

Ectoparasite Infestation of Egyptian Fruit Bats (*Rousettus aegyptiacus*) in Kagera, Tanzania: First Records of Fleas, Mites, and Ticks

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SUMMARY

Bats are recognized as important hosts of diverse ectoparasite communities that play significant roles in parasite ecology and pathogen transmission. However, knowledge of bat-associated arthropods in Tanzania remains limited. This study investigated the ectoparasites infesting cave-dwelling Egyptian fruit bats (*Rousettus aegyptiacus*) in the Kagera Region, northwestern Tanzania. A descriptive cross-sectional survey was conducted in four caves of Kyerwa districts, Muleba districts and Bukoba rural. Among 193 bats examined six fleas were recovered from five bats, two mites from two bats, and two ticks from two bats. Morphological examination identified three groups of ectoparasites: fleas (*Thaumapsylla* sp.), ticks (*Amblyomma* sp.), and mites (*Meristaspis* sp.). Flea specimens were further characterized using mitochondrial cytochrome c oxidase subunit I (COI) gene sequencing, and phylogenetic analysis confirmed their identity as *Thaumapsylla breviceps* (*T. breviceps*). To our knowledge, this represents the first documented record of *T. breviceps*, *Amblyomma* sp., and *Meristaspis* sp. infesting *R. aegyptiacus* in the Kagera Region of Tanzania. These findings expand the current knowledge of bat-associated ectoparasite diversity in East Africa and provide a baseline for future studies on parasite ecology, host-parasite associations, and the ectoparasite diversity.

Keywords: *Rousettus aegyptiacus*, ticks, fleas, mites, Kagera, Tanzania

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INTRODUCTION

Bats (Order *Chiroptera*) are among the most successful and widely distributed mammalian groups, playing vital roles in ecosystems through insect control, pollination, and seed dispersal (Kunz et al., 2011; Ramírez-Francel et al., 2022). Their diverse roosting habits and social structures, especially in cave environments, support the formation of large, dense colonies, which create

favorable conditions for a wide range of organisms to thrive (Fenton & Simmons, 2014).

Bats host a wide range of ectoparasites, including blood-feeding (hematophagous) and tissue-feeding (histiophagous) arthropods such as fleas (*Siphonaptera*), ticks (*Ixodida*), bat flies (*Nycteribiidae* and *Streblidae*), mites (*Spinturnicidae*), and other arthropods that often exhibit a high degree of bat host specificity

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(Andrianiana et al., 2025; Corduneanu et al., 2025). These ectoparasites form complex host–parasite associations and can influence bat ecology, population dynamics, and pathogen transmission. By feeding directly on their hosts, ectoparasites may cause adverse health effects, including blood loss, skin irritation, and anemia (Szentiványi et al., 2019).

Bat ectoparasites are increasingly recognized for their role in the transmission and maintenance of bacterial and viral pathogens (Szentiványi et al., 2024). These arthropods can function as both reservoirs and vectors, facilitating pathogen circulation within bat populations and, in some cases, across species (Dick & Dittmar, 2014). The detection of diverse *Bartonella* and *Rickettsia* lineages, as well as viruses including Marburg virus, Kaeng Khoi virus, Kanyawara virus, Mahlapitsi virus, and Wolkberg virus, in bat-associated ectoparasites across different studies highlights their potential role in the maintenance and dissemination of emerging zoonotic pathogens within bat populations (Dietrich et al., 2016; Sanches et al., 2025; Xu et al., 2022). Despite these findings, significant knowledge gaps remain regarding the contribution of bat ectoparasites to

pathogen spillover and the ecological mechanisms underlying disease transmission within and between host species.

Despite their recognized ecological and epidemiological importance, the diversity and distribution of bat ectoparasites remain poorly documented in Tanzania. The Kagera Region represents a biogeographically important transition zone between the Lake Victoria Basin and the highland ecosystems of Rwanda and Uganda, yet baseline data on bat ectoparasites from this region remain scarce. This knowledge gap limits biodiversity assessments and hinders the evaluation of potential public health risks associated with bat populations.

To the best of our knowledge, this study provides the first documented record of the flea *T. breviceps*, ticks of the genus *Amblyomma*, and mites of the genus *Meristaspis* infesting cave-dwelling Egyptian fruit bats (*R. aegyptiacus*) in the Kagera Region, Tanzania. By combining morphological and molecular identification, this study expands knowledge of bat ectoparasite diversity in Tanzania and provides a baseline for future studies of parasite biodiversity, host–parasite associations, and pathogen surveillance in East Africa.

MATERIALS AND METHODS

Study area

The Kagera Region is located in northwestern Tanzania, bordering Uganda, Rwanda, and Burundi, with Lake Victoria forming its eastern boundary (Mmbaga et al., 2024). This study was conducted in three districts—Kyerwa, Muleba, and Bukoba Rural—which together represent a diverse ecological gradient ranging from cave-rich highlands and rocky outcrops to humid, plantation-dominated lowlands (Figure 1). The region has a tropical climate with seasonal rainfall that supports dense vegetation and widespread small-scale farming, particularly banana and coffee cultivation.

These environmental conditions provide suitable habitats for cave-dwelling Egyptian fruit bats, which depend on dark, stable, and protected daytime roosts. Natural features such as caves and rock crevices, together with human-made structures such as abandoned buildings and mines, provide important shelter. Because these roosting sites often occur near farms and homes, bats commonly share the landscape with local communities while foraging in nearby agricultural areas (Kinimi et al., 2025).

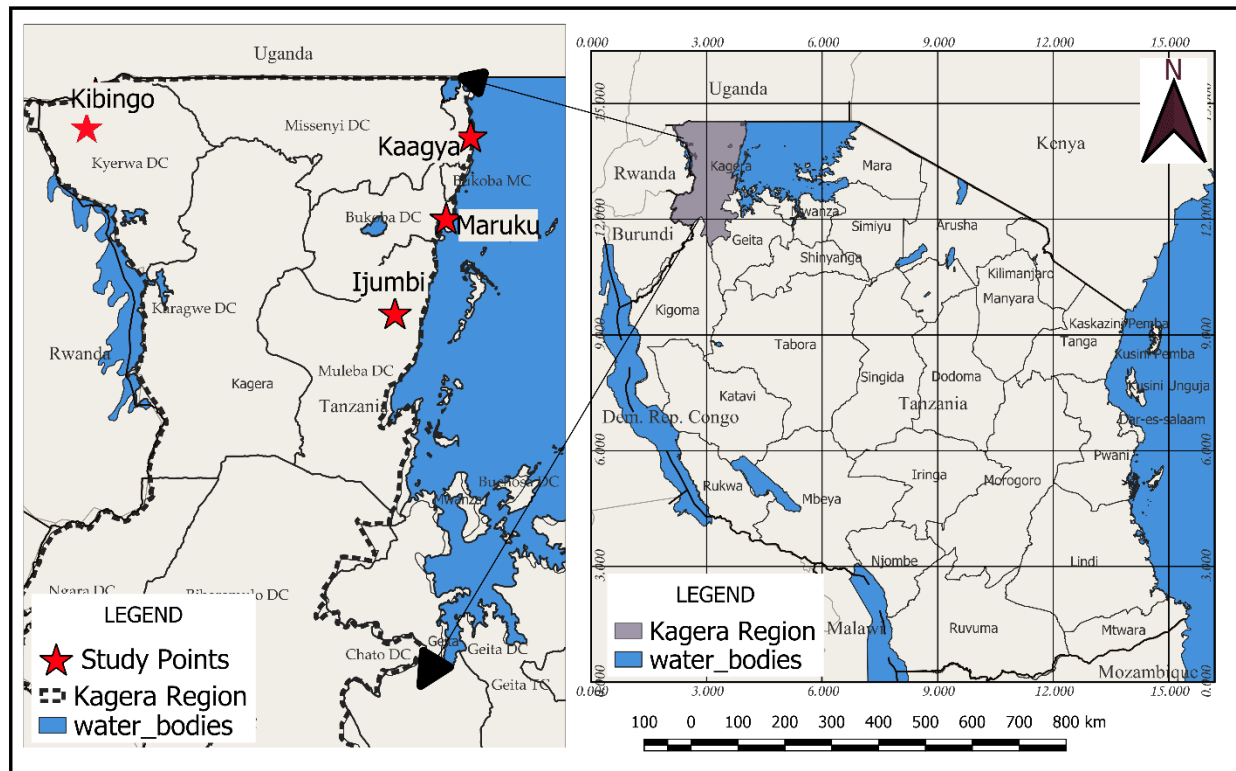


Figure 1. Map of Tanzania showing the four selected study villages, indicated by red stars.

Study design and sampling strategy

A descriptive cross-sectional study was conducted to investigate the diversity and occurrence of bat ectoparasites in the Kagera Region, Tanzania. Field sampling was conducted from 14 to 17 May 2025 in three districts. The study sites comprised four villages known to harbour cave-dwelling bat populations. Sites were selected from historical records and information provided by local community members familiar with bat roosts. Within each village, all accessible caves were systematically identified, mapped, and documented. The geographic coordinates of each cave were recorded using a Global Positioning System (GPS) receiver. The sampled caves were located in Rugasha Village (Kyerwa District; 36M 241772E, 9883446N), Butahyaibega and Katangalala villages (Bukoba Rural District; 36M 364830E, 9840122N and 36M 373390E, 9868621N, respectively), and Ibare Village (Muleba District; 36M 346874E, 9807109N).

Sample collection

Bats were captured at cave entrances using mist nets and handled by trained veterinarians and ecologists wearing appropriate personal protective equipment (PPE). Strict biosafety measures were followed to reduce the risk of pathogen transmission between bats and humans and to minimize disturbance to the animals (Islam et al., 2025). Each bat was briefly restrained for examination, and its sex, age, and lactation status were recorded. Approximately 1 mL of blood was collected from the cephalic (wing) vein using sterile venipuncture techniques in each cave of the three districts, after which ectoparasites were collected using fine-tipped forceps and preserved in 70% ethanol (Barbier & Gracioli, 2016; Moretti et al., 2021). Specimens were maintained at approximately 4 °C in a portable cooling system during transport to the Parasitology Laboratory at Sokoine University of Agriculture (SUA), where they were stored until analysis.

Morphological identification

External morphological features of the collected ectoparasites were examined under a stereoscopic microscope (Opta-Tech SK series, Wrocław, Poland) at 40x magnification. Ectoparasites preserved in 70% ethanol were carefully retrieved, gently blotted dry using absorbent tissue paper, and placed directly onto the microscope stage for observation. Digital micrographs were captured using the integrated imaging system. Ectoparasites were identified to the lowest possible taxonomic level using established morphological keys (Amarga et al., 2017; Delfinado & Baker, 1963; Nicholson et al., 2019).

Molecular identification

Sample preparation

The six bat flea specimens collected from five bats across three districts during the study were divided into three pools, each comprising two specimens collected from different individual bats within the same district. The specimens were pooled to obtain sufficient material for DNA extraction and PCR amplification, given the small size and limited number of flea specimens available. Each pool was homogenized in phosphate-buffered saline (PBS) using a sterile mortar and pestle to ensure thorough tissue disruption. Bat blood samples were processed separately; prior to DNA extraction, they were allowed to equilibrate to room temperature. All procedures were performed in a Class II biological safety cabinet (Telstar, Terrassa, Spain). Molecular analysis was performed only on flea specimens and individual bat blood samples, whereas mites and ticks were excluded due to limited number of specimens reliable for DNA extraction and downstream molecular characterization.

Parasite genome detection for genetic characterization

Genomic DNA was extracted from three pooled flