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Ectoparasites of wild rodents in forest sites invaded and uninvaded by *Maesopsis eminii* in Amani nature forest reserve, Tanzania

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ABSTRACT

Parasites are important component of communities in a forest ecosystem with profound effects on trophic interactions such as food web. Modification of the forest structure (e.g. changes in species composition and abundance of key species) can have a strong impact on the occurrence, diversity, and abundance of parasites, with subsequent repercussions for ecosystem functioning. In this study, we compared the occurrence and abundance of wild rodents' ectoparasites from forest sites invaded and uninvaded by an invasive tree, Maesopsis eminii in Amani Nature Forest Reserve, Tanzania. Three large plots (40 m × 100 m) were randomly established in each forest sites invaded and uninvaded by M. eminii. In each plot, 50 Sherman traps were systematically placed at 10 m interval for capturing wild rodents through a capture-mark-recapture technique. Wilcox rank sum test was used to compare for differences in the abundance of infested rodents and ectoparasites between the invaded and uninvaded forest sites. A total of 297 individual rodents were captured and screened for ectoparasites, including 174 rodents from uninvaded forest site and 123 rodents from invaded forest site. The number of infested rodents were significantly (W = 8592, P < 0.001) greater in uninvaded forest site (66.27%) than in the invaded forest site (36.2%). Furthermore, a significant greater number of Echinolaelaps echidninus (W = 1849, P < 0.01) and *Dinopsyllus ellobius* (W = 2800.5, P < 0.05) ectoparasites were found in uninvaded as compared to the invaded forest sites. The results of this study suggest that the invasion and dominance by, M. eminii in Amani Nature Reserve has created unfavorable conditions for rodents and ectoparasites and therefore impacting the diversity and function of the forest ecosystem. We recommend prevention of further introduction of the M. eminii outside their natural range and mitigating the impact of the established M. eminii in Amani Forest Nature Reserve.

1. Introduction

Parasites are organisms that live at the expense of certain individual host during their life cycle (Galván et al., 2012). They account for up to half of all animal species on Earth (Poulin, 2014), and play critical roles such as maintaining their host population in biological systems and ecological interaction (Maizels and McSorley, 2016), making them an important component of global biodiversity (Veitch et al., 2020). When parasites inhabit the surfaces of another organism, they are referred as ectoparasites (Hanafi-Bojd et al., 2007), and rodents are among the most important hosts of ectoparasites globally (Fagir et al., 2014). Although ectoparasites rely on their hosts for their survival (Krasnov et al., 2006), they are also influenced by their surroundings (Guerra et al., 2002).

Thus, ectoparasites such as fleas and mites depend on the surrounding environment of their hosts when they are not feeding on the host (Babyesiza et al., 2023) since environmental conditions limit species occurrence in local communities (Gómez-Rodríguez et al., 2015). Generally, environment acts as an important determinant of the occurrence and abundance of parasites (Krasnov et al., 2006) as host-ectoparasite association is the result of both parasites, hosts and environmental factors (Linardi and Krasnov, 2013). Therefore, occurrence and abundance of ectoparasites are not just a function of host-parasite relationships but also parasite-environment relationships (Mize et al., 2011).

The influence of the environment on invertebrate especially parasite diversity and distribution is becoming more important globally (Bordes

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et al., 2013), as environmental changes due to anthropogenic activities has altered biological systems, and consequently, affecting parasite population (Acevedo-Whitehouse and Duffus 2009; Chaisiri et al., 2010). The effects of the environmental changes including vegetation change on parasite populations can be positive or negative (Ostfeld et al., 2005). For example, the invasion of Japanese honeysuckle (*Berberis thunbergii*) has reduced the abundance of parasites of the genus *Ixodes* in Connecticut, USA (Williams et al., 2017). The invasion of Common water hyacinth, *Eichhornia crassipes* (currently *Pontederia crassipes*) aided in the presence and increase of *Schistosoma* parasites in invaded areas of Lake Victoria, Mwanza, Tanzania (Plummer, 2005; Mack and Smith, 2011). In addition, invasive plant, *Lantana camara*, attracted and caused the increase in population of *Glossina* parasites in invaded areas of Africa (Syed and Guerin, 2004).

Maesopsis eminii is an invasive tree species threatening forests of Eastern Arc Mountains (EAM) (Hall et al., 2010; Mwendwa et al., 2020). The tree has been found to change the structure of the forest ecosystem (Musila and Leonhartsberger, 2006) and has significantly reduced the abundance of wild rodents in the invaded forest sites (Musese et al., 2023). The decline in host populations may have significant impact on parasite populations, including their occurrence and abundance (Dunn et al., 2009). However, there is limited information on how the interaction between M. eminii and wild rodents may affect the occurrence and abundance of ectoparasites of wild rodents. This study focused on, assessing the occurrence of ectoparasites (infestation) on host rodents and the ectoparasites' composition and load (abundance) in forest sites invaded and uninvaded with M. eminii in Amani Forest Nature Reserve. It was hypothesized that forest sites invaded with M. eminii will have lower number of infested rodent hosts and lower ectoparasites' abundance as compared to uninvaded forest sites.

2. Material and methods

2.1. Study area

The research was carried out at Amani Nature Forest Reserve (ANFR), in northeastern Tanzania. The forest is located along 5⁰ 06' S and 38° 38' E at 920–1150 m. a.s.l, (Fig. 1), covering approximately an area of 83,600 ha (Newmark and Stanley, 2011). Because of its proximity to the Indian Ocean, ANFR receives a lot of rain, with an annual rainfall of about 1918 mm and an annual mean temperature of 20.6°C (URT, 2017). The reserve is part of the Eastern Arc Chain of Mountains which are small, isolated block of natural rain forests remaining in Kenya and Tanzania known for their rich and unique biodiversity (Newmark, 2002; URT, 2017). Intense mechanical logging between mid-1960s to 1986 damaged large part of the AFNR (Hamilton and Mwasha, 1989), which permitted the invasion and spreading of introduced tree species *Maesopsis eminii* in large areas through forest edges and tree-fall gaps (Newmark, 2002).

Maesopsis eminii was introduced in AFNR for restoration purposes to fill forest gaps and clear-felled areas after expansion of peasant's agriculture and large scale logging operations in the 1960's (Dawson et al., 2008). However, tree-dwelling birds such as silvery-cheeked hornbill (*Bycanistes brevis*) naturally dispersed *M. eminii* in forests (Cordeiro et al., 2004), making it dominant in significant area of the AFNR (Hall et al., 2010). It is therefore reported as among the highly successful invasive woody plants in AFNR (Gereau et al., 2016) thereby negatively impacting both flora and fauna of the AFNR (Dawson et al., 2009; Musese et al., 2023).

2.2. Study design and rodent trapping

The present study design adopted the design for rodent trapping utilized by Musese et al. (2023). A total of six, 40 m \times 100 m plots, were



Fig. 1. A Map showing the location of invaded and uninvaded forest sites in Amani Nature Forest Reserve, Tanzania. Top left in an insert of map of Tanzania and bottom is an insert of Map of Amani Forest Nature Reserve.

