Sokoine University of Agriculture

MSc. Dissertation

Bionomics, Blood-Host Plasticity and Its Effect on Host-Choice and Feeding Behaviour of Tsetse Species from Selected Human-Wildlife Interface in Tanzania

Filbert Ewald Mdee

April 2024

Bionomics, Blood-Host Plasticity and Its Effect on Host-Choice and Feeding Behaviour of Tsetse Species from Selected Human-Wildlife Interface in Tanzania

A Dissertation Submitted to Sokoine University of Agriculture in Fulfilment of the Requirements for the Degree of Masters of Science in Public Health Pest Management

By

Filbert Ewald Mdee

Supervisors

Prof. Ladslaus L. Mnyone
Dr. Eliakunda Mafie

Department of Veterinary Medicine and Public Health
College of Veterinary Medicine and Biomedical Sciences
Sokoine University of Agriculture (SUA), Morogoro, Tanzania

April 2024

EXTENDED ABSTRACT

Tsetse flies are vectors of trypanosome parasites which cause human African trypanosomiasis (HAT) in human and African animal trypanosomiasis (AAT) in livestock across Sub-Saharan Africa. These flies feed exclusively on animals' blood. It is during blood-feeding process of these flies; un-infected host get infected by parasites carried by infected vector. Several tsetse controls programs have been implemented so as to minimize the incidences of trypanosome infections; however, the human-wildlife interfaces remain as the risk areas where both livestock and human being can be infected with trypanosome parasites. Therefore, surveillance and control of these flies is important so as to minimize the African trypanosomiasis' risks in these areas. This study assessed the species composition, abundance and phylogenetic relatedness of wild collected tsetse flies from selected human-wildlife-livestock interface in Tanzania. Variation in host choice and feeding behaviours of predominant species' (*Glossina morsitans*) siblings whose parents were blood-fed on different host species were also investigated. The tsetse flies were trapped seasonally in two selected wards within Morogoro Rural district. The study wards and villages were purposively selected targeting those which are bordering to protected areas. In each ward, baited NZI, Pyramidal and Biconical traps were deployed at 200m distance apart from each other for 72 hours before rotating to the next trapping sites. Trapped flies were collected from the traps after 24 hours then identified morphologically and later confirmed using the Polymerase Chain Reaction (PCR). Moreover, a colony of tsetse flies were established from pupa obtained from tsetse and vector control centre, Tanga. Hatched flies were maintained on selected hosts blood until the offsprings were obtained for the experiments. The host-choice and feeding behaviours experiments were carried out in large semi-field cage containing four small equal size screen cages. During the experiments, individual host was placed in a screen cage which allowed flies to enter through openings on each side. The groups of

flies (20 per replicate) colour-marked differently basing on their parents' bloodmeal hosts, were released from the centre of large semi-field cage and left to forage for 24 hours before being collected, then, sorted basing on the location, feeding status and parents' bloodmeal. The total of 784 tsetseflies were collected; *Glossina pallidipes* (n=371; 47.32%) and *Glossina morsitans morsitans* (n=413; 52.68%). Of these, 96 flies (80-female, 16-male) were blood-fed; 57(48-female and 9-male) *G. pallidipes* and 39(32-female, 7-male) *G.m. morsitans*. Overall abundance of collected tsetse significantly varied across surveyed wards (χ^2=4.597, df=1, p= 0.032), villages (χ^2=9.491, df=3, p= 0.023), habitats (χ^2=17.239, df=2, p<0.001), months (χ^2=13.507, df=3, p= 0.004) and deployed traps (χ^2=6.348, df= 2, p= 0.04). About 78.82% of tsetse flies were collected from Kisaki ward (n=618; p<0.001) and 21.17% (n=166; p=0.032) from Bwakila chini. The highest proportion of these flies were collected in Mbojoge village (62%; n= 489) followed by Kiperege (18%; n=141) and Sebo (16%; n=129). NZI traps collected the highest proportion of tsetse flies (n=422; 54%; 4.98 FTD) followed by Pyramidal traps (n=281; 36%; 4.01 FTD) and Biconical traps (n=81; 10%; 1.87 FTD). Similarly, the large proportion of tsetse flies (78.06%) were collected in bushed grassland habitat (n=612; 55.41 FTD) followed by woodland habitat (16.45%; n=129; 20.56 FTD) and farmland (5.5%; n=43; 7.17 FTD). The phylogenetic analysis revealed genetic relatedness of tsetse flies collected in Tanzania with those collected from Nigeria and Senegal. Furthermore, a total of 213 flies (72.95% of the recovered) were attracted to the hosts. The number of flies attracted to different hosts varied significantly (χ^2_4= 33.685, p= 0.0001); Rodent (n=80, p=0.006), Rabbit (n=59, p=0.331), Guinea pig (n=49, p=0.057) and squirrel (n=25, p=0.005). The number of flies attracted to their parent's blood meal source varied significantly (χ^2_{12} = 56.476, p<0.001); rabbits (n= 35, 59.32%, p<0.001), rodent (n=25, 31.25%, p=0.043) and guinea pig (n= 19, 38.78%, p=0.45). But, only 39 flies (18.31% of total attracted) bloodfed on the hosts; Guinea pigs (n=10, 25.64%), Rodents (n=23, 58.97%), Rabbits (n=6, 15.38%) and

Squirrels (n=0,0.0%). There was significant variation in number of flies that fed successively across hosts (χ^2_4=49.478, p<0.001). The present study recommends NZI and Pyramidal traps for tsetse fly control at the interface and wet season as appropriate season for conducting control activities. Also, the study confirms the presence of the hosts' differential attractiveness to flies but failed to explain observed behaviours in relation to genetic inheritance. Therefore, future studies are recommended to investigate the effect of bloodmeal sources on tsetse fly siblings' behaviours across filial generations using small mammals.

Keywords: Tsetse fly, wildlife-livestock-human interface, NZI, Pyramidal, Abundance, *Glossina morsitans***,** Rabbits, Guinea pigs, Rodents and Squirrels**,** blood-fed, attracted.

IKISIRI KUU

Tsetse ni wabebaji wa vimelea vya trypanosome ambavyo husababisha ugonjwa wa malale (HAT) na nagana (AAT) katika mifugo katika eneo la Kusini mwa Jangwa la Sahara. Wadudu hawa hula damu ya wanyama pekee. Ni wakati wa kula damu; mnyama asiye na maambukizi hupata maambukizi kutoka kwa vimelea vilivyobebwa na tsetse mwenye maambukizi. Programu kadhaa za kudhibiti tsetse zimefanywa ili kupunguza visa vya maambukizi ya trypanosoma; hata hivyo, maeneo ya mwingiliano kati ya binadamu na wanyama pori bado ni maeneo hatari ambapo mifugo na binadamu wanaweza kuambukizwa na vimelea vya trypanosoma. Kwa hiyo, ufuatiliaji na udhibiti wa wadudu hawa ni muhimu ili kupunguza hatari ya ugonywa wa malale katika maeneo haya. Utafiti huu ulitathmini aina ya spishi, wingi, na uhusiano wa kifailogenetiki wa tsetse waliokusanywa katika maeneo ambayo wanyamapori, binadamu na mifugo inayatumia nchini Tanzania. Tofauti katika chaguo la wanyama na tabia za kula za aina ya tsetse (*Glossina morsitans*) ambao wazazi walikua wanalishwa damu za aina tofauti za wanyama pia zilichunguzwa. Tsetse walitegwa kwa msimu miwili katika kata mbili zilizochaguliwa ndani ya wilaya ya Morogoro. Katika kila kata, mitego ya aina ya NZI, Pyramidal na Biconical zilitegwa kwa umbali wa mita 200 kutoka kwa kila mmoja kwa masaa 72 kabla ya kusonga kwenye maeneo mengine ya kutega. Tsetse walionaswa walikusanywa kutoka kwenye mitego baada ya masaa 24, kisha kutambuliwa kwa njia ya morfolojia na baadaye kuthibitishwa kwa kutumia Polymerase Chain Reaction (PCR). Uchunguzi wa tabia ya uchaguzi wa wanyama na ulaji yalifanyika katika. kizimba kikubwa cha nusu-shamba kilichokuwa na mabanda manne madogo ya ukubwa sawa Wakati wa majaribio, mnyama alikuwa amewekwa katika banda dogo ambalo liliwaruhusu tsetse kuingia kupitia matundu yaliyokua kila upande. Kikundi cha tsetse (20 kwa kila jaribio) kilichopakwa rangi tofauti kulingana na wazazi wao walikula damu ya mnyama yupi, kiliruhusiwa kutoka katikati ya kizimba kikubwa cha tsetse na kuachwa kutafuta chakula kwa masaa 24

kabla ya kukusanywa, kisha kugawanywa kulingana na eneo, hali ya matumbo yao, na damu walizolishwa wazazi wao. Jumla ya tsetse 784 walikusanywa; *Glossina pallidipes* (n=371; 4732%) na *Glossina morsitans morsitans* (n=413; 5268%). Kati ya hao, tsetse 96 (80-mwanamke, 16-kiume) walikua wamekula damu za wanyama; 57 (48-wakike 9-wakiume) *G. pallidipes* na 39 (32-wakike, 7-wakiume) *G.m. morsitans*. Kwa ujumla idadi ya tsetse waliokusanywa kwa kiasi kikubwa walitofautiana katika kata zilizofanyiwa utafiti (χ^2=4.597, df=1, p= 0.032), vijiji (χ^2=9.491, df=3, p= 0.023), makazi (χ^2=17.239, df=2, p<0.001), miezi (χ^2=13.507, df=3, p= 0.004) na mitego iliyotumiwa (χ^2=6.348, df= 2, p= 0.04). Asilimia 78.82 ya tsetse walikusanywa kutoka kata ya Kisaki (n=618; p<0.001) na asilimia 21.17 (n=166; p=0.032) kutoka Bwakila chini. Idadi kubwa zaidi ya tsetse hawa ilikusanywa katika kijiji cha Mbojoge (62%; n=489), ikifuatiwa na Kiperege (18%; n=141) na Sebo (16%; n=129). Mitego aina ya NZI ilikusanya idadi kubwa zaidi ya tsetse (n=422; 54%; 4.98 FTD), ikifuatiwa na mitego ya piramidi (n=281; 36%; 4.01 FTD) na mitego ya biconical (n=81; 10%; 1.87 FTD). Vivyo hivyo, idadi kubwa ya tsetse (78.06%) ilikusanywa katika maeneo ya nyasi (n=612; 55.41 FTD) ikifuatiwa na maeneo ya msitu (16.45%; n=129; 20.56 FTD) na ardhi ya kilimo (55%; n=43; 7.17 FTD). Uchambuzi wa kifailojenetiki ulionyesha uhusiano wa kinasaba kati ya tsetse waliokusanywa Tanzania na wale waliokusanywa Nigeria na Senegal. Zaidi ya hayo, jumla ya tsetse 213 (72.95% ya waliokusanywa tena) walivutiwa na wanyama tofauti. Idadi ya tsetse wanaovutwa kwa wanyama tofauti ilikuwa tofauti kwa kiasi kikubwa ($\chi^2$4= 33685, p= 0.0001); panya (n=80, p=0.006), sungura (n=59, p=0.331), pimbi (n=49, p=0.057) na chindi (n=25, p=0.005) Idadi ya tsetse waliovutwa na chanzo cha damu ya wazazi wao ilikuwa tofauti kwa kiasi kikubwa ($\chi^2$12 = 56.476, p<0.001); sungura (n= 35, 5932%, p<0.001), panya (n=25, 31.25%, p=0.043) na pimbi (n= 19, 38.78%, p=0.45). Lakini, ni tsetse 39 tu (18.31% ya jumla ya waliovutiwa kwa wanyama) waliokula damu kwa wanyama; pimbi (n = 10, 25.64%), panya (n = 23, 58.97%), Sungura (n = 6, 15.38%) na chindi (n = 0,0.0%). Kulikuwa na utofauti katika idadi ya tsetse

ambao walifanikiwa kula damu katika wanyama ($\chi^2 4 = 49.478$, $p<0.001$). Majibu ya utafiti huu yanapendekeza mitego aina ya NZI na piramidi kwa udhibiti wa tsetse kwenye maeneo ambayo binabamu, wanyamapori na mifugo inayatumia kwa pamoja na msimu wa mvua kama msimu unaofaa kwa kufanya shughuli za udhibiti. Pia, utafiti unathibitisha uwepo wa mvuto tofauti wa wadudu aina ya tsetse lakini haujafanikiwa kuelezea tabia zilizoonekana kuhusiana na urithi wa jeni. Kwa hiyo, utafiti wa baadaye unapendekezwa kuchunguza athari za vyanzo vya damu kwa tabia za ndugu wa tsetse katika vizazi vya watoto kwa kutumia wanyama wadogo.

Maneno muhimu: Tsetse, wanyamapori-mifugo-binadamu, NZI, Piramidi, idadi ya tsetse, *Glossina morsitans*, sungura, pimbi, panya na chindi, ulaji wa damu, kuvutia

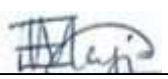
DECLARATION

I, **Filbert Ewald Mdee,** hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work done within the period on registration and it has neither been nor currently submitted for a higher degree award in any other institution.

Filbert Ewald Mdee **Date**
(MSc. Candidate)

The above declaration is confirmed by;

Prof. Ladslaus L. Mnyone **Date**
(Supervisor)



Dr. Eliakunda Mafie **Date**
(Supervisor)

LIST OF PAPERS AND MANUSCRIPT

Paper I: Mdee, F.E.; Lyatuu, J.; Msoffe,V.T.; Mafie, E.; Mnyone, L.L. 2 Species composition, abundance and phylogenetic relatedness of wild tsetse flies collected from selected human-wildlife interface in Tanzania.

Status: Submitted to the Journal of Parasite Epidemiology and Control

Paper II: Mdee, F.E.; Lyatuu, J.; Mafie, E.; Mnyone, L.L. Host Choice and Feeding Behaviours of Glossina morsitans Offspring Whose Parents Were Fed on Different Host Species. Parasitologia 2024, 4, 38–46. https://doi.org/10.3390/parasitologia4010003

Status: Published in the Journal of Parasitologia. Volume 4 (1), 38-46; https://doi.org/10.3390/parasitologia4010003

COPYRIGHT

ACKNOWLEDGEMENTS

All the glory and thanks should go to the almighty God for the blessings vested upon me throughout my MSc studies.

I sincerely appreciate the financial support from the Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE II IRPM-BTD).

I extend my special thanks to my research supervisors, Prof. Ladslaus L. Mnyone and Dr. Eliakunda Mafie, for their constructive comments, guidance and overall supervision from the start up to the final write ups of this research work. I have learnt plenty of things throughout the processes.

I also appreciate the love, encouragement and support from my beloved parents which in one way or the other simplified the whole process of studying.

Finally, I am indebted to Mr. Venance Msoffe, Mr. Mustapha Halfani, Mr. Deusdedit Malulu, Mr. Ginethon Mhamphi, Mr. Mwinyi Masala and all staff at the Institute of Pest Management (IPM) for their technical support and encouragement throughout my research work.

DEDICATION

This research work is dedicated to all scientific community working tirelessly to achieve the desired minimum threshold of tsetse populations and impact thereof across Sub-Saharan Africa.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AAT	Animal African Trypanosomiasis
ACE	Africa Centre of Excellence
AD	Apparent Density
DA	Diminazene diaceturate
DNA	Deoxyribonucleic acid
FAO	Food Agriculture Organization
FTD	Fly Trap Density
GIS	Geographic Information System
GLMMs	Generalized Linear Mixed Models
GPS	Global Position System
HAT	Human African Trypanosomiasis
HDX	Humanitarian Data Exchange
IAEA	International Atomic Energy Agency
IPM	Institute of Pest Management
ISM	Isometamedium chloride hydrochloride
PA	Protected Area
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
SOP	Standard Operating Procedure
SSA	sub-Saharan Africa
SUA	Sokoine University of Agriculture
TVLA	Tanzania Veterinary Laboratory Agency
WHO	World Health Organization

STRUCTURE OF THE DISSERTATION

This dissertation is structured into FOUR chapters. Chapter ONE entails the background information on tsetse flies' abundance and distribution, the burden of African Trypanosomiasis (AT) in Africa and Tanzania, tsetse flies' behaviours and control, problem statement and justification, research objectives and research questions. Chapter TWO entails the first manuscript titled 'Species composition, abundance and phylogenetic relatedness of the wild tsetse flies collected from selected human-livestock-wildlife interface in Tanzania**'**. Chapter THREE contains the second manuscript titled 'Host-choice and feeding behaviour of *Glossina morsitans'* offspring whose parents were fed on different host species'. Chapter FOUR entails the general discussion as well as the main conclusions and recommendations.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Abundance and Distribution of Tsetse Flies

Tsetse flies are vectors of the protozoan trypanosome parasites that cause sleeping sickness in humans and nagana in domestic animals (Vreysen *et al.*, 2012). There are 31 species and subspecies of tsetse flies which are grouped to three subgenera based on morphological and ecological characteristics: Austenina (Fusca group), Nemorhina (Palpalis group), and Glossina (Morsitans groups) (Cecchi *et al.*, 2008). The fusca group is composed of several species including *Glossina fusca, G. tabaniformis, G. medicorum, G. longipennis, G. brevipalpis*, while, Morsitans group is composed of *G. morsitans, G. pallidipes, G. longipalpis, G. swynnertoni, and G. austeni.* The Palpalis group consists of *G. palpalis, G. tachinoides, and G. fuscipes* (Bouteille & Buguet, 2014). Only about 10 of the 31 species have been proven to have public health and veterinary significance (Gooding and Krafsur, 2005; Vreysen *et al*., 2012; Kweka *et al.,* 2017). Those species include *G. pallidipes, G. brevipalpis, G. m. morsitans, G.m. centralis, G.p. palpalis, G. fuscipes fuscipes, G. tachinoides, Glossina palpalis gambiensis, Glossina fuscipes quanzensis, and G. swynnertoni*.

Tsetse inhabits only sub-Saharan Africa (SSA) between latitudes 14° N and 20° S (WHO, 2013) infesting an area of about 8-11 million km^2 across 38 countries (Krafsur, 2003; Vreysen *et al.*, 2012). In Tanzania, it is estimated that tsetse flies infest about 15.5% of the total area (Malele, 2011), where the dominant species are the savannah species, which among others include *Glossina pallidipes*, *Glossina swynnertoni* and *G. morsitans morsitans*. Other species are present but with limited distribution and these include among others *Glossina austeni*, *Glossina brevipalpis*, *Glossina longipennis, Glossina fuscipes martinii* and *G. fuscipes fuscipes* (Malele *et al*., 2007; Malele, 2011). This non-uniform distribution is generally determined by climatic and demographic pressure (Salekwa *et al.*,

2014; Dicko *et al.*, 2015; Nnko *et al.*, 2021). Moreover, occurrence and dispersal of tsetseflies is affected by factors such as humidity, altitude, vegetation/shades, availability and host density (Soberon & Peterson, 2005; Salekwa *et al.*, 2014). Among these factors, temperature is considered the major driver of tsetse occurrence and distribution (Nnko *et al.*, 2021).

1.2 Burden of African Trypanosomiasis

Tsetse flies pose a significant health risk to both humans and animals particularly in sub-Saharan due to human and animal movements (Simarro *et al.*, 2012). In addition, tsetse leads into economic losses resulting from loss of manpower, livestock death, and decrease in animal productivity. Approximately 40 to 70 million people (Simarro *et al*., 2012; Ngonyoka *et al*., 2017) and 60 million livestock (Cecchi *et al*., 2014) in SSA are at risk of tsetse-borne trypanosome infection. In Tanzania, about 4 million people in rural communities are at risk of acquiring sleeping sickness which results into approximated annual loss of about 8 million USD (Nnko *et al.*, 2021). The high risk is arguably contributed by an exclusive hematophagous nature of both the male and female tsetse flies and their tendency to blood feed on several vertebrate disease reservoirs (Holmes, 2013; Vale *et al.*, 2014).

1.3 Tsetse Flies' Behaviours

Unlike other insects which produce many eggs (r-strategists), tsetse flies are k-strategists due to their low rate of reproduction (Veterinärmedizin, 2012). According to Loke and Randolph, (1995), the body fat content of arthropods influences the daily blood intake hence determines their behaviours. Several insects including tsetse flies possess learning ability which also determines their host choice (Bouyer *et al.,* 2005). It is possible that the first encountered host (blood meal) strongly influences host choice on subsequent feeding cycles of many blood-feeding vectors (Bouyer *et al.,* 2007).

The host choice of tsetse flies is influenced by several factors including the geographical location and animal population present in a particular habitat or ecosystem (Nyingilili *et al.,* 2016). The feeding pattern and preference of tsetse flies vary from place to place and evolve rapidly over time (Farikou *et al*., 2010). Although tsetse are flexible in terms of their blood-feeding but they largely feed on warthog, giraffe and elephant, buffalo, impala, goats cattle (Clausen *et al*., 1998; Muturi *et al*., 2011; Nyingilili & Malele, 2016) and on some opportunistic hosts such as bats and rats (Gaithuma *et al*., 2020). The feeding behaviour of these flies is attributed to various host-related factors such as diurnal behaviour, odour, size, shape, colour and fly-defence reactions (Veterinärmedizin, 2012). Their feeding success and host choice are determined by factors such as changes in the environment, fauna, host availability (Clausen *et al.,*1998), host defensive behaviour, hosts immune responses to arthropod saliva, blood nutritive value and the cost of digestion (Lyimo & Ferguson, 2009).

