, Ribas et al., 2013, Rungwe, 2022). to identify *Trichuris* species. The molecular identification of *Trichuris* sp. was performed using Cytochrome B, ITS1 and ITS2 genes, with the deliberate choice of the ITS marker as it is a recommended for species-specific identification as compared to other markers such as cyclooxygenase one (CO1) of the mitochondrial DNA (Bhat et al., 2022b). Cytochrome B was added as the gene of selection for mitochondrion genes as compared to CO1, which during PCR optimization failed to amplify. A combination of mitochondrion genes and nuclear genes is needed when genetic markers of closely related species are used in determining the species level relationship to avoid the alignment ambiguity in conjunction with morphometric measurements (Ballard and Rand, 2005). Proper and complete understanding of the nematode diversity is necessary in designing and implementing management programs/strategies (Bogale et al., 2020). The individual gene sequences obtained from the study

were also submitted to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) to obtain accession numbers and to make the sequences available to the broader scientific community.

1.4 Aims

The study aimed to:

1. Record the nematode diversity associated with *Mastomys* spp. (*Mastomys coucha* and *M. natalensis*) in the Savanna biome.
2. Assess the role of host and habitat related factors on the infection and species richness of nematodes in *Mastomys* spp. across a wildlife-human/domestic animal interface in the Savanna biome.
3. Confirm the taxonomic placement of *Trichuris* spp. that occur in *Mastomys natalensis* in the Savanna biome.

1.5 Objectives

1. Identify and enumerate nematode species recorded in *Mastomys* spp. (*M. coucha* and *M. natalensis*) collected from three habitat types (village, crops and natural).
2. Assess the association between presence of *Trichuris* sp. and host species *(M. coucha* and *M. natalensis*), sex (male and female) and habitat type (crop, village and natural) for the rodents.
3. Conduct measurements of selected morphological characters for nematodes identified as *Trichuris* sp. in *M. natalensis*.
4. Characterize *Trichuris* sp. recorded in *M. natalensis* by sequence and phylogenetic analyses of cytochrome B, ITS 1 and ITS 2 genes.

1.6 Hypotheses

Null hypothesis 1: Nematode diversity is similar in the two Mastomys spp. amongst the three habitat types.

Null hypothesis 2: *Trichuris* sp. recorded in *M. natalensis* is not different from rodent associated *Trichuris* spp. recorded elsewhere in Africa.

2. LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview of *Mastomys* species in Africa and Southern Africa. It reviews nematodes of the genus *Mastomys*, highlighting their morphology, prevalence, geographical and ecological specificity. The chapter also discusses identification of nematodes, particularly the morphological and molecular charaterisation of nematodes.

2.2 *Mastomys* species in Africa and South Africa

The genus *Mastomys* comprises small to medium-sized rodents widely distributed across Africa (Musser and Carleton, 1993). *Mastomys* is one of the most extensive rodent genera in sub-Saharan Africa (Monadjem et al., 2015). Eight *Mastomys* species have been reported across about 30 African countries (Hánová et al., 2021). These species exhibit geographical and ecological specificity: *M. natalensis, M. kollmannspergeri*, and *M. erythroleucus* inhabit non-forested areas with widespread distribution in central, western, southern, and eastern African regions. On the other hand, *M. coucha* is limited to Southern Africa, *M. awashensis* in Ethiopia, *M. angolensis* in Angola and Southern Democratic Republic Congo, *M. huberti* in western Africa wetlands and *M. shortridgei* in Southwestern Africa.

In Senegal, *M. natalensis* exclusively exhibits commensal behavior, while *M. erythroleucus* demonstrates opportunistic habitat selection, with greatest preference for savanna habitats as compared to cohabitating in human settlements (Brouat and Duplantier, 2007, Duplantier et al., 1996). In Nigeria where both *M. natalensis* and *M. erythroleucus* occur, *M. natalensis* preferred natural habitats as compared to *M. erythroleucus* which prefers human settlements. However, in the absence of *M. erythroleucus*, *M. natalensis* occurs in human settlements (Dobrokhotov, 1982). These findings clearly demonstrate the semi commensal nature of *M. natalensis* as previously reported by Coetzee (1975) for the same *Mastomys* species in South Africa. In Ethiopia, *M. awashensis* was reported to be a semi commensal species that occupy both natural and village habitats with a higher prevalence in village/modified habitats (Assefa and Chelmal, 2019). In Southern Africa, *M. coucha* is found in natural habitats within savanna and grassland vegetation (Monadjem et al., 2015).

Mastomys natalensis, commonly called the Natal multimammate mouse, is one of the most wide spread rodent species in Southern Africa (Calvet, 2014). Despite their nocturnal nature, *M. natalensis* exhibit diurnal activity, in houses with no electricity, increasing disease transmission risks (Lalis and Wirth, 2018). Regarded as an agricultural pest in southern Africa due to its prolific breeding (Isaacson, 1975), *M. natalensis* serves as a reservoir host for many zoonotic diseases such as bubonic plague and lassa fever (Green et al., 1980). In South Africa the distribution of *M. natalensis* largely overlaps with *M. coucha*, the southern multimammate mouse (Dippenaar, 1993). Although the two rodent species may co-occur, they appear to exhibit different climatic preferences. For example, *M. coucha* prefers areas with <700 mm annual rainfall, while *M. natalensis* occurs in higher rainfall areas (> 600 mm p.a.) (Gordon, 1987). In addition, *M. coucha* is usually located in the central and northeastern regions of South Africa with a high altitude and moderate rainfall patterns serving as an important agricultural pest (Venturi et al., 2004). Despite the potential co-occurrence of the two species, there is a lack of studies specifically testing their habitat preferences in South Africa.

Given the increasing human population and land use changes in Africa, the rodent problems are intensifying as contact between people and wild rodents increases (Belmain, 2006). There is, therefore, a need to increase surveillance of commensal rodent populations and their associated parasites to safeguard public health and understand the ecological diversity.

2.3 Nematodes of rodents

Helminths are a diverse group of invertebrates encompassing acanthocephalans, trematodes, cestodes and nematodes (Morand et al., 2001). Nematodes represent the highest proportion of helminth species in many vertebrate hosts, especially mammals (Poulin and Leung, 2011), and are prevalent in naturally occurring rodent species (Behnke et al., 2001).

Within small mammals and specifically rodents, phylum Nematoda is represented by five major clades that constitute seven orders: Trichurida, Ascaridida, Oxyurida, Spirurida, Rhabdiasoidea, Strongylida and Rhabditida (Table 1) (Paul and Mark, 2002). Nematodes can have an indirect (heteroxenous) cycle or direct life (monoxenous) cycle (Anderson, 2000). However, both life

cycles first start in a definitive host, where maturation and reproduction takes place, followed by a pre-parasitic stage that is composed of a larva either in the external environment or inside an intermediate host (Morand and Krasnov, 2006). Transmission of nematodes usually follows four principles: a) Monoxeny which can be primary, characterized by absence of an intermediate host in the evolution life cycle of the nematode such as that exhibited by trichostrongyloids, oxyuroids and ancylostomatoids; or secondary characterized by the final host serving the purpose of the intermediate host, usually exhibiting a phase of visceral larval migrans for example in ascariods, Toxocara spp. b) Heteronexy which is characterized by nematodes having both vertebrate and invertebrate intermediate hosts and many superfamiles poses this kind of life cycle. c) Paratensis in which the nematode is carried by a transport host in which no development stages of the nematode occur until it is taken up by the intermediate host, another paratenic host or final host. d) Precocity characterized by development of the nematode parasites beyond expected stages within the intermediate hosts (Anderson, 1988). Direct transmission is facilitated by behaviors such as coprophagy, grooming and ingestion of contaminated feed (Anderson, 2000, Carlberg and Lang, 2004, Taffs, 1976b). Table 1 summarizes the transmission patterns for nematodes.

Further, nematodes also vary in the level of host specificity. Generalist nematode parasites constitute more than 50% of the ecosystem and are capable of infecting multiple, often closely related host species (Walker and Morgan, 2014). Nematodes that have a generalist host range (euryxenous) are highly adaptable and are usually locally abundant and regionally widespread (i.e., large geographical ranges) (Krasnov et al., 2004, Poulin et al., 2011). A good example is *Mastopharus muris* which parasitizes rodents, marsupials and carnivores (Rojas and Digiani, 2003). In contrast, some nematode species are host specialists and occur on single host species or congeneric species. The specialized categories, as outlined by Feliu et al. (1997) and Sasal et al. (2001), encompass Oioxenous specialists, which are specific to a particular host species, exemplified by *Syphacia frederici* (Roman, 1945). Stenoxenous specialists, found within a specific host genus, such as *Syphacia obvelata* (Rudolphi, 1802); and digoxenous specialists, specific to a particular host family, as illustrated by *Trichuris muris* in mice and rats (Mair et al., 2021).. The life history characteristics of a nematode species play an important role in the density, dispersal and geographic distribution of the nematode species (Brouat et al., 2007a). In addition, the

importance of climatic conditions in shaping a nematode species' distribution may be related to the time spent in the external environment (Brooker, 2007).

Table 1 : Nematode families, and their life histories, that are commonly associated with small mammals.

Family example	Infective stage	Infection route	Intermediate host	Need for paratenic host
Trichuridae	Egg (L3)	Oral	No	No
Trichinellidae	L_1	Oral	No	Yes
Trichonstrongylidae	L_3	Oral/Skin	No	No
Metastrongylidae	L_3	Oral	Yes	Yes
Ascarididae	Egg (L_2)	Oral	Yes/No	Yes/No
Oxyuridae	L_3	Oral	No	No
Physalopteridae	L_3	Oral	Yes	No
Onchocercidae	L_3	Skin	Yes	No

Adopted from (Morand and Krasnov, 2006).

2.4 Host and habitat related factors that influence occurrence of nematodes

Nematodes have a dual environment that is the host and the external environment, both of which can influence nematode abundances (Spickett et al., 2017a). Although several host factors can play a role, it does appear that there are certain factors that are of a more general importance (e.g. host geographical range size, host body size and sex) (Kamiya et al., 2014). Host species that have a narrower geographical range and low population density have less contact with free-living infective stages and or intermediate hosts and can subsequently harbor fewer nematodes species (Bordes and Morand, 2011). In contrast, hosts that have large geographic ranges, multiple vegetation types and habitats are exposed to more diverse habitats and food resources, which can facilitate contact with nematodes and result in higher nematode infections and higher species richness (Spickett et al., 2019a). Larger bodied hosts provide more resources and space that promote parasite proliferation (Morand et al., 2015), with body size positively correlating with hosting of closely related nematode parasites (Morand and Poulin, 2002, Serge et al., 1996).

Nematode abundance and prevalence vary between host sexes and are most times biased towards males, due to larger body sizes, with a few exceptions (Grzybek et al., 2015). In males, testosterone-induced immunosuppression makes reproductively active male rodents more susceptible to parasite infection than females (Poulin, 1996, Schalk and Forbes, 1997). Likewise, immunosuppression in reproductive females also causes increased parasite loads (Vandegrift and Hudson, 2009).

The development and distribution of nematode species are also influenced by environmental factors (climate and vegetation structure) as demonstrated by studies that found a strong positive correlation between climatic variables (rainfall and relative humidity) and nematode species richness and abundance (Froeschke et al., 2010). Abiotic parameters such as temperature, pH, humidity and salinity affect the survival of free-living nematode life stages in the outside environment (Pietrock and Marcogliese, 2003). The free-living stages of nematodes rely on their stored energy reserves for survival before finding a suitable host and the external environment influences their survival in microhabitats (Brooks et al., 2006, Pietrock and Marcogliese, 2003). For example lower winter temperatures slow down the egg development process while higher temperatures increase the egg development rates (Hudson et al., 2006). Extremely high temperatures result in desiccation of nematode eggs and larvae, and these environmental factors remain variable at times going beyond the optimal nematode survival ranges in various habitats (Stromberg, 1997).

The vegetation structure, particularly percentage vegetation cover, plays an important role in maintaining adequate microclimatic conditions (e.g higher soil moisture and milder temperature ranges) favourable for the development and survival of free-living nematode life stages (Anderson, 2000, Morand and Krasnov, 2006, Ribas and Casanova, 2006). Vegetation structure also influences rodent diversity and occurrence as rodents depend on the vegetation to provide nesting and food resources and cover against predators (Anke and Ulrich, 2006). For example in South Africa, the prevalence and abundance of strongyloid nematodes in *R. pumilio* was positively associated with higher vegetation cover (Froeschke and Matthee, 2014).

Anthropogenic habitat transformation often results in a change in the natural vegetation structure with the extent of transformation causing variations in the microclimatic conditions and resource availability consequently impacting rodent and helminth diversity (Ramahlo et al., 2022). For instance, rodents from human settlements display significantly lower helminth infections than those from forest and peri-domesticated habitats (Chaisiri et al., 2017). As demonstrated by Froeschke and Matthee (2014), landscape characteristics greatly influenced both host parameters and helminth infections on *R. pumilio*, with nematode infection being highest in hosts obtained from crop fragments as compared to urban and livestock fragments. Further, Spickett et al. (2017a) recorded biome-related difference and in particular higher nematode infection in *R. pumilio* in the more mesic Fynbos compared to the more xeric Succulent Karoo biome. Nevertheless, there is a current research gap regarding the influence of vegetation types and habitat transformation on the nematode diversity and infection in the summer -rain-fall region in South Africa.

2.5 Occurrence of nematodes in African rodents.

In depth knowledge of helminth diversity and specifically nematodes in rodents across the African continent remains limited. Despite some studies being conducted in Africa, especially for Northern and Eastern African countries, the scale remains constrained (Jrijer et al., 2015, Mawanda et al., 2020, Ribas et al., 2013). For instance, a study in Nigeria recorded nematodes (three genera), cestodes (three genera) and acanthocephala (1 genus and species) in *Rattus rattus* obtained from commensal areas in Southwestern Nigeria, highlighting the need for increased investigation (Mafiana et al., 1997).