systematically established in forest sites. Three plots were established in forest sites invaded by M. eminii, and three in forest sites uninvaded by M. eminii. Selection of the study sites and the number of plot replicates followed Musese et al. (2023). In each plot a total of 50 trapping stations were established in five line transects measuring 100 m in length. The transects were established parallel to each other and spaced 10 m apart. In each transect, 10 trapping stations placed at an interval of 10 m were established, Sherman trap baited with peanut butter was set at each trapping station. This gives a total of 150 trapping stations per forest site invasion status, and an overall of 300 trapping stations for the whole study area. Trapping stations were then marked with unique codes written on ribbons for easy identification. Traps were hidden with dry leaf litter to protect them from rain and direct sunlight, and were set for three consecutive nights at each site per month, for 24 months from April 2020 through March 2022 and inspected once per day between 7:00 a.m. and 11:00 a.m. Mortelliti and Boitani (2006) and Musese et al. (2023) have also found success in inspecting traps once a day, with no heat or cold deaths reported. The Capture- Mark Recapture (CMR) technique was used to capture rodents and each rodent captured was permanently marked with the unique number codes generated by the CMR software MARK as explained by Borremans et al. (2015). Captured rodents were removed from the trap using a cotton bag and handled using protective gear particularly gloves and masks. Rodent species were morphologically identified by an expert from the Institute of Pest Management at the Sokoine University of Agriculture following Happold (2013), Monadjem et al. (2015) and Musese et al. (2023). Once captured, information such as date of capture, trapping station, site of capture, and identification of trapped rodents were recorded. After completion of ectoparasites screening, captured rodents were released to their trapping station. Throughout the entire process rodent handling followed the American Society of Mammologists (ASM) guidelines for the use of wild mammals in research and education, particularly, Animal Care and Use Committee of the American Society of Mammologists (Sikes, 2016).

2.3. Ectoparasites collection and identification

Each captured rodent was anesthetized with a wad of cotton soaked with ether following the procedure recommended by De Mendonça et al. (2020) and visually examined for ectoparasites as described by Guernier et al. (2014) and monitored in separate recovery cage until they recover from anesthesia before released to their trapping station (Geiger et al., 2008). Ectoparasites were manually collected by brushing rodent's fur with a clean toothbrush on a white plastic tray and carefully picked with fine forceps (Patterson et al., 2013). To avoid the possibility of ectoparasites being assigned to the wrong rodent host, traps were cleaned after each rodent capture (Bittencourt and Rocha, 2003). Ectoparasites were stored in labeled vials containing absolute ethanol (Calvani et al., 2020) corresponding to the rodent host from which they were collected. The samples were then transported to the laboratory for further morphological identification using a compound microscope with the aid of reported descriptions and taxonomic keys (Furman 1972; Ahmed 2006; Baak-Baak et al., 2016). To confirm the morphologically identified flea and mite species, molecular identification was done at the department of Parasitology and Institute of Pest Management at Sokoine University of Agriculture. The molecular identification of ectoparasites followed the following procedures devised by Gebrezgiher et al. (2023); DNA from the whole body of individual fleas and mites were extracted using the Quick-DNATM Miniprep Plus Kit following the manufacturer's instructions. Nano spectrophotometer at 260 and 280 nm wavelengths was used to determine the purity and concentration of the extracted DNA. For fleas' identification, the cytochrome oxidase subunit II (cox 2) gene was amplified using the primer sequences following Zhu et al. (2015): forward primer (F-Leu: TCTAATATGGGCAGATTAGTGC) and reverse primer (R-Lys: GAGACCAGTACTTGCTTTCAGTCATC. For mite's identification, the cytochrome oxidase subunit I (cox1) gene was amplified using primer sequences following Folmer et al. (1994): forward primer (cox1-F: GTTTTGGGATATCTCTCATAC) and reverse primer (cox1-R: GAGCAACAACATAATAAGTAT).

PCR amplification was performed using AccuPower® PCR PreMix from Bioneer (Bioneer Corporation, Daejeon, Republic of Korea). For fleas, the PCR reaction mixture consisted of 2 μ L of template DNA, 0.5 μ L of forward primer, 0.5 µL of reverse primer, and 17 µL of nuclease free water in a micro-tube containing AccuPower® PCR PreMix concentrate, making a total reaction volume of 20 µL was used. Cycling conditions included an initial denaturation at 950C for 5 min followed by 40 cycles of 940C for 40 s, 560C for 45 s, and 72 0C for 45 s. To complete the extension, a final extension at 720C for 5 min was performed. For mites, a total of 20 μL of PCR reaction mixture consisted of 2 μL of extracted DNA, 1 μ L of forward primer, 1 μ L of reverse primer, and 16 μ L of nuclease-free water in a micro-tube containing AccuPower® PCR Pre-Mix concentrate was used. Cycling conditions involved initial denaturation at 950C for 5 min followed by 40 cycles of 950C for 40 s, 470C for 40 s, and 720C for 30s. To complete the extension, a final extension at 720C for 5 min was performed.

After the PCR reaction procedures, a 1.5% agarose gel was prepared by dissolving 1.5 g of agarose into 100 mL of sodium borate buffer and heated until the agarose had dissolved completely, and then stained with 4 µL of EZ-Vision® In-Gel Solution. A volume of 4 µL of each sample was then loaded into each well of the gel, and 4 μ L of DNA ladder was loaded into the first well in order to indicate the size of any fragments. The voltage was then set to 100 V, and run the electrophoresis for 40 min. DNA fragments image was captured using Bio-Rad's Gel Doc™EZ Imaging System. Nine amplicons, three for fleas and three for mites, were sequenced at Macrogen Europe (Amsterdam, The Netherlands). To obtain consensus sequences, raw sequence data were cleaned, edited and assembled using Geneious Prime version 2022.1.1 software (Geneious,). For completion of the process, the obtained nucleotide sequences were aligned with other ectoparasite reference sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program (Morgulis et al., 2008).

2.4. Data analysis

The abundance of ectoparasites was estimated as total number of the individual ectoparasites species collected per total number of infested rodents (Rózsa et al., 2000) while the host density (individual count per trapping area (n/1.5ha)) and prevalence (number infested host per number of examined hosts) were computed following Kiene et al. (2020) and Va' zquez et al. (2000) respectively. Since the abundance of ectoparasites did not conform to a normal distribution, a Wilcox rank sum test using a "wilcox.test" function was used to test for differences in abundance of ectoparasites and infestation in rodents between the invaded and uninvaded forest sites. All statistics were calculated using R v4.2.2 (R Core Team, 2020) from which statistical significance was inferred if p-value was ≤ 0.05 .

3. Results

3.1. Rodent species density and their infestation status

A total of 297 rodents were captured and examined for ectoparasites, of which 174 were captured from uninvaded and 123 from invaded forest sites (Table 1). A total of 156 rodents were found infested with various ectoparasites (Table 1). There were greater overall host density and prevalence of ectoparasite infestation in uninvaded forest site; however, the infestation was only in a single species *Montemys delectorum* (Table 1). While host density and ectoparasites prevalence was lower in the invaded forest site, it was detected that three species specifically *Montemys delectorum*, *Beamys hindei*, and *Lophuromys kilonzoi* were infested (Table 1). Furthermore, the results revealed that the number of infested rodents per trapping station in invaded forest site

Table 1

Rodent species density and prevalence of ectoparasites infestation on rodent hosts in Amani Forest Nature Reserve.

Rodent species name	Density (count/1.5ha)		Prevalence (%)	
	Uninvaded	Invaded	Uninvaded	Invaded
Aethomys chrysophilus	1	0	0	0
Beamys hindei	3	3	0	60
Grammomys dolichurus	0	1	0	0
Lophuromys kilonzoi	2	1	0	100
Montemys delectorum	111	77	66.27	36.21
Total	117	82	63.2	37.4

was significantly lower than the number of infested rodents per traps in uninvaded forest site (W = 8592, P < 0.001). As the infestation was more dominant to one host, rodent species *M. delectorum* in forest sites in both invaded and uninvaded forests, analysis on the differences in presence of ectoparasites between rodent host species was not considered.

3.2. Ectoparasites composition and abundance

During this study, a total of 749 ectoparasites belonging to three ectoparasites species were collected from captured rodents (Table 2). The collected ectoparasites were specifically two flea species; Ctenophthalmus calceatus cabirus and Dinopsyllus ellobius, and one mite specie Echinolaelaps echidninus. The Molecular results confirmed that C. calceatus cabirus revealed 92% identity of similarity with OP857547.1 gene sequence and D. ellobius revealed 95.75% identity of similarity with EU335993.1 gene sequence results while the mite specie E. echidninus revealed 87.53% identity of similarity with OP954302.1 gene sequence in the GenBank. The total abundance of ectoparasites per rodents was greater on rodents captured in uninvaded forest sites (five ectoparasites per rodent) than from rodents captured in invaded forest sites (four ectoparasites per rodent), however the differences were not statistically significant (W = 2367, P > 0.05). Among all ectoparasites species collected, mites, E. echidninus was the most abundant and common ectoparasites of rodents in both invaded and uninvaded forest site. However, rodents captured from uninvaded forest sites had significantly (W = 1849, P < 0.01) greater abundance of *E. echidninus* (500-E. echidninus/100 rodents) than rodents capture in invaded forest sites (300- E. echidninus/100 rodents). While Dinopsyllus ellobius was found significantly (W = 2800. f5, P < 0.05) more abundant on rodents captured in uninvaded forest sites (20-D. ellobius/100 rodents), than rodents in invaded forest site (10-D. ellobius/100 rodents), Ctenophthalmus calceatus cabirus was statistically (W = 3136.5, P < 0.001) more abundant on rodents captured in invaded forest sites (43-C. calceatus cabirus/100 rodents) as compared to rodents captured in uninvaded forest site (5-C. calceatus cabirus/100 rodents).

4. Discussion

Similar to our hypothesis, we confirmed differences in ectoparasites presence on rodents between the forest sites, with low infested rodents captured from invaded forest sites than uninvaded forest site; an implication that invaded forest site is probably not suitable to

Table 2

Mean number of ectoparasite species collected from invaded and uninvaded forest sites in Amani Forest nature Reserve.

Species	Mean number of ectoparasites/100 rodents		W	P-value
	Uninvaded	Invaded		
Echinolaelaps echidninus Dinopsyllus ellobius Ctenophthalmus calceatus cabirus	500 10 5	300 20 43	1849 2801 3137	<0.01 <0.05 <0.001

ectoparasites. This is supported by Mize et al. (2011), that, some rodents may be infested with few or no parasites due to external environment which is not suitable to certain life stages of potential parasites. On the other hand, presence of ectoparasites on the host depends on finding the right host in the right environments (Krasnov et al., 2006). Torchin and Mitchell (2004), concluded that, individual animals in invaded areas are generally less infested compared to individuals from uninvaded areas since vegetation changes influences host abundance and diversity. Suggestively in our study area, M. eminii invasion in AFNR has significantly changed the forest structure such as canopy cover, natural understory scrub in the invaded area (Dawson et al. (2009), which has also affected presence of important ectoparasites' hosts particularly rodents (Musese et al., 2023) thus probably affected the presence of ectoparasites in the invaded forest site. Krasnov et al. (2006) proposed that, environmental factors particularly vegetation species are strongly associated with presence of ectoparasites in a certain habitat.

Mites, E. echidninus were the most abundant ectoparasites in both invaded and uninvaded forest sites of AFNR; this could be partially explained by its capability of parasitising a wide range of rodent species which favoring its survival and spread (Mawanda et al., 2020). Similarly, dominance of Echinolaelaps echidninus on wild rodents has also been reported by Wei et al. (2010) in South Western China and Mawanda et al. (2020) in South Western Uganda. According to Montasser (2006) and Mawanda et al. (2020), E. echidninus is a well-known mite infesting domestic rats and wild rodents, a suggestion that this mite species is generalist species for rodent hosts (Nieri-Bastos et al., 2004; Changbunjong et al., 2010). Supportively, Yonas et al. (2011) and Cruz et al. (2012) pointed out that, mites have greater tolerance of the habitat, thus most abundant ectoparasite species in both invaded and uninvaded forest sites during this study. Similarly, Gebrezgiher et al. (2023) reported mites as common ectoparasites of rodents captured from the Mount Meru, Tanzania. This suggests that mites could be the common ectoparasite species of the mountainous forests, according to Farská et al. (2014), mites are the most abundant ectoparasites of the forest ecosystem. Although mites were the most abundant ectoparasites species in both invaded and uninvaded forest site but we recorded more mites in the uninvaded forest sites as compared to invaded forest site, a suggestion that the abundance of mites declined in the invaded forest site. Our results are similar to Koutika et al. (2007), and Skubała (2012) who reported a significant low abundance of ectoparasites mostly mites in plant invaded areas. Similarity, Malloch et al. (2020) reported declined abundance of the microarthropod including mites in invaded plots of Greater Toronto Area, Canada. Another study, Torchin and Mitchell (2004) summarized that, invasive plants reduces parasites in the invaded area. According to Krasnov et al. (2006), vegetation has major influence on composition of ectoparasites. Low mites' abundance in invaded forest of AFNR can probably be due to changes in vegetation structure due to M. eminii invasion. According to Hall et al. (2010) and Dawson et al. (2009), M. eminii has significantly changed the vegetation structure such as reducing plant species composition and natural understory scrub of AFNR. According to Musese et al. (2023) M. eminii invasion has significantly reduced the abundance of wild rodents in the invaded forest sites in ANFR. Thus, it can be argued that there is a link between M. eminii invasion and the reduction in overall presence and abundance of mites on rodent hosts in ANFR.

This study observed significantly more *Ctenophthalmus calceatus cabirus* in invaded forest site, than uninvaded forest site; which is probably a result of environmental disturbances in invaded forest sites. We couldn't find supporting literature on the impact of plant invasion on fleas, however, Friggens and Beier (2010) reported significantly greater flea load in disturbed areas particularly agricultural lands and periurban as compared to natural undisturbed forests. This justifies that, forest disturbances attributed by *M. eminii* invasion in invaded forest sites of AFNR has probably facilitated greater *C. calceatus cabirus* abundance in the invaded forest site. Therefore, the differences in abundance of mites and fleas between the invaded and uninvaded forest site suggests that

the effects of the environmental changes on parasite populations can be positive or negative (Ostfeld et al., 2005).

5. Conclusion

The study found that the number of infested rodents and ectoparasites particularly *E. echidninus* and *D. ellobius* were substantially greater in uninvaded than invaded forest sites, an implication that *M. eminii* invasion has possibly altered the presence and abundance of ectoparasites in invaded forest site. The study suggests that, the *M. eminii* invaded forest is not a hospitable environment for ectoparasites presence and abundance. Furthermore, the study found a substantially greater an abundance of flea species *C. calceatus* in invaded forest site, a specie which is enhanced by environmental disturbances, thus suggesting the negative impact of *M. eminii* invasion in the invaded forest sites. In light of these results, we therefore recommend prevention of further introduction of the *M. eminii* outside its natural range and mitigating the impact of the established *M. eminii*.

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Ethical consideration

Research ethical approval was sought from the ethical committee and decision board of the Sokoine University of Agriculture [SUA] with Ref. No. ADM/R.1/8/57, Tanzania Wildlife Research Institute (TAWIRI) referenced, RCA 2020/198 and Tanzania Commission for Science and Technology (COSTECH) with permit number 2020-434-NA-2020-198. All rodent species investigated are not on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) list, nor the Red List of International Union for Conservation of Nature (IUCN). All rodent collection procedures were specifically approved by Tanzania Wildlife Research institute (TAWIRI).

Data availability statement

Data can be obtained from the corresponding author upon a reasonable request.

CRediT authorship contribution statement

Leticia J. Musese: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. Amani S. Kitegile: Conceptualization, Methodology, Supervision, Writing – review & editing. Charles J. Kilawe: Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors have declared no conflicts of interest.

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Appendix A. Supplementary data

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