1.4 Tsetse Flies' Control

African trypanosomiasis is most frequently and widely controlled by targeting tsetse vectors. The success of this strategy largely depends on the availability of accurate baseline data on various aspects of the targeted species (Leak *et al.,* 2008). For instance, the knowledge of the distribution patterns of tsetse flies is vital for better understanding of the transmission dynamics of trypanosomiasis, therefore, coordinated control efforts and regular monitoring of tsetse distribution is highly encouraged (Gashururu *et al.*, 2021). This control approach so far has proved to be the most reliable way of minimizing disease risk both in humans and animals (Hordofa & Haile, 2017). Another control approach involves targeting trypanosomes by trypanocidal drugs such as suramin, pentamidine, melarsoprol, Diminazene diaceturate (DA), Isometamidium chloride hydrochloride (ISM) and homidium bromide (Kazibwe and Matovu, 2011; Shiferaw *et al*., 2015; Bengaly *et al*., 2018).

Strategic tsetse vector control specifically in human-livestock-wildlife interface areas may minimize the risk of trypanosome transmission in both animal and human from the wildlife (Salekwa *et al.*, 2014). Therefore, several bait technologies which uses a 'pull' and 'pull-push' tactics are been deployed in many places to control tsetse flies (Wachira *et al*., 2021). With all technologies, proper identification of the predominant tsetse fly species as well as Trypanosoma species, is important for the management of AAT and HAT (Luziga *et al*., 2017). But, due to challenges that are faced in identifying hosts directly in the field, an indirect method such as analysis of insects' gut content is used to know sources of blood meals which also reflect the trypanosome transmission cycle. Furthermore, assessment of the effects of blood meals on vector' life history (Foster, 1957) and different behaviours may contribute toward the improvement of vector control programs.

1.5 Problem Statement and Study Justification

Increased frequency of tsetse-reservoirs-human interactions due to human encroachment and pressure in protected areas (PA) increases the risk of trypanosomes transmission in many adjacent areas (Thompson, 2013). Since many new PAs are established, the disease reservoirs in those areas are also increasing. Consequent to these factors and the general increase in disease burden across many endemic countries including Tanzania, the on-going control efforts, require robust surveillance system and tools as well as control strategies. Considering that the transmission risk is largely driven by the escalating interaction between tsetse vector species, animals and humans, acquiring in-depth understanding of the spatial-temporal diversity, abundance, feeding status and other behaviours is crucial in enhancing the desired improvements. Unfortunately, these parameters remain grossly understudied in Tanzania. Furthermore, tsetse vector species keep on changing over time in terms of behaviours. Therefore, the establishment of baseline information will provide an excellent premise for regular follow-up and rejuvenation of surveillance and control toolbox.

Therefore, we assessed species composition and abundance, phylogenetic relatedness of wild collected tsetse flies, and the effects of blood-meal sources on host-choice as well as feeding behaviour in one predominant tsetse vector species from the selected human-wildlife interface in Tanzania. The results of this study will improve our understanding of the trypanosome transmission cycle and tsetse behaviours as influenced by different blood meal sources.

1.6 Objectives

1.6.1 Main objective

To assess the species composition, abundance, phylogenetic relatedness and the effects of different blood-meal sources on host-choice and blood-feeding of predominant tsetse vector species from selected human-wildlife interface in Tanzania

1.6.2 Specific objectives

i. To determine species composition, abundance and phylogenetic relatedness of wild collected tsetse flies from selected human-wildlife interface in Tanzania;
ii. To determine whether the offspring of tsetse fed on different hosts vary in terms of their host-choice and feeding success behaviours;
iii. To determine whether host-choice and feeding success of adult tsetse flies varies with nutritional properties of the blood meal source.

1.6.3 Research questions

i. What is the species composition and abundance of the wild-caught tsetse flies at the human-wildlife interface in Tanzania?
ii. Are the wild collected tsetse flies in the study area phylogenetically related with the available tsetse flies' sequences in the GenBank?

iii. How do predominant tsetse vector' offspring vary in terms of their host choice and feeding success behaviours in a controlled semi-field environment?

iv. How does hosts' Haemoglobin (Hb) concentration and total plasma protein correlate with the number of flies attracted to the hosts and successfully feed?

References

Bengaly, Z., Vitouley, S. H., Somda, M. B., Zongo, A., Têko-Agbo, A., Cecchi, G., Adam, Y., Sidibé, I., Bayala, B., Belem, A. M. G., Van Den Abbeele, J., & Delespaux, V. (2018). Drug quality analysis of isometamidium chloride hydrochloride and diminazene diaceturate used for the treatment of African animal trypanosomosis in West Africa. *BMC Veterinary Research*, *14*(1), 1–8. https://doi.org/10.1186/s12917-018-1633-7

Bouteille, B., Louis, F. J., & Buguet, A. (2014). Trypanosomoses africaines : maladie du sommeil. *EMC - Maladies infectieuses 2014; 11*(1), 1-12.

Bouyer, J., & Guerin, D. C. S. M. P. M. (2007). *Learning Affects Host Preference in Tsetse Flies*. pp. 27–29.

Bouyer, J., Cuisance, D., Messad, S., & Guerin, P. (2005). Learning affects host preference in tsetse flies. *Revue d'• levage et de M,decine V,t,rinaire Des Pays Tropicaux*, *58*(1/2), 27-29. z:%5C6608.pdf

Cecchi, G., Mattioli, R. C., Slingenbergh, J., & De La Rocque, S. (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical and Veterinary Entomology*, *22*(4), 364–373. https://doi.org/10.1111/j.1365-2915.2008.00747.x

Cecchi, G., Paone, M., Feldmann, U., Vreysen, M. J., Diall, O., & Mattioli, R. C. (2014). Assembling a geospatial database of tsetse-transmitted animal trypanosomosis for Africa. *Parasites and Vectors*, *7*(1), 1–10. https://doi.org/10.1186/1756-3305-7-39

Clausen, P. H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F., & Staak, C. (1998). Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. *Medical and Veterinary Entomology*, *12*(2), 169–180. https://doi.org/10.1046/j.1365-2915.1998.00097.x

Dicko, A. H., Percoma, L., Sow, A., Adam, Y., Mahama, C., Sidibé, I., Dayo, G. K., Thévenon, S., Fonta, W., Sanfo, S., Djiteye, A., Salou, E., Djohan, V., Cecchi, G., & Bouyer, J. (2015). A

spatio-temporal model of African animal trypanosomosis risk. *PLoS Neglected Tropical Diseases*, *9*(7), 1–20. https://doi.org/10.1371/journal.pntd.0003921

Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G., & Geiger, A. (2010). Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica*, *116*(1), 81–88. https://doi.org/10.1016/j.actatropica.2010.06.002

Foster, R. (1957). Observations on laboratory colonies of the tsetse flies glossina morsitans West. and glossina austeni newstead. *Parasitology*, *47*(3–4), 361–374. https://doi.org/10.1017/S0031182000022058

Gaithuma, A., Yamagishi, J., Hayashida, K., Kawai, N., & Namangala, B. (2020). Blood meal sources and bacterial microbiome diversity in wild- caught tsetse flies. *Scientific Reports*, 1–10. https://doi.org/10.1038/s41598-020-61817-2

Gashururu, R. S., Githigia, S. M., Gasana, M. N., Habimana, R., Maingi, N., Cecchi, G., Paone, M., Zhao, W., Masiga, D. K., & Gashumba, J. (2021). An update on the distribution of Glossina (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda. *Parasites and Vectors*, *14*(1), 1–13. https://doi.org/10.1186/s13071-021-04786-3

Gooding, R. H., & Krafsur, E. S. (2005). Tsetse genetics: Contributions to biology, systematics, and control of tsetse flies. *Annual Review of Entomology*, *50*(69), 101–123. https://doi.org/10.1146/annurev.ento.50.071803.130443

Holmes, P. (2013). Tsetse-transmitted trypanosomes - Their biology, disease impact and control. *Journal of Invertebrate Pathology*, *112*(SUPPL.1), S11–S14. https://doi.org/10.1016/j.jip.2012.07.014

Hordofa, K. S., & Haile, G. (2017). A review on epidemiological distribution, impacts and integrated control approach of tsetse fly. *Journal of Parasitology and Vector Biology*, *9*(9), 122–131. https://doi.org/10.5897/JPVB2015.0213

Kazibwe, A. J. N., & Matovu, E. (2011). *Drug resistance in human*

African trypanosomiasis. pp. 1037–1047.

Krafsur, E. S. (2003). Tsetse fly population genetics: An indirect approach to dispersal. *Trends in Parasitology*, *19*(4), 162–166. https://doi.org/10.1016/S1471-4922(03)00034-5

Kweka, E. J., Kimaro, E. E., Kimaro, E. G., Nagagi, Y. P., & Malele, I. I. (2017). Major Disease Vectors in Tanzania: Distribution, Control and Challenges. *Biological Control of Pest and Vector Insects*. https://doi.org/10.5772/67109

Leak, S., Ejigu, D., & Vreysen, M. (2008). Collection of Entomological Baseline Data for Tsetse Area-Wide Integrated Pest Management Programmes. In *Fao*. ftp://ftp.fao.org/docrep/fao/011/i0535e/i0535e.pdf%5Cnhttp://www.mendeley.com/download/personal/4558981/5529372041/f6b153743490f7b3a77e6ef58a9eda4a1780e7cd/dl.pdf

Loke, H., & Randolph, S. E. (1995). Reciprocal regulation of fat content and flight activity in male tsetse flies (*Glossina palpalis*). *Physiological Entomology*, *20*(3), 243–247. https://doi.org/10.1111/j.1365-3032.1995.tb00007.x

Luziga, C., Muya, C., Mramba, F., Byamungu, M., Mbata, G., & Mtambuki, A. (2017). A tsetse Glossina pallidipes harbors the pathogenic trypanosomes circulating in Liwale district, Tanzania. *Veterinary Parasitology: Regional Studies and Reports*, *9*(June), 93–97. https://doi.org/10.1016/j.vprsr.2017.06.002

Lyimo, I. N., & Ferguson, H. M. (2009). Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends in Parasitology*, *25*(4), 189–196. https://doi.org/10.1016/j.pt.2009.01.005

Malele, I. I. (2011). Fifty years of tsetse control in Tanzania: challenges and prospects for the future. *Tanzania Journal of Health Research*, *13*(5), 399–406. https://doi.org/10.4314/thrb.v13i5.9

Malele, I. I., Kinung'hi, S. M., Nyingilili, H. S., Matemba, L. E., Sahani, J. K., Mlengeya, T. D. K., Wambura, M., & Kibona, S. N. (2007). Glossina dynamics in and around the sleeping

sickness endemic Serengeti ecosystem of northwestern Tanzania. *Journal of Vector Ecology*, *32*(2), 263. https://doi.org/10.3376/1081-1710(2007)32[263:gdiaat]2.0.co;2

Ngonyoka, A., Gwakisa, P. S., Estes, A. B., Salekwa, L. P., Nnko, H. J., Hudson, P. J., & Cattadori, I. M. (2017). Patterns of tsetse abundance and trypanosome infection rates among habitats of surveyed villages in Maasai steppe of northern Tanzania. *Infectious Diseases of Poverty*, *6*(1), 1–12. https://doi.org/10.1186/s40249-017-0340-0

Nnko, H. J., Gwakisa, P. S., Ngonyoka, A., Sindato, C., & Estes, A. B. (2021). Potential impacts of climate change on geographical distribution of three primary vectors of african trypanosomiasis in tanzania's maasai steppe: G. m. morsitans, g. pallidipes and g. swynnertoni. *PLoS Neglected Tropical Diseases*, *15*(2), 1–17. https://doi.org/10.1371/journal.pntd.0009081

Nyingilili, H. S., & Malele, I. (2016). *Diversity of Blood Meal Hosts in Glossina Pallidipes and Its Role in the Epidemiology of Trypanosomiasis at a Localized Area in Serengeti National Park*. *January*.

Salekwa, L. P., Nnko, H. J., Ngonyoka, A., Estes, A. B., Agaba, M., & Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in simanjiro, Northern Tanzania. *Livestock Research for Rural Development*, *26*(12).

Salekwa, L. P., Nnko, H. J., Ngonyoka, A., Estes, A. B., Agaba, M., & Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in simanjiro, Northern Tanzania. *Livestock Research for Rural Development*, *26*(12).

Shiferaw, S., Muktar, Y., & Belina, D. (2015). A review on trypanocidal drug resistance in Ethiopia. *Journal of Parasitology and Vector Biology*, *7*(4), 58–66. https://doi.org/10.5897/JPVB2015.0190

Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Ruiz-Postigo, J. A., Fèvre, E. M., Mattioli, R. C., & Jannin, J. G. (2012). Estimating and Mapping the Population at Risk of Sleeping Sickness. *PLoS Neglected Tropical Diseases*, *6*(10).

https://doi.org/10.1371/journal.pntd.0001859

Soberon, J., & Peterson, A. T. (2005). Interpretation of Models of Fundamental Ecological Niches and Species' Distributional Areas. *Biodiversity Informatics*, *2*(0), 0–10. https://doi.org/10.17161/bi.v2i0.4

Thompson, R. C. A. (2013). Parasite zoonoses and wildlife: One health, spillover and human activity. *International Journal for Parasitology*, *43*(12–13), 1079–1088. https://doi.org/10.1016/j.ijpara.2013.06.007

Vale, G. A., Hargrove, J. W., Solano, P., Courtin, F., Rayaisse, J. B., Lehane, M. J., Esterhuizen, J., Tirados, I., & Torr, S. J. (2014). Explaining the Host-Finding Behavior of Blood-Sucking Insects: Computerized Simulation of the Effects of Habitat Geometry on Tsetse Fly Movement. *PLoS Neglected Tropical Diseases*, *8*(6). https://doi.org/10.1371/journal.pntd.0002901

Veterinärmedizin, F. (2012). Tsetse (Diptera : Glossinidae) Bloodmeal Analysis by PCR and Species Differentiation by MALDI TOF MS as Contributions to Rational Vector Control zur Erlangung des Grades eines PhD of Biomedical Sciences.

Vreysen, M. J. B., Seck, M. T., Sall, B., & Bouyer, J. (2013). Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology*, *112*(SUPPL.1), S15–S25. https://doi.org/10.1016/j.jip.2012.07.026

Wachira, B. M., Kabaka, J. M., Mireji, P. O., Okoth, S. O., Nganga, M. M., Changasi, R., Obore, P., Ochieng', B., Murilla, G. A., & Hassanali, A. (2021). Characterization of a composite with enhanced attraction to savannah tsetse flies from constituents or analogues of tsetse refractory waterbuck (Kobus defassa) body odor. *PLOS Neglected Tropical Diseases*, *15*(6), e0009474. https://doi.org/10.1371/journal.pntd.0009474

World Health Organization. (2013). Control and surveillance of human African trypanosomiasis. *World Health Organization Technical Report Series*, *984*, 1–237

CHAPTER TWO

2.0 PAPER ONE

2.1 Species Composition, Abundance and Phylogenetic Relatedness of Wild Tsetse Flies Collected from Selected Human-Wildlife Interface in Tanzania

Filbert E. Mdee[1, 5*], Jeremiah Lyatuu[1], Venance T. Msoffe[2], Eliakunda M. Mafie[3], Ladslaus L. Mnyone[2, 4*]

[1]Department of Veterinary Medicine and Public health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.
[2]The Institute of Pest Management, Sokoine University of Agriculture, P.O. Box 3110, Morogoro, Tanzania.
[3]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.
[4]Deputy Director of Science Technology and Innovation, Ministry of Education Science and Technology.
[5]African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development, Sokoine University of Agriculture, Morogoro P.O. Box 3110, Tanzania.

[*]Corresponding authors: fillmdee@gmail.com and llaurent@sua.ac.tz

STATUS: submitted to the Journal of Parasite Epidemiology and Control

2.1.1 Abstract

The successful control of tsetse flies largely depends on understanding of their species composition, abundance and diversity. This study assessed the species composition, abundance and apparent density of wild collected tsetse flies from selected human-wildlife-livestock interface in Tanzania. Seasonal trapping using baited NZI, Pyramidal and Biconical traps was done across selected wards: Kisaki and Bwakila chini. Traps were set at 200m apart and rotated to the next sites after 72 hours. Collected flies were identified morphologically and letter confirmed using the Polymerase Chain Reaction (PCR). Only two *Glossina* species; *Glossina pallidipes* (n=371; 47.32%) and *Glossina morsitans morsitans* (n=413; 52.68%) were identified. Among them, 96 flies (80-female, 16-male) were bloodfed, 57(48-female and 9-male) *G. pallidipes* and 39(32-female and 7-male) *G.m. morsitans*. Tsetse fly abundance varied across wards (χ^2=4.597, df=1, p= 0.032), villages (χ^2=9.491, df=3, p= 0.023), habitats (χ^2=17.239, df=2, p<0.001), months (χ^2=13.507, df=3, p= 0.004) and deployed traps (χ^2=6.348, df= 2, p= 0.04). The highest proportion of tsetse flies were collected in Kisaki ward (78.82%; n=618; p<0.001) followed by Bwakila chini (21.17%; n=166; p=0.032). Similarly, the highest proportion of tsetse flies were collected in Mbojoge village (62%; n= 489) followed by Kiperege (18%; n=141) and Sebo (16%; n=129). NZI traps collected the highest proportion of tsetse flies (n=422; 54%; 4.98 FTD) followed by Pyramidal traps (n=281; 36%; 4.01 FTD) and Biconical traps (n=81; 10%; 1.87 FTD). Moreover, the majority of tsetse flies (78.06%) were collected in bushed grassland habitat (n=612; 55.41 FTD) followed by woodland habitat (16.45%; n=129; 20.56 FTD) and farmland (5.5%; n=43; 7.17 FTD). The phylogenetic analysis revealed genetic relatedness of tsetse flies collected in Tanzania with those collected from Nigeria and Senegal. In conclusion, the proportion of tsetse flies varied with study areas, traps and type of vegetation. The majority of tsetse flies were collected in grassland habitat and NZI and Pyramidal were the most effective collection tools. These findings warrant further studies aimed to assess tsetse

flies' blood-meal sources and the infection status to establish the prevalence to inform existing trypanosome control programs.

Keywords: Tsetse fly, wildlife-livestock-human interface, traps, abundance, diversity

2.1.2 Introduction

Tsetse flies are cyclical vectors of flagellated protozoa parasites that cause sleeping sickness in humans (HAT) and nagana (AAT) in domestic animals (Vreysen *et al*., 2013; Gashururu *et al*., 2021). Tsetse flies are confined between latitudes 14° N and 20° S (Bouteille & Buguet, 2016) hence inhabiting only the areas from Sahara to Somali desert in the northern part and Kalahari to Namibian deserts in the Southern part (Vreysen *et al*., 2013). There are about 31 known species and subspecies of tsetse flies described to date (Gooding & Krafsur, 2005; Cecchi *et al.*, 2008). These species are sub-divided into three subgenera based on morphological and ecological characteristics: Austenina (Fusca group), Nemorhina (Palpalis group), and Glossina (Morsitans groups)(Cecchi *et al.*, 2008). Nemorhina species prefer vegetations close to watercourses such as riverine forest, protected forests, vegetation along lakes and mangroves. Glossina species prefer dry savannah woodland while Austenina species preferentially inhabit dense forest belts (Van den Bossche *et al*., 2010).

The dominant tsetse fly species in East Africa including Tanzania are savannah species including *G. morsitans, G. pallidipes and G. swynnertoni* (Bouteille & Buguet, 2016). The other species, *G. austeni*, *G. brevipalpis*, *G. longipennis, G. fuscipes martinii* and *G. fuscipes fuscipes* are also present but with limited distribution (Moloo, 1993; Malele *et al.*, 2007; Kweka *et al*., 2017). The limited distribution is mainly determined by climatic and demographic pressure (Dicko *et al*., 2015; Nnko *et al*., 2021). Moreover, tsetse occurrence and dispersal is affected by humidity, altitude, vegetation/shades availability and host density (Soberon &

Peterson, 2005; Salekwa *et al*., 2014). Of these factors, temperature constitutes the major driver for tsetse occurrence and distribution in sub-Saharan Africa (Nnko *et al*., 2021). All tsetse flies are intrinsically capable of transmitting pathogenic trypanosome parasites, but to date, only 6-10 species have proven public health and veterinary significance (Gooding and Krafsur, 2005; Vreysen *et al*., 2013; Kweka *et al.*, 2017). Notable examples of such species include *G. pallidipes, G. brevipalpis, G. m. moristans and G. swynertoni, G.m. centralis, G.p. palpalis, G. fuscipes fuscipes, G. tachinoides, and Glossina palpalis gambiensis* (Malele *et al*., 2003; Malele *et al*., 2011; Auty *et al*., 2012).

Tsetse fly infests an area of about 8-11 million km^2 across 38 sub-Saharan countries (Krafsur, 2003;Holmes, 2013; Vreysen *et al*., 2013). Thus, posing the risk of tsetse-borne trypanosome infection to approximately 60 to 70 million people (Simarro *et al.*, 2012;Holmes, 2013) and 50 to 60 million cattle (Cecchi and Mattioli, 2009; Holmes, 2013; Cecchi *et al*., 2014) in the entire tsetse-endemic region. Moreover, tsetse-born trypanosome infection risk sometimes affects other non-endemic countries whose citizens visits tsetse-endemic foci for several reasons (Simarro *et al*., 2012). In Tanzania, tsetse flies infest about 15.5% of the total area hence risking approximately 4 million people (Malele, 2011). This high risk is arguably contributed by an exclusive hematophagous nature of both the male and female tsetse flies and their tendency to blood feed on several vertebrate disease reservoirs (Holmes, 2013; Malele, 2011; Vale *et al*., 2014).

Among the root cause of poverty in Sub-Saharan Africa is trypanosomes infection. The Animal African Trypanosomiasis (AAT) alone leads to about 7.98 million US$ annual loss (Malele, 2011), hence affecting the efforts invested to attain food self-sufficiency. Therefore, the control methods which reduces the number of tsetse vectors are highly encouraged (Vreysen *et al*., 2013) due to the proven ability to reduce disease risk in human and animals (Hordofa

& Haile, 2017). Another control approach targets trypanosomes by trypanocidal drugs such as suramin, pentamidine, melarsoprol, Diminazene diaceturate (DA), Isometamidium chloride hydrochloride (ISM) and homidium bromide (Barrett *et al*., 2011; Shiferaw *et al*., 2015; Bengaly *et al*., 2018).

Increased frequency of tsetse-reservoirs-human interactions due to human encroachment and pressure in protected areas (PA) maximizes the risk of trypanosome transmission in many adjacent areas (Thompson, 2013). As many new Protected Areas are established, the number of disease reservoirs in those areas also increase. Considering that the transmission risk is largely driven by the escalating interaction between tsetse vector species, animals and a human, acquiring in-depth understanding of the species richness, abundance and apparent density is crucial in enhancing the desired improvements. Unfortunately, these parameters remain grossly understudied in central and southern regions of Tanzania. Moreover, the tsetse control interventions available to date are not sufficient on their own, hence attracting a need to develop new supplementary sampling tools, surveillance system and control strategies deployable in resource-limited settings. In view of that, understanding tsetse species, and abundance is vital toward making rational decision that will guide the control of tsetse flies and trypanosomiasis (Muturi e*t al*., 2011). Therefore, the present study assessed species composition, abundance and phylogenetic relatedness of wild tsetse flies collected in selected wildlife-human-livestock interface in Tanzania.

2.1.3 Materials and methods

2.1.3.1 Study site

The study was conducted in Morogoro Rural district in Morogoro region, Tanzania. The district is located between latitude 6° 54' 0" S, and longitude 37° 53' 59" E, at an altitude of 509m a.s.l and covers an area of about 12 457.44 km^2. Morogoro Rural district is bordered to Pwani region in the north and east, Kilombero district to the south,

Kilosa district to the southwest, Mvomero and Morogoro urban district to the west (Ng'ida *et al*., 2019). The annual rainfall ranges from 700 to 1 000 mm. The short-wet season occurs from mid of October to the end of December, long wet season from February to the mid-May and the dry season occurs from June to October. The temperature ranges from 21°C to 26°C. Crop cultivation is the main economic activity; and the main crops are rice, maize, banana, beans, soybeans and horticultural crop (Mgode *et al*., 2014).

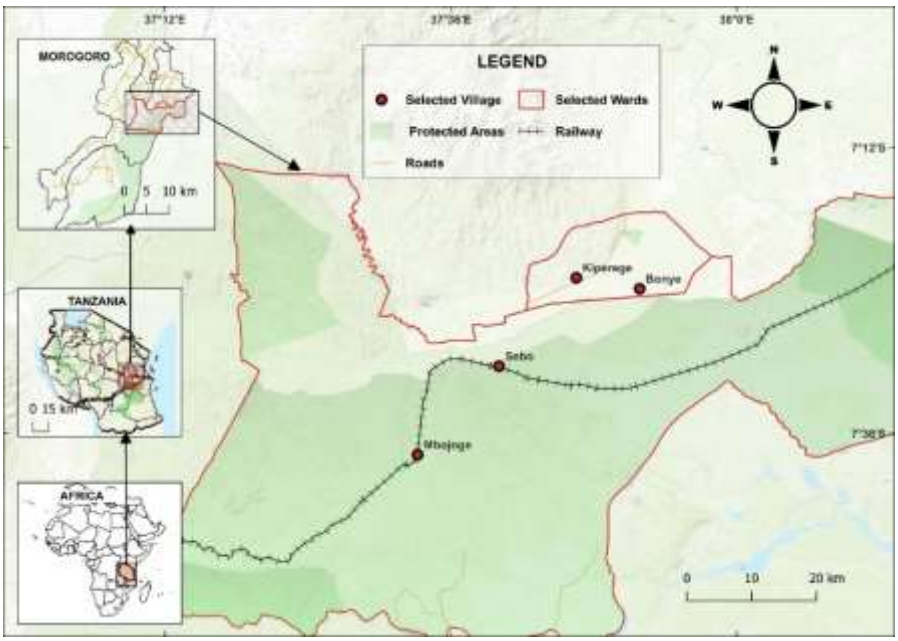


Figure 2.1: A map showing trapping sites: Kisaki and Bwakila chini wards in Morogoro region. The map was developed using QGIS software version 3.26.1 and data from DIVA-GIS and The Humanitarian Data Exchange (HDX), freely available at https://www. diva-gis.org/datadown and https://data. humdata.org/ dataset/cod-ab-tza respectively.

2.1.3.2 Tsetse fly trapping

Trapping was done across season (wet and dry). The NZI, Pyramidal, and Biconical traps baited with acetone (100mg/h), 1-octen-3-ol (0.5mg/h), 4-methyl phenol (1mg/h), and 3-n-propyphenol (0.1mg/h) were deployed following procedures described by Auty *et al*. (2016). Four villages (two in each ward) were purposively selected as trapping sites targeting the area bordering protected areas but with human–wildlife-livestock interaction. The selected villages included; Mbojoge and Sebo (in Kisaki ward), while in Bwakila chini the villages were, Kiperege and Bonye. The individual traps were set at 200 m distance apart. All set traps were maintained in one trapping site, and then rotated across the trapping points every three days (72 hours). The traps were emptied every 24 hours from 10:00 am to 11:00 am (Figure 2.2). The GPS coordinates of all trapping sites and points were recorded using GPS device (Garmin GPSMAP 60CSx).

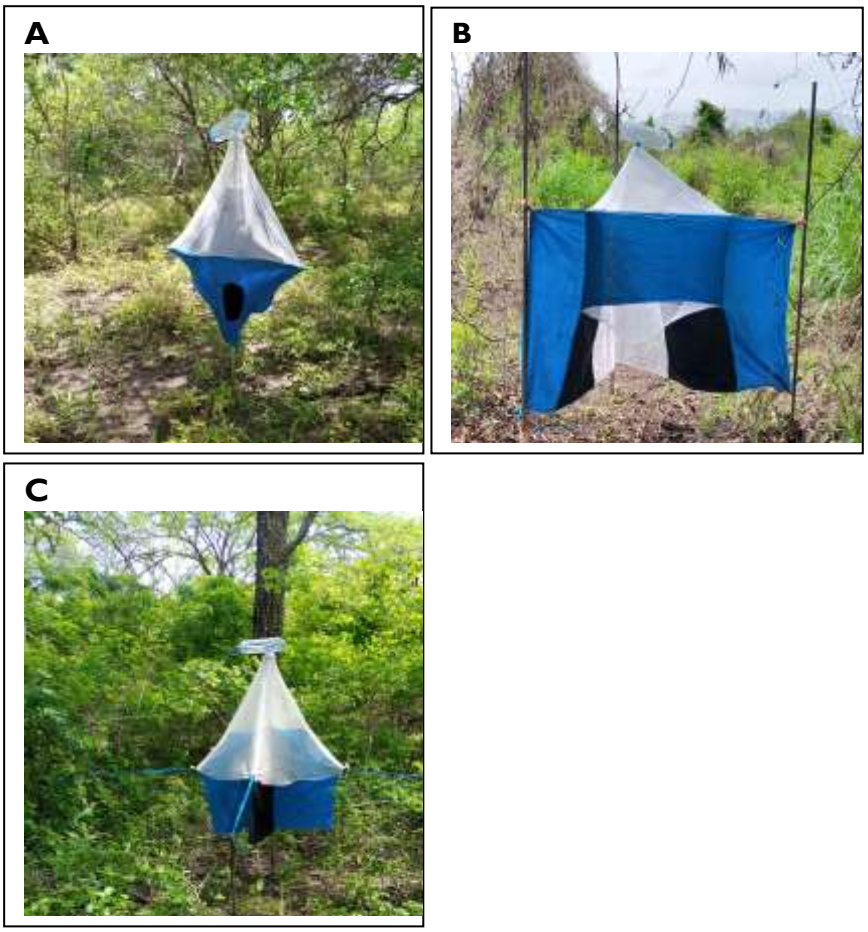


Figure 2.2: Baited traps deployed in study area, **A**)Biconical trap**,** **B**)Nzi trap, and **C**) Pyramidal trap

2.1.3.3 Morphological Identification and storage of tsetse flies

Collected tsetse flies were morphologically identified using the taxonomic keys developed by Leak *et al.* (2008). Trapped tsetseflies were firstly identified in the field using hand lens and letter confirmed in the laboratory using stereomicroscope. Before identification, all live tsetse flies were killed by placing them in the cool box for 2-3 minutes then categorized according sex and feeding status.

Individual flies were stored in well labelled 1.5 mL Eppendorf tubes containing silica gel for molecular identification following Standard Operating Procedures (SOP) developed by FAO/IAEA,(2018).

2.1.3.3 Molecular identification of tsetse flies

2.1.3.3.1 Sample preparation

Fifteen (15) tsetse flies individually stored in Eppendorf tubes were selected for molecular identification. These specimens were washed in a phosphate-buffered saline, air dried for 5 min using tissue paper and separately grinded by a sterile motor and pestle. Resulting samples were re-suspended in 100 µl of PBS in 1.5 mL micro-centrifuge tube, and homogenized manually with a sterile micropipette tip.

2.1.3.3.2 DNA extraction

In a sample homogenate, 100 µl of autoclaved 0.5% Tween-20 in 1X PBS solution were added then mixed by gentle momentary vortexing. Obtained mixtures, were incubated for 20 min at room temperature then centrifuged at 16,000 rpm for 2 min. The supernatant was discarded in order to remain with pellets. The pellets were re-suspended in 100 µl 1X PBS and centrifuged again at 16,000 rpm for 2 min and then the supernatant was discarded. By gentle vortexing for 5 sec, the remaining pellets were re-suspended in 2:1 of sterile deionized water (PCR water) to 20% w/v Chelex-100 resin suspension. Obtained sample suspensions were then incubated in water bath on floating rack for 15 min and centrifuged at 16,000 rpm, for 5 min then transferred the supernatant into pre-labelled 1.5 mL microfuge tube. The mixture was further centrifuged at 16,000 rpm for 10 min and resulting supernatant (extracted DNA template) transferred into a new well-labelled 1.5 mL Eppendorf tubes then stored at -20˚C while awaiting further laboratory analysis. Nano drop spectroscope was used to measure the amount of DNA concentration and contamination of an Aliquots (1 µl) of each extracted DNA sample.

2.1.3.3.3 PCR amplification

The amplification of an Internal Transcribed Spacer (ITS) 1 DNA fragment was done using primers sequence; Glossina forward primer (GITS-F: GTGATCCACCGCTTAGAGTGA) and reverse primer (GITS-R: GCAAAAGTTGACCGAACTTGA) in a Polymerase chain reaction (PCR). The PCR was performed in a 20µl mixture consisted of extracted DNA (2µl), forward primer (1µl), reverse primer (1µl) and nuclease free water (16µl) in micro-tube containing AccuPower® PCR PreMix concentrate. The thermocycling conditions were as follows: denaturation at 95◦C for 5 min, followed by 35 cycles of amplification at 95◦C for 1min, 62◦C for 1 min and 72 ◦C for 1 min and extension at 72 ◦C for 7 min.

2.1.3.3.4 Gel-electrophoresis of the PCR products

The PCR products were then subjected to electrophoresis in 1.5% agarose gel (prepared by dissolving 1.5g agarose into 100 ml of 1x Sodium borate then heated in microwave prior to staining using 4µl of GelRed® Nucleic Acid Gel Stain). Then, 4µl of the amplified PCR products was loaded into each wall of the gel after loading 4µl of 100bp DNA ladder into the first wall. 100V was then subjected to the system and allowed electrophoresis to run for 35 minutes. The resulting DNA fragments were observed as grey bands against a black background. Identification of the species were done basing on the gel electrophoresis results following the Standard Operating Procedures for Identification of Tsetse species from Wild Populations (FAO/IAEA, 2018).

2.1.3.3.5 Sequence analysis

Two amplicons (ID: 43 and 94) were shipped to Macrogen Europe (Meibergdree 57, 1105 BA, Amsterdam the Netherland) for sequencing. After purification of the samples, the PCR amplicons were sequenced using BigDye Terminator Cycle Sequencing Kit (Applied Bio systems, Foster City, CA, USA) and a genetic analyzer (ABI 3730xl System from Applied Bio systems). Cleaning, editing

and assemblage of the raw sequences was performed in Geneious Prime software (version 2023.1.2)(Biomatters, 2013).

2.1.3.4 Phylogenetic analysis

The resulted nucleotide sequences were subjected to Basic Local Alignment Search Tool (BLAST) to determine their genetic relatedness by comparing with the existing tsetsefly sequences published in a GenBank database. Partial sequence nucleotides from samples were aligned with selected reference ITS 1 gene nucleotide from the GenBank using ClusterW in MEGA 11 software (Tamura *et al*., 2021). Furthermore, the phylogenetic analysis was done in the same software using the Maximum Likelihood method, utilizing bootstrap test method with 1000 replicates (Felsenstein, 1985). Finally, the phylogenetic tree was generated based on the partial nucleotide sequences of tsetse flies' ITS 1 gene from two samples and 8 nucleotide sequences were retrieved from references strains obtained from the GenBank.

2.1.3.5 Data analysis

Data were entered, organized and cleaned in Microsoft Excel 2021 before analysis. The average apparent density (AD) was calculated using the formula: $AD = \sum F_t / (T \times D)$, where, $\sum F_t$ is the total number of tsetse flies caught; T is the number of trap deployed and D is the number of days the trap has been in place (Waiswa *et al*., 2006; Gashururu *et al*., 2021; Opiro *et al*., 2021).

The variation in tsetsefly abundance across different parameters such as wards, villages, seasons, traps, species and habitat were analyzed using a Generalized Linear Mixed Models (GLMMs) in R statistical software version 4.2.2. Due to unequal variance and non-normal distribution of count data confirmed with Bartlett's test and Shapiro-Wilk test respectively, a negative binomial distribution (*glmer.nb* function of the lme4 package) was used to account for over-dispersion of the data. Collection date was set as random effect in all models. Plots were created using ggplot2 package.

2.1.4 Results

2.1.4.1 Tsetse flies' abundance

A total of 784 tsetse flies (584 female and 200 males) were trapped across the four study villages. Of those, 96 flies (12.24% of total catch; 16-male, 80-female) were blood fed (Table 2.1 and 2.3), while 688 flies (87.76%; 184-male, 504-female) were not fed.

Only two *Glossina* species were identified, *Glossina pallidipes* (1100bp) and *Glossina morsitans morsitans* (900 bp) (Figure 2.3). *Glossina morsitans morsitans* (n=413, 52.68%) and *Glossina pallidip*es were equally abundant (n=371, 47.32%) (χ^2=1.577, df=1, p= 0.209). Among the blood-fed flies, 40.62% (n=39; 32 female and 7 male) were *G.m.morsitans* and 59.38% (n=57; 48 female and 9 male) were *G.pallidipes* (χ^2=5.678, df=1, p=0.017).

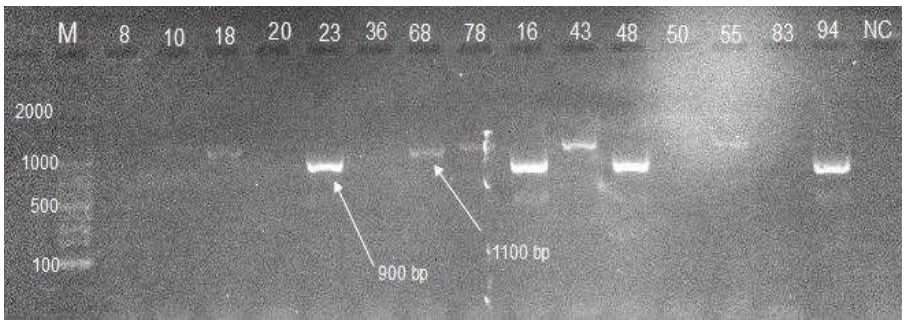


Figure 2.3: Agarose gel electrophoresis showing ITS polymerase chain reaction product, amplified from the DNA extracted from wild collected adult tsetse flies

The two wards surveyed recorded significant different tsetse flies' abundance (χ^2=4.597, df=1, p= 0.032) with about 3-folds higher collected in Kisaki (n=618; 78.82%; p=0.032) relative to those collected in Bwakila chini (n=166; 21.17%; p<0.001). About 82% of *G.m. morsitans* (n=340, p=0.07) and 75% of *G. pallidipes* (n=278, p=0.822) were collected from Kisaki, while 18% (n=73, p=0.004) and 15% (n=93, p<0.001) of each species respectively were collected in Bwakila chini. Similar number of both *G.m. morsitans* (χ^2=3.404,

df=1, p= 0.07) and *G. pallidipes* (χ^2= 0.0502, df=1, p= 0.823) were collected across the study wards.

The total tsetse flies' abundance varied across villages (χ^2=9.491, df=3, p=0.023) with significantly higher number recorded in Mbojoge (n=489, 62.37%, p<0.001) and Kiperege (17.98%; n=141; p=0.15) while Sebo (16.45%; n=129; p=0.12) and Bonye (n=25,3.2%, p=0.08) recorded the least abundance (Figure 2.4). Similarly, *G.m. morsitans* (χ^2=33.067, df=3, p<0.001) and *G. pallidipes* (χ^2=18.392; df=3; p<0.001) abundances significantly varied between villages (Table 2.1).

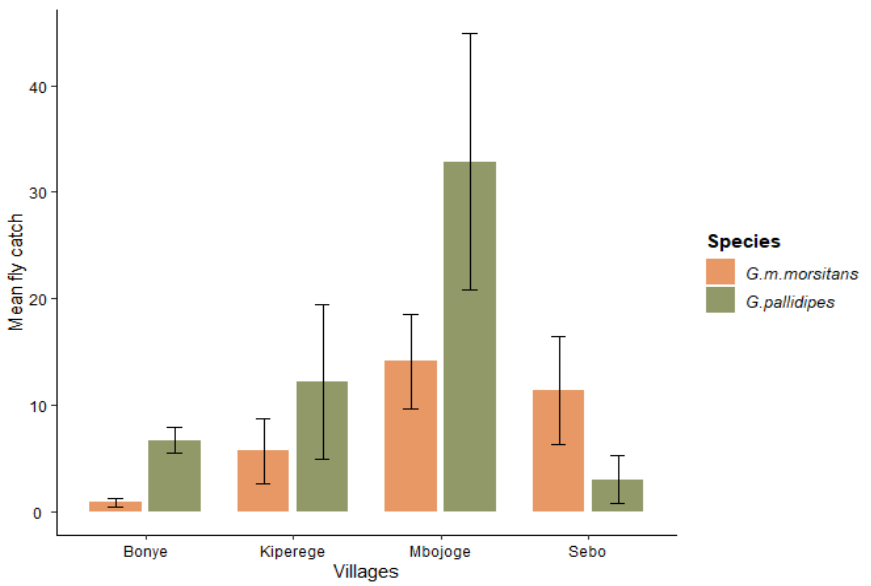


Figure 2.4: A bar plot showing average abundance of collected tsetse species across the study villages

The difference in tsetse fly catches across seasons was not significant (χ^2=0.178, df=1, p= 0.673). However, higher fly catch occurred during wet season (n=499; 64%; p<0.001) compared to dry season (n= 285; 36%; p=0.67). Likewise, the variation in the number of *G.m. morsitans* (χ^2=0.3199, df=1, p=0.572) and *G. pallidipes*

(χ^2=0.212, df=1, p= 0.645) collected across seasons was not significant. Also, there was no significant variation in the number of blood-fed flies by species across season (χ^2=0.068, df=1, p=0.794). However, 68% of the fed flies were trapped during wet season; *G.m. morsitans* (n=30; 31.35%) and *G. pallidipes* (n=36; 37.5%) while 31% during dry season; *G.m. morsitans* (n=9; 9.38%) and *G. pallidipes* (n=21; 21.88%). Note the distribution of tsetse flies catch in each surveyed month (Table 2.1).

Table 2.1: Tsetse fly abundance by species across season, feeding status, traps, wards, villages, habitats, and months

Characteristic	**Overall catch** N = 784	***G.m. morsitans*** N = 413	***G. pallidipes*** N = 371
Season			
Dry season	285 (36%)	99 (24%)	186 (50%)
Wet season	499 (64%)	314 (76%)	185 (50%)
Feeding status			
Blood fed	96 (12%)	39 (9.4%)	57 (15%)
Non-blood fed	688 (88%)	374 (91%)	314 (85%)
Kind of trap			
Biconical	81 (10%)	52 (13%)	29 (7.8%)
NZI	422 (54%)	201 (49%)	221 (60%)
Pyramidal	281 (36%)	160 (39%)	121 (33%)
Ward			
Bwakila chini	166 (21%)	73 (18%)	93 (25%)
Kisaki	618 (79%)	340 (82%)	278 (75%)
Village			
Bonye	25 (3.2%)	5 (1.2%)	20 (5.4%)
Kiperege	141 (18%)	68 (16%)	73 (20%)
Mbojoge	489 (62%)	226 (55%)	263 (71%)
Sebo	129 (16%)	114 (28%)	15 (4.0%)
Habitat			
Bushed grassland	612 (78%)	285 (69%)	327 (88%)
Farm land	43 (5.5%)	14 (3.4%)	29 (7.8%)
Woodland	129 (16%)	114 (28%)	15 (4.0%)
Month			
April	104 (13%)	57 (14%)	47 (13%)
February	395 (50%)	257 (62%)	138 (37%)
July	18 (2.3%)	9 (2.2%)	9 (2.4%)
June	267 (34%)	90 (22%)	177 (48%)

The traps success varied significantly across deployed traps (χ^2=6.348, df= 2, p= 0.042). The overall trap success in descending order was as follows; NZI traps (n=422;54%;4.98 FTD), Pyramidal traps (n=281;36%;4.01 FTD) and Biconical traps (n=81;10%;1.87 FTD) (Table 2.2). The same order of traps performance occurred when assessing the number of fed flies collected using three kinds of traps, but the variation between them was not significant (χ^2=3.3044, df=2, p=0.1916).

The distribution of tsetse catches varied across habitats (χ^2=17.239, df=2, p<0.001). The highest fly catch occurred in bushed grassland habitat (n=612; 78.06%; 55.41 FTD), while, the lowest occurred in farmland habitat (n=43; 5.48%; 7.17 FTD). Most *G.m. morsitans* (n=285; 69%; p<0.001) and *G. pallidipes* (n=327; 88%; p<0.001) were caught in bushed grassland (Table 2.2). Fed flies were mostly caught in grassland habitat compared to other habitats (p=0.01) (Table 2.2). Figure 2.5 shows that, most bloodfed flies were collected from Mbojoge village, using NZI traps in bushed grassland during wet season.

Table 2.2: Distribution of tsetse catches and apparent densities by sex across different traps deployed, season, wards, villages and habitat types

Variable		Overall fly catch	Catch(M±SE)	Overall FTD	Male FTD	Female FTD
Trap	NZI	422	5.41 ± 0.63	4.98	1.26	3.72
	Pyramidal	281	4.07 ± 0.62	4.01	1.17	2.84
	Biconical	81	1.80 ± 0.36	1.87	0.37	1.50
Season	Wet season	499	4.06 ± 0.45	4.27	1.16	3.11
	Dry season	285	4.13 ± 0.60	2.72	0.62	2.11
Ward	Bwakila chini	166	2.41 ± 0.50	19.72	4.03	15.69
	Kisaki	618	5.02 ± 0.41	63.41	17.61	45.80
Village	Mbojoge	489	5.43 ± 0.58	42.85	11.50	31.35
	Sebo	129	3.91 ± 0.75	20.56	6.11	14.44
	Bonye	25	1.13 ± 0.23	4.17	0.50	3.67
	Kiperege	141	3.38 ± 0.83	15.56	3.53	12.03
Vegetation	Bushed grassland	612	4.98 ± 0.50	55.41	14.20	41.21
	Farmland	43	1.19 ± 0.20	7.17	1.33	5.83
	Woodland	129	3.91 ± 0.75	20.56	6.11	14.44
Grand total		784		83.13	21.64	61.49

Table 2.3: The distribution of the number of tsetse flies by species across sex and feeding status

Species	Sex		Feeding Status	
	Female	Male	Blood-fed	Non-blood-fed
G.m.morsitans	276 (67%)	137 (33%)	39 (9.4%)	374 (91%)
G.pallidipes	308 (83%)	63 (17%)	57 (15%)	314 (85%)
Total	584 (74%)	200 (26%)	96 (12%)	688 (88%)

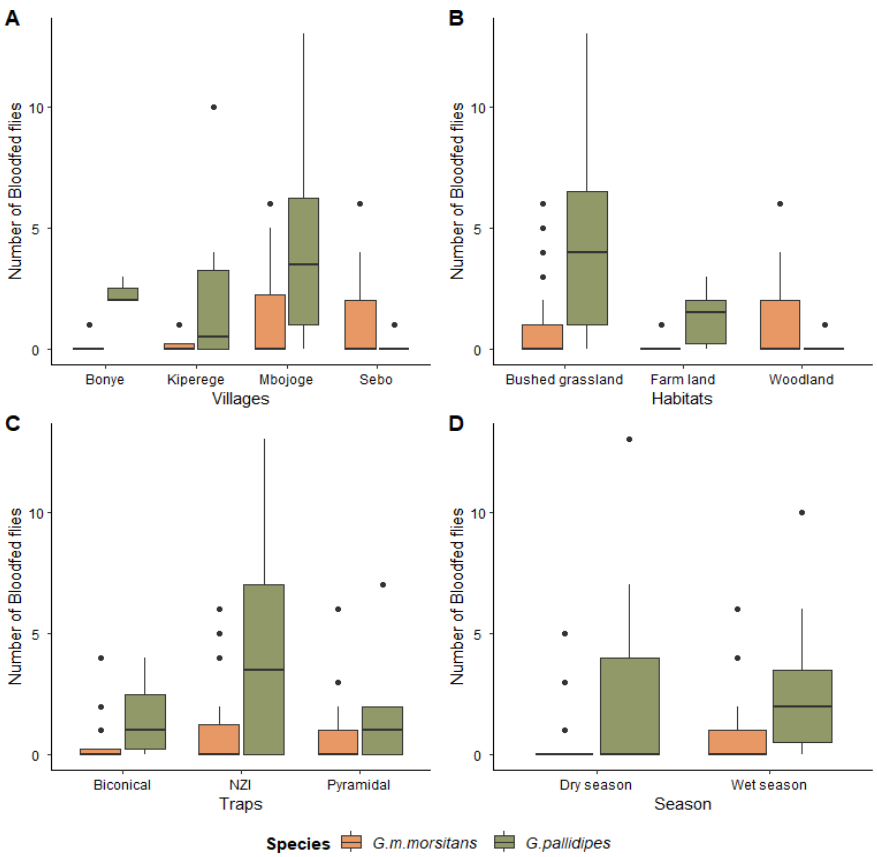


Figure 2.5: The distribution of blood-fed tsetse flies across A: Villages, B: Habitats, C: Traps and D: Seasons

2.1.4.2 Nucleotide sequence and phylogenetic analysis

The sample DNA sequences (ID: 43 and 94) submitted in GenBank revealed several related *Glossina* species with a percentage identity ranging from 95.75 to 80.75 (Table 2.4). The GenBank lacked exact sequences for the two sample's sequences, hence available nearly identical sequences were used to evaluate the evolutionary relatedness.

The Basic Local Alignment Search Tools revealed eight amplicons retrieved from NCBI GenBank to be relatedness with our sequenced

samples (Table 2.4). The phylogenetic tree developed using retrieved sequences showed that *G.m. morsitans* (ID: 94) were closely related with *Glossina palpalis* with accession number LR813463.1 and LR813462.1 from Nigeria by an identity above 95%. Moreover, *Glossina pallidipes* (ID: 43), was identical to *Glossina morsitans submorsitans* (accession number; GQ255906.1) from Senegal by 91.52% (Figure 2.6).

Table 2.4: BLAST result of *Glossina pallidipes* (ID: 43) and *Glossina morsitans morsitans* (ID: 94) showing matching species' base pairs, gene used, percentage identity and accession number

Sample	Base pairs	Blast results			
		Matching species	Gene	% maximum identity	Accession number
43	628	*Glossina morsitans submorsitans*	ITS 1	91.52	GQ255906.1
	537	*Glossina palpalis palpalis*	ITS 1	84.38	LR813473.1
	443	*Glossina palpalis palpalis*	ITS 1	84.91	LR813461.1
	544	*Glossina furcipes furcipes*	ITS 1	83.89	HQ387129.1
	473	*Glossina furcipes furcipes*	ITS 1	82.35	EU591939.1
	452	*Glossina tachnoides*	ITS 1	80.75	EU591936.1
94	1027	*Glossina palpalis*	ITS 1	95.75	LR813463.1
	1010	*Glossina palpalis*	ITS 1	95.74	LR813462.1

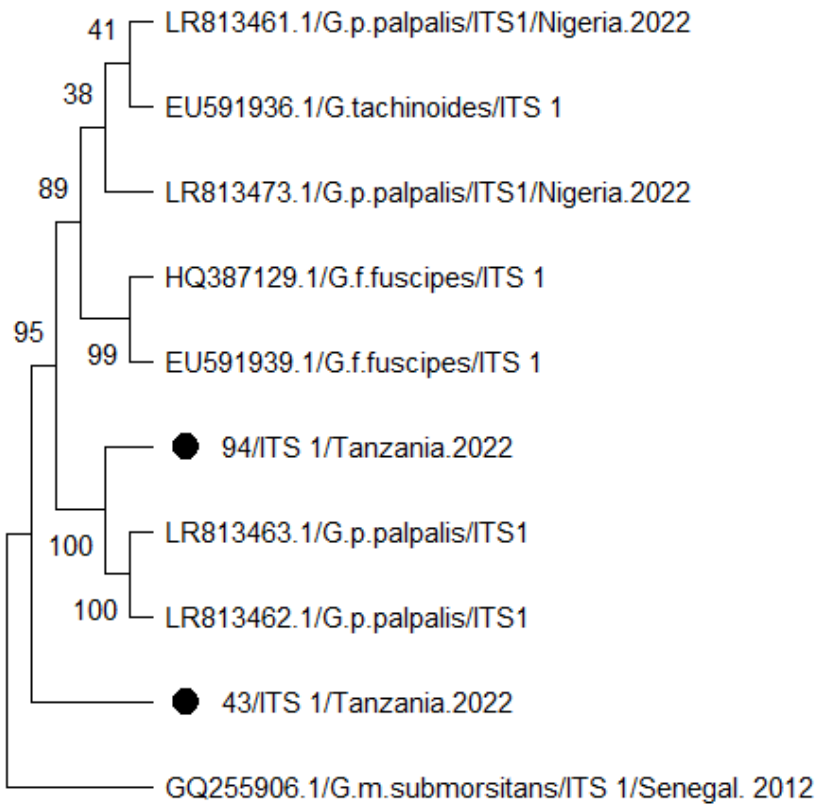


Figure 2.6: The phylogenetic tree showing the evolutionary relatedness of *G. m. morsitans* (ID: 94) and *G. pallidipes* (ID: 43) with retrieved sequences from NCBI GenBank

2.1.5 Discussion

Several studies have assessed tsetse flies abundance and related risks around and within protected areas in Tanzania (Muturi *et al.*, 2011; Salekwa *et al*., 2014; *Luziga et al*., 2017), but most of them were conducted in the northern regions and very few in the southern and central regions. This study assessed the species composition, abundance, and phylogenetic relatedness of wild collected tsetse flies from selected human-wildlife-livestock interface bordering Nyerere National Park, Tanzania.

Two *Glossina* species, *Glossina pallidipes* and *Glossina morsitans morsitans,* were identified during the survey. These species dominate the Selous ecosystem (Malele *et al*., 2016) where the surveyed interface is located. Proportionally, *G.m. morsitans* were more abundant than *G. pallidipes*, likely due to the presence of their preferred hosts and suitable habitats for them to survive. The species, *G.m. morsitans* prefer feeding on warthogs (Gaithuma *et al.*, 2020), and *G. pallidipes* prefer feeding on warthog, giraffe and elephant (Muturi *et al*., 2011; Nyingilili & Malele, 2016). But, on the absence of their preferred hosts, both species blood-feed on cattle, goats and human (Clausen *et al*., 1998). Several wild animals' marks (foot prints and faeces) such as for elephants, Impala, lions and baboons were observed across the study areas. This indicates the presence of the wild hosts visiting the interface. In addition, cattle and goats frequently crossed the trapping sites heading to or from the grazing areas. There could be more *Glossina* species in the area, but since the study focused on the interface only two species were collected.

Most tsetse flies were collected from Kisaki ward than Bwakila chini. The villages within each ward recorded significant different fly abundance. The highest fly catch occurred in Mbojoge villages. This can be attributed to the distance of the protected area to the surveyed villages and hosts availability in these sites. This corroborates with the results from several studies conducted elsewhere (Salekwa *et al*., 2014; Ngonyoka *et al*., 2017; Luziga *et al*., 2017; Gashururu *et al*., 2021). These studies report the decrease in tsetse abundance with an increase in the distance from the PA boundary. The trapping sites in Kisaki were relatively closer to the boundary relative to Bwakila chini. On the other hand, large herds of livestock crossing and grazing in the interface could have influenced tsetse flies abundance. Ngonyoka *et al*. (2017) reports that, available hosts at the interface play a great role in sustaining existing tsetse fly population. In addition to human being, several wildlife and livestock were observed during trapping period. Among

them, Impala, livestock and goats were dominant especially in Kisaki ward. This interaction on the other hand poses significant risk to both human and livestock. Therefore, future studies should assess the tsetse flies' infection status as a way to combat tsetseflies and trypanosome risks.

Most flies were caught during wet season compared to dry season. This can be attributed to availability of favourable weather condition during wet season and tsetse flies' behaviour. Tsetse flies survival and reproduction mainly depend on average temperature and relative humidity (Nnko *et al*., 2021). During the dry season, tsetse flies aggregate in dense vegetation and disperse during the rainy season (Leak *et al*., 2008) thus affecting tsetse catches (Nnko *et al*., 2017). It is therefore more likely to record high fly abundance when trapping during wet season than dry season. Furthermore, hosts especially wild animals travel short distance searching for good pasture during wet season. Hence, the blood-feeding flies including tsetse flies, which depend on these hosts, were highly collected during wet season.

NZI traps showed the highest trap success among all kinds of traps deployed in this study. It attracted the highest proportion of both identified tsetsefly species in all trapping sites followed by Pyramidal trap and Biconical (Table 2.1). Such variations have also been demonstrated in other studies (Malele *et al*., 2016). The variation in the designs of deployed traps could have offered easy entrance of attracted flies to the collection cages compared to other kind of traps, but this was not assessed in this study.

The abundance of tsetse flies varied across the surveyed habitats. Most flies were collected from bushed grassland habitat followed by woodland and very few in a farmland. This observation corroborates with several studies conducted elsewhere (Reid *et al.*, 2000; Salekwa *et al*., 2014; Ngongolo *et al*., 2019). The two species identified; *G.m. morsitans,* and, *G. pallidipes***,** inhabits mainly open

woodland, grass land and bush-land (Leak *et al*., 2008). Therefore, recording the highest fly abundance in bushed grassland is likely due to the availability of bloodmeal sources. Tsetse flies in bushed grassland and woodland habitats, can feed on wild animals and livestock, which often visits the area for grazing. In a farmland habitat, there is habitat distraction and strict protection for livestock to graze, hence only human being and perhaps less preferred wildlife (examples; reptiles,rodents and birds) visit the areas.

Only 96 tsetse flies (12.24% of total catch; 80- female, 16- male) were bloodfed. Among them, 57 flies (48-female, 9- male) were *G. pallidipes* and 39 flies (32- female: 7- male) were *G.m. morsitans*. Observed small proportion of fed flies could be attributed the tsetseflies' behaviors. Mosst engorged tsetse flies often remain in shades and resting sites and the hungry roam around reaching for bloodmeal sources. This finding is supported with the study done by Farikou *et al*. (2010), which reported only 4.7% of the total fly catch with blood in their guts. Therefore, targeting the fed population using traps could end up catching larger proportion of the hungry flies. Furthermore, the highest abundance of bloodfed flies occurred during wet season in a bushed-grassland habitat and were trapped using NZI trap (Figure 2.5). This finding cement on the frequent visitation of the hosts in bushed grassland habitats and the minimum dispersal of both flies and their potential hosts during wet season.

The study report 80.75 to 91.52 percentage identity between *Glossina pallidipes* (43) and related sequences available at the GenBank. While, *G.m.morsitans* (ID: 94) *had* percentage identity of 95.74 to 95.74. This indicates greater homology existing between *G.m.morsitans* and *G.palpalis*.

A bootstrap value of 70% is considered significant evidence for phylogenetic grouping (Hillis & Bull, 1993). Since, *Glossina pallidipes* (43) and *G.m. morsitans* (ID: 94) met above criteria, it therefore indicates the phylogenetic relatedness. The letter is closely

related with *G.palpalis* (accession numbers LR813463.1 and LR813462.1) from Nigeria. Hence, it is possible that they share common ancestry. While, prior appeared to be closely related with *G.m. submorsitans* from Senegal (accession number; GQ255906.1) (Figure 2.6). There could be more species related to sequenced samples when using COII gene but few related samples were retrieved from the GenBank when using ITS 1 gene perhaps due to samples in a database. Future studies could use other gene markers in addition to ITS 1 to show phylogenetic relatedness of these species in Tanzania.

2.1.6 Conclusion

The study reported the decrease in tsetse fly abundance with the distance from the protected area boundary. It also reports highest fly catch during wet season and NZI and Pyramidal traps as the most effective traps. On the other hand, the study shows the phylogenetic relatedness of tsetse flies collected in Tanzania with those collected from Nigeria and Senegal. Finally, the study recommends the future studies to be done in and out of the protected area while focusing on assessing trypanosome infection status in both tsetse flies and livestock at the surveyed human-wildlife interface.

2.1.6 Ethical consideration

Research permits were obtained from Tanzania Wildlife Research Institute and the Tanzania Commission for Science and Technology (permits number: 2022-735-NA-2022-082) and from the Sokoine University of Agriculture Research and Publication Committee (reference number SUA/DRRTC/R/186/18).

2.1.7 Acknowledgement

The authors sincerely appreciate the financial support from The Africa Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE II IRPM-BTD) for sponsoring this study. Special thanks should go to all village chairpersons in the surveyed villages for their support during field

trapping of the tsetse flies. They also appreciate the advice and support from staffs of the Institute of Pest Management (IPM) and Tanzania Veterinary Laboratory Agency (TVLA) Tanga.

2.1.8 Author's contribution

Conceptualization: Filbert E. Mdee, Jeremiah Lyatuu, Eliakunda M. Mafie, Ladslaus L. Mnyone

Methodology: Filbert E. Mdee, Jeremiah Lyatuu, Venance T. Msoffe, Ladslaus L. Mnyone

Field trapping: Filbert E. Mdee, Jeremiah Lyatuu

Data analysis: Filbert E. Mdee, Venance T. Msoffe, Ladslaus L. Mnyone

Supervision: Eliakunda M. Mafie, Ladslaus L. Mnyone

Writing – original draft: Filbert E. Mdee, Ladslaus L. Mnyone

Writing – review & editing: Filbert E. Mdee, Eliakunda M. Mafie, Ladslaus L. Mnyone

References

Auty, H., Anderson, N. E., Picozzi, K., Lembo, T., Mubanga, J., Hoare, R., Fyumagwa, R. D., Mable, B., Hamill, L., Cleaveland, S., & Welburn, S. C. (2012). *Trypanosome Diversity in Wildlife Species from the Serengeti and Luangwa Valley Ecosystems*. *6*(10). https://doi.org/10.1371/journal.pntd.0001828

Auty, H., Cleaveland, S., Malele, I., Masoy, J., Lembo, T., Bessell, P., Torr, S., Picozzi, K., & Welburn, S. C. (2016). Quantifying heterogeneity in host-vector contact: Tsetse (Glossina swynnertoni and G. pallidipes) host choice in Serengeti National Park, Tanzania. *PLoS ONE*, *11*(10), 1–19. https://doi.org/10.1371/journal.pone.0161291

Barrett, M. P., Vincent, I. M., Burchmore, R. J., Kazibwe, A. J., & Matovu, E. (2011). Drug resistance in human African trypanosomiasis. *Future Microbiology*, *6*(9), 1037–1047. https://doi.org/10.2217/fmb.11.88

Bengaly, Z., Vitouley, S. H., Somda, M. B., Zongo, A., Têko-Agbo, A., Cecchi, G., Adam, Y., Sidibé, I., Bayala, B., Belem, A. M. G., Van Den Abbeele, J., & Delespaux, V. (2018). Drug quality analysis of isometamidium chloride hydrochloride and diminazene diaceturate used for the treatment of African animal trypanosomosis in West Africa. *BMC Veterinary Research*, *14*(1), 1–8. https://doi.org/10.1186/s12917-018-1633-7

Biomatters. (2013). Geneious Prime 2022.1 User Manual. *Data Base*, *3304*(January), 1–148.

Bouteille, B., Louis, F. J., & Buguet, A. (2014). Trypanosomoses africaines: maladie du sommeil. *EMC - Maladies infectieuses 2014; 11*(1):1-12 [Article 8-504-A-20]. EMC - Maladies Infectieuses. 11. 1-12.

Cecchi, G., Mattioli, R. C., Slingenbergh, J., & De La Rocque, S. (2008). Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical and Veterinary Entomology*, *22*(4), 364–373. https://doi.org/10.1111/j.1365-2915.2008.00747.x

Cecchi, G., Paone, M., Feldmann, U., Vreysen, M. J., Diall, O., & Mattioli, R. C. (2014). Assembling a geospatial database of tsetse-transmitted animal trypanosomosis for Africa. *Parasites and Vectors*, *7*(1), 1–10. https://doi.org/10.1186/1756-3305-7-39

Cecchi, R., & Mattioli, G. (2009). Geospatial datasets and analyses for an environmental approach to African trypanosomiasis. In *Programme Against African Trypanosomiasis*.

Clausen, P. H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F., & Staak, C. (1998). Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. *Medical and Veterinary Entomology*, *12*(2), 169–180. https://doi.org/10.1046/j.1365-2915.1998.00097.x

Daroff, R. B., & Oxfor, A. P. (2016). *Chapter 391 . Human African trypanosomiasis*. *2*(March).

Dicko, A. H., Percoma, L., Sow, A., Adam, Y., Mahama, C., Sidibé, I., Dayo, G. K., Thévenon, S., Fonta, W., Sanfo, S., Djiteye, A., Salou, E., Djohan, V., Cecchi, G., & Bouyer, J. (2015). A spatio-temporal model of African animal trypanosomosis risk. *PLoS Neglected Tropical Diseases*, *9*(7), 1–20. https://doi.org/10.1371/journal.pntd.0003921

FAO/IAEA. (2018). Standard Operating Procedures for Identification of Tsetse Species from Wild Populations and Laboratory Colonies Standard. *Vienna, Austria*, pp. 1–22.

Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G., & Geiger, A. (2010). Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica*, *116*(1), 81–88. https://doi.org/10.1016/j.actatropica.2010.06.002

Felsenstein, J. (1985). *Confidence limits on phylogenies: an approach using the bootstrap*. *39*(4), 783–791.

Gaithuma, A., Yamagishi, J., Hayashida, K., Kawai, N., & Namangala, B. (2020). Blood meal sources and bacterial microbiome diversity in wild- caught tsetse flies. *Scientific Reports*, *2020,* 1–10. https://doi.org/10.1038/s41598-020-

61817-2

Gashururu, R. S., Githigia, S. M., Gasana, M. N., Habimana, R., Maingi, N., Cecchi, G., Paone, M., Zhao, W., Masiga, D. K., & Gashumba, J. (2021). An update on the distribution of Glossina (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda. *Parasites and Vectors*, *14*(1), 1–13. https://doi.org/10.1186/s13071-021-04786-3

Gooding, R. H., & Krafsur, E. S. (2005). Tsetse genetics: Contributions to biology, systematics, and control of tsetse flies. *Annual Review of Entomology*, *50*(69), 101–123. https://doi.org/10.1146/annurev.ento.50.071803.130443

Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, *42*(2), 182–192. https://doi.org/10.1093/sysbio/42.2.182

Holmes, P. (2013). Tsetse-transmitted trypanosomes - Their biology, disease impact and control. *Journal of Invertebrate Pathology*, *112*(SUPPL.1), S11–S14. https://doi.org/10.1016/j.jip.2012.07.014

Hordofa, K. S., & Haile, G. (2017). A review on epidemiological distribution, impacts and integrated control approach of tsetse fly. *Journal of Parasitology and Vector Biology*, *9*(9), 122–131. https://doi.org/10.5897/JPVB2015.0213

Krafsur, E. S. (2003). Tsetse fly population genetics: An indirect approach to dispersal. *Trends in Parasitology*, *19*(4), 162–166. https://doi.org/10.1016/S1471-4922(03)00034-5

Kweka, E. J., Kimaro, E. E., Kimaro, E. G., Nagagi, Y. P., & Malele, I. I. (2017). Major Disease Vectors in Tanzania: Distribution, Control and Challenges. *Biological Control of Pest and Vector Insects*, 2017, 1-11. https://doi.org/10.5772/67109

Leak, S., Ejigu, D., & Vreysen, M. (2008). Collection of Entomological Baseline Data for Tsetse Area-Wide Integrated Pest Management Programmes. In: *Fao*.

ftp://ftp.fao.org/docrep/fao/011/i0535e/i0535e.pdf%5Cnhttp://www.mendeley.com/download/personal/4558981/5529372041/f6b153743490f7b3a77e6ef58a9eda4a1780e7cd/dl.pdf

Luziga, C., Muya, C., Mramba, F., Byamungu, M., Mbata, G., & Mtambuki, A. (2017). A tsetse Glossina pallidipes harbors the pathogenic trypanosomes circulating in Liwale district, Tanzania. *Veterinary Parasitology: Regional Studies and Reports*, *9*(June), 93–97. https://doi.org/10.1016/j.vprsr.2017.06.002

Malele, I., Craske, L., Knight, C., Ferris, V., Njiru, Z., Hamilton, P., Lehane, S., Lehane, M., & Gibson, W. (2003). The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. *Infection, Genetics and Evolution*, *3*(4), 271–279. https://doi.org/10.1016/S1567-1348(03)00090-X

Malele, I. I. (2011). Fifty years of tsetse control in Tanzania: challenges and prospects for the future. *Tanzania Journal of Health Research*, *13*(5), 399–406. https://doi.org/10.4314/thrb.v13i5.9

Malele, I. I., Kinung'hi, S. M., Nyingilili, H. S., Matemba, L. E., Sahani, J. K., Mlengeya, T. D. K., Wambura, M., & Kibona, S. N. (2007). Glossina dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania. *Journal of Vector Ecology*, *32*(2), 263. https://doi.org/10.3376/1081-1710(2007)32[263:gdiaat]2.0.co;2

Malele, I. I., Magwisha, H. B., Nyingilili, H. S., Mamiro, K. A., Rukambile, E. J., Daffa, J. W., Lyaruu, E. A., Kapange, L. A., Kasilagila, G. K., & Lwitiko, N. K. (2011). *Multiple Trypanosoma infections are common amongst Glossina species in the new farming areas of Rufiji district , Tanzania*. pp. 1–8.

Malele, I. I., Ouma, J. O., Nyingilili, H. S., Kitwika, W. A., Malulu, D. J., Magwisha, H. B., & Kweka, E. J. (2016). Comparative

performance of traps in catching tsetse flies (Diptera: Glossinidae) in Tanzania. *Onderstepoort Journal of Veterinary Research*, *83*(1), 1–7. https://doi.org/10.4102/ojvr.v83i1.1057

Mgode, G. F., Katakweba, A. S., Mhamphi, G. G., Fwalo, F., Bahari, M., Mdangi, M., Kilonzo, B. S., & Mulungu, L. S. (2014). Prevalence of leptospirosis and toxoplasmosis: a study of rodents and shrews in cultivated and fallow land, Morogoro rural district, Tanzania. *Tanzania Journal of Health Research*, *16*(3), 1–7. https://doi.org/10.4314/thrb.v16i3.11

Moloo, S. K. (1993). *THE DISTRIBUTION OF GLOSSINA SPECIES IN AFRICA AND*. *4*, 511–527.

Muturi, C. N., Ouma, J. O., Malele, I. I., Ngure, R. M., Rutto, J. J., Mithöfer, K. M., Enyaru, J., & Masiga, D. K. (2011). Tracking the feeding patterns of tsetse flies (glossina genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS ONE*, *6*(2), 1–6. https://doi.org/10.1371/journal.pone.0017284

Ng'ida, F. D., Kotoroi, G. L., Mwangi, R., Mabelele, M. M., Kitau, J., & Mahande, M. J. (2019). Knowledge and practices on breast cancer detection and associated challenges among women aged 35 years and above in Tanzania: A case in morogoro rural district. *Breast Cancer: Targets and Therapy*, *11*, 191–197. https://doi.org/10.2147/BCTT.S199889

Ngongolo, K., Mtoka, S., & Rubanza, C. D. (2019). *Ecological Factors and the Prevalence of Trypanosome Infections and Its Economic Implications for Livestock Industry in Simanjiro, Tanzania : A Review*. *36*(3), 1–11. https://doi.org/10.9734/IJTDH/2019/v36i330145

Ngonyoka, A., Gwakisa, P. S., Estes, A. B., Salekwa, L. P., Nnko, H. J., Hudson, P. J., & Cattadori, I. M. (2017). Patterns of tsetse abundance and trypanosome infection rates among habitats of surveyed villages in Maasai steppe of northern Tanzania. *Infectious Diseases of Poverty*, *6*(1), 1–12.

https://doi.org/10.1186/s40249-017-0340-0

Nnko, H. J., Gwakisa, P. S., Ngonyoka, A., Sindato, C., & Estes, A. B. (2021). Potential impacts of climate change on geographical distribution of three primary vectors of african trypanosomiasis in tanzania's maasai steppe: G. m. morsitans, g. pallidipes and g. swynnertoni. *PLoS Neglected Tropical Diseases*, *15*(2), 1–17. https://doi.org/10.1371/journal.pntd.0009081

Nnko, H. J., Ngonyoka, A., Salekwa, L., Estes, A. B., Hudson, P. J., Gwakisa, P. S., & Cattadori, I. M. (2017). Seasonal variation of tsetse fly species abundance and prevalence of trypanosomes in the Maasai Steppe, Tanzania. *Journal of Vector Ecology*, *42*(1), 24–33. https://doi.org/10.1111/jvec.12236

Nyingilili, H. S., & Malele, I. (2016). *Diversity of Blood Meal Hosts in Glossina Pallidipes and Its Role in the Epidemiology of Trypanosomiasis at a Localized Area in Serengeti National Park*. *January*.

Opiro, R., Opoke, R., Angwech, H., Nakafu, E., Oloya, F. A., Openy, G., Njahira, M., Macharia, M., Echodu, R., Malinga, G. M., & Opiyo, E. A. (2021). Apparent density, trypanosome infection rates and host preference of tsetse flies in the sleeping sickness endemic focus of northwestern Uganda. *BMC Veterinary Research*, *17*(1), 1–12. https://doi.org/10.1186/s12917-021-03071-w

Reid, R. S., Kruska, R. L., Deichmann, U., Thornton, P. K., & Leak, S. G. A. (2000). *Human population growth and the extinction of the tsetse fly*. *77*, 227–236.

Robinson. (2003). *Fao Animal Production and Health Paper*.

Salekwa, L. P., Nnko, H. J., Ngonyoka, A., Estes, A. B., Agaba, M., & Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in simanjiro, Northern Tanzania. *Livestock Research for Rural Development*, *26*(12).

Shiferaw, S., Muktar, Y., & Belina, D. (2015). A review on

trypanocidal drug resistance in Ethiopia. *Journal of Parasitology and Vector Biology*, *7*(4), 58–66. https://doi.org/10.5897/JPVB2015.0190

Simarro, P. P., Cecchi, G., Franco, J. R., Paone, M., Diarra, A., Ruiz-Postigo, J. A., Fèvre, E. M., Mattioli, R. C., & Jannin, J. G. (2012). Estimating and Mapping the Population at Risk of Sleeping Sickness. *PLoS Neglected Tropical Diseases*, *6*(10). https://doi.org/10.1371/journal.pntd.0001859

Simarro, P. P., Franco, J. R., Cecchi, G., Paone, M., Diarra, A., Ruiz Postigo, J. A., & Jannin, J. G. (2012). Human African trypanosomiasis in non-endemic countries (2000-2010). *Journal of Travel Medicine*, *19*(1), 44–53. https://doi.org/10.1111/j.1708-8305.2011.00576.x

Soberon, J., & Peterson, A. T. (2005). Interpretation of Models of Fundamental Ecological Niches and Species' Distributional Areas. *Biodiversity Informatics*, *2*(0), 0–10. https://doi.org/10.17161/bi.v2i0.4

Tamura, K., Stecher, G., & Kumar, S. (2021). *MEGA11 : Molecular Evolutionary Genetics Analysis Version 11*. *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thompson, R. C. A. (2013). Parasite zoonoses and wildlife: One health, spillover and human activity. *International Journal for Parasitology*, *43*(12–13), 1079–1088. https://doi.org/10.1016/j.ijpara.2013.06.007

Vale, G. A., Hargrove, J. W., Solano, P., Courtin, F., Rayaisse, J. B., Lehane, M. J., Esterhuizen, J., Tirados, I., & Torr, S. J. (2014). Explaining the Host-Finding Behavior of Blood-Sucking Insects: Computerized Simulation of the Effects of Habitat Geometry on Tsetse Fly Movement. *PLoS Neglected Tropical Diseases*, *8*(6). https://doi.org/10.1371/journal.pntd.0002901

Van den Bossche, P., Rocque, S. de La, Hendrickx, G., & Bouyer, J. (2010). A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. *Trends in*

Parasitology, *26*(5), 236–243. https://doi.org/10.1016/j.pt.2010.02.010

Vreysen, M. J. B., Seck, M. T., Sall, B., & Bouyer, J. (2013). Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology*, *112*(SUPPL.1), S15–S25. https://doi.org/10.1016/j.jip.2012.07.026

Waiswa, C., Picozzi, K., Katunguka-Rwakishaya, E., Olaho-Mukani, W., Musoke, R. A., & Welburn, S. C. (2006). Glossina fuscipes fuscipes in the trypanosomiasis endemic areas of south eastern Uganda: Apparent density, trypanosome infection rates and host feeding preferences. *Acta Tropica*, *99*(1), 23–29. https://doi.org/10.1016/j.actatropica.2006.06.005

World Health Organization. (2013). Control and surveillance of human African trypanosomiasis. *World Health Organization Technical Report Series*, *984*, 1–237.

CHAPTER THREE

3.0 PAPER TWO

3.1 Host-choice and feeding Behaviour of *Glossina morsitans'* Offspring Whose Parents Were Fed on Different Host Species

Filbert E. Mdee[1], [2]*, Jeremiah Lyatuu[1], Eliakunda M. Mafie[3], Ladslaus L. Mnyone[2], [4]*

[1]Department of Veterinary medicine and public health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.
[2]The Institute of Pest Management, Sokoine University of Agriculture, P.O. Box 3110, Morogoro, Tanzania.
[3]Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Morogoro, Tanzania.
[4]Deputy Director of Science Technology and Innovation, Ministry of Education Science and Technology

*Corresponding authors: fillmdee@gmail.com and llaurent@sua.ac.tz,

Submitted to Parasitologia Journal.

Received: 21 September 2023; Revised: 11 January 2024; Accepted: 12 January 2024; Published: 15 January 2024.

parasitologia

MDPI

Article

Host Choice and Feeding Behaviours of *Glossina morsitans* Offspring Whose Parents Were Fed on Different Host Species

Filbert E. Mdee [1,2,3,*], Jeremiah Lyatuu [1,2], Eliakunda Mafie [4] and Ladslaus L. Mnyone [1,3,5,*]

[1] African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development, Sokoine University of Agriculture, Morogoro P.O. Box 3110, Tanzania
[2] Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro P.O. Box 3019, Tanzania
[3] Institute of Pest Management, Sokoine University of Agriculture, Morogoro P.O. Box 3110, Tanzania
[4] Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro P.O. Box 3019, Tanzania; eliakunda.mafie@sua.ac.tz
[5] Department of Science, Technology and Innovation, Ministry of Education Science and Technology, Dodoma P.O. Box 10, Tanzania
* Correspondence: fillmdee@gmail.com (F.E.M.); lauren@sua.ac.tz (L.L.M.)

Abstract: The success of any tsetse control program depends on the knowledge of their behaviour. This study assessed the host choice and feeding behaviours of *Glossina morsitans* siblings whose parents were bloodfed on rabbits, guinea pigs, rodents, and squirrels. Each individual host was placed in a screen cage, which allowed flies to enter through openings on each side. The groups of flies (20 per replicate), which were colour-marked differently based on their parents' blood meal hosts, were released from the centre of large semi-field cage. The released flies were aspirated after 24 h and then sorted based on their location, feeding status, and parents' blood meal. A total of 213 flies (72.95% of those recovered) were attracted to the hosts. The numbers of flies attracted to different hosts varied significantly ($\chi^2_4 = 33.685$, $p = 0.0001$): rodents ($n = 80$, $p = 0.006$), rabbits ($n = 59$, $p = 0.331$), guinea pigs ($n = 49$, $p = 0.057$), and squirrels ($n = 25$, $p = 0.005$). The numbers of flies attracted to their parent's blood meal source varied significantly ($\chi^2_{12} = 56.476$, $p < 0.001$): rabbits ($n = 35$, 59.32%, $p < 0.001$), rodents ($n = 25$, 31.25%, $p = 0.043$), and guinea pigs ($n = 19$, 38.78%, $p = 0.45$). But only 39 flies (18.31% of the total attracted) bloodfed on the hosts, including guinea pigs ($n = 10$, 25.64%), rodents ($n = 23$, 58.97%), rabbits ($n = 6$, 15.38%), and squirrels ($n = 0$, 0.0%). There was significant variation in the number of flies that fed successively across hosts ($\chi^2_4 = 49.478$, $p < 0.001$). The findings from this study confirm the presence of differential attractiveness of the hosts to flies and the so-called "Hopkins host selection principle" or "pre-imaginal conditioning". Therefore, the study attracts the need for detailed investigation on the influence of blood meal sources on tsetse fly siblings' behaviours across filial generations using small mammals or other large mammal species.

Keywords: *Glossina morsitans*; rabbits; guinea pigs; rodents; squirrels; bloodfed; attracted

check for updates

Citation: Mdee, F.E.; Lyatuu, J.; Mafie, E.; Mnyone, L.L. Host Choice and Feeding Behaviours of *Glossina morsitans* Offspring Whose Parents Were Fed on Different Host Species. *Parasitologia* **2024**, *4*, 38–46. https://doi.org/10.3390/parasitologia4010003

Academic Editor: Maurizio Francesco Brivio

Received: 21 September 2023
Revised: 11 January 2024
Accepted: 12 January 2024
Published: 15 January 2024

1. Introduction

Tsetse flies (Diptera, Glossinidae, *Glossina*) are blood-sucking, cyclical vectors of protozoan trypanosomes that cause sleeping sickness in humans (HAT) and nagana (AAT) in domestic animals [1,2]. Tsetse flies only inhabit sub-Saharan Africa [3] from the Kalahari to the Namibian deserts in the southern part and from the Sahara to the Somali desert in the northern part [1]. About 33 species and subspecies of these arthropods have been identified thus far [4] and subdivided into three subgenera, namely Austenina (Fusca group), Nemorhina (Palpalis group), and Glossina (Morsitans group) [5]. The savannah tsetse flies, *Glossina morsitans*, *G. pallidipes*, and *G. swynnertoni*, are the most dominant species in East African regions, including Tanzania [6]. There are also species that are

occurring with limited distribution, and these include *Glossina brevipalpis*, *Glossina longipennis*, *Glossina fuscipes martinii*, and *G. fuscipes fuscipes* [7–9]. Of all the species identified, only 6–10 species have public health and veterinary significance [1,4,9]. Examples of those species include *G. pallidipes*, *G. brevipalpis*, *G. m. morsitans*, and *G. szunnertoni* [10,11]. The major vectors of human African trypanosomiasis (HAT) in East Africa include *G. pallidipes* and *G. szunnertoni* [8,12].

Like other animals with learning ability, insects can also learn and adjust their intrinsic and extrinsic behaviours accordingly [13–16]. Such a learning ability helps insects to locate and assess the quality of resources such as food, breeding sites, and mates [15]. For instance, *Anopheles arabiensis* mosquitoes and *Lutzomyia whitmani* sandflies can return to the site and host where they were originally collected [14,17,18]. Similarly, *Glossina* species can return to the same host for a second blood meal whenever the feeding interval is within two days [15]. Such behaviours are sometimes referred to as host fidelity and site fidelity behaviours. Another study has proposed the so-called "Hopkins host selection principle" or pre-imaginal conditioning [18]. This concept explains that the larvae of the flies become attracted to their parents' food sources, possibly due to olfactory cues experienced during development.

The feeding behaviours of tsetse flies are genetically determined [19,20]. They are mostly opportunistic feeders; however, in the absence of a preferred host, they adapt to feeding on available hosts [20]. Their choice to feed on a specific host is influenced by multiple factors, such as the shape of the host, the colour of the host, odour emanation, the size of the host, and host availability [20,21]. Understanding these and other behaviours is critical in designing and implementing surveillance and control strategies [15,16,22]. In-depth knowledge of host choice and feeding behaviours of tsetse flies could be important, as it may influence parasite transmissions in different vectors [15], thus facilitating the formation of new strategies to minimize vector–host contact, especially in settings where humans, wildlife, and livestock interact. Similarly, such knowledge may be useful during the monitoring and evaluation of tsetse surveillance and control programs [21]. Despite their urgent and adverse impacts on public health, the behaviours of most hematophagous insects, including tsetse flies, are understudied. Therefore, in this study, we investigated the host choice and feeding behaviours of *Glossina morsitans*, one of the most predominant tsetse vector species in Tanzania and elsewhere in Africa.

2. Materials and Methods

2.1. Rearing the Tsetse Flies

A colony of *Glossina morsitans* was established using pupae from the Tsetse and Vector Control Centre, Tanga, Tanzania. The pupae were transferred into large, fine-meshed emergence cages and rearing cages and therein maintained under ambient conditions at 25 ± 2 °C with 70 ± 2% RH and a 12 h photo phase within an insectary at the Institute of Pest Management (IPM), Sokoine University of Agriculture, Tanzania. Emerging flies were sorted by sex and transferred to separate cages with either 25 flies (7.5 × 5 × 4 cm) or 40 flies (13.5 × 8 × 4.5 cm). Virgin female flies were mated with 6- to 8-day-old virgin males in separate cages at 1:3 male-to-female ratios [23,24]. While in the insectarium, different cohorts of adult tsetse, consisting of at least 80–100 tsetse flies, were respectively blood-fed on a guinea pig (*Cavia porcellus*), rodent (*Cricetomys gambianus*), rabbit (*Oryctolagus cuniculus*), and squirrel (*Paraxerus ochraceus*). Prior to feeding the flies, hosts were shaved on one side of the abdomen then cleaned using warm water. During blood-feeding, hosts were humanely restrained, then one cage (which contained 10–15 flies) was attached on the abdomen of the hosts to allow the flies to blood-feed for 10 min. The same process of blood-feeding was conducted for 3 h on Monday, Wednesday, and Friday every week from 11:00 am to 2:00 pm for five weeks.

2.2. Experimental Setup

A large semi-field cage made of inert mosquito nets (size: 245 cm × 185 cm × 203 cm) was constructed and placed inside a room (size: 336 cm × 195 cm × 308 cm) with ambient conditions similar to those of the rearing insectary. Then, four small screen cages of the same size (size: 62 cm × 42 cm × 62 cm) were fixed one in each of the four sides of the semi-field cage (Figure 1). The host screen cages were made of metal bars and improvised with four openings, one on each of their four sides, through which the host-seeking tsetse would access the hosts. The openings were tapered such that tsetse flies visiting the large cages were unable to leave. The large semi-field and screen cages were regularly checked for intactness to prevent experimental tsetse flies from escaping to the outside.

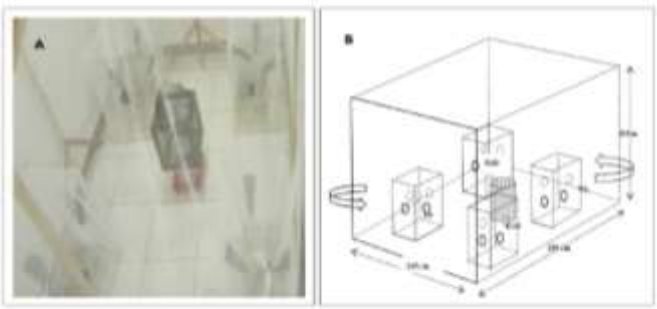


Figure 1. Host-choice experimental setup with four small screen cages containing different host species. During the experiment, the position of the four cages were alternated such that each host occupied all four different positions within the large semi-field cage. (**A**) Shows four screen cages and releasing cage positioned within large semi-field cage. (**B**) Shows the experimental layout.

2.3. Assessing the Host Choice and Feeding Success of Offspring Whose Mothers Were Fed on Different Host Species

Using a 4 by 4 Latin Square design, 4 host species (1 guinea pig, 1 rodent, 1 rabbit, and 1 squirrel) were placed in each of the four screen cages inside the large semi-field cage (Figure 1). In each replicate, four cohorts of offspring (20 tsetse flies each) obtained from mothers, for *Glossina morsitans* species, blood-fed on the different hosts and labelled with different colours of fluorescent powder, were released simultaneously at the centre of the large semi-field cage. Before releasing, the tsetse flies were starved for 72 hours to maximize their physiological demand for blood and urge for host-seeking. After release, the tsetse flies were left to forage for 24 hours and recaptured independently from all the four screen cages and elsewhere in the semi-field system using aspirators. Recaptured tsetse flies were identified and categorized as fed, unfed, live, or dead, as well as the location where released flies were collected (hosts' screen cage). This experiment was repeated four times, and each time hosts were randomly rotated across the four cages such that all hosts occupied all positions in the large semi-field cages. To assess the feeding success of released flies, the proportion of live or dead engorged flies obtained in host-choice experiment were compared. By observing the abdomen, engorged recaptured flies were sorted out, counted, and recorded.

2.4. Determination of Haemoglobin (Hb) Concentrations and Total Plasma Protein of Blood Samples from the Experimental Host Species

Haemoglobin concentration and total plasma protein were assessed in all hosts before they were deployed in the experiment. Prior to blood samples' collection, hosts were anaesthetized shortly using diethyl ether for about 2 min. Using micro-capillary tubes,

blood samples (2 mL per host) were drawn from the retro-orbital sinus and transferred into two well-labelled EDTA K3 2.5 mL tubes. These samples were shipped to the laboratory for analysis inside a cool box with recyclable ice.

Haemoglobin concentration (Hb) was determined using the Cyanomethemoglobin method. The blood samples were gently mixed before taking 0.02 mL of the sample using a pipette. Excess blood on the pipette surface was wiped using clean tissue paper. The individual samples were then transferred into test tubes containing 5 mL Drabkin's reagents. The tubes containing samples were stoppered then gently mixed, and then left for 10 min for maximum colour development. The samples were then poured into the cuvette, where absorbance at 540 nm versus a reagent blank was compared [25]. Haemoglobin concentration (mg/mL) and percentage (%) were obtained and recorded accordingly.

The Biuret method was used to determine total blood protein for all collected blood samples following the Erba Total Protein protocol. This method involves the formation of a blue-violet ion complex resulting from the reaction between the peptide bonds of protein and copper II ions in the alkaline solution.

2.5. Data Analysis

The data were entered, cleaned, and organized in Microsoft Excel 2010 prior to statistical analysis. The variation in the total number of flies that entered screen cages and the proportion of flies which returned to the same host were analysed using a generalized linear mixed model (GLMM) in R statistical software version 4.2.2. The hetero-scedasticity and non-normal distribution of count data were confirmed using Bartlett's test and the Shapiro–Wilk test, respectively. Hence, a negative binomial distribution (glmer.nb function of the lme4 package) was used to account for the over-dispersion of the data. An initial model fixed the number of flies entering screen cages as a dependent factor predicted by fixed factors: Hb concentration, total blood protein, screen cage, blood meal sources used to maintain parents, average temperature, and relative humidity. The cage position was set as a random effect in all models. Insignificant fixed predictors such as Hb concentration, total plasma protein, average temperature, and relative humidity were removed from the model until the lower Akaike information criterion (AIC) was achieved.

2.6. Ethical Approval

Ethical clearance for conducting this particular study was obtained from the Sokoine University of Agriculture Research and Publication Committee (reference number SUA/DRRTC/R/186/18), and the Tanzanian Commission for Science and Technology (reference number: 2022-735-NA-2022-082).

3. Results

3.1. Choice of Adult Tsetse Fly, Glossina Morsitans, on Different Host Species

A total of 320 adult *G.morsitans* were released for the host choice experiment, of which 292 (91.25% of the released) were recovered and 28 (8.75% of released flies) unrecovered. Out of the recovered adult tsetse, 213 (72.95%) were attracted to different hosts: rodent (n = 80, 27.4%), rabbit (n = 59, 20.21%), guinea pig (n = 49, 16.78%), and squirrel (n = 25, 8.56%). The remaining flies (n = 51, 17.47% of recovered flies) were collected in the large semi-field cage (Table 1). The number of flies attracted to individual hosts varied significantly regardless of position ($\chi^2_4 = 33.685$, $p = 0.0001$). Rodents attracted the highest number of flies ($p = 0.006$), followed by rabbits ($p = 0.331$), guinea pigs ($p = 0.057$), and squirrels ($p = 0.005$) (Table 1). Nevertheless, the difference in the number of flies attracted to guinea pigs and rodents ($p = 0.001$), rabbits and rodents ($p = 0.003$), guinea pigs and squirrels ($p = 0.002$), rabbits and squirrels ($p < 0.001$), and rodents and squirrels ($p < 0.001$) was statistically significant.

Table 1. The distribution of offspring flies attracted to their parent's bloodmeal sources (guinea pig, rabbit, and squirrel).

Bloodmeal Source	Host Cages									
	Guinea Pig		Rabbit		Rodent		Squirrel		Out	
	n	%	n	%	n	%	n	%	n	%
Guinea pig	19	38.78	12	20.34	11	13.75	7	28.00	19	24.05
Rabbit	8	16.33	35	59.32	16	20.00	1	4.00	18	22.78
Rodent	16	32.65	8	13.56	25	31.25	10	40.00	15	18.99
Squirrel	6	12.24	4	6.78	28	35.00	7	28.00	27	34.18
TOTAL	49	100.00	59	100.00	80	100.00	25	100.00	79	100.00

The number of offspring attracted to their parent's blood meal source varied significantly regardless of position ($\chi^2_{12} = 56.476$, $p < 0.001$). Furthermore, the highest number of offspring flies attracted to their parent's bloodmeal source was observed in rabbits (n = 35, 59.32%, $p < 0.001$), rodents (n = 25, 31.25%, $p = 0.043$), and guinea pigs (n = 19, 38.78%, $p = 0.45$). Considering the contribution of offspring from different bloodmeal sources attracted to individual hosts in the host-choice experiment, rodents attracted more flies from squirrel (n = 28, 35%) and rodent blood (n = 25, 31.25%); guinea pigs attracted more flies from guinea pig blood (n = 19, 38.78%) and rodent blood (n = 16, 32.65%); rabbits attracted more flies from rabbit blood (n = 35, 59.32%) and guinea pigs (n = 12, 20.34%); finally, squirrels attracted more flies from rodent blood (n = 10, 40%) (Table 1). The distribution of the mean number of flies that were attracted to the parent's blood meal source is shown in Figure 2 and Table 1. Interestingly, 34.18% (n = 27) of the offspring flies that remained outside of the host's cages or screen cages were those from parents which bloodfed on squirrels (Figure 2). Unlike the significant variation in the number of offspring flies attracted to rabbits, guinea pigs, and rodents ($p < 0.001$), no significant variation was observed in the number of flies attracted to squirrels ($\chi^2_3 = 4.9624$, $p = 0.1746$).

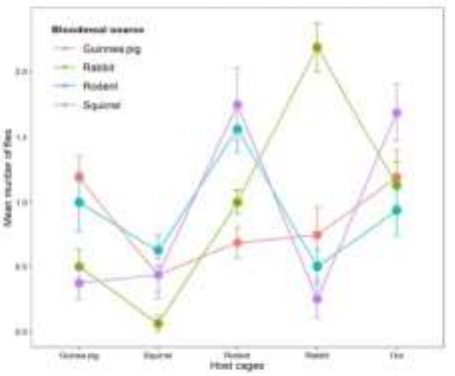


Figure 2. The average number of offspring flies that were attracted to their parent's bloodmeal source (guinea pig, squirrel, rodent, and rabbit).

3.2. Feeding Success of Tsetse Flies Attracted to Different Hosts

Of the flies attracted to different hosts, only 39 flies (18.31%, alive = 6, dead = 33) successively bloodfed on hosts. The number of flies that were attracted and successfully fed varied across the different hosts ($\chi^2_4 = 49.478$, $p < 0.001$): guinea pigs (n = 10, 25.64%), rodents (n = 23, 58.97%), and rabbits (n = 6, 15.38%). None of the flies attracted to squirrels

were bloodfed. Most of the flies that were attracted and successively fed on rodents (n = 13, 56.52%) originate from parents maintained on blood from squirrels (Table 2).

Table 2. The distribution of the number flies bloodfed successively on different hosts (guinea pig, squirrel, rodent, and rabbit).

Host Cages	Blood Meal Sources				
	Guinea Pig	Rabbit	Rodent	Squirrel	Total
Guinea pig	1	0	7	2	10
Rabbit	1	3	1	1	6
Rodent	0	1	9	13	23
Squirrel	0	0	0	0	0
Total	2	4	17	16	39

3.3. Haemoglobin Concentration (Hb) and Total Plasma Protein in Different Hosts

Of all hosts used in the choice experiment, squirrels had the highest Hb concentration (mean: 19.32 ± 0.51 g/dL), while rabbits had the least (mean: 14.515 ± 0.05 g/dL). Furthermore, rodents had the highest total plasma protein (mean: 75.17 ± 0.497 g/dL), and squirrels had the least among all (mean: 7.756 ± 0.028 g/dL) (Table 3). There was a statistically significant difference in both mean Hb concentration ($\chi^2_3 = 155.24$, $p < 0.001$) and total blood protein ($\chi^2_3 = 302.91$, $p < 0.001$) between hosts. The number of flies attracted to specific hosts is not significantly correlated with either the host's haemoglobin concentration ($r(1) = -0.03$, $p = 0.5368$) or the total plasma protein ($r(1) = 0.05$, $p = 0.3431$). Furthermore, the number of bloodfed flies positively correlated with the host's haemoglobin concentration (Hb) ($r(1) = 0.17$, $p = 0.002$) and was insignificantly correlated with the total plasma protein ($r(1) = 0.04$, $p = 0.478$).

Table 3. The average haemoglobin (Hb) and total plasma protein concentration of different attracted and successfully bloodfed flies.

Host Type	Hb Concentration (g/dL)		Total Plasma Protein (g/dL)		Number of Flies (n, %)	
	Mean	Std. Error	Mean	Std. Error	Attracted	Bloodfed
Guinea pig	14.730	0.071	52.695	0.053	49 (23.00%)	10 (25.64%)
Rabbit	14.515	0.050	65.225	0.003	59 (27.7%)	6 (15.38%)
Rodent	17.335	0.244	75.170	0.497	80 (37.56%)	23 (58.97%)
Squirrel	19.320	0.505	7.756	0.028	25 (11.74%)	0 (0.000%)

4. Discussion

This study assessed the variation in the host choice and feeding success behaviours of *Glossina morsitans* siblings whose parents were maintained from guinea pigs, rabbits, rodents, and squirrels.

The results show the variation in the proportion of attracted tsetse flies across individual hosts. Rodents attracted the highest proportion of released flies (27.4%), followed by rabbits (20.21%), guinea pigs (16.78%), and squirrels (8.56%). This can be explained by the variation in the level of hosts' attractiveness to the flies. Hosts' cues, such as the odour emanating from hosts' bodies, trigger fly-searching behaviours, while host shape, colour, and size determine their choice of specific host [20,26]. It is likely that rodents and rabbits had relatively larger bodies than squirrels and guinea pigs, which influenced their level of attractiveness to flies. A similar finding was also reported in other studies [27,28], where hosts with a larger body size attracted a relatively larger proportion of flies than hosts with a smaller body size. For example, most tsetse flies were attracted to cattle and donkeys compared to monitor lizards, goats, and sheep. Moreover, rodents had buff-grey pelage, and rabbits had black pelage, squirrels had dull yellowish-brown pelage, and guinea pigs had yellow-white pelage. Considering tsetse flies' preference for black or dark colours [29–32], it is possible that the deployed host colours influenced the flies' host choice. For example, one of these studies reported a higher proportion of flies being attracted to hosts with a relatively dark pelage than those with yellow pelage/colour [30].

Likewise, rabbits attracted the highest proportion (59.32%) of flies originating from parents that were maintained on rabbits, followed by rodents, which attracted more of the flies (31.25%) whose parents bloodfed on rodents. This can be attributed to hosts' differential attractiveness to released flies. Similar studies conducted elsewhere, which deployed teneral flies, reported similar results where hosts with larger body sizes (cows) attracted more flies than those with smaller bodies (lizards) [28]. The study conducted on mosquitoes reported evidence that host choice for mosquitoes can be explained by physiological or behavioural conditioning rather than genetic variability [33]. This may be true in the case of deployed tsetse flies, where feeding of the flies on experimental hosts was conducted in only one generation. Hence, there could be less chance for parents' behaviour to be inherited by their siblings if this process existed at all.

Interestingly, about 34% of the flies whose parents bloodfed on squirrels remained in the large semi-field cage (they did not visit any screen cage that contained hosts). This can be explained by the variation in the flies' physical fitness and their ability to detect hosts. Several studies have reported the influence of blood quality on the physiology and biology of flies [34,35]. These studies reported the impact of bloodmeal sources on mosquitoes' feeding rates, survivorship, and fecundity [34], as well as the variation in feeding activities and reproductive capacity and efficiency [36]. Since hosts' haematological properties influence blood nutrition [35] and are known to vary across species [37], this could have influenced flies' physical fitness. Squirrels had the least total plasma protein and the largest haemoglobin (Hb) concentration among all of the hosts (Table 3). Most of these offspring that remained in the large cage were originating from parents that were maintained on squirrels. Future studies may perhaps focus on assessing the influence of hosts' haematological parameters on tsetse siblings' behaviours.

Despite the higher proportion of attracted flies on rabbits and rodents, only 28.75% (23 flies) and 10.17% (6 flies) of attracted flies fed successfully on these hosts, respectively. This finding can be explained by the variation in the level of hosts' defensive behaviour, which affects flies' feeding success [38–41]. It is possible that the hosts deployed in this study varied in the level of their defensive behaviours, thereby affecting flies' feeding success. This finding agrees with the study which reported reduced feeding success for tsetse flies due to hosts' defensive behaviour [38]. And the study which reported a relatively higher feeding rate of *G.pallidipes* on adult cattle compared to young cattle due to variation in the level of their defensive behaviours [39]. In addition to the above factors, the nature of the hosts' furs could have also affected flies' feeding success. The rabbits' bodies, unlike those of rodents, were covered with long, dense furs, which could have minimized the surface area available for the attracted flies to feed on. Finally, the taste system determines attracted flies' biting decisions [22]. The difference in chemical signatures that results from the host's dermal secretions or metabolism of microbiota could have influenced flies' biting decisions. But, since these factors were not assessed in this study, we lack evidence to confirm their influence on observed behaviours. Future studies can be performed to assess the influence of host chemical signatures on the behaviours of tsetse fly siblings using similar host species in Tanzania.

5. Conclusions

This study reports the varied proportion of tsetse flies' siblings that were attracted to and successfully bloodfed on different hosts that were used to maintain their parents. It was hard to confirm the presence of the inherited behaviours of the parents in their siblings; however, only host-related factors explained the observed variations in the deployed tsetse flies' siblings. Future studies need to be conducted to assess the same behaviours using more species of tsetse flies and small mammals, which, on the other hand, could be alternative blood hosts for these flies in the absence of their preferred hosts. The resulting findings will inform tsetse control programs on the possible ways of altering offspring choice and feeding behaviours as a way of controlling African trypanosomiasis.

Author Contributions: Conceptualization, F.E.M., J.L., E.M. and L.L.M.; methodology, F.E.M., E.M. and L.L.M.; formal analysis, F.E.M. and L.L.M.; writing—original draft preparation, F.E.M.; writing—review and editing, F.E.M., E.M. and L.L.M.; visualization, F.E.M.; supervision, E.M. and L.L.M. All authors have read and agreed to the published version of the manuscript.

Funding: The financial support for conducting this study was obtained from The African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development (ACE II IRPM-BTD) ACE II-Credit number 5799-TZ at Sokoine University of Agriculture, Morogoro, Tanzania.

Institutional Review Board Statement: The study was conducted in accordance with the Sokoine University of Agriculture and the Tanzanian Commission for Science and Technology research ethics. The study ethical clearances were approved by the Institutional Ethics Committees of Sokoine University of Agriculture (reference numbers: SUA/DRDTC/R/186/18) and the Tanzanian Commission for Science and Technology for studies involving animals (reference numbers: 2022-735-NA-2022-082).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors sincerely appreciate the advice and support from staffs of the Institute of Pest Management (IPM) and the Tanzania Veterinary Laboratory Agency (TVLA), Tanga.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vreysen, M.J.B.; Seck, M.T.; Sall, B.; Bouyer, J. Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *J. Invertebr. Pathol.* **2013**, *112* (Suppl. S1), S15–S25. [CrossRef] [PubMed]
2. Gashururu, R.S.; Githigia, S.M.; Gasana, M.N.; Habimana, R.; Maingi, N.; Cecchi, G.; Paone, M.; Zhao, W.; Masiga, D.K.; Gashumba, J. An update on the distribution of *Glossina* (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda. *Parasites Vectors* **2021**, *14*, 294. [CrossRef] [PubMed]
3. World Health Organization. *Control and Surveillance of Human African Trypanosomiasis*; World Health Organization: Geneva, Switzerland, 2013; pp. 1–237.
4. Gooding, R.H.; Krafsur, E.S. Tsetse genetics: Contributions to biology, systematics, and control of tsetse flies. *Annu. Rev. Entomol.* **2005**, *50*, 101–123. [CrossRef]
5. Cecchi, G.; Mattioli, R.C.; Slingenbergh, J.; De La Rocque, S. Land cover and tsetse fly distributions in sub-Saharan Africa. *Med. Vet. Entomol.* **2008**, *22*, 364–373. [CrossRef] [PubMed]
6. Bouteille, B. Human African Trypanosomiasis. In *Encyclopedia of the Neurological Sciences*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2014; Volume 2, pp. 618–624. [CrossRef]
7. Moloo, S.K. Distribution of Glossina species in Africa. *Acta Trop.* **1985**, *42*, 275–281.
8. Malele, A.I.I.; Kinung, S.M.; Nyingilili, H.S.; Matemba, L.E.; Sahani, J.K.; Mlengeya, T.D.K.; Wambura, M.; Kibona, S.N. Glossina dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania Glossina dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania. *J. Vector Ecol.* **2007**, *32*, 265–268. [CrossRef]
9. Kweka, E.J.; Kimaro, E.E.; Kweka, J.; Kimaro, E.; Kimaro, E.G.; Nagagi, Y.P.; Malele, I.I. Major Disease Vectors in Tanzania: Distribution, Major Disease Vectors in Tanzania: Distribution, Control and Challenges Control and Challenges. In *Biological Control of Pest and Vector Insects*; IntechOpen: London, UK, 2017.
10. Malele, I.; Craske, L.; Knight, C.; Ferris, V.; Njiru, Z.; Hamilton, P.; Lehane, S.; Lehane, M.; Gibson, W. The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. *Infect. Genet. Evol.* **2003**, *3*, 271–279. [CrossRef]
11. Malele, I.I.; Magwisha, H.B.; Nyingilili, H.S.; Mamiro, K.A.; Rukambile, E.J.; Daffa, J.W.; Lyaruu, E.A.; Kapunge, L.A.; Kasilagila, G.K.; Lwitiko, N.K. Multiple Trypanosoma infections are common amongst Glossina species in the new farming areas of Rufiji district, Tanzania. *Parasites Vectors* **2011**, *4*, 217. [CrossRef]
12. Makhulu, E.E.; Villinger, J.; Adunga, V.O.; Jones, M.M.; Kimathi, E.M.; Mararo, E.; Osaido, J.W.; Musa, A.A.; Wambua, L. Tsetse blood-meal sources, endosymbionts and trypanosome-associations in the Maasai mara national reserve, a wildlife-human-livestock interface. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0008267. [CrossRef]
13. Jaenike, J. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* **1990**, *21*, 243–273. [CrossRef]
14. Vinauger, C.; Lahondère, C.; Cohuet, A.; Lazzari, C.R.; Riffell, J.A. Learning and Memory in Disease Vector Insects. *Trends Parasitol.* **2016**, *32*, 761–771. [CrossRef] [PubMed]
15. McCall, P.J.; Kelly, D.W. Learning and memory in disease vectors. *Trends Parasitol.* **2002**, *18*, 429–433. [CrossRef] [PubMed]
16. Campbell-Lendrum, D.H.; Brandão-Filho, S.P.; Ready, P.D.; Davies, C.R. Host and/or site loyalty of *Lutzomyia whitmani* (Diptera: Psychodidae) in Brazil. *Med. Vet. Entomol.* **1999**, *13*, 209–211. [CrossRef] [PubMed]

17. Bouyer, J.; Pruvot, M.; Bengaly, Z.; Guerin, P.M.; Lancelot, R. Learning influences host choice in tsetse. *Biol. Lett.* **2007**, *3*, 113–117. [CrossRef] [PubMed]
18. Barron, A.B. The Life and Death of Hopkins' Host-Selection Principle. *J. Insect Behav.* **2001**, *14*, 725–737. [CrossRef]
19. Weitz, B. The feeding habits of Glossina. *Bull. World Health Organ.* **1963**, *28*, 711–729.
20. Onyiah, J.A. Mechanism of host selection by tsetse flies. *Int. J. Trop. Insect Sci.* **1980**, *1*, 31–34. [CrossRef]
21. Clausen, P.H.; Adeyemi, I.; Bauer, B.; Breloeer, M.; Salchow, F.; Staak, C. Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. *Med. Vet. Entomol.* **1998**, *12*, 169–180. [CrossRef]
22. Barrozo, R.B. Food recognition in hematophagous insects. *Curr. Opin. Insect Sci.* **2019**, *34*, 55–60. [CrossRef]
23. Camara, K.; Ilboudo, K.; Salou, E.W.; Gimonneau, G. Evaluation of different blood-feeding frequencies on *Glossina palpalis* gambiensis performance in a mass-rearing insectary. *Parasites Vectors* **2021**, *14*, 46. [CrossRef]
24. Opiyo, E.; Parker, A.G.; Mohammed, A.H. *Standard Operating Procedures for Mass Rearing Tsetse Flies*; FAO/IAEA: Vienna, Austria, 2006; Volume 239.
25. Posner, L.P.; Erb, H.N.; Gleed, R.D. The HemoCue® for point-of-care hemoglobin measurement and packed cell volume estimation in cats. *J. Vet. Emerg. Crit. Care* **2005**, *15*, 22–25. [CrossRef]
26. Nagagi, Y.P.; Silayo, R.S.; Kweka, E.J. Advancements in bait technology to control Glossina swynnertoni Austen, the species of limited distribution in Kenya and Tanzania border: A review. *J. Vector Borne Dis.* **2017**, *54*, 16–24. [PubMed]
27. Boyt, W.P.; Mackenzie, P.K.I.; Pilson, R.D. The relative attractiveness of donkeys, cattle, sheep and goats to *Glossina morsitans* morsitans Westwood and *G. pallidipes* Austen (Diptera: Glos-sinidae) in a middle-veld area of Rhodesia. *Bull. Entomol. Res.* **1978**, *68*, 497–500. [CrossRef]
28. Bouyer, J.; Cuisance, D.; Messad, S.; Guérin, P.M. Learning Affects Host Preference in Tsetse Flies. *Rev. D'elevage Medecine Veterinaire Pays Trop.* **2005**, *58*, 27–29. [CrossRef]
29. Green, C.H. The use of two-coloured screens for catching *Glossina palpalis* palpalis. *Bull. Entomol. Res.* **1989**, *79*, 81–93. [CrossRef]
30. Gibson, G.; Torr, S.J. Visual and olfactory responses of haematophagous Diptera to host stimuli. *Med. Vet. Entomol.* **1999**, *13*, 2–23. [CrossRef]
31. Torr, S.J. The host-orientated behaviour of tsetse flies (*Glossina*): The interaction of visual and olfactory stimuli. *Physiol. Entomol.* **1989**, *14*, 325–340. [CrossRef]
32. Mwandawiro, C.; Boots, M.; Tuna, N.; Suwonkerd, W. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; Royal Society of Tropical Medicine and Hygiene: London, UK, 2000; Volume 94, pp. 238–242.
33. Phasomkusolsil, S.; Tawong, J.; Monkanna, N.; Pantuwatana, K.; Damdangdee, N.; Khongtak, W.; Kertmanee, Y.; Evans, B.P.; Schuster, A.L. Maintenance of mosquito vectors: Effects of blood source on feeding, survival, fecundity, and egg hatching rates. *J. Vector Ecol.* **2013**, *38*, 38–45. [CrossRef]
34. Al-rashidi, H.S.; Alghamdi, K.M.; Al-otaibi, W.M.; Al-Solami, H.M.; Mahyoub, J.A. Saudi Journal of Biological Sciences Effects of blood meal sources on the biological characteristics of *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae). *Saudi J. Biol. Sci.* **2022**, *29*, 103448. [CrossRef]
35. Khan, S.A.; Kassim, N.F.A.; Webb, C.E.; Aquel, M.A.; Ahmad, S.; Malik, S.; Hussain, T. Human blood type influences the host-seeking behavior and fecundity of the Asian malaria vector *Anopheles stephensi*. *Sci. Rep.* **2021**, *11*, 24298. [CrossRef]
36. Hawkey, C.M.; Bennett, P.M.; Gascoyne, S.C.; Hart, M.G.; Kirkwood, J.K. Erythrocyte size, number and haemoglobin content in vertebrates. *Br. J. Haematol.* **1991**, *77*, 392–397. [CrossRef] [PubMed]
37. Walker, E.D.; Edman, J.D. The influence of host defensive behavior on mosquito (diptera: Culicidae) biting persistence. *J. Med. Entomol.* **1985**, *22*, 370–372. [CrossRef] [PubMed]
38. Takken, W.; Smallegange, R.C.; Vigneau, A.J.; Johnston, V.; Brown, M.; Mordue-Luntz, A.J.; Billingsley, P.F. Larval nutrition differentially affects adult fitness and *Plasmodium* development in the malaria vectors *Anopheles gambiae* and *Anopheles stephensi*. *Parasites Vectors* **2013**, *6*, 345. [CrossRef]
39. Schofield, S.; Torr, S.J. A comparison of the feeding behaviour of tsetse and stable flies. *Med. Vet. Entomol.* **2002**, *16*, 177–185. [CrossRef] [PubMed]
40. Torr, S.J.; Mangwiro, T.N.C. Interactions between cattle and biting flies: Effects on the feeding rate of tsetse. *Med. Vet. Entomol.* **2000**, *14*, 400–409. [CrossRef]
41. Lyimo, I.N.; Ferguson, H.M. Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends Parasitol.* **2009**, *25*, 189–196. [CrossRef]

CHAPTER FOUR

4.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 General Discussion

The present study reports the species composition, abundance, and phylogenetic relatedness of tsetse flies collected in selected human-wildlife interface bordering Nyerere National Park, Tanzania. Furthermore, it reports on the variation of the host choice and feeding success behaviours of *Glossina morsitans* siblings whose parents were maintained from guinea pig, rabbit, rodent, and squirrel.

The field survey resulted into identification of two *Glossina* species namely, *Glossina pallidipes* and *Glossina morsitans morsitans*. These are the key species inhabiting the Selous ecosystem (Malele *et al*., 2016). Proportionally, *Glossina morsitans morsitans* (n=413, 52.68%) were most abundant species compared to *Glossina pallidipes* (n=371, 47.32%). Among the factors influencing the abundance of tsetse flies, host availability and habitat characteristics play a great role. Specifically, *G.m.morsitans* prefer feeding on warthogs (Gaithuma *et al.*, 2020), and *G.pallidipes* prefers feeding on warthog, giraffe and elephant (Muturi *et al*., 2011; Nyingilili & Malele, 2016). But, on the absence of their preferred hosts, these *Glossina* species feeds on available hosts such as livestock and human being. During the survey, several livestock herds and human being were spotted. It is likely that both serve as alternative sources of bloodmeals to tsetseflies in the interface. Furthermore, wildlife signs such as footprints and faeces were observed, hence suggesting the presence of wild hosts which sustain existing population of tsetse flies.

The large proportion of the tsetse flies were collected from Kisaki ward compared to Bwakila chini. Similarly, the villages selected in Kisaki ward recorded highest tsetse fly abundance compared to

those in Bwakila chini. To mention, Mbojoge was highly infested among all the villages. Several factors can be attributed to this finding. Among them, the distance of the trapping sites from the PA boundary (Salekwa *et al*., 2014; Luziga *et al*., 2017; Ngonyoka *et al*., 2017; Gashururu *et al*., 2021) and host availability (Ngonyoka *et al*., 2017;Postigo *et al*., 2017). Kisaki and its villages are much closer to the PA boundary with larges herd of livestock observed, hence attributed to the higher abundance of tsetse flies in these areas.

There was an influence of seasons on tsetse fly abundance. Higher abundance was recorded during wet season compared to dry season. This is likely due to presence of the weather conditions which favours tsetseflies survival and reproduction. Tsetse flies largely depend on temperature and humidity for their survival (Nnko *et al*., 2021). Average temperature and humidity favour their survival, hence higher abundance during set season. Furthermore, tsetse flies aggregate in dense vegetation during dry season and disperse during rainy season (Robinson, 2003). This behaviour affects tsetse fly catch catches (Nnko *et al*., 2017). Similar behaviours can be observed in host animals, which disperse during dry season when searching for better pasture and aggregate during rainy season. Therefore, the seasonal variation of the tsetse fly abundance is likely due to tsetse flies or hosts' behaviours.

Observed influence of habitat on tsetse flies' abundance, highlights on the importance of selecting sites for trapping tsetseflies. In this case bushed grassland was highly infested with both *G.m.morsitans,* and, *G.pallidipes* (Table 2.2). These flies occurs mostly in open woodland, grass land and bush-land (Fao,1982; Leak *et al*., 2008). This is likely due to the availability of hosts in these areas elsewhere (Reid *et al.*, 2000; Salekwa *et al*., 2014; Ngongolo *et al*., 2019).

Of the three deployed traps, NZI showed the highest performance followed by Pyramidal and Biconical (Table 2.1). All the traps had similar colours but different designs. Furthermore, they were both

set in same habitats. Thus, traps colours and habitats cannot be attributed to the trap success. Perhaps their designs, however, it was not assessed in this study. As per visual observation during trapping, we noted the easy entrance of the flies into NZI trap than other kinds of traps. Trapping performance of different kind of traps in different habitats is reported in the study done by Malele *et al*., (2016).

Only 12.24% of total fly caught were bloodfed (Figure 2.3). Observed small proportion can be explained by tsetse flies' behaviours. Most bloodfed tsetse flies often remain in shades and resting sites while the hungry flies around searching for bloodmeal sources. The similar finding was reported by Farikou *et al*. (2010), where only 4.7% of the flies caught were bloodfed.

In general, there were variations in host attractiveness to tsetse siblings even though they are not preferred hosts for *G.morsitans*. The Rodents attracted the largest proportion of flies followed by Rabbit, Guinea pig and squirrel. Several cues are involved in host's attractiveness to flies, namely; Odour emanated from hosts' body and visual cues (Onyiah, 1980). These factors determine tsetse flies' choice. Rodents (had buff-grey pelage) and Rabbits (had black pelage) were relatively larger in terms of the body size compared to Squirrel (dull yellowish-brown pelage) and Guinea pig (yellow-white pelage). The large hosts attracted most flies than the small hosts. Similar finding was reported by Bouyer *et al.* (2007), where cattle attracted relatively higher proportion of teneral flies than monitor lizard. Also, Ox and donkey attracted most flies compared to goats and sheep (Boyt *et al.*, 1978).

When referring to parent's bloodmeal sources, rodents attracted the highest proportion of flies originating from parents that were maintained on rodent followed by rabbit which attracted more of the flies whose parent's bloodfed on rabbit. This can be explained by inheritance of parents' feeding behaviours and hosts differential

attractiveness. The prior was not assessed in study, however, the later agrees with the results reported by Bouyer and Guerin (2007) where hosts with bigger body size attracted the larger proportion of teneral flies. Also, mosquitoes offspring preferred cow to pig in a choice situation (Mwandawiro *et al*., 2000).

About 34% of the flies whose parent's bloodfed of squirrels did not visit any screen cage that contained hosts. This is likely due to the variation of the flies' physical fitness and their ability to detect hosts as a results of different blood nutrition. Previous studies by Phasomkusolsil *et al.* (2013) and Al-rashidi *et al*. (2022), reported the impact of bloodmeal sources on mosquitoes' feeding rates, survivorship, fecundity, reproductive capacity and efficiency of mosquitoes bloodfed on different hosts.

There was variation in number of tsetse flies successfully fed on deployed host species. Observed results can be explained by the variation of the defensive behaviours among hosts. Squirrels were the most defensive hosts among all, hence none of attracted flies fed successfully. Similar finding was reported by Schofield and Torr (2002), where small proportion of attracted tsetseflies successfully fed on defensive host. Moreover, Torr and Mangwiro (2000) reported relatively higher feeding rate of *G.pallidipes* on adult cattle compared to the young cattle.

4.2 General Conclusion

This study identified *G.pallidipes* and *G.morsitans* dominating the surveyed human-livestock-wildlife interface bordering Nyerere national Park-Tanzania. The study also highlights the variation of *G.morsitans'* host choice and feeding behaviours of tsetseflies siblings when subjected to different hosts used to bloodfed their parents. In general, flies' abundance decreased with an increase of the distance from the PA boundary. And NZI traps were the most successful traps for collecting all tsetse fly species. No concrete evidence was found on the influence of different bloodmeal sources

on tsetseflies host-choice and feeding success, however, host size and defensive behaviours were pointed out to influence the hosts' choice and feeding behaviours of tsetseflies.

4.3 Recommendations

i. Survey should be designed to assess tsetse flies' abundance and diversity both in and out of the protected area using different kinds of traps.
ii. The surveillance of animal and human trypanosomiasis is recommended at the wildlife-livestock-human interface.
iii. For effective tsetse control, NZI and pyramidal traps in combination with other vector control methods should be deployed.
iv. There is need for detailed investigation on the influence of bloodmeal sources on tsetse fly siblings' behaviours across filial generations.

References

Al-rashidi, H. S., Alghamdi, K. M., Al-otaibi, W. M., Al-solami, H. M., & Mahyoub, J. A. (2022). Saudi Journal of Biological Sciences Effects of blood meal sources on the biological characteristics of Aedes aegypti and Culex pipiens (Diptera: Culicidae). *Saudi Journal of Biological Sciences*, *29*(12), 103448. https://doi.org/10.1016/j.sjbs.2022.103448

Bouyer, J., & Guerin, D. C. S. M. P. M. (2007). Learning Affects Host Preference in Tsetse Flies. pp. 27–29.

Bouyer, J., Pruvot, M., Bengaly, Z., Guerin, P. M., & Lancelot, R. (2007). Learning influences host choice in tsetse. *Biology Letters, 3*(2), 113–117. https://doi.org/10.1098/rsbl.2006.0578

Boyt, W. P., Mackenzie, P. K. I., & Pilson, R. D. (1978). The relative attractiveness of donkeys, cattle, sheep and goats to Glossina morsitans morsitans Westwood and G. pallidipes Austen (Diptera: Glossinidae) in a middle-veld area of Rhodesia. November 1971, pp. 497–500.

Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G., & Geiger, A. (2010). Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica, 116*(1), 81–88. https://doi.org/10.1016/j.actatropica.2010.06.002

Gaithuma, A., Yamagishi, J., Hayashida, K., Kawai, N., & Namangala, B. (2020). Blood meal sources and bacterial microbiome diversity in wild- caught tsetse flies. *Scientific Reports, 2020,* 1–10. https://doi.org/10.1038/s41598-020-61817-2

Gashururu, R. S., Githigia, S. M., Gasana, M. N., Habimana, R., Maingi, N., Cecchi, G., Paone, M., Zhao, W., Masiga, D. K., & Gashumba, J. (2021). An update on the distribution of Glossina (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda. *Parasites and Vectors, 14*(1), 1–13. https://doi.org/10.1186/s13071-021-04786-3

Leak, S., Ejigu, D., & Vreysen, M. (2008). Collection of Entomological Baseline Data for Tsetse Area-Wide Integrated Pest Management Programmes. In: *Fao.* ftp://ftp.fao.org/docrep/fao/011/i0535e/i0535e.pdf%5Cnhttp://www.mendeley.com/download/personal/4558981/5529372041/f6b153743490f7b3a77e6ef58a9 eda4a1780e7cd/dl.pdf

Luziga, C., Muya, C., Mramba, F., Byamungu, M., Mbata, G., & Mtambuki, A. (2017). A tsetse Glossina pallidipes harbors the pathogenic trypanosomes circulating in Liwale district, Tanzania. Veterinary Parasitology: Regional St.

Malele, I. I., Ouma, J. O., Nyingilili, H. S., Kitwika, W. A., Malulu, D. J., Magwisha, H. B., & Kweka, E. J. (2016). Comparative performance of traps in catching tsetse flies (Diptera: Glossinidae) in Tanzania. *Onderstepoort Journal of Veterinary Research, 83*(1), 1–7. https://doi.org/10.4102/ojvr.v83i1.105

Muturi, C. N., Ouma, J. O., Malele, I. I., Ngure, R. M., Rutto, J. J., Mithöfer, K. M., Enyaru, J., & Masiga, D. K. (2011). Tracking the feeding patterns of tsetse flies (glossina genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS ONE, 6*(2), 1–6. https://doi.org/10.1371/journal.pone.0017284

Mwandawiro, C., Boots, M., Tuna, N., & Suwonkerd, W. (2000). Transactions of the royal society of tropical medicine and hygiene (2000)94,238-242. 238–242.

Ngongolo, K., Mtoka, S., & Rubanza, C. D. (2019). Ecological Factors and the Prevalence of Trypanosome Infections and Its Economic Implications for Livestock Industry in Simanjiro, Tanzania: *A Review. 36*(3), 1–11. https://doi.org/10.9734/IJTDH/2019/v36i330145

Ngonyoka, A., Gwakisa, P. S., Estes, A. B., Salekwa, L. P., Nnko, H. J., Hudson, P. J., & Cattadori, I. M. (2017). Patterns of tsetse abundance and trypanosome infection rates among habitats of surveyed villages in Maasai steppe of northern

Tanzania. *Infectious Diseases of Poverty, 6*(1), 1–12. https://doi.org/10.1186/s40249-017-0340-0

Nnko, H. J., Gwakisa, P. S., Ngonyoka, A., Sindato, C., & Estes, A. B. (2021). Potential impacts of climate change on geographical distribution of three primary vectors of african trypanosomiasis in tanzania's maasai steppe: G. m. morsitans, g. pallidipes and g. swynnertoni. *PLoS Neglected Tropical Diseases, 15*(2), 1–17. https://doi.org/10.1371/journal.pntd.0009081

Nyingilili, H. S., & Malele, I. (2016). Diversity of Blood Meal Hosts in Glossina Pallidipes and Its Role in the Epidemiology of Trypanosomiasis at a Localized Area in Serengeti National Park. January

Onyiah, J. A. (1980). Mechanism of host selection by tsetse flies. *International Journal of Tropical Insect Science, 1*(01), 31–34. https://doi.org/10.1017/s1742758400000096

Phasomkusolsil, S., Tawong, J., Monkanna, N., Pantuwatana, K., Damdangdee, N., Khongtak, W., Kertmanee, Y., Evans, B. P., & Schuster, A. L. (2013). Maintenance of mosquito vectors: effects of blood source on feeding, survival, fecundity, and egg hatching rates. June, pp. 38–45.

Postigo, J. A. R., Gashururu, R. S., Githigia, S. M., Gasana, M. N., Habimana, R., Maingi, N., Cecchi, G., Paone, M., Zhao, W., Masiga, D. K., Gashumba, J., Feldmann, U., Vreysen, M. J. B., Diall, O., Mattioli, R. C., Id, M. M. N., Changasi, R., Obore, P., Ochieng, B., & Masiga, D. K. (2017). Seasonal variation of tsetse fly species abundance and prevalence of trypanosomes in the Maasai Steppe, Tanzania. *Journal of Vector Ecology, 42*(1), 24–33. https://doi.org/10.1111/jvec.12236

Reid, R. S., Kruska, R. L., Deichmann, U., Thornton, P. K., & Leak, S. G. A. (2000). *Human population growth and the extinction of the tsetse fly. 77,* 227–236.

Robinson. (2003). Fao Animal Production and Health Paper.

Salekwa, L. P., Nnko, H. J., Ngonyoka, A., Estes, A. B., Agaba, M., & Gwakisa, P. S. (2014). Relative abundance of tsetse fly species and their infection rates in simanjiro, Northern Tanzania. *Livestock Research for Rural Development, 26*(12)

Schofield, S., & Torr, S. J. (2002). A comparison of the feeding behaviour of tsetse and stable flies. *Medical and Veterinary Entomology, 16*(2), 177–185. https://doi.org/10.1046/ j.1365- 2915.2002.00361.x

Torr, S. J., & Mangwiro, T. N. C. (2000). Interactions between cattle and biting flies: Effects on the feeding rate of tsetse. *Medical and Veterinary Entomology, 14*(4), 400–409. https://doi.org/10.1046/j.1365-2915.2000.00257.x

APPENDICES

Appendix 1: Tsetse survey data collection sheet

Date of entry _____ District name_____

Ward name _____

GPS Accuracy _____

Village_____

KIND OF TRAP (V)

NZI	
Biconical	
pyramidal	
Sticky panel	

EM Hrs.	Trap no:	Longitude (x-coordinate)	Latitude (y-coordinates)	Altitude (m)	Vegetation	Start time	End time	Tsetse flies			Engorged		Hungry	Remarks
								Species	M	F	M	F		

NOTE: M=Male, F=Female, EM=Emptying

Appendix 2: Data collection sheet for designed behavioural experiments

Host choice and feeding success data recording sheet																																			
Rep	V-col	Number of recaptured flies and their status																				Relative humidity						Temperature							
		Goat cage				Squirrel cage				Rodent cage				Rabbit cage				Out				H0			H1			T0			T1				
		T	D	H	B	T	D	H	B	T	D	H	B	T	D	H	B	T	D	H	B	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av		
R1	Green																																		
	Pink																																		
	Yellow																																		
	Red																																		

DATE: _____

TIME: _____

Note: Total, **D**=Died, **H**=Hungry/Non-Blood Fed, **B**=Blood Fed, **GREEN**=Maintained on Goat, **BLUE**=Maintained on Squirrel, **YELLOW**=Maintained on Rodent, **RED**=Maintained on Rabbit, **H0 =** Initial relative humidity, **H1=** Final relative humidity**, T0=** Initial Temperature, **T1=** Final temperature

Appendix 3: SUA Ethical clearance approval

SOKOINE UNIVERSITY OF AGRICULTURE
DIRECTORATE OF RESEARCH, TECHNOLOGY TRANSFER AND CONSULTANCY

Our Ref:	RPGS/R/ETHICS	Our Date	15/12/2017
Your Ref:	SUA/MPP/D/2020/0003/05	Your Date	03/12/2021

EVALUATION OF THE RESEARCH PROPOSALS FOR ETHICAL CLEARANCE

All research projects at SUA are subject to evaluation for Ethical Clearance in accordance with the SUA Code of Conduct for Research Ethics (2012).

PRELIMINARY INFORMATION (BE COMPLETED BY APPLICANT)

Name of applicant:
MDEE,FILBERT EWALD

Affiliation: 1. SUA Staff ☐ 2. Postgraduate student: ☒

3. Undergraduate student : ☐ 4. Research Associate: ☐

Research Supervisor/Contact person at SUA:
Prof. LADSLAUS MNYONE (PhD), DR. MAFIE ELIAKUNDA (PhD)

Department/Unit/Centre:
DEPARTMENT OF VETERINARY MEDICINE AND PUBLIC HEALTH

College/Institute
COLLEGE OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES

Title of project (must be similar to that entered in the application form)
BIONOMICS, BLOOD-HOST PLASTICITY AND ITS EFFECT ON HOST SEEKING AND FEEDING BEHAVIOUR OF TWO PREDOMINANT TSETSE SPECIES FROM SELECTED HUMAN-WILDLIFE INTERFACE IN TANZANIA

Study Start Date
20-Dec-21

Proposed Study end Date:
30-Sep-22

STATEMENT OF RESEARCH ETHICAL APPROVAL

1. *This project has been considered and has been **Approved/Not Approved** by the Department/College Research and Publication Committee, Department/College/Unit

Signature: ..... Name: DR. ABDUL A.S. KATAKWEBA

Date: 19/12/2021

(Chairperson, Research & Publication Committee)

2. This project has been considered and has been **Approved/Not Approved** by the Ethical Committee DPRTC

Signature: ..... Name: Dr. Doreen Ndesi

Date: 05/01/2022

(Chairperson, Ethics Committee, DPRTC)

3. This project has been considered and **Approved/Not Approved** by the Senate Research and Publication Committee (SRPC), Sokoine University of Agriculture

Signature: ..... Name: PROF. ESD KARIMURIBO

Date: 06/01/2022

(Chairperson, SRPC)

Director
Postgraduate studies, Research, Technology transfer and Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro

Postal address:	Telephone:	Fax:	Telex:	e-mail Address:
P.O. Box 3151 Morogoro, Tanzania	+255 23 2640013	+255 56 4388	55308 UNIVMOG TZ	drpgs@suanet.ac.tz

* All special Projects (Undergraduate studies research be evaluated and approved by the Department/College Research and Publication Committee, Department/College/Unit and reported to REC/DPRTC. Only Applications from Postgraduate, Research Associates and Staff be forwarded to University wide REC

Appendix 4: COSTECH research permit

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY

TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY P.O. Box 4302 DAR-ES-SALAAM

RESEARCH PERMIT

Permit No.	2022-735 -NA-2022-082
Date issued	22nd August, 2022
Researcher's Name	Filbert Mdee
Nationality	Tanzanian
Research Title	Bionomic, blood-host plasticity and its effects on host seeking and feeding behaviour of two predominant tsetse species from selected human-wildlife interface in Tanzania
Research Area(s)	Iringa, Morogoro
Validity	From: 22nd August, 2022 to 21st August, 2023
Contacts of local collaborator *(with affiliated institution)*	

PROGRAM OFFICER

FOR: DIRECTOR GENERAL

IMPORTANT REQUIREMENTS

- *A PI who wishes to continue with a research beyond the expiry date of the research permit should write to COSTECH two months before the operational permit's expiry date, to request for an extension or renewal of the permit.*
- *Research permit that involves collecting human, plant or animal materials / data that will be exported outside Tanzania must submit a signed Material Transfer Agreement (MTA), Data Transfer Agreement (DTA) between Tanzania host institution and the foreign counterpart. The MTA/DTA will indicate terms for collecting, storing/managing, transporting, disposal or returning of the materials/DATA to Tanzania after the closure of the research project.*
- *Any patent or intellectual property and royalty emanating from any research approved by the National Research Registration Committee (NRRC) shall be owned as stipulated in the research proposals and in accordance with the IP policy of the respective research institutions.*
- *All researchers are required to report to a Regional Administrative Secretary (RAS) of the study area and present the introduction letter and activity schedule(plan) prior starting any research activity.*
- *All researchers are required to submit quarterly progress reports and all relevant publications made after completion of the research.*
- *All communications should be addressed to COSTECH Director General through clearance@costech.or.tz; dg@costech.or.tz or +255 (022) 2700749; +255 (022) 2771358. Terms and conditions of the permit are found at www.costech.or.tz*

Tanzania Commission for Science and Technology, Ali Hassan Mwinyi Road, P.O. Box 4302, Dar Es Salaam.
General line: +255(022) 277 1358, Fax: COSTECH, E-mail: dg@costech.or.tz,
Website: http://www.costech.or.tz/

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY

In reply please quote: **RCA 2022/082** **Date: 22nd August, 2022**

Permanent Secretary,
President's Office,
Regional Administration and Local Government,
P.O. Box 1923,
DODOMA.

Dear Sir/Madam,

INTRODUCTION LETTER ON RESEARCH PERMIT

I wish to introduce **Filbert Mdee, a Tanzanian** who has been granted Research Permit **No.** 2022-735-NA-2022-082 dated **22nd August, 2022.**

2. The permit allows him/her to conduct research titled **"Bionomic, blood-host plasticity and its effects on host seeking and feeding behaviour of two predominant tsetse species from selected human-wildlife interface in Tanzania"** under the terms and conditions as per the National Research Registration and Clearance Guidelines of 2022. The research will be conducted in **Iringa, Morogoro** regions.

3. COSTECH is therefore kindly requesting you to introduce the researcher(s) to relevant Regional Administrative Officer(s) and support with any necessary assistance and guidance under national laws and regulations.

4. Thank you for your cooperation

.....

Dr. Bugwesa Katale
FOR: DIRECTOR GENERAL

CC: Regional Administrative Secretary: **Iringa, Morogoro**

BIONOMICS, BLOOD-HOST PLASTICITY AND ITS EFFECT ON HOST-CHOICE AND FEEDING BEHAVIOUR OF TSETSE SPECIES FROM SELECTED HUMAN-WILDLIFE INTERFACE IN TANZANIA

ORIGINALITY REPORT

15%	12%	9%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.suaire.sua.ac.tz Internet Source	2%
2	Submitted to Sokoine University of Agriculture Student Paper	1%
3	suaire.suanet.ac.tz Internet Source	1%
4	doaj.org Internet Source	<1%
5	etd.aau.edu.et Internet Source	<1%
6	Suzana M. Thomas, Geofrey E. Soka, Loth S. Mulungu. "Influence of vegetation structure, seasonality, and soil properties on rodent diversi community assemblages in west Mount Kilimanjaro, Tanzania", Ecology and Evolution, 2022 Publication	<1%

7	link.springer.com Internet Source	<1%
8	Mouhamadou M. Dieng, Kiswend-sida M. Dera, Percy Moyaba, Gisele M. S. Ouedraogo et al. "Prevalence of Trypanosoma and Sodalis in Wild Populations of Tsetse Flies: Impact on Sit Programmes for Tsetse Eradication", Research Square Platform LLC, 2021 Publication	<1%
9	hdl.handle.net Internet Source	<1%
10	ir-library.ku.ac.ke Internet Source	<1%
11	theses.gla.ac.uk Internet Source	<1%
12	www.mdpi.com Internet Source	<1%
13	erepository.uonbi.ac.ke:8080 Internet Source	<1%
14	researchonline.jcu.edu.au Internet Source	<1%
15	discol.umk.edu.my Internet Source	<1%
16	www.ajol.info	

	Internet Source	<1%
17	era.ed.ac.uk Internet Source	<1%
18	journals.plos.org Internet Source	<1%
19	Submitted to University of Stellenbosch, South Africa Student Paper	<1%
20	Submitted to University of Westminster Student Paper	<1%
21	livrepository.liverpool.ac.uk Internet Source	<1%
22	Upendo RICHARD, Robert Modest BYAMUNGU, Flora MAGIGE, Fortunatus B.S. MAKONDA. "Microhabitat, altitude and seasonal influence on the abundance of non-volant small mammals in Mount Rungwe forest nature reserve", Global Ecology and Conservation, 2022 Publication	<1%
23	eprints.soton.ac.uk Internet Source	<1%
24	www.iaea.org Internet Source	<1%

25	dspace.unza.zm Internet Source	<1%
26	Submitted to Loughborough University Student Paper	<1%
27	dalspace.library.dal.ca Internet Source	<1%
28	dspace.nm-aist.ac.tz Internet Source	<1%
29	etd.auburn.edu Internet Source	<1%
30	erepository.uonbi.ac.ke Internet Source	<1%
31	www.tva.or.tz Internet Source	<1%
32	S. J. Torr. "Interactions between cattle and biting flies: effects on the feeding rate of tsetse", Medical and Veterinary Entomology, 12/2000 Publication	<1%
33	Frances S. Sivakoff, Scott P. Prajzner, Mary M. Gardiner. "Urban heavy metal contamination limits bumblebee colony growth", Journal of Applied Ecology, 2020 Publication	<1%

34	Kalinga Chilongo, Tawanda Manyangadze, Samson Mukaratirwa. " Effects of Human Settlements and Spatial Distribution of Wing Vein Length, Wing Fray Categories and Hunger Stages in (Diptera: Glossinidae) and (Diptera: Glossinidae) in Areas Devoid of Cattle in North-Eastern Zambia ", Journal of Medical Entomology, 2021 Publication	<1 %
35	Kat S. Rock, Chris M. Stone, Ian M. Hastings, Matt J. Keeling, Steve J. Torr, Nakul Chitnis. "Mathematical Models of Human African Trypanosomiasis Epidemiology", Elsevier BV, 2015 Publication	<1 %
36	healthdocbox.com Internet Source	<1 %
37	Zakaria Bengaly, Sèna Hervé Vitouley, Martin Bienvenu Somda, André Zongo et al. "Drug quality analysis of isometamidium chloride hydrochloride and diminazene diaceturate used for the treatment of African animal trypanosomosis in West Africa", BMC Veterinary Research, 2018 Publication	<1 %
38	www.researchgate.net Internet Source	<1 %

39	www.scielo.br Internet Source	<1 %
40	R. WALL. "The mating behaviour of tsetse flies (Glossina): a review", Physiological Entomology, 6/1993 Publication	<1 %
41	Submitted to Sultan Qaboos University Student Paper	<1 %
42	peercommunityjournal.org Internet Source	<1 %
43	researcharchive.vuw.ac.nz Internet Source	<1 %
44	Bengisu S. Subasi, Veit Grabe, Martin Kaltenpoth, Jens Rolff, Sophie A.O. Armitage. " How frequently are insects wounded in the wild? A case study using ", Cold Spring Harbor Laboratory, 2023 Publication	<1 %
45	Soumaïla Pagabeleguem, Sophie Ravel, Ahmadou H. Dicko, Marc J. B. Vreysen et al. "Influence of temperature and relative humidity on survival and fecundity of three tsetse strains", Parasites & Vectors, 2016 Publication	<1 %
46	Zoe Giagkazoglou, Andrew M. Griffiths, Anastasia Imsiridou, Archontia Chatzispyrou	<1 %

et al. "Flying under the radar: DNA barcoding ray wings in Greece detects protected species and umbrella labelling terms", Food Control, 2021
Publication

47	biblio.ugent.be Internet Source	<1 %
48	docslib.org Internet Source	<1 %
49	Faraja D. Ng'ida, Gladness L. Kotoroi, Rose Mwangi, Mabula Mabelele, Jovin Kitau, Michael J. Mahande. " Knowledge and practices on breast cancer detection and associated challenges among women aged 35 years and above in Tanzania: a case in Morogoro Rural District ", Breast Cancer: Targets and Therapy, 2019 Publication	<1 %
50	elifesciences.org Internet Source	<1 %
51	Hargrove, John W., and M. Odwell Muzari. "Artificial Warthog Burrows Used to Sample Adult and Immature Tsetse (Glossina spp) in the Zambezi Valley of Zimbabwe", PLoS Neglected Tropical Diseases, 2015. Publication	<1 %

52	Peter Van den Bossche, Stéphane de La Rocque, Guy Hendrickx, Jérémy Bouyer. "A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis", Trends in Parasitology, 2010 Publication	<1 %
53	Submitted to The Mwalimi Nyerere Memorial Academy Student Paper	<1 %
54	Submitted to University of Liverpool Student Paper	<1 %
55	etda.libraries.psu.edu Internet Source	<1 %
56	library.oapen.org Internet Source	<1 %
57	Godfrey C. Katusi, Marie R. G. Hermy, Samwely M. Makayula, Rickard Ignell et al. "Seasonal variation in abundance and blood meal sources of primary and secondary malaria vectors within Kilombero Valley, Southern Tanzania", Parasites & Vectors, 2022 Publication	<1 %
58	Joseph N Aikambe, Ladslaus L Mnyone. " Retrospective Analysis of Malaria Cases in a Potentially High Endemic Area of Morogoro	<1 %

Rural District, Eastern Tanzania

", Research and Reports in Tropical Medicine, 2020
Publication

59	Submitted to University of Bedfordshire Student Paper	<1 %
60	Bernard Bouteille, Alain Buguet. "The detection and treatment of human African trypanosomiasis", Research and Reports in Tropical Medicine, 2012 Publication	<1 %
61	Faith I. Ebhodaghe, Armanda D. S. Bastos, Michael N. Okal, Daniel K. Masiga. "Entomological assessment of tsetse-borne trypanosome risk in the Shimba Hills human-wildlife-livestock interface, Kenya", Frontiers in Veterinary Science, 2022 Publication	<1 %
62	Mgode, Georgies F., Abdul S. Katakweba, Ginethon G. Mhamphi, Frank Fwalo, Mohamed Bahari, Mashaka Mdangi, Bukheti S. Kilonzo, and Loth S. Mulungu. "Prevalence of leptospirosis and toxoplasmosis: a study of rodents and shrews in cultivated and fallow land, Morogoro rural district, Tanzania", Tanzania Journal of Health Research, 2014. Publication	<1 %

86	"Measuring Arthropod Biodiversity", Springer Science and Business Media LLC, 2021 Publication	<1 %
87	A. G. S. Chandu, P. P. Sengupta, S. S. Jacob, K. P. Suresh, S. K. Borthakur, G. Patra, P. Roy. "Seroprevalence of Trypanosoma evansi in cattle and analysis of associated climatic risk factors in Mizoram, India", Journal of Parasitic Diseases, 2020 Publication	<1 %
88	C. Vinauger, L. Buratti, C. R. Lazzari. "Learning the way to blood: first evidence of dual olfactory conditioning in a blood-sucking insect, Rhodnius prolixus. I. Appetitive learning", Journal of Experimental Biology, 2011 Publication	<1 %
89	Claus Thomas, Venance Msoffe, Natalie Van Houtte, Ginethon Mhamphi et al. "Prevalence and Seasonal Variation of Trichuris Worms Infection in Mastomys natalensis in Morogoro and Iringa Regions, Tanzania", Parasitologia, 2023 Publication	<1 %
90	Eliane Tihon, Hideo Imamura, Frederik Van den Broeck, Lieve Vermeiren, Jean-Claude Dujardin, Jan Van Den Abbeele. "Genomic analysis of Isometamidium Chloride	<1 %

91	G. Gibson, S. J. Torr. "Visual and olfactory responses of haematophagous Diptera to host stimuli", Medical and Veterinary Entomology, 2002 Publication	<1 %
92	Genet B. Gebrezgiher, Rhodes H. Makundi, Abdul A. S. Katakweba, Steven R. Belmain, Charles M. Lyimo, Yonas Meheretu. "Arthropod Ectoparasites of Two Rodent Species Occurring in Varied Elevations on Tanzania's Second Highest Mountain", Biology, 2023 Publication	<1 %
93	J. O. Ouma, J. G. Marquez, E. S. Krafsur. "Patterns of genetic diversity and differentiation in the tsetse fly Glossina morsitans morsitans Westwood populations in East and southern Africa", Genetica, 2006 Publication	<1 %
94	Lyimo, I.N.. "Ecological and evolutionary determinants of host species choice in mosquito vectors", Trends in Parasitology, 200904 Publication	<1 %
95	Simegnew Adugna Kallu, Joseph Ndebe, Yongjin Qiu, Ryo Nakao, Martin C. Simuunza. "Prevalence and Association of Trypanosomes and Sodalis glossinidius in Tsetse Flies from the Kafue National Park in Zambia", Tropical Medicine and Infectious Disease, 2023 Publication	<1 %

96	academic.oup.com Internet Source	<1 %
97	afribary.com Internet Source	<1 %
98	archive.org Internet Source	<1 %
99	atrium.lib.uoguelph.ca Internet Source	<1 %
100	citeseerx.ist.psu.edu Internet Source	<1 %
101	coek.info Internet Source	<1 %
102	digitum.um.es Internet Source	<1 %
103	edocs.fu-berlin.de Internet Source	<1 %
104	expeditiorepositorio.utadeo.edu.co Internet Source	<1 %
105	gala.gre.ac.uk Internet Source	<1 %
106	ir.uz.ac.zw:8080 Internet Source	<1 %
107	journalcra.com Internet Source	<1 %
108	mafiadoc.com Internet Source	<1 %
109	nuxeo.edel.univ-poitiers.fr Internet Source	<1 %

110	scholar.ufs.ac.za Internet Source	<1 %
111	spiral.imperial.ac.uk Internet Source	<1 %
112	www.biorxiv.org Internet Source	<1 %
113	www.frontiersin.org Internet Source	<1 %
114	www.nature.com Internet Source	<1 %
115	www.researchintouse.com Internet Source	<1 %
116	Clive Alfred Spinage. "African Ecology", Springer Science and Business Media LLC, 2012 Publication	<1 %
117	G. Mhamphi Ginethon, S. Katakweba Abdul, W. Massawe Apia, H. Makundi Rhodes et al. "Prevalence of Bartonella spp. in rodent and shrew species trapped in Kigoma and Morogoro Regions, Tanzania: A public health concern", African Journal of Microbiology Research, 2023 Publication	<1 %
118	Richard S. Gashururu, Samuel M. Githigia, Methode N Gasana, Richard Habimana et al. "An update on the distribution of Glossina (tsetse flies) at the wildlife-human-livestock interface of Akagera National Park, Rwanda", Research Square, 2021 Publication	<1 %

119	"Olfaction in vector-host interactions", Wageningen Academic Publishers, 2010 Publication	<1 %
120	Joshua B. Benoit, Geoffrey M. Attardo, Brian L. Weiss. "Tsetse flies (Glossinidae)", Elsevier BV, 2020 Publication	<1 %
121	Kostas Bourtzis, Rosemary Susan Lees, Jorge Hendrichs, Marc J.B. Vreysen. "More than one rabbit out of the hat: Radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations", Acta Tropica, 2016 Publication	<1 %
122	Malal M Diop, Fabrice Chandre, Marie Rossignol, Angélique Porciani, Mathieu Chateau, Nicolas Moiroux, Cédric Pennetier. "Sub-lethal insecticide exposure affects host biting efficiency of Kdr-resistant *Anopheles gambiae*", Peer Community Journal, 2021 Publication	<1 %

FILBERT EWALD MDEE,
P. O. Box 3015,
MOROGORO.
27th September, 2023.

Director,
Postgraduate Studies, Research, Technology transfer and Consultancy,
Sokoine University of Agriculture,
P.O. Box 3151,
MOROGORO.

u.f.s **Principal,**
College of Veterinary Medicine and Biomedical Sciences,
Sokoine University of Agriculture,
P.O Box 3015,
MOROGORO.

u.f.s. **Head,**
Department of Veterinary Medicine and Public Health,
Sokoine University of Agriculture,
P.O. Box 3015,
MOROGORO.

u.f.s. Dr. Eliakunda Mafie (Supervisor), Forwarded for consideration
Department of Veterinary Microbiology, 27/09/2023
Parasitology and Biotechnology,
Sokoine University of Agriculture,
P.O. Box 3015,
MOROGORO.

Dear Sir/Madam,

RE: SUBMISSION OF THE SOFT COPY OF THE DISSERTATION VIA EMAIL

Reference is made to the above heading.
I, Mdee, Filbert Ewald with Registration Number MPP/D/2020/0003, do hereby submit the soft copy of my dissertation in a Word document titled **"BIONOMICS, BLOOD-HOST PLASTICITY AND ITS EFFECT ON HOST-CHOICE AND FEEDING BEHAVIOUR OF TSETSE SPECIES FROM SELECTED HUMAN-WILDLIFE INTERFACE IN TANZANIA"** via the email address **vetmed@sua.ac.tz.** A copy has also been sent to **mdegela@sua.ac.tz** (Head, Department of Veterinary Medicine and Public Health), as well as to my Supervisors, today, the .... of August, 2023. in fulfilment of the requirements for the degree of Master of Science in Public Health Pest Management of Sokoine University of Agriculture for further actions according to the University regulations.

Yours sincerely,

.....

Mdee, Filbert Ewald

FILBERT EWALD MDEE,
P. O. Box 3015,
MOROGORO.
27th September, 2023.

Director,
Postgraduate Studies, Research, Technology transfer and Consultancy,
Sokoine University of Agriculture,
P.O. Box 3151,
MOROGORO.

u.f.s **Principal,**
College of Veterinary Medicine and Biomedical Sciences,
Sokoine University of Agriculture,
P.O Box 3015,
MOROGORO.

u.f.s. **Head,**
Department of Veterinary Medicine and Public Health,
Sokoine University of Agriculture
P.O. Box 3015,
MOROGORO.

u.f.s Dr. Eliakunda Mafie (Supervisor), Forwarded for consideration
Department of Veterinary Microbiology, 27/09/2023
Parasitology and Biotechnology,
Sokoine University of Agriculture,
P.O. Box 3015,
MOROGORO.

Dear Sir/Madam,

RE: REQUEST FOR A RELEASE LETTER

Reference is made to above heading.

I **Mdee, Filbert Ewald** with Registration Number MPP/D/2020/0003, do hereby request for a release letter after submission of the soft copy of word document file of the dissertation titled **"BIONOMICS, BLOOD-HOST PLASTICITY AND ITS EFFECT ON HOST-CHOICE AND FEEDING BEHAVIOUR OF TSETSE SPECIES FROM SELECTED HUMAN-WILDLIFE INTERFACE IN TANZANIA"** today, 27th September, 2023 for examination as per Sokoine University of Agriculture regulations.

Yours sincerely,

Mdee, Filbert Ewald