In Southeastern Senegal several nematode species, including *Trichuris* species, were recorded in *Mastomys erythroleucus* and *M. natalensis* with *M. natalensis* individuals having higher nematode species diversity (Brouat and Duplantier, 2007). Limitations in species-level identification for most of the nematode species (*Pseudophy-saloptera* sp., *Anatrichosoma* sp., *Abbreviata* sp., *Neoheligmonella* sp., *Heligmonina* sp., *Limucolaria* sp., *Syphacia* sp. and *Trichuris* sp.) was a notable challenge due to use of conventional microscopy. In Senegal, another study that aimed at assessing the influence of habitat type (village and natural) on the helminth diversity found a *Neoheligmonella* species (nematode) to be the most abundant in the natural habitats while *Trichuris* sp., *Syphacia* sp. and *Neoheligmonella* sp. dominated in *Mastomys* rodents (*M.*

natalensis and *M. erythroleucus*) in village habitats (Brouat and Duplantier, 2007). This study also used conventional microscopy for nematode identification which is less specific in species identification when used in isolation. Similarly, a study in Bwindi, impenetrable forest, Southwestern Uganda, investigated nematode diversity in four rodent families, 17 genera and 24 rodent species following coproscopic examination and identified *Nippostrongylus* sp., *Ascaris* sp., *Strongyloides* sp. And *Trichuris* sp. (Mawanda et al., 2020). These studies highlight the over-reliance on conventional microscopy in most African studies and emphasize the need for an integrated approach that includes advanced diagnostic techniques, such as the more specific and sensitive molecular analyses, to explore nematode species diversity on the continent.

In Southern Africa, limited information is available on the diversity, geographic distribution and host range of nematodes of naturally occurring rodents. Baseline studies in South Africa suggest that there is a wealth of nematode species that is yet to be described (Froeschke and Matthee, 2014, Julius et al., 2018, Spickett et al., 2019a). The challenge of distinguishing between different types of helminths extends beyond just nematodes and encompasses various other helminth classes. This highlights the inadequacy of relying solely on conventional microscopy for taxonomic purposes, emphasizing the necessity to expand research efforts in this field. To further understand the helminth diversity in South Africa, a regional scale study was undertaken in 26 localities across the country (Spickett et al. (2019a). The study recorded fifty-five helminth taxa in 13 rodent species, with nematodes being the most species rich (n=15), followed by cestodes (n=9) and one acanthocephala (Spickett et al., 2019a). Several undescribed nematode species, including a species in the genus *Trichuris* from *R. pumilio* and *R. dilectus* were also recorded.

A recent study in the savanna biome, South Africa, recorded a 30% prevalence of helminths in *Mastomys* spp. (n=73) the majority being nematodes of two genera *Trichuris* and *Syphacia* spp. based on morphological features (Rungwe, 2022). The study recommended the use of molecular means to conclusively identify the *Trichuris* sp. occurring in the study area. These studies highlight a need to expand the current information on the nematode diversity associated with naturally occurring rodents in South and southern Africa. More importantly, future studies must combine morphological and molecular techniques to confirm species identifications and to describe newly

discovered species. The most common nematodes observed in Africa will be described further in this section.

2.5.1 *Syphacia* nematodes recorded in African rodents

The nematode Order Oxyurida has two genera *Syphacia* and *Aspiculuris* and are referred to as pinworms (Khalil and Abdelmottaleb, 2014). Pinworms possess a small to medium sized body, characterized by a head with three lips on the anterior portion and a well-developed oesophagus that has a single bulb towards the posterior end (Taffs, 1976a). Hosts are infected by ingestion of the embryonated eggs. *Syphacia* sp. particularly, are associated with high infection rates due to the eggs adhering to the host (Anderson, 2000). Male *Syphacia* possess mamelons on their ventral side which are used to trap females during copulation after which males die (Abdel-Gaber, 2016). Initially pinworms were considered nonpathogenic however *Syphacia obvelata* and *Aspiculuris tetraptera* were linked to various health complications. In mice pinworm infection has been proven to influence host susceptibility to other intestinal nematodes (Michels et al., 2006).

The genus *Syphacia* (family Oxyuridae) has a worldwide distribution and has been reported to have a zoonotic potential (Yamaguti, 1961). The best-known species in the genus *Syphacia* Seurat, 1916 are *S. obvelata* (Rudolphi, 1802) and *S. muris* Yamagut, (1935). *Syphacia obvelata* are associated with domestic and wild rodents, while *S. muris* occur mainly in *Rattus* species (Behnke et al., 2022). Morphological features such as egg size, location of mamelons, excretory pore and tail length enable differentiation of *Syphacia muris* from *S. obvelata* (Hussey, 1957). In *S. muris* the vulva is more posterior as compared to *S. obvelata* (Khalil and Abdelmottaleb, 2014). Much of the literature about the genus *Syphacia* in wild rodents remains scanty in Africa however some studies have been conducted in South and southern Africa. On the African continent, *Syphacia nigerians* was first reported by Baylis (1928) from five rodent species (*Taterillus gracilis, Gerbiliscus kempi, Praomys tulbergi, Mastomys erythroleucus,* and *Lemniscus striatus*) all obtained from different localities in Nigeria (Baylis, 2009). *Syphacia* sp. were recovered from *Rattus rattus* captured from Abeokuta, South Western Nigeria and prevalence was 2.1% (Mafiana et al., 1997). In Egypt, Cambieri (1957) observed *S. obvelata* in the large intestines of *Avicanthis niloticus* and this was based on morphometric descriptions. *Syphacia obvelata* Yamaguti (1941) was also recorded in central Tunisia in various rodent species (Jrijer et al., 2023). In Southern

Africa, *S. obvelata* was the most common nematode species recorded in *R. pumilio* captured along a climate gradient (Froeschke et al., 2010). *Syphacia minuta* was the most common helminth parasitizing the spiny mouse (*Acomys spinosissimus*) in Limpopo province, South Africa, with higher prevalence during the drier and cooler seasons as opposed to the wet and warmer seasons (Lutermann et al., 2014). Studies by Julius et al. (2018) recovered *S. obvelata* in *M. coucha* captured from both formal and informal localities in Gauteng province, South Africa. Baseline studies on helminths of South African rodents by Spickett et al. (2019a) recorded *S. nigeriana, S. obvelata* and unknown *Syphacia* spp. in 11 of the rodent species. Recently Rungwe (2022) recorded a *Syphacia* species assumed to be *S. obvelata* based on morphological and morphometric identification as the most prevalent helminth with the highest number occurring in *Mastomys* spp. captured across a wildlife-human/domestic animal interface in Mpumalanga province, South Africa.

2.5.2 *Abbreviata* nematodes recorded in African rodents

The nematode Order Spirurida has six genera: *Physaloptera* Railliet, 1893, *Abbreviata* Travassos, 1920, *Rasheedia* Moravec and Justine, 2018, *Heliconema* Travassos, 1919, *Paraleptus* Wu, 1927 and *Proleptus* Dujardin, 1845 (Anderson et al., 1974, De Ley and Blaxter, 2002, Moravec and Justine, 2018, Railliet, 1893). The majority of vertebrates are susceptible hosts of species within the genus *Abbreviata* Travassos,1920 (Family: Physalopteridae and subfamily: Physsalopterinae). The genus comprises of a large number of species that were added over the years. In particular, by 1860s about 18 species had been described to belong to the genus (Ortlepp, 1992, Pereira et al., 2014) and Schulz (1927) named 23 species into the genus with more species that have been added however no updated written literature exists (Morgan, 1945a).

Abbreviata sp. seem to be more common in reptiles, where they occur in the stomach as medium sized white worms (Jones, 1988b). Morphologically, the oral opening is encircled by two large pseudolabia, each of which has a single lobe. These pseudo labial lips have specific dental features including one tooth on the outer side, one tooth on the inner side and two double teeth closer to the center. Additionally, there are two external papillae and one amphid present. Sexual dimorphism occurs and in males, the tail is characterized by broad, wing like structures known as the lateral alae just proximal to the cloaca. The number of sessile genital papillae on the ventral

side of the tail vary but usually three before the cloaca. The ventral surface of the tail is covered with rows of tubercles. The spicules in males are usually unequal in length. In females the vulva is typically located in the anterior half of the body. The uterus branches into two or four and at times more branches. The eggs are usually oval in shape, have a smooth surface and possess a thick shell and embryonated at deposition (Morgan, 1945a). The Physalopterids are pathogenic with major associated lesions occurring at the site of attachment to the host and clinically they have been associated with mucosal ulcerations, inflammation of the stomach mucosa walls and oesophagitis in non-human primates with unknown public health concerns (Strait et al., 2012). Given the large size of the *Abbreviata* individuals at heavy infection in small hosts obstruction of the GIT may be possible. Literature about pathogenicity in rodents remains scant.

Abbreviata sp. has been identified in South Africa hosted by rodent species such as *Lemniscomys rosalia, Micaelamys namaquensis* and *R. dilectus* (Spickett et al. (2019a). It is apparent that *L. rosalia* hosts the highest number of *Abbreviata* sp. and that the *Abbreviata* sp. in *R. dilectus* is more prevalent in eastern summer rainfall regions where habitats have a moderate balanced moisture all year round (Spickett et al., 2017a).

2.5.3 *Trichuris* nematodes recorded in African rodents

The Order Trichurida consists of three genera: *Capillaria*, *Trichinella* and *Trichuris*. African literature has reported and described occurrence of all three. For example, *Trichinella spiralis* based on microscopic examination were reported to occur in South African hosts from Kruger National park such as *M. natalensis*, lion, African bush rat, warthog, buffalos among others (Young and Kruger, 1967), while Archer et al. (2017) recorded *Capillaria hepaticum* from *R. norvegicus* captured in Durban, South Africa. However, for purposes of this thesis, more focus was on the genus *Trichuris*. Family Trichiuridae are long and slender worms at the anterior with the name *Trichuris* translating into whip tail however in reality it is the head which is whip (Zachary, 2017). To differentiate between *Trichuris* species, various features such as the presence/absence of specular tubes, distribution and shape of the spines of the specular sheath, spicule length, shape and position of the distal cloaca tube are useful. Additionally, the morphology of the vulva, along with classic morphometric characteristics are regarded of high discriminatory value (Babero and Murua, 1990, Gomes et al., 1992, María del Rosario and Juliana, 2006, Rossin and Malizia, 2005,

Spakulova, 1994, Suriano and Navone, 1994). Several *Trichuris* spp. that are associated with rodents are cryptic (morphological features are identical across spp.) (Babero et al., 1976, Feliu et al., 2000a, Tiner, 1950).

Globally about 80 *Trichuris* species have been documented to infect various mammalian hosts including domestic and wild animals (Mair et al., 2021). The major transmission route is by ingestion of eggs and infective larvae from soil (Brouat et al., 2007b, Feliu et al., 2000b). The diversity of *Trichuris* species in African rodents remains unknown as most studies only report it as *Trichuris* sp. based on conventional morphometrics (Froeschke and Matthee, 2014, Julius et al., 2018, Rungwe, 2022, Spickett et al., 2019a). Based on a literature review conducted up to 2003, it was found that up to that point, approximately 10 *Trichuris* sp. had been documented among African rodents through morphological identification with majority of the early discoveries concentrated in South Africa (Ribas et al., 2013). Further studies have identified and described more *Trichuris* spp. in other parts of the continent based on a combination of morphological classification and molecular techniques with some of the species being similar to those reported earlier on by Ribas et al. (2013).

The genus has been reported in rodents from various African countries such as, Nigeria where *Trichuris* sp. eggs were recovered from the African Giant Rat (*Cricetomys gambianus*) and Grasscuter rat (*Thryonomys swinderianus*) (Ayinmode et al. (2015). Earlier studies in Nigeria recorded *T. muris* hosted in *R. rattus* (Mafiana et al. (1997), while in Uganda *Trichuris* sp. was recorded from multiple rodents species (Mawanda et al. (2020). In Senegal *Trichuris* sp. was recorded in *M. natalensis* and *M. erythroleucus* (Brouat and Duplantier, 2007) while in South Africa *Trichuris* sp. was recorded in several rodents species from several localities (Froeschke and Matthee 2014; Julius et al., 2018; Spickett et al., 2019; Rungwe (2022). All the above-mentioned studies made use of morphological characteristics to identify the *Trichuris* sp. In Tanzania, Ribas et al. (2013) molecularly identified two *Trichuris* species *T. mastomysi* in *M. natalensis* and *T. carlieri* s.l in *Gerbilliscus vicinus* indicating host specificity exhibited in the genus.

Molecular results from a study by Ribas et al. (2017) conducted on pooled samples obtained from six west African countries recorded three genetic clades of *Trichuris* sp. with clade one containing

only samples obtained from four countries (Burkina Faso, Guinea, Senegal and Mali). The second clade contained *T. mastomysi* that was similar to the one earlier on recorded in Tanzania by Ribas et al. (2013)) and then *T*. sp. *Mastomys* Benin. Clade three had two non-identified previously described species denoted as a new species and named as *T. duplanteri* n. sp. from Mauritania which suggests the existence of more unknown species in the genus. Table 2 highlights *Trichuris* sp. that have been reported in African rodents using either morphological or molecular techniques. Literature review from Ribas et al. (2013) reported *Trichuris* spp. (*T. petteri* Quentin (1966) and *T. mastomysi* Verster (1960) indicated in the (Table 2) were earlier on omitted from considerations based on conventional identification. However, on molecular analysis, *T. mastomysi* was confirmed as an existent species in *M. natalensis* of Tanzania (Ribas et al., 2013) and the same study molecularly confirmed *T. mastomysi* and *T. carlieri* s.l as two distinctive species exhibiting host specificity however their various morphological features were overlapping with those of those of *T. muris*.

The genus is pathogenic and different species exist for different susceptible hosts however the disease process is similar in all, the whip head attaches to the mucosa of the caecum, colon and rectum so as to obtain nourishment. This results into blood loss from the host although it rarely progresses to anaemia, and blood stained diarrhea (Zachary, 2017). Infection by *Trichuris* results into goblet cell hyperplasia hence increased mucous production in both resistant and susceptible animals (Artis et al., 2004). As *Trichuris* sp. parasitize the intestinal mucosa of hosts it causes enteritis and weight loss (Hansen, 1969).

These findings indicate that the genus *Trichuri*s has a widespread occurrence in African rodents and that the current diversity is severely underestimated. Further, there is huge benefit in using molecular analyses in addition to morphological and morphometric techniques to characterize the genus.

Table 2: *Trichuris* species reported in African rodents and reporting country.

Species	Country	Molecular confirmation
T. calieri Gedoelst (1916), *T. vondwei* Ortlepp (1938), *T. contorta* Rudolphi (1819), Hall, 1916 *T. parvispicularis* Clapham (1945), *T. petteri* Quentin (1966), *T. gerbillis* Bernard (1969) *T. gundi* Bernard (1969), *T. mastomysi* pedetei Verster (1960) *T. procaviae* Verster (1960), *T. hyracis* Ezzat (1954) *T. pettteri* Quentin (1966)	South Africa South Africa South Africa South Africa South Africa, Tunisia South Africa, Tunisia South Africa, Tanzania, Mauritania South Africa South Africa	 No No No No No No Yes
Trichuris carlieri Gedoelst, 1916 sensu lato	Tanzania	Yes
Trichuris muris Schrank (1788)	Nigeria, Tanzania,	Yes
Trichuris duplantieri	Mauritania	Yes
Trichuris hystricis Kreis, 1938	Tunisia	No
Trichuris infundibus Linstow, 1906	Not clearly defined in Africa	No
Trichuris sp. (non-identified)	Senegal	Yes
Trichuris sp.	Uganda, South Africa	No

2.5.4 Other nematode species previously recorded in African rodents

Several other nematode species are also associated with rodents in Africa including *Strongyloides ratti*, *Ascaridia* spp. *Nippostrongylus brasilensis*. *Trichosomoides crassicauda,* and *Heterakis spumosa* which were recovered by coprophagic examination in Nigeria from three rodent species (*Rattus norvegicus*, *Cricetomys gambianus* and *Thryonomys swinderianus*) (Ayinmode et al.,

2015). *Heligmonina spira*, *Neoheligmonella capensis* and *Trichostrongylus probulurus* were recorded from *R. pumilio* in the Western Cape province of South Africa (Froeschke and Matthee, 2014). *Trichosomoides crassicauda*, *Protospirura* sp., *Strongyloides ratti*, *Mastophorus muris* and *Nippostrongylus brasilensis* (most abundant) were recorded from four rodent species (*R. norvegicus*, *R. rattus*, *R. tanezumi* and *M. coucha*) in the Gauteng Province in South Africa (Julius et al., 2018). Lastly, *Heligmonina spira*, *Neoheligmonella capensis*, *Trichostrongylus probulurus*, *Protospirura* sp. and *Streptoharagu*s sp. among others were recorded from 13 rodent species across multiple localities in South Africa (Spickett et al., 2019a). The diversity of nematodes in African rodents spans different genera and species, emphasizing a need for in depth studies utilizing both morphological and molecular techniques.

2.6 Methods used for the identification of nematodes

Various factors influence the accuracy of any technique used in nematode identification and this varies from absence of specific identification features, small size of the worms, presence of a high biodiversity of nematodes in each sample among others (Chitwood, 2003, Floyd et al., 2002). This calls for not only more work on nematode identification but also advancement in nematode identification techniques.

In the past, identification relied on features like body length, the structure of reproductive organs, features of mouth and tail, alongside other physical attributes. However, this method of taxonomic classification based on morphology may not always be sufficient enough due to absence of distinct differences among closely related groups and together with the decreasing availability of expert taxonomists (Oliveira et al., 2011). In case of large sample sizes, the morphological identification technique is a very tiring and demanding physical endeavor (Bogale et al., 2020). Two main techniques currently employed for nematode identification are classical morphological identification and molecular identification using polymerase chain reaction (PCR).

2.6.1 Classical morphological identification

Also known as conventional or morphometric method, the technique employs the analysis of anatomical and morphological differences under a light microscope (Bogale et al., 2020). It is relatively affordable with the principle of associating identified morphological features to expected

functions thus ruling out the most probable role (Oliveira et al., 2011). Morphological analysis and morphometrics have been used to identify nematodes up to genus level since the 19th century (Roeber et al., 2013) and remain relevant today. Morphological and the physical measurable characteristics (biometrical features) of *Trichuris* sp. have been insufficient for identification of congeners for many years (Liu et al., 2012)**.** Complexity arises when in a genera or species with similar morphological features happen in form of mixed infections or congeneric diversity in the same genus exists such as *Trichuris* sp. The length of the spicule was reported as an important character for *Trichuris* sp. identification (Gomes et al., 1992, Spakulova, 1994). The failure of this method to effectively distinguish between closely related nematode species remains a major constraint for biologists/researchers using the approach (Seberg et al., 2003, Vogler and Monaghan, 2007).

Characterization and identification of nematodes using anatomical features by measurement is very difficult for non-skilled personnel yet and a huge concern is the declining number of experienced taxonomists. In addition, the known primary key identification features are not well illustrated hence a gap exists for their improvement before they can be used as reference points (Lichtenfels et al., 1997). The greatest advantage of this technique is that the method lies in minimal cost when there is an experienced taxonomist available (Floyd et al., 2002). In certain nematode generally, sexual reproductive organs of adult males have been used for species-level identification (Gasser et al., 2008, Mattiucci and Nascetti, 2008). In the genus *Syphacia*, the number of post-natal papillae, spicule length, shape of cephalic plate, presence and absence of cervical alae, presence or absence of ornamentations on the gubernaculum, number and position of the mamelons among other features are used to morphologically distinguish between species (Dewi et al., 2016). Morphologically, the genus *Trichuris* has a characteristic whip shape with a thin threadlike elongated anterior body and a broad posterior end that is usually coiled, other features used for identification as described by (Feliu et al., 2000b, Ribas et al., 2013, Robles, 2011).

In female *Trichuris* sp. García-Sáncheza et al. (2019) reported that the morphometric measurement combinations of various individuals of different species yielded similar measurements. In the genus *Trichuris*, grossly females are larger than the males. Factors such as level of infection,

nematode predilection site in the host and sex of the host influence the size of nematodes as found in *Heaemochus contotus* where nematode size was larger in low infection and also larger in female hosts as compared to the male hosts. The findings can be attributed to the influence of competition for resources among the nematodes and also the effect of male and female host hormones, reproductive status among others on *H. contortus* (Kuchai et al., 2012). This may explain why morphometrics for various nematodes species always have ranges for reference figures as the hosts characteristics at time of capture vary hence lack of a single gold standard figure even for a single species. The technique serves purposes in identification of known sp. and a partial description of new species (Seesao et al., 2017). Use of egg larval culture under morphological and morphometric identification up to genus level and certain genera up to species (*Nematodirus* sp.) (Höglund et al., 2013) can be used however it is time consuming and in addition an experienced taxonomists is needed (Roeber et al., 2012, Van Wyk et al., 2004, von Samson-Himmelstjerna et al., 2002). In addition, egg larval culture cannot also be used for identification of the non-reproductive life stages of nematodes.

2.6.2 Molecular identification by polymerase chain reaction (PCR)

Within any given species, the ribosomal deoxyribonucleic acid (rDNA) is universally conserved providing genetic homogeneity and serving as valuable markers (Gerbi, 1986). Through polymerase chain reaction (PCR) targeted regions of the genetic material (DNA or rDNA) are amplified in vitro, facilitating detection of unknown and known species as well as diagnosis (Seesao et al., 2017). Specifically designed primers allow the desired targeted DNA sequences to be amplified (Amiteye, 2021). Genetic markers, derived from nuclear or mitochondrial DNA regions, vary in effectiveness and degree of precision depending on the level of variation in the DNA sequence (Blasco-Costa et al., 2016). Mitochondrial genomes are more suitable as genetic markers in lower organism identification and taxonomy due to their higher variability (Allio et al., 2017, Blouin, 2002, Hwang and Kim, 1999). On the contrary, the highly conserved regions of the ribosomal Ribonucleic Acid (rRNA) are very useful in deciphering higher organism taxonomy (Choudhary et al., 2015, Hwang and Kim, 1999). In nematode identification, mitochondrial genome markers for protein coding such as Cytochrome Oxidase 1 (Cox1) and Cytochrome B (cytB) along with nuclear genome marker Internal Transcribed Spacer (ITS) serve as primers for PCR (Chan et al., 2021, Hwang and Kim, 1999). Phenotypic plasticity causes natural variation

(Kiontke and Fitch, 2010) additional to cryptic diversity in species which can only be identified by molecular based techniques (Derycke et al., 2013, Derycke et al., 2008).

To efficiently amplify the desired nematode genome using PCR, there is a requirement for quality gene extraction from the sample by removing all inhibitors and then need for sufficient quantities of the DNA to be amplified to increase the sensitivity and specificity of the PCR (Rådström et al., 2004, Seesao et al., 2014). Diagnosis and identification of nematodes by use of PCR allows differentiation of active and passive infections and detection of any life cycle stage of the nematode. This is because genetic material is universal and can be extracted from any stage of the nematodes (McKeand, 1998) and yield similar results. Lack of positive controls which are needed to check for the presence of inhibitors may hinder the detection of unknown species. The advancement of DNA extraction technologies has made PCR a method of choice for almost any sample even faecal due to development of inhibitor removal technologies (Reslova et al., 2021). Presumed single species can always be revealed by molecular approaches (Anderson, 1988).

Comparative analysis of the coding and non-coding regions of rDNA is a new tool for phylogenic tree construction of organisms nematodes inclusive (Subbotin et al., 2001). Molecular techniques use genetic markers such as the first and second internal transcribed spacers (ITS 1 and ITS 2) of the rDNA and the ribosomal RNA subunit (16S) of the mitochondrial genome (Callejon et al., 2012). Of greater interest is the ribosomal ITS 2 that has proven a great valuable resource for species identification a factor attributed to high specificity derived from being located between the conserved regions of the rDNA so research uses universal primers targeting the 5.8S and 28S rDNA genes for helminth identification (Callejón and Cutillas, 2017, Callejón et al., 2015, Chilton et al., 1995, Hoste et al., 1993). Phylogenetic studies of closely related nematode species have demonstrated the use of ITS of nuclear ribosomal (rDNA) as ideal markers for differentiation (Zhu et al., 1988). ITS marker analysis is preferred in species specific identification as compared to other markers such as cyclooxygenase one (cox1) of the mitochondrial DNA (Bhat et al., 2022b). When determining species level relationships using markers of closely related species, the rapidly and highly evolving sequences of rDNA including ITS they are prone to alignment ambiguity and to overcome such it is important to use markers for both mitochondrial and nuclear genes in a combination with other variables such as morphological features (Ballard and Rand, 2005). DNA

polymorphisms of *Trichuris muris* isolates from various locations in Europe have been confirmed by molecular analysis of the ITS 1 and ITS 2 of the rDNA (Callejon et al., 2010). PCR methods can be conventional, quantitative, Restriction Fragment Length Polymorphism (RFPL) PCR among others (Nisa et al., 2022).

2.7 Genome sequence database approaches

Sequences are produced by analysis of PCR products using software methods such as Sanger's method, pyrosequencing, and next generation sequencing among others. The sequences outputs can then be compared with other stored genome sequences from various electronic databases which are usually free for online analysis and repository submission of new identifies genome sequences that are then made available for use such as Nucleotide (http://www.ncbi.nlm.nih.gov/nucleotide). The databases have been developed to analyze sequences and genome related information obtained from molecular methods by using bioinformatic techniques to decrypt nematode diversity and a smaller extent trematodes (Bhat et al., 2022a). Examples of these include Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) which is general for all DNA or protein sequences. Databases that are specific to nematodes include; NemaBlast (http://nematode.net/NemaBlast.html) and NemaBrowse (http://nematode.net/NemaBrowse.html) (Nisa et al., 2022) among others. The various methods are needed to be able to identify species.

2.8 Summary

The genus *Mastomys*, comprising small to medium-sized rodents, is widely distributed across Africa. In Southern Africa, *M. natalensis*, also called the Natal multimammate mouse, overlaps with *M. coucha*, the southern multimammate mouse, but with different climatic preferences. These rodents serve as reservoirs of zoonotic disease, amongst them gastrointestinal nematodes. Rodent nematodes, are a diverse group of invertebrates, consisting of various clades and orders, exhibit different life cycles, transmission patterns, habitat and host preferences. Knowledge of nematode diversity and distribution as well as associated risk factors in *Mastomys* species in Southern Africa, is limited. In particular, the molecular characterization of the genus *Trichuris* remains poorly studied and warrants attention. The present study, therefore, aimed to investigate nematode diversity in *Mastomys* species rodents in three habitats and characterize *Trichuris* sp. using

morphometric and molecular techniques at a wildlife-domestic/human interface in the Savanna biome in Mnisi communal area, Mpumalanga South Africa.

3. MATERIALS AND METHODS

3.1 Ethical approval and biosafety

The study adhered to research ethics outlined by the University of Pretoria and complied with "The Care and Use of Animals for Scientific Purposes" standards. Approvals for the study were granted by the Research Ethics Committee (REC) and Animal Ethics Committee (AEC) of the Faculty of Veterinary Science, University of Pretoria (reference number REC134-22) (Appendix 1 and 2). Additional permission to conduct the research was obtained under Section 20 of the Animal Disease Act 1984 from the Department of Land Reform and Rural Development of the Republic of South Africa (reference 12/11//1/1/6; 2833SdIR) (Appendix 3). The study strictly followed standard operating procedures and biosafety protocols in line with the respective laboratories' regulations in the Department of Veterinary Tropical Diseases (DVTD), University of Pretoria, South Africa.

3.2 Study area, study design and sample size

Rodents were trapped in the Mnisi One Health Platform community (Figure 1). This area is located in the northeastern Savanna biome of the Bushbuckridge Municipal area, Mpumalanga Province, South Africa. This biome is characterized by hot and wet summers with mild and cool winters and an annual rainfall average of 550 mm and mean annual temperatures of 21 ^{0}C (Murapa, 2018). The area spans about 29500 hectares of communal land, bordered by private and provincial conservation areas (Berrian et al., 2016). The Mnisi community primarily depends on livestock farming as the main agricultural activity and exhibits a strong interdependence between humans, domestic animals, and wildlife (Jongejan et al., 2020; Berrian et al., 2016; David et al., 2013). The study area represents three habitats crop, village and natural/reserve habitats as indicated in the Table 3. The crop habitats were characterized by cultivated grounds with food plants such as maize and located in proximity in selected areas (yellow stars on the map) with the village habitats. The village habitats were demarcated from the natural reserve by the red border line on the map and these included home steads in the selected localities where communities had constructed home steads and other communal social facilities while natural habitats (green areas on the map) included vegetation in which no human activity had not been done.

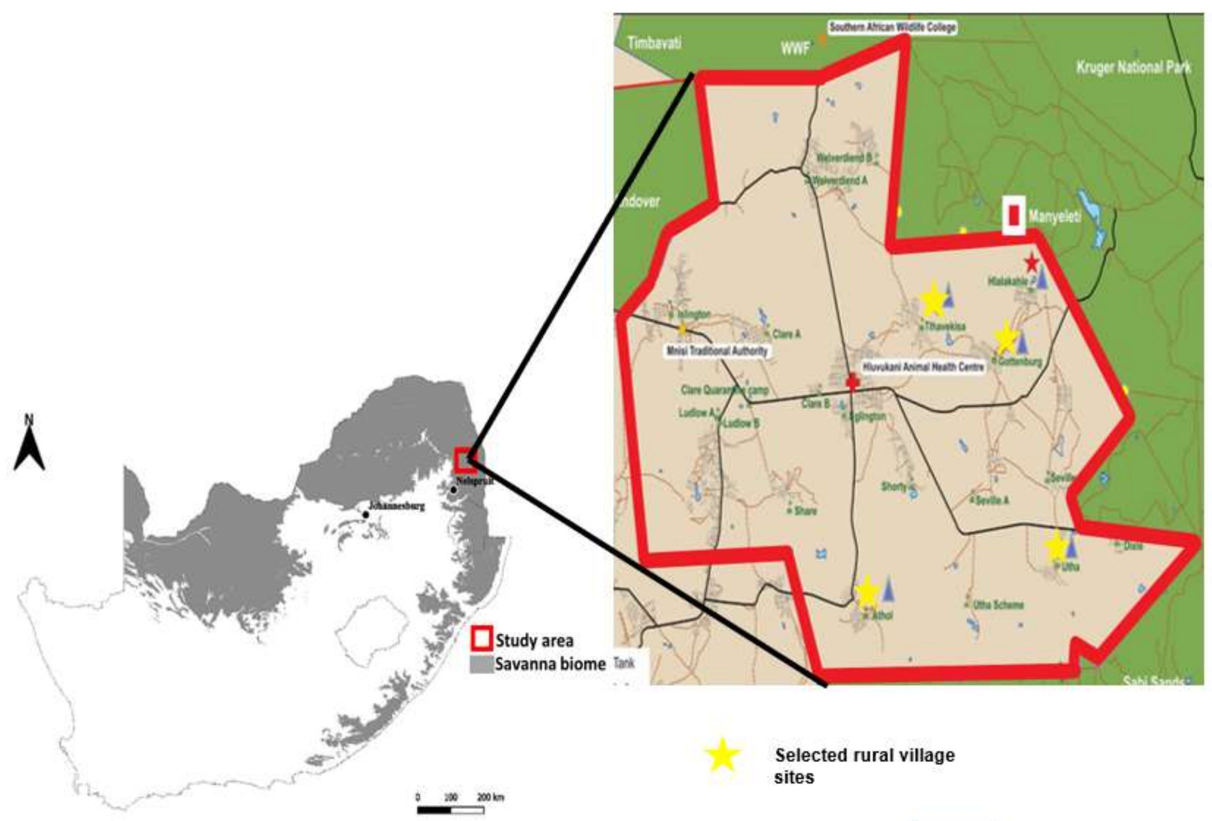

Figure 1: A map showing some of the rodent sampling localities in the Mnisi community, Mpumalanga Province, South Africa during October 2020.

Table 3: Global Positioning System (GPS) coordinates of the study sampling sites of rodents across a wildlife-human domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Habitat type	Name	GPS Coordinates
Crop	Utg (Utha)	-24,703277, 31,443616
Crop	Atg (Athol)	-24,702248, 31,346744
Crop	Thg (Thlavekisa)	-24,632923, 31,379908
Village	Ut (Ultha)	-24,703277, 31,443616
Village	At (Athol)	-24,702248, 31,346744
Village	ThV (Thlavekisa)	-24,632923, 31,379908
Village	Gt (Gottenberg)	-24,634509, 31,420916
Natural (Reserve)	Site 1	-24,614067, 31,448468
Natural (Reserve)	Site 2	-24,603532, 31,439627
Natural (Reserve)	Site 3	-24,602595, 31,416024
Natural (Reserve)	Site 4	-24,702248, 31,396797

The current study made use of GIT samples from *Mastomys* spp. that were obtained in October 2020. The samples formed part of a larger collaborative project that focused on rodent and vector-borne zoonotic diseases in the Mnisi community, Mpumalanga province, South Africa, and spanned several years (2019-2022). The *Mastomys* individuals were molecularly typed using nuclei acid-based methods (Bastos et al., 2005).

The sample size for the study was estimated at 95% confidence interval using the formula by Thrusfield (2005).

$$n_0 = \frac{\{1.96^2 \times P_{exp} \times (1 - P_{exp})\}}{d^2}$$

Where P_{exp} was the expected prevalence and d was the desired precision (margin of error of prevalence). A P_{exp}value of 30% (P_{exp}) was adopted from a previous study in the same study area by Rungwe (2022) and the margin of error, d, was 0.09 ,

$$n_0 = \frac{3.8416 \times 0.30 \times 0.7}{0.0081} = 99.6 \text{ samples}$$

3.3 Trapping and processing of rodents for GITs extraction

Rodents were trapped using the Sherman-type live traps following established procedures (Matthee et al., 2020). Trapping activities were part of a larger study on rodents. Traps were deployed over a span of four consecutive days at different locations within each of the three habitat types. Subsequently, the captured rodents underwent humane euthanasia with halothane at the Hans Hoheisen Wildlife Research Station (HHRWS), situated in Kruger National Park. Identification was conducted at both genus and species levels, relying on morphological characteristics as described by Skinner and Chimimba (2005) and Monadjem et al. (2015). Furthermore, confirmation of the identifications was accomplished through a species-specific multiplex PCR based on the cytochrome B gene, following the protocol outlined by Bastos et al. (2005).. Further processing of the rodents followed a protocol by Herbreteau et al. (2011). The sex was also determined, followed by extraction of the GITs. The GITs were preserved in 70% ethanol, and then transported to the DVTD Research and Training laboratories under a red cross movement permit from Department of Agriculture, Land Reform and Rural Development, Mpumalanga Province (Rungwe, 2022).

3.4 Processing of the GITs for nematode collection

The stored GITs were first sorted to identify those from *Mastomys* sp. (*M. natalensis* and *M. coucha*) whose identity had been confirmed as described above, and a total of 100 rodent GIT samples were randomly selected to include 46 from the village habitat, 27 for crop habitat and 27 for natural habitat. The GIT of each rodent was placed in a petri dish containing 10 ml of physiologic saline solution and then split into sections (stomach, small intestines, caecum and colon) with the aid of a scalpel and tissue forceps. Each section was transferred to a separate petri dish containing 10 ml of physiologic saline solution, teased open gently using a pair of curved Mayo dissecting scissors, a scalpel and tissue forceps, and examined for helminths under a stereoscopic and light microscope. Nematodes were identified from the Agricultural Research Council-Onderstepoort Veterinary Research laboratories up to the genus level based on morphometrics. Careful examination, scraping and washing of the mucosa of each section, and breakdown of solidified ingesta were done to ensure removal of any adhering helminth. All

nematodes (total counts) recovered from each section of the GIT were then rinsed with physiologic saline solution, correctly identified, and fixed in 70% alcohol in labelled plastic tubes. Fecal floatation was done on fecal samples obtained from the caecum of each GIT sample using concentrated sugar solution and then observed under a light microscope to identify the nematode eggs following the guide by Thienpont et al. (1986). The tally of nematodes and identified eggs was documented in a laboratory logbook. Subsequently, this information was transcribed to an Excel spreadsheet after their initial morphological identification up to the genus level, relying on taxonomic references provided by Feliu et al. (2000b), Gasser and Monti (1997), Hussey (1957), Khalil and Abdelmottaleb (2014), and Ribas et al. (2013).

3.5 Identification of nematodes and morphometric measurements for *Trichuris* species

The worm recovery, initial morphological identification, and *Trichuris* sp. processing were done at DVTD laboratory. Out of the recovered *Trichuris* sp. specimens, a total of seven mature *Trichuris* sp. individuals recovered from *M. natalensis* were mounted in lactophenol for measurements three being male and four females. The remaining complete *Trichuris* sp. individuals were reserved for DNA extraction of those recovered from *M. natalensis* since worms mounted in lactophenol could not be used in DNA extraction under the study protocol. Seven *Abbreviata* sp. individuals were mounted in lactophenol selected randomly to represent each infested *Mastomys* spp.

The mounted nematodes were then morphologically identified and measurements for *Trichuris* sp. individuals made at the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) following descriptions by previous authors (Feliu et al., 2000b, Gasser and Monti, 1997, Hussey, 1957, Khalil and Abdelmottaleb, 2014, Ribas et al., 2013, Bursey and Brooks, 2011, Jones, 1988a, Morgan, 1945b). Morphometric characters measured included; total body length, anterior body length, posterior body length, spicule length, spicule width at the base, vaginal length, egg length and egg width as recorded by (Feliu et al., 2000b, Robles, 2011) for they showed variation in *Trichuris* sp. as compared to other measurable parameters.

All measurements were done in micrometers (µm) and later converted to millimeters (mm) since most of the reference figures were in mm. Only microscopically identified *Trichuri*s specimens

were measured for the study since it was aimed at confirming *Trichuris* sp. taxonomy using morphometric and molecular techniques.

3.6 DNA extraction from presumed *Trichuris* sp. specimens

Following morphological identification of *Trichuris* spp. from ARC-OVR, DNA extraction and PCR were conducted at DVTD laboratories. Genomic DNA was extracted from eight whole individuals morphologically identified as *Trichuris* sp. obtained from eight infected *M. natalensis* rodents using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Eight 2-ml microcentrifuge tubes were labelled with numbers 1 to 8, and then a corresponding worm, three 3-mm borosilicate glass beads (Merck, Darmstadt, Germany) and 180 µl of buffer ATL from the DNA extraction kit were added. The mixture was homogenized twice for 18 seconds at 5200 revolutions per minute (rpm) using a Precellys 24 (Bertin Technologies, Montigny, France). Samples were then cooled on ice for 1 minute and vortexed using a VM-1000 mixer (mrc Laboratory Instruments, Essex, UK) for 10 seconds.
About 30 µl of proteinase K were added to each tube mixture and then incubated at 56º C overnight on a heating block (Labnet International Inc, Woodbridge, USA), followed by brief centrifugation for 1 minute using a bench centrifuge (Inqaba Biotech, Hamburg, Germany). About 200 µl of the resultant mixture for each sample was pipetted into new microcentrifuge tubes and 200 µl of AL lysis buffer was added. The mixture was heated on a heating block for 10 minutes at 56 ºC. Then 200 µl of 99.6% ethanol were added to each sample and vortexed for 30 seconds. The resultant mixture was then transferred to new spin columns and centrifuged at 8000 rpm for 1 minute and the filtrate was discarded. About 500 µl of AW1 buffer was added to the filter membranes of the spin columns and centrifuged for 1 minute at 8000 rpm. The filtrate was discarded, and the spin column with its contents transferred to a new collection tube and then 500 µl of AW2 were added for each sample. The spin column in the collection tube was centrifuged at 12700 rpm for 3 minutes and the filtrate discarded. The extracted DNA was then eluted by adding 100µl of AE buffer to the spin column placed in a 1.5 mL Eppendorf tube, and then incubated at room temperature for 1 minute. This was followed by centrifuging at 8000 rpm for a minute. The DNA was stored at -20 ºC until needed for PCR analysis.

3.7 PCR for *Trichuris* species

Four nematode target genes: mitochondrial cytochrome oxidase subunit 1 gene (Cox 1 mtDNA) and cytochrome B (cytB) and ribosomal universal first internal transcribed spacer (ITS 1) and second internal transcribed spacer (ITS 2) were selected for the characterization of *Trichuris* spp. with primer sets targeting various genera of *Trichuris* as previously described in literature (Table 4). The primer sets used in this study were synthesized and supplied by Inqaba Biotec (Pretoria, South Africa). The ITS regions although highly conserved exhibit a high degree of sequence variation between different species, a characteristic of great importance for our phylogenetic analyses, as earlier reported by Callejon et al. (2010).

Table 4: Description of primers used during PCR analysis of *Trichuris* sp. recovered from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Target gene	Primers name and corresponding sequence (5'……..3')	Amplicon length (bp)	Reference
Cox 1 mtDNA	CO1Fmod: TGR TTT TTT GGI CAY CCI GAR G	˜500 bp	(Guardone et al., 2013)
	CO1Rmod: CAC TAC ATA GTA DGT RTC RTG		
ITS 1	NC5: GTA GGT GAA CCT GCG GAA GGA TCA TT	˜500 bp	(Eberhardt et al., 2019, Gasser et al., 1996)
	5.8SR: GAG TGT CAC GTC GTT CTT CA		(Rivero et al., 2023, Rivero et al., 2022)
ITS 2	TrF: CTC GTA GGT CGT TGA AGA AC	˜500 bp	(Nissen et al., 2012)
	NC2: TTA GTT TCT TTT CCT CCG CT		(Gasser et al., 1993, Nissen et al., 2012)
Cytochrome B	D769: GAG TAA TTT TTA TAA TRC GRG AAG T	˜570 bp	(Callejon et al., 2015, Rivero et al., 2022)
	D770: AAT TTT CAG GRT CTC TRC TTC AAT A		

For each of the four genes, each reaction mixture contained 12.5 µl of Phusion Flash High-Fidelity PCR master mix (Thermo Scientific, Johannesburg, South Africa), 0.5 µM each for forward and reverse primers, 7.5 µl of nuclease free water, and 2.5 µl of template DNA to make a total volume of 25 µl for each reaction. Each PCR reaction was run in triplicate to ensure consistency and reliability of results. Nuclease free water was used as a negative control. We did not include a positive control because no positive reference sample was available. The PCR conditions for amplification of the four target genes are shown in Table 5. The PCR products were visualized by agarose gel (stained with ethidium bromide) electrophoresis at 120 V for 35 min.

Table 5: PCR conditions for the amplification of four target genes for *Trichuris* sp. recovered from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Stage	Temperature (ºC)	Duration	No. of cycles
Initial denaturation	98	10 sec	1
Denaturation	98	1 sec	35
Annealing	54 for Cox 1; 55 for ITS-1; 50 for cytB; 55 for ITS-2	1 min	
Extension	72	6 sec for Cox 1 and CytB; 8 sec for ITS-1 and ITS-2	
Final extension	72	1 min	1
Incubation	4	∞	

3.8 PCR purification, sequencing and sequence analysis

The triplicate PCR products from each sample for the three genes (ITS-1, ITS-2, CytB) were added in one tube and then purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. No amplification was obtained for the Cox 1 gene, even after optimization, and therefore not continued. The purified PCR product was eluted in 50 µl of the elution buffer and then visualized by running on a 2% agarose gel (stained with ethidium bromide) at 120V for 35 minutes alongside a 100-bp molecular marker, GeneRuler (ThermoScientific, Johannesburg, South Africa). About 10 µl of each purified PCR sample was

submitted to the Central Analytical Facility of Stellenbosch University in Cape Town, South Africa for sequencing.

3.8.1 Sequence analysis

Sanger sequencing on the purified amplicons was conducted using the respective reverse and forward primers that were used in the PCR amplification of each gene. The AB1 files were quality trimmed, assembled and cleaned using CLC Genomics Workbench version 7.5.1 (QIAGEN, Hilden, Germany). The Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for taxonomic classification of the sequences and to retrieve homologous reference sequences. The obtained sequences of each gene, together with corresponding reference sequences were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) version 7 (Katoh and Standley, 2013) and then visualized in BioEDIT version 7.2 (Hall, 1999).

3.9 Phylogenetic analysis

Phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 (Tamura and Kumar, 2021) employing the General Time Reversible model with 1000 bootstrap replications.

3.10 Data Analysis

Descriptive statistics were used to establish the proportion of infested rodents as well as median and mean for helminth counts (overall, *Trichuris* sp., *Abbreviata* sp.) from the host GITs. For qualitative data (presence, absence), association between presence of helminths (all worm species) and each of the predictor factors: habitat (crop, natural, village), rodent sex (male, female), rodent species (*M. natalensis*, *M. coucha*) were determined in univariate analyses using the chi-squared test (Table 8). All variables from univariate analysis irrespective of p-value were combined in a multivariable binomial Generalised Linear Model that employed a stepwise elimination procedure (forward and backward), with an Akaike Information Criterion, to determine risk factors for helminth infection (presence or absence) (Table 9). For quantitative data, a zero-inflated negative binomial Generalised Linear Model was applied to determine the relationships between *Trichuris* sp. counts and predictor variables: species, habitat and sex of rodent (Table 10). Data analysis was

performed using the packages “doBy”, “pscl”, “MASS” and “lmtest” in R statistical software version 4.2.1 (RCoreTeam, 2022).

4. RESULTS

4.1. Nematode prevalence and infection rates in *Mastomys* species

Of the 100 *Mastomys* individuals that were examined 60 were males and 40 females. Most of the rodents were *M. natalensis* (68) and 27 were *M. coucha* while taxonomic molecular identification of five rodents could not be confirmed from the study samples. Both rodent species occurred in all the three habitats, but the village habitat harbored the highest number of *M. natalensis* (n=40), followed by crop (n=26) and natural habitat (n=3). For *M. coucha*, the natural habitat had the highest number (n=22) followed by the village (n=4) and the crop habitat (n=1) had the lowest number. Nematodes were recorded and with an overall prevalence of 20.00%.

A total of 46 nematodes representing two species, *Trichuris* sp. and *Abbreviata* sp. were recorded. *Trichuris* sp. exhibited higher abundance (0.31 ± 0.22) compared to *Abbreviata* sp. (0.15 ± 0.14) (Table 6) while a mean abundance of 0.23 ± 0.18 was recorded for both nematodes. *Trichuris* sp. was primarily recorded in the caecum, while *Abbreviata* sp. was recorded in the stomach. Single species infections were common, with only one rodent harboring a mixed infection of both nematodes (Table 7). Nematodes were more prevalent in *Mastomys* spp. from the natural habitat (22.22%) followed by village (19.58%) and crop habitat type (18.52%). *Trichuris* sp. was recorded in all three habitat types with the highest prevalence in crops (14.81%) followed by village (13.00%) (Table 7). *Abbreviata* sp. was most prevalent in the natural habitat (18.50%) and absent in the crop habitat. Female and male *Mastomys* spp. showed a similar nematode prevalence of 20%. Overall *M. coucha* had a higher nematode prevalence (25.93%) dominated by *Abbreviata* sp. (22.20%) as compared to *M. natalensis* with a nematode prevalence of 19.10%, the majority being *Trichuris* sp. (16.18%). In the present study *Trichuris* sp. was predominantly recorded in the caecum of *M. natalensis* in contrast to Rungwe (2022) who detected *Trichuris* sp. predominantly in the caecum and small intestine of *Mastomys* spp.,with a prevalence of 8.20%.

Table 6: Nematode abundance and at 95% CI prevalence in *Mastomys* spp. (n = 100) across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Nematode Species	Total abundance	Mean abundance ± SE	Prevalence (%)
Trichuris sp.	31.00	0.31 ± 0.22	12.00
Abbreviata sp.	15.00	0.15 ± 0.14	8.00
Total	46.00	0.23 ± 0.18	20.00

Table 7: Nematode abundance and prevalence from *Mastomys* spp. (n = 100) per habitat type, host sex and rodent sp. captured across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

Variable	**No. examined**	**Nematodes abundance (prevalence %)**	***Mastomys* with only *Trichuris* sp. infections (%)**	***Mastomys* with only *Abbreviata* sp. infections (%)**	***Mastomys* with Mixed infections (%)**
Habitat type					
Village	46.00	9 (19.57)	6 (13.00)	3 (6.50)	0 (0.00)
Crop	27 .00	5 (18.52)	4 (14.81)	0 (0.00)	1 (3.60)
Natural	27.00	6 (22.22)	1(3.70)	5 (18.50)	0 (0.00)
Sex					
Male	60.00	12 (20.00)	7 (11.90)	4 (6.80)	1 (1.70)
Female	40.00	8 (20.00)	4 (10.00)	4 (10.00)	0 (0.00)
Rodent species					
M. natalensis	68.00	13 (19.12)	11 (16.18)	1 (1.47)	1 (1.47)
M. coucha	27.00	7 (25.93)	1 (3.70)	6 (22.20)	0 (0.00)
Non conclusive	5.00		0 (0.00)	0 (0.00)	0 (0.00)

4.2 Descriptive output and associations between prevalence and abundance and predictor variables

4.2.1 Descriptive output and association between presence of worms in the GI of rodents and host or habitat types

Univariate analysis revealed no significant relationship between the presence of helminth parasites and host sex or habitat type ($p > 0.05$). The proportions of infected hosts were similar ($p > 0.05$) between males (20.00%) and females (20.00%), and among crop (17.90%), natural (22.20%) and village (19.60%) habitats (Table 8). The presence of nematodes was not significantly ($p > 0.05$) related to rodent species (Table 8 and 9).

Table 8: Univariate association between host factors and presence of nematode parasites recovered from the gastrointestinal tracts of *Mastomys* spp. (n = 100) across a wildlife-human /domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

Variable	Category	No. of infested rodents (%)	P value
Host Sex	Female (n=40)	8 (20.00)	
	Male (n=60)	12 (20.00)	0.94
Habitat	Crop (n=28)	5 (17.90)	
	Natural (n=27)	6 (22.20)	0.90
	Village (n=46)	9 (19.60)	0.88
Rodent species	*M. natalensis* (n=69)	13 (18.80)	
	M. coucha (n=27)	7 (25.90)	0.52

Table 9: Multivariable binomial Generalised Linear model regarding association between presence of nematode parasites in the gastrointestinal tract of *Mastomys* spp. (n = 100) across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Variable	Category	No. of infested rodents (%)	Odds ratio	P value
Rodent species	*M. natalensis* (n=69)	13 (18.80)		
	M. coucha (n=27)	7 (25.90)	1.51	0.44

4.2.2 Quantitative relationship between *Trichuris* spp. worm counts and rodent host/environmental factors

None of the three variables (habitat type, host sex and rodent species) assessed in the zero-inflated negative binomial Generalised Linear Model showed a statistically significant relationship (p > 0.05) with *Trichuris* abundance in *Mastomys* spp. (Table 10). However, *M. coucha* individuals were 1.5 times more likely to be infested than *M. natalensis* (OR>1.51). (Table 9). *Mastomys. natalensis* rodent species were more likely to have higher *Trichuris* worm counts than *M. coucha* species (Exp-coeff>1000) (Table 10). Furthermore, the odds that an individual rodent would be in the "Certain Zero" group was 1000 times higher in *M. natalensis* group than in *M. coucha* group (p=0.95). Overall, *Trichuris* abundance in individual rodents was low, with a maximum of five worms (Table 10).

Table 10: A zero-inflated negative binomial Generalized Linear Model with modelling that zero values are dependent on habitat and rodent species for *Trichuris* worms recovered from gastrointestinal tracts from *Mastomys* spp. (n = 100) in Mnisi Community, Mpumalanga Province, South Africa in October 2020

Variable	**Category**	**Mean (range) of *Trichuris* species counts**	**Exp(coef.) and p-values of the -count model**	**Exp(coef.) and p-value of- zero-inflated model**
Sex	Male (n=60) (ref)	0.300 (0 - 4.00)		
	Female (n=40)	0.325 (0 – 5.00)	1.17 (p=0.81)	
Habitat	Natural (n=27) (ref)	0.037 (0 – 1.00)		
	Crop (n=28)	0.357 (0 – 4.00)	0.00 (p=0.95)	<0.00 (p=0.95)
	Village (n=46)	0.435 (0 – 5.00)	0.00 (p=0.95)	<0.00 (p=0.95)
Rodent species	*M. coucha* (n=27) (ref)	0.037 (0 – 1.00)		
	M. natalensis (n=69)	0.435 (0 – 5.00)	184370.00 (p=0.92)	80922301.00 (p=0.95)

4.3 Morphology of nematodes recovered

4.3.1 Morphology of *Abbreviata* species

Abbreviata sp. individuals recovered only comprised of adult females (n = 15). They were generally white cylindrical in body shape with same width from cranial to caudal end (Fig 2e), a cervical head with cuticular inflation (Fig 2b) with two pseudolabia (Fig 2f) each possessing two double submedian teeth (external lateral and internal lateral tooth), short buccal cavity with an oesophagus divided into an anterior short muscular portion (fig 2b) and a long posterior glandular portion. A nerve ring that was anterior to the junction of the muscular and glandular oesophagus was present. The uterus was divided into four portions and contained thick ovoid eggs. Majority were recovered from the stomach of the hosts.

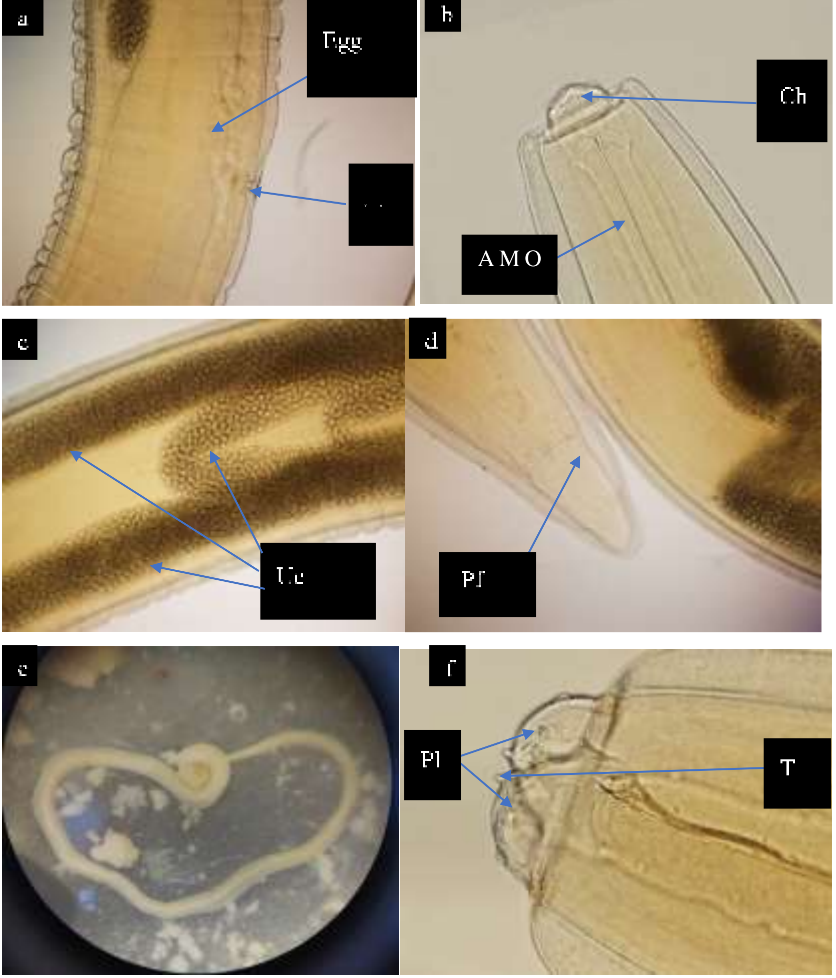

Figure 2: *Abbreviata* sp. recovered from *Mastomys* spp. recovered across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020. Vu- Vulva, Pf- Posterior end of female *Abbreviata* sp., Ue- Uteri portions engorged with thick ovoid eggs, AMO- Anterior portion of the muscular oesophagus, Ch- Cervical head, Pl- Pseudo labia, T- Teeth

4.3.2 Morphology of *Trichuris* species

A total of 31 *Trichuris* sp. individuals were recovered, comprising 19 females and 11 males. In one of the specimens, the posterior segment was damaged during recovery and in some worms the damage was on the anterior end, rendering sex determination through morphological means impossible. All the worms had a characteristic whip shape with a narrow thread like anterior portion and a thick posterior portion (Fig 3f) which coiled heavily for males (Fig 3e) and terminated into a curved spicule surrounded by a sheath that was ornamented with spines (Fig 3c).

The vulva opening of the females was located on the lateral side almost mid worm (fig 3a), the uterus of reproductive specimens was heavily loaded with bi-operculated ovoid eggs (Fig d). These features are common of the *Trichuris* genus hence the need for further advanced molecular diagnostic methods to decipher the genus up to species level. A very low egg count was recorded on coprological examination.

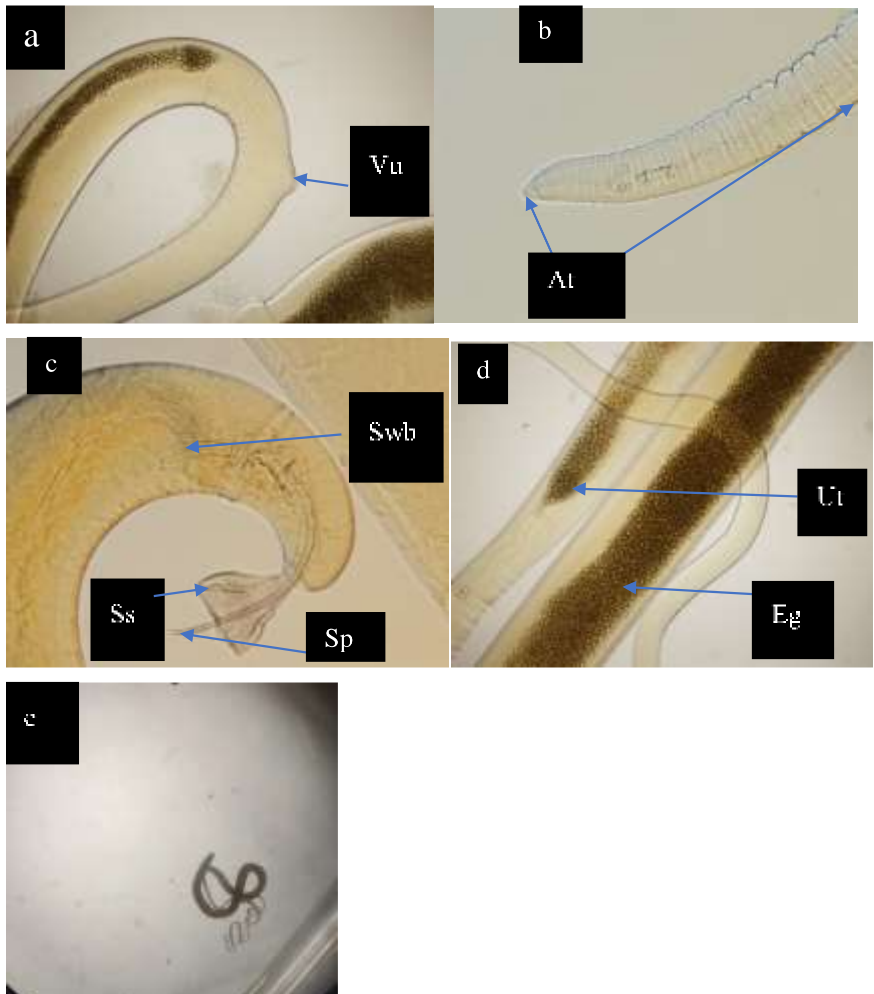

Figure 3: *Trichuris* sp. recovered from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020. At- Part of Anterior portion of *Trichuris* sp., Sp-Male spicule, Ut- uterus fully packed with eggs, Swb- Spicule Width at Base, Eg-Eggs, Vu-Vulva opening located mid body of the worm, Ss- spicule sheath.

4.3.3 Morphometrics of *Trichuris* species

For the males, the average total body length was 21.80 mm, anterior body length, posterior body length, spicule length, and spicule width at the base are shown in (Table 11). Females had an average body length of 27.80 mm and other measurements are indicated in Table 11.

Table 11: Morphometric measurements for *Trichuris* sp. (3 males and 4 females) for selected characters obtained from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020

Sex	Character	mean	Min-Max	Median	SD
Male (n=3)	Total body length	21.80	18.11-25.49	21.80	3.69
	Anterior length	15.16	12.91-17.40	15.16	2.25
	Posterior length	7.64	5.20-9.60	6.65	1.45
	Spicule length	0.83	0.80-0.88	0.85	0.04
	Spicule width at base	0.04	0.04-0.04	0.04	0.00
Female (n=4)	Total body length	27.80	22.6-33	27.80	5.20
	Anterior length	15.35	12.20-18.50	15.35	3.15
	Posterior length	13.93	10.40-16.50	14.40	2.20
	Vaginal length	0.15	0.13-0.16	0.15	0.01
	Egg Length	0.06	0.06-0.06	0.06	0.00
	Egg Width	0.03	0.03-0.03	0.03	0.00

4.4. Conventional PCR amplification and amplicon size confirmation

All the eight samples were successfully amplified for the ITS-1 and ITS-2 genes, while seven of the eight samples were amplified with the cytochrome B gene (Figure 4). No success was obtained with amplification of the cox 1 gene for all the eight samples.

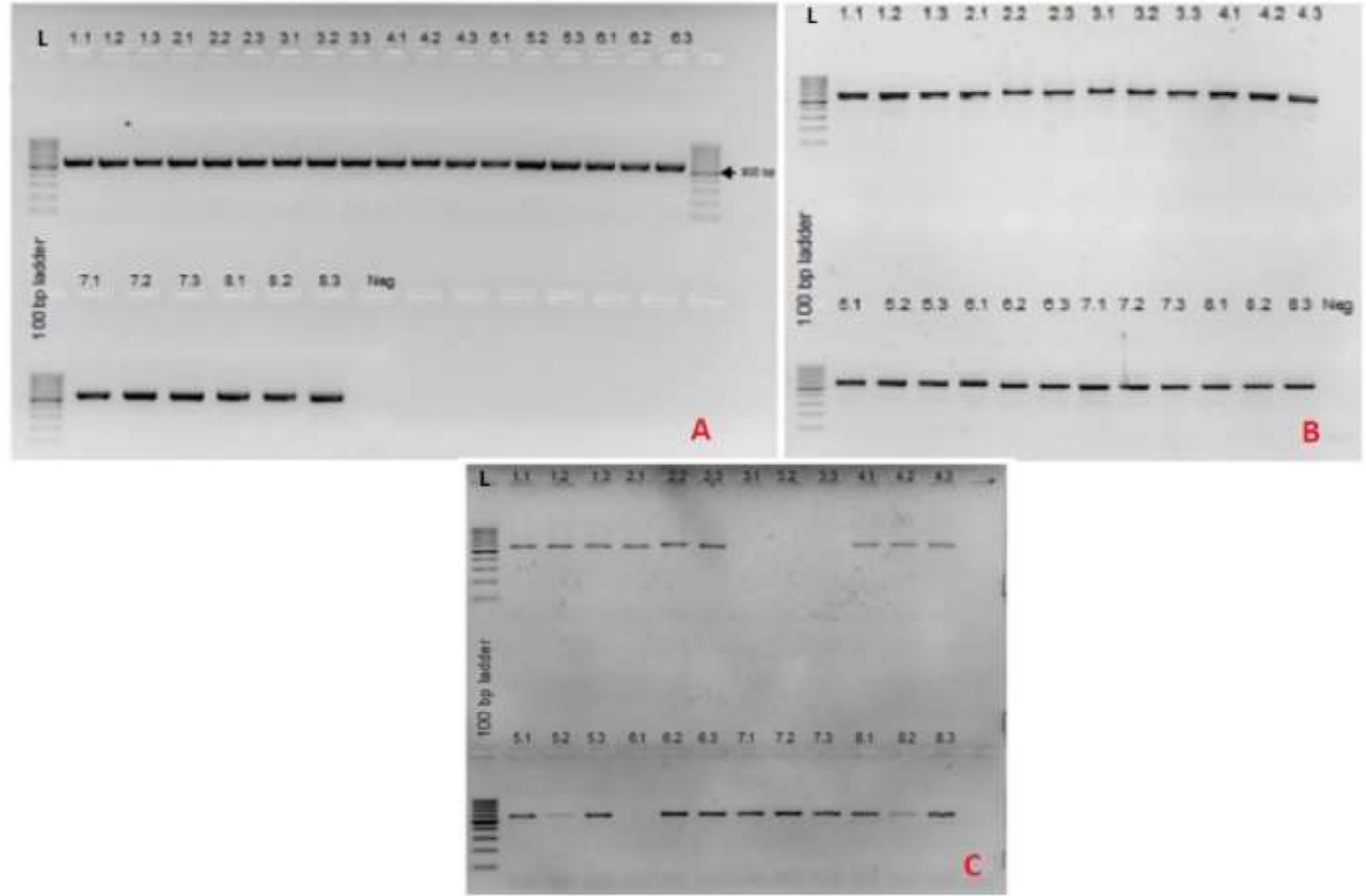

Figure 4: Conventional PCR products of eight *Trichuris* sp. samples (each run in triplicate) visualized on agarose gel stained with ethidium bromide. A, PCR amplification for ITS 1 gene fragment, while B and C are for the ITS 2 and cytochrome B genes, respectively. Lane L indicates the 100 bp DNA gene ladder, while lanes 1-8 represent the eight *Trichuris* species samples obtained from eight *M. natalensis*; , Lane Neg, negative control.

4.5 Sequence analysis

A total of eight sequences (seven for the cytB) were obtained for each gene comprising of a forward and reverse set. The obtained sequence lengths were 571 to 582 bp for cytB gene, 527 to 564 bp for the ITS 1 gene and 548 to 589 bp for the ITS 2. The obtained ITS 1 sequences were 80.4 to 80.5% identical to *T. muris* sequences (accession numbers FN543136 and LC171641; query cover 85-86%) from European countries, while ITS 2 sequences 78.8 to 82.9% to 84.2% identical to *Trichuris carlieri* s. l. (JX683522) sequence from Tanzania or 78.9% to 82.5% identical to *T. muris* (KU575094) sequence from Europe. The cytB sequences were 83.9% and

86.5% to *Trichuris arvicolae* (LM994698) from Spain and 83.8% to 84.0% to *Trichuris* sp. ETH392 (MZ229686) from Ethiopia.

Table 12: Genetic relatedness of the obtained *Trichuris* sequences to published sequences. The *Trichuris* spp. were isolated from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

Sample ID	Morphological identity	ITS 1 identity	ITS 2 identity	Cytochrome B identity
1	*Trichuris* sp.	80.50% (query cover 85.00%) to *Trichuris muris* (FN543136) 80.42% (query cover 85%) to *Trichuris muris* (LC171641)	83.58% (query cover 85.00%) to *Trichuris carlieri* s. l. (JX683522) 82.46% (query cover 69%) to *Trichuris muris* (KU575094)	86.53% (query cover 88.00%) to *Trichuris arvicolae* (LM994698) 84.01% (query cover 99.00%) to *Trichuris* sp. ETH392 (MZ229686)
2	*Trichuris* sp.	80.50% (query cover 86.00%) to *Trichuris muris* (FN543136) 80.42% (query cover 86%) to *Trichuris muris* (LC171641)	83.62% (query cover 89.00%) to *Trichuris carieli* s.l (JX683522) 82.49% (query cover 72.00%) to *Trichuris muris* (KU575094)	86.53% (query cover 87.00%) to *Trichuris arvicolae* (LM994698) 84.01% (query cover 98.00%) to *Trichuris* sp. ETH392 (MZ229686)
3	*Trichuris* sp.	80.50% (query cover 86.00%) to *Trichuris muris* (FN543136)	83.75% (query cover 83.00%) to *Trichuris carieli* s.l (JX683522)	- -

		80.42% (query cover 86.00%) to *Trichuris muris* (LC171641)	78.86% (query cover 95.00%) to *Trichuris muris* (KU575094)	
4	*Trichuris* sp.	80.50% (query cover 80.00%) to *Trichuris muris* (FN543136)	83.62% (query cover 85.00%) to *Trichuris carieli* s.l (JX683522)	86.53% ((87% query cover) *Trichuris arvicolae* (LM994698)
		80.42% (query cover 80.00%) to *Trichuris muris* (LC171641)	78.72% (query cover 96.00%) to *Trichuris muris* (KU575094)	84.01% (query cover 98.00%) to *Trichuris* sp. ETH392 (MZ229686)
5	*Trichuris* sp.	80.50% (query cover 86.00%) to *Trichuris muris* (FN543136)	82.96% (query cover 84.00%) to *Trichuris carieli* s.l (JX683522)	86.53% (query cover 88.00%) to *Trichuris arvicolae* (LM994698)
		80.42% (query cover 86%) to *Trichuris muris* (LC171641)	81.71% (query cover 69.00%) to *Trichuris muris* (KU575094)	84.01% (query cover 99.00%) to *Trichuris* sp. ETH392 (MZ229686)
6	*Trichuris* sp.	80.50% (query cover 86.00%) to *Trichuris muris* (FN543136)	83.93% (query cover 84.00%) to *Trichuris carieli* s.l (JX683522)	86.53% (query cover 87.00%) to *Trichuris arvicolae* (LM994698)
		80.42% (query cover 86.00%) to *Trichuris muris* (LC171641)	79.07% (query cover 95.00%) to *Trichuris muris* (FN543136)	84.01% (query cover 98.00%) to *Trichuris* sp. ETH392 (MZ229686)

7	*Trichuris* sp.	80.50% (query cover 84.00%) to *Trichuris muris* (FN543136)	83.69% (query cover 86.00%) to *Trichuris carieli* s.l (JX683522)	86.53% (query cover 86.00%) to *Trichuris arvicolae* (LM994698)
		80.42% (query cover 84%) to *Trichuris muris* (LC171641)	78.79% (query cover 98.00%) to *Trichuris muris* (FN543136)	83.77% (query cover 98.00%) to *Trichuris* sp. ETH392 (MZ229686)
8	*Trichuris* sp.	80.50% (query cover 85.00%) to *Trichuris muris* (FN543136)	84.17% (query cover 85.00%) to *Trichuris carieli* s.l (JX683522)	83.85% (query cover 86.00%) to *Trichuris arvicolae* (LM994698)
		80.42% (query cover 85%) to *Trichuris muris* (LC171641)	79.34% (query cover 97.00%) to *Trichuris muris* (FN543136)	83.77% (query cover 99.00%) to *Trichuris* sp. ETH392 (MZ229686)

4.6 Phylogenetic analyses

The genetic relationships between the new *Trichuris* sp. specimens and corresponding published sequences of the three genes ITS 1, ITS 2 and cytB are shown in Figures 5 to7. The obtained sequences consistently grouped in a separate clade from published sequences, suggesting a novel *Trichuris* sp from *Mastomys* rodents in South Africa (Figures 5 to 7).

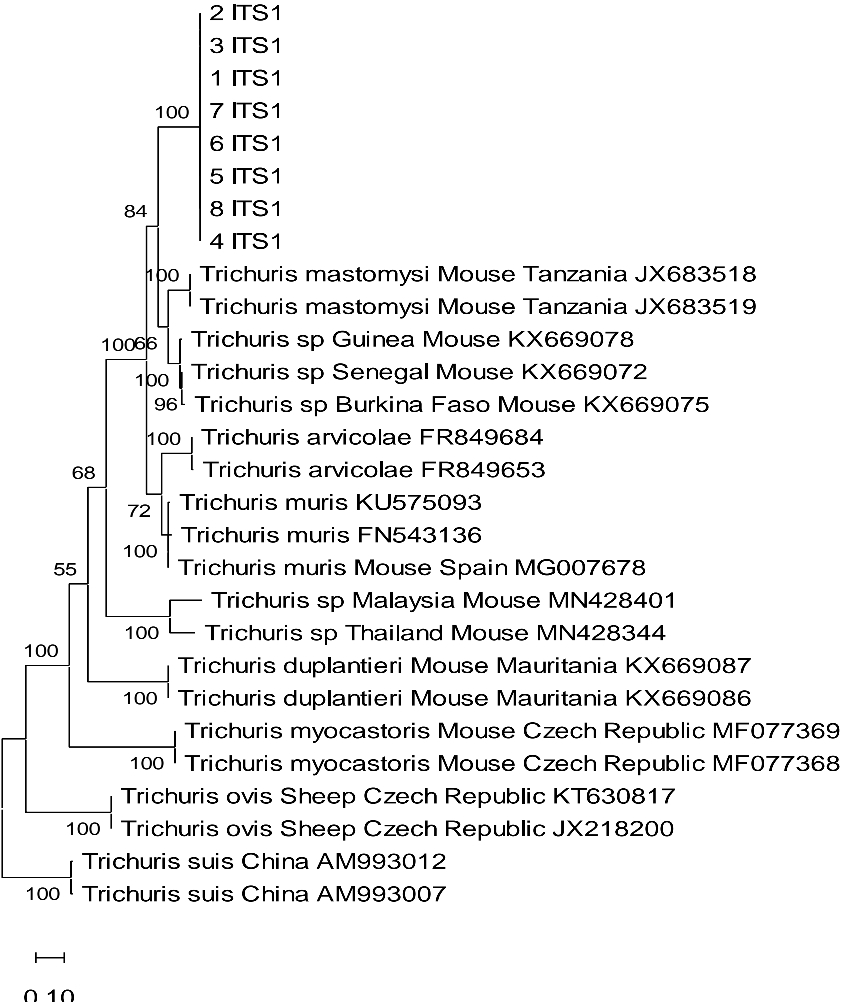

Figure 5: Phylogenetic analysis of *Trichuris* spp. ITS1 sequences obtained in this study with published sequences. The tree was generated using a maximum likelihood method with 1000 bootstrap replicates as implemented in Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. Scores at nodes represent corresponding bootstrap support. The scale bar is proportional to the genetic distance in terms of nucleotide substitutions per site. The ITS 1 sequences obtained in present study are labelled 1 ITS 1 to 8 ITS 1. The tree was rooted using *Trichuris suis* sequences from China. The *Trichuris* specimens were isolated from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

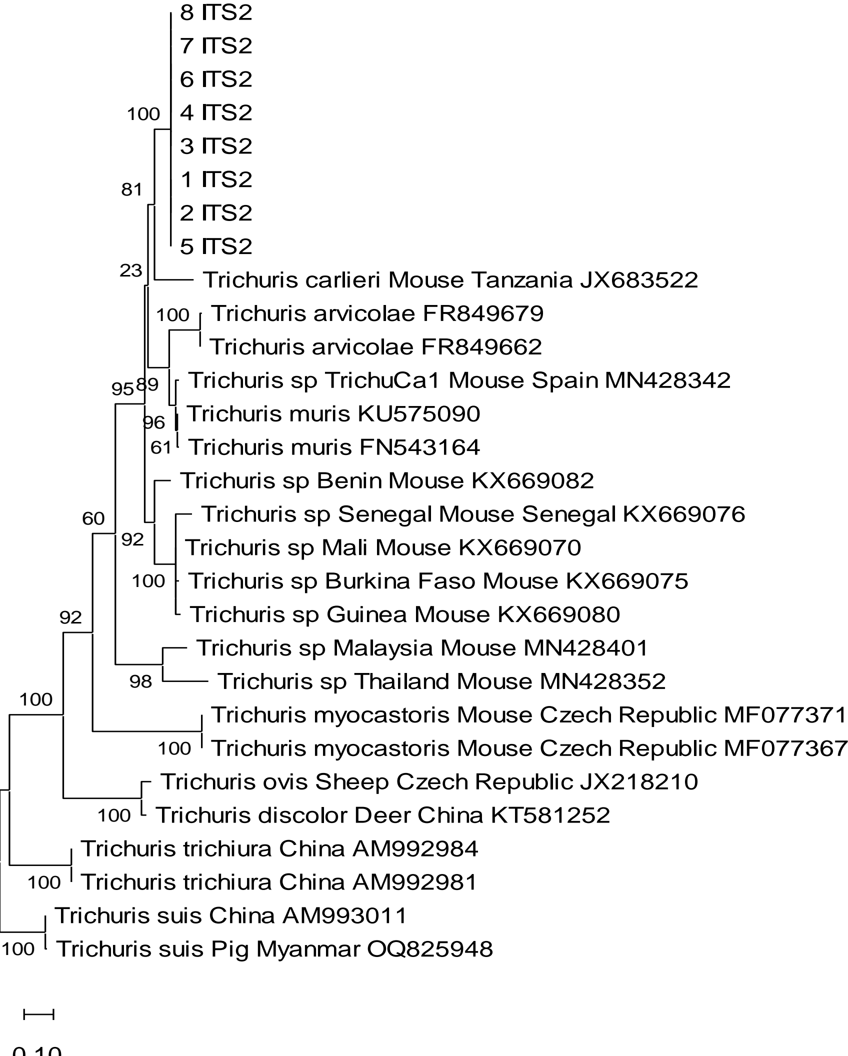

Figure 6: Phylogenetic analysis of *Trichuris* spp. ITS 2 sequences obtained in this study with published sequences. The tree was generated using a maximum likelihood method with 1000 bootstrap replicates as implemented in Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. Scores at nodes represent corresponding bootstrap support. The scale bar is proportional to the genetic distance in terms of nucleotide substitutions per site. The ITS 2 sequences obtained in present study are labelled 1 ITS 2 to 8 ITS 2. The tree was rooted using *Trichuris suis* sequences from China and Mynmar. The *Trichuris* specimens were isolated from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

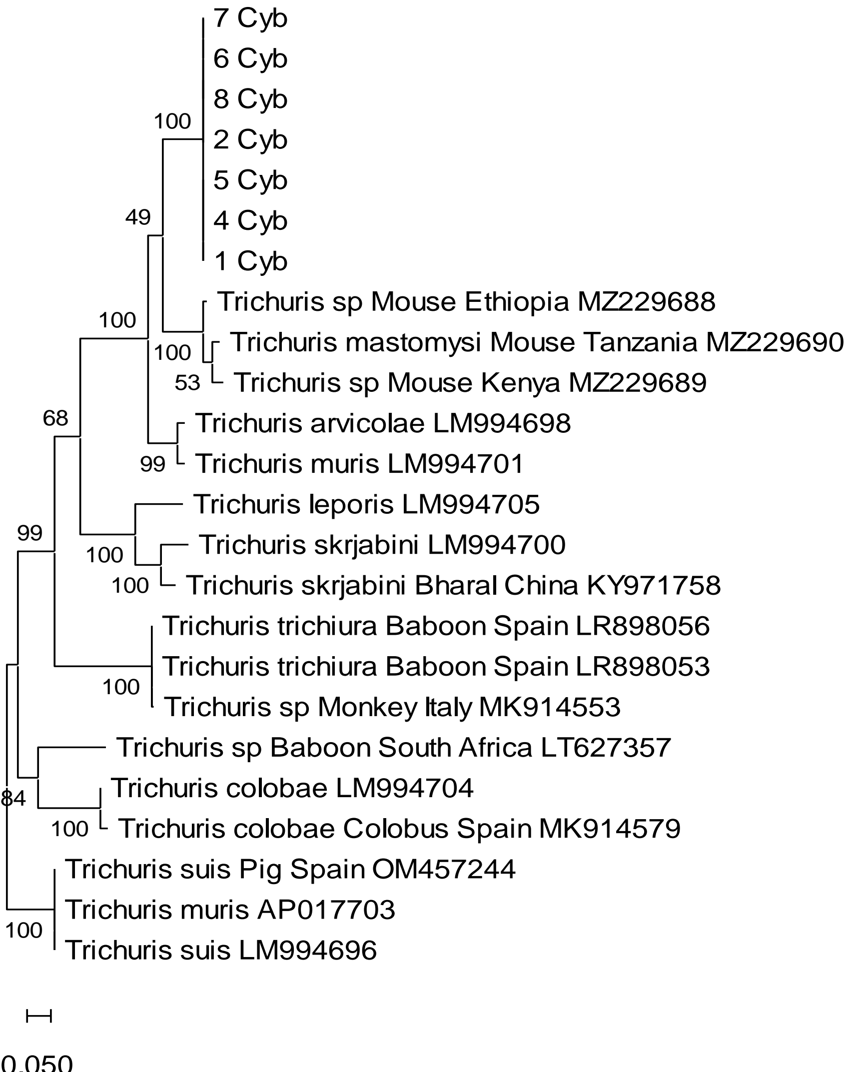

Figure 6: Phylogenetic analysis of *Trichuris* spp. cytochrome B sequences obtained in this study with published sequences. The tree was generated using a maximum likelihood method with 1000 bootstrap replicates as implemented in Molecular Evolutionary Genetics Analysis (MEGA) software version 11.0. Scores at nodes represent corresponding bootstrap support. The scale bar is proportional to the genetic distance in terms of nucleotide substitutions per site. The cytochrome B sequences obtained in present study are labelled 1 CyB to 8 CyB. The tree was rooted using *Trichuris muris* and *Trichuris suis* sequences. The *Trichuris* specimens were isolated from *M. natalensis* across a wildlife-human/domestic animal interfaces, Mpumalanga province, South Africa in October 2020.

5. DISCUSSION

The present study aimed to assess nematode diversity in *Mastomys* rodents (Rodentia: Muridae) and to characterize using morphological and molecular methods *Trichuris* sp. in the Savanna biome, South Africa. This was achieved by determining the prevalence of nematodes, investigating the influence of habitat type, host species and sex on nematode abundance and confirming the *Trichuris* sp. in Mnisi community using molecular methods.

5.1 Nematode diversity associated with *Mastomys* species

The overall nematode prevalence in the present study was low (20.00%) with a higher prevalence of *Trichuris* sp. in *M. natalensis* and *Abbreviata* sp. in *M. coucha*. In comparison, Rungwe (2022) reported a prevalence of 30.10% in the same area in September 2019, recording *Trichuris* sp. and *Syphacia* sp. Several factors may have likely contributed to this difference including variations in nematode life histories, climatic conditions and rodent species composition, which most likely influenced nematode transmission, abundance and survival (Anderson et al., 1998, Anderson, 2000, Andersen et al., 1966). The life cycle can be direct or indirect and this determines the nematode abundance with higher localised infections usually recorded for direct life cycles (Anderson, 2000). Additonally, ife history traits such as host habitat preferences have been indicated to impact nematode infection and biodiversity (Froeschke et al., 2010). Climate influences the habitat characteristics and this has been reported to greatly influence the helminth communities in *Mastomys* spp. hosts (Brouat et al., 2007b). Host specificity, as influenced by habitat preference, diet, and human activities, among others, may further impact nematode diversity (Chaisiri et al., 2017).

Abbreviata sp. requires an arthropod intermediate host to complete its life cycle (King et al., 2013), potentially favouring natural habitats. In contrast, *Trichuris* sp. have a direct life cycle facilitating transmission through the faecal oral route (Horton, 2014). Similarly, *Syphacia* species has a characteristic oxyurid life cycle which does not only depend on exposure to the external environment or host for completion (Dewi et al., 2016). Climatic conditions, particularly precipitation, influence nematode abundances (Froeschke et al., 2010) and it is possible that the pre-rain season (September-December) in the savanna biome was drier in during the current sampling period (October 2020) compared to September 2019. In addition, climatic conditions can

also influence the abundance and diversity of intermediate hosts (Haukisalmi and Henttonen, 1990, Morley and Lewis, 2008), which will further impact the survival and abundance of *Abbreviata* sp. The *Mastomys* individuals included in the Rungwe (2022) study were only identified morphologically but not typed molecularly. This potential difference in rodent species composition and abundance between the two studies would most likely influence nematode species diversity and relative abundances (Spickett et al., 2019b).

The current study recorded the highest number of *Abbreviata* sp. in *M. coucha* and only one *M. natalensis* was infested with *Abbreviata* sp., which was consistent with this nematode's host preference. The absence of *Abbreviata* sp. in the previous study by Rungwe (2022) could potentially be explained by the absence of the preferred host, *M. coucha*, though rodent speciation was not performed in that study. The overall low *Abbreviata* sp. abundance could be attributed to host preference, with all previous studies having never reported *Abbreviata* sp. in *Mastomys* sp. (Spickett et al., 2019a, Spickett et al., 2017a, Spickett et al., 2017b), most likely due to this nematode's preference of larger hosts such as reptiles and amphibians over small mammals such as rodents (King et al., 2013). Human activities in the village and crop habitats could destroy the habitat for the intermediate host needed in transmission of *Abbreviata* sp. The overall relatively low nematode counts registered in the current and (Rungwe, 2022)'s study may be attributed to sampling in the pre-planting season, which is climatically non ideal for helminth survival. In contrast, a nematode prevalance of 44.10% was recorded with the most prevalent being *S. obvelata* in *R. pumilio* South Africa (Froeschke et al., 2010) while Jrijer et al. (2023) reported an overall prevalence of 50% for *Gongylomema neoplasticum* in *Meriones shawi* of Tunisia. Differences in rodent hosts sampled and geographic location of these studies could likely contribute to the observed variations with the current study.

The difference in predilection site of *Trichuris* sp. as compared with Rungwe (2022) may be attributed to the infection being in its initial stages of establishment in the host in the previous study, thereby targeting sites with weaker immune defense mechanisms. The caecum has a stronger immune defense mechanism to overcome before infection establishment (Behnke et al., 2001) most likely explaining the lower infection level in the current study than in (Rungwe, 2022).

In South Africa, *Abbreviata* spp. have been previously recorded in several rodent hosts including *L. rosalia*, *M. namaquensis,* and *R. dilectus* (Spickett et al., 2019a, Spickett et al., 2017a, Spickett et al., 2017b). Concurrently, *Trichuris* spp. have been recorded in rodent hosts such as *M. coucha*, *M. natalensis*, *R. dilectus* and *R. pumilio* (Rungwe, 2022, Spickett et al., 2019a, Spickett et al., 2017a). These rodents, chartecterised as omnivores, can ingest the intermediate host, with the exception of the herbivorous *M. coucha* and *M. namaquensis*, which are usually infected by ingestion of the infective larval stage of *Abbreviata* sp. on contaminated vegetation. However, it is important to note that the feeding habits of the rodents vary based on environmental conditions, food availability and other habitat factors (Sassi et al., 2011).

Nematode abundance was not affected by habitat, host sex and species, in sync with Rungwe (2022) who also reported lack of influence of sex, habitat and breeding status on nematode prevalence and abundance. In contrast, Froeschke and Matthee (2014) reported habitat influences on helminth prevalence and species abundance with the highest infection and helminth diversity recorded in crop habitat as compared with the natural habitat, urban and livestock grazed areas. Furthermore, helminth assemblages were found to be influenced by both host behaviors and environmental factors but at varying levels following analysis of two closely related hosts (*R. pumilio* and *R. dilectus*), a difference in assemblage was recorded for both species per different locality (Spickett et al., 2017b). The low parasite infections, in the present study, might have influenced distinct patterns and statistical analyses of host species, with potential impact on nematode prevalence and distribution.

The recovery of only female *Abbreviata* sp. coupled with the presence of egg-engorged uteri in the present study suggests sexual dimorphism or parthenogenesis in this nematode. The life cycle of *Abbreviata* sp., however, remains poorly described in literature (Elmahy and Harras, 2019). Cuticular inflation, pseudo labia and sub median teeth possessed by *Abbreviata* sp. are believed to be used in attachment and feeding. The absence of association between nematode infection level and habitat type, host species and sex could be attributed to the smaller sample size per category attributable to the low nematode prevalence recorded in the current study. These findings are different from previous studies which show association between host factors and environmental factors on helminth assemblages in rodent hosts (Spickett et al., 2017b). Variations in sample size

and nematode diversity between the two studies could likely have contributed to the observed differences.

5.2 Morphometric measurements and molecular analyses of *Trichuris* species

Average total body length measurements of *Trichuri*s sp. in the present study for males (21.80 mm) was longer than that obtained by Rungwe (2022) (17.39 mm) but shorter than that recorded for females (27.80 mm) by the same author. Despite both studies using *Trichuris* sp. samples from the same study area, the difference could be attributed to the use of few individuals for measurements hence reflecting normal variation. The present study measured seven *Trichuris* sp. individuals (three males and four females) while Rungwe (2022) examined four *Trichuris* sp. individuals (two females and two males). The measurements of the characters in the present study were in ranges obtained by Feliu et al. (2000a) for *T. muris* and *T. arvicolae* captured in Spain. Spicule length (0.83mm) obtained in the current study was consistent with that of *T. mastomysi, T. carlieri* s.l and that of *T. muris* (0.58 mm-0.99 mm) recovered from African rodents (Ribas et al., 2013). The male ratio of posterior-anterior length recorded for the study was 1:1.98 slightly higher than the one reported for clade one of west African *Trichuris* sp. (1:1.29-1.39) and (1:1.48-1:1.50) for clade three (Ribas et al., 2017). Average egg length (0.06 mm) and width (0.030 mm) were slightly greater than those reported by Ribas et al. (2017) for a *Trichuris* sp. from West Africa (egg length of 0.050 mm and an average of 0.026 - 0.030 mm egg width). The morphometric comparison of African female *Trichuris* sp. remains scanty and these findings contribute to the reference literature. The present findings clearly indicate that the species diversity of *Trichuris* cannot be confirmed by only conventional morphometric methods as emphasized in previous studies (Feliu et al., 2000b, Liu et al., 2012, Ribas et al., 2013).

The current study corroborates previous studies suggesting that morphometric characteristics may not confirm the identity of female *Trichuris* species, nor can they differentiate between males at the species level (Callejón and Cutillas, 2017, Cutillas et al., 2009, Cutillas et al., 2014, García-Sánchez et al., 2019). Morphological characters clearly lack diagnostic specificity for species identification (Cavallero et al., 2015). However, combining morphological and biometrical characters may enhance diagnostic specificity for species as demonstrated by Robles (2011). The presence of *Mastomys* spp. in all the three habitats considered in the current study is aligned with

earlier studies, confirming the semi-commensal nature of the genus and co-existence of the two *Mastomys* spp. (Dippenaar, 1993).

5.3 Molecular and phylogenetics of *Trichuris* species

Lack of amplification of the CO1 gene at PCR optimization in the current study may be due to the annealing temperature that was used. This therefore, prompted the use of only three genes to characterize *Trichuris* sp. in the present study. Variable amplicon length in the different genes may be indicative of the great genetic diversity and potential mutations (insertions or deletions) in the gene sequences. This is characteristic of the congeneric nature of *Trichuris* sp.

Molecular results indicated that the *Trichuris* sp. recovered in the study fall into very distinct genetic clades on assessment with ribosomal DNA genes (ITS 1 and ITS 2) or mitochondrial gene cytB. The ITS 1 sequences recovered were in a separate clade but closer to another clade containing *T. mastomysi* (JX683519) and *T. mastomysi* (JX683518) obtained from Tanzania in Morogoro and Berega respectively, hosted by *M. natalensis*. For ITS 2 the sequences were in a separate clade but closer to *T. carlieri* s.l (JX683522) from *G. vicinus* in Maguha Tanzania (Ribas et al., 2013). This observed genetic diversity and variable sequence similarities with the known *Trichuris* sp. suggests the presence of distinct strains or subspecies within the genus *Trichuris* populations that occur in the Mnisi community. These findings contribute to understanding of nematode diversity and evolution in the Mnisi community geographic region which may represent a unique genetic lineage within *Trichuris* genus despite having genetic similarities to *Trichuris* sp. from other regions. It also confirms the specific nature of *Trichuris* sp. and the ability of the genus to infect various host species corroborated by the reference gene sequences in the current study.

While mtDNA phylogenetic studies utilize 12 protein coding genes, these analyses essentially reflect the mitochondrial gene tree evolutionary history, which is inherited as a single locus. In contrast, ribosomal RNA genes, integral to evolutionary studies, are situated on a non-independent single locus. Thus, conducting separate trees of only mitochondrial or ribosomal RNA gene comparisons does not provide independent tests of evolutionary history. For in depth understanding of evolutionary relationships within *Trichuris* sp., a preferred approach involves conducting phylogenetic analyses based on a combination two independent loci (mtDNA genes

and rRna genes) (Callejon et al., 2013, Liu et al., 2012). The present study used two rRNA genes (ITS 1 and ITS 2) and one mitochondrial gene (cytochrome B) to identify *Trichuris* sp., and the aligning of results of morphometric and molecular methods clearly demonstrates the need to use both techniques in taxonomy. Findings from the current study bring out the rich diversity of *Trichuris* sp. on the African continent and demonstrate the possibility of more undocumented species or subspecies in the genus.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Trichuris sp. and *Abbreviata* sp. were the only nematode species infecting *Mastomys natalensis* and *M. coucha* rodents across a wildlife-human/domestic animal interface in the Savanna biome. Infections were generally low level and nematode prevalence, and abundance were not affected by habitat and host factors. A previously undocumented *Trichuris* sp. was identified in *M. natalensis*, which was a novel finding. The current study emphasizes the importance of using an integrated approach of morphological measurements and molecular analysis to accurately categorize *Trichuris* sp.

6.2 Recommendations

There is a need for a larger sample size per habitat type in future research on nematode diversity and the influence of the predictor variables such as (habitat type, host sex and species) on nematode abundance. Further studies on nematodes must also focus on *Abbreviata* sp. to address the scanty literature on the biology of its lifecycle. Optimization and use of the CO1 gene in further studies is encouraged to enable evolutionary histories of nematodes to be studied. Expanding the rodent host range in future studies is recommended to allow a more in-depth study of nematode diversity at the wildlife-human/domestic animal interface and potential public health threat of the novel *Trichuris* sp.

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8. APPENDICES

Appendix 1: Research Ethics Committee Approval

Faculty of Veterinary Science
Research Ethics Committee

6 March 2023

CONDITIONALLY APPROVAL

Ethics Reference No	**REC134-22**
Protocol Title	**Diversity of nematodes in the rodent genus Mastomys and molecular characterization of Trichuris species across a wildlife-human/domestic animal interface in the savanna biome, South Africa**
Principal Investigator	**Dr JM Mutesasira**
Supervisors	**Dr MC Marufu**

Dear Dr JM Mutesasira,

We are pleased to inform you that your submission has been conditionally approved by the Faculty of Veterinary Sciences Research Ethics committee, subject to other relevant approvals.

Please note the following about your ethics approval:

1. Please use your reference number (REC134-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application for post graduate studies (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

NOTES: Conditionally approved pending the following:

1. Obtaining ALL other relevant approvals.

2. Upload the 2023 letter of collaboration (for the use of the samples).

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Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Faculty of Veterinary Science

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

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Appendix 2: Animal Ethics Committee Approval

Faculty of Veterinary Science
Animal Ethics Committee

21 April 2023

Approval Certificate
New Application

AEC Reference No.: REC134-22
Title: Diversity of nematodes in the rodent genus Mastomys and molecular characterization of Trichuris species across a wildlife-human/domestic animal interface in the savanna biome, South Africa
Researcher: Dr JM Mutesasira
Student's Supervisor: Dr MC Marufu

Dear Dr JM Mutesasira,

The **New Application** as supported by documents received between 2023-02-10 and 2023-03-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-03-27.

Please note the following about your ethics approval:

1. The use of species is approved:

Samples	Number
Rodent - GIT Stored- Historic/Retrospective (V023-19)	100

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-04-21.
3. Please remember to use your protocol number (REC134-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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Lefapha la Disaense tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Appendix 3: Section 20 Approval

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development Private Bag X138, Pretoria 0001
Enquiries: Ms Mama Laing • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: MamaL@dalrrd.gov.za Website: www.dalrrd.gov.za
Reference: 12/11/1/1/6 (2833 SdlR)

Responsible person: Dr Jesse Mukisa Mutesasira
Institution: Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
Old Soutpan Road
Onderstepoort
0110
Email: jessemukisabryt@gmail.com , chris.marufu@up.ac.za
Tel: 012 529 8460

Dear Dr Jesse and Dr Chris

PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "Diversity of nematodes in Mastomys and molecular characterization of Trichuris species across a wildlife-human/domestic animal interface in the Savanna biome, South Africa."

Your application, dated 30/11/2022, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform the research project or study stipulated above, refers.

I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa.
2. The research project is approved as per the application form dated 30 November

2022 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this research project under this Section 20 permit. Please apply in writing to MamaL@dalrrd.gov.za.

3. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to MamaL@dalrrd.gov.za.
4. No part of this research project may begin until the valid ethical approval has been obtained in writing from the relevant South African authority.
5. All biological or potentially infectious material must be packaged and transported in accordance to the International Air Transport Association (IATA) requirements and/or the National Road Traffic Act, 1996 (Act No. 93 of 1996).
6. Only the following samples may be used in this research project:
   6.1. Biobank samples from the gastrointestinal tracts from Rodents (Mastomys) stored at DVTD Research and Training Laboratories (Helminthology laboratory).
7. Department of Veterinary Tropical Diseases, University of Pretoria - Biohazard Safety level 2 laboratory will be used for the extraction of DNA only from those nematodes morphologically identified as Trichuris.
8. All potentially infectious material utilised or generated during or by the research project is to be destroyed and disposed of by a waste disposal company registered to remove biohazardous waste at completion of the research project.
9. Any incidence or suspected incidence of a controlled or notifiable disease in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984), must be reported immediately to the responsible state veterinarian.
10. All documents or publications that may be made available to the public and that contain the outcomes of the testing of controlled and notifiable animal diseases in South Africa in terms of the Animal Diseases Act 1984 (Act no 35 of 84), must be screened and approved by the Director: Animal Health prior to their release and/or publication. These must be sent to MamaL@dalrrd.gov.za and the Section 20 permit reference number and research project title must be included in the email.
11. It is the responsibility of the researcher and relevant laboratory or facility managers to ensure that the human safety aspects of this research project are adequately addressed.
12. Records must be kept for five years for auditing purposes.

SUBJECT: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984) 12/11/1/1/6 (2833 SdIR)

Title of research/study: Diversity of nematodes in Mastomys and molecular characterization of Trichuris species across a wildlife-human/domestic animal interface in the Savanna biome, South Africa.

Researcher: Dr Jesse Mukisa Mutesasira

Institution: Department of Veterinary Tropical Diseases

Permit Expiry Date: 2024/12/31

Our ref Number: 12/11/1/1/6 (2833 SdIR)

Your ref: REC134-22

Kind regards,

DR. MPHO MAJA

DIRECTOR: ANIMAL HEALTH

Date: 2023-04-19

Appendix 4: Turnitin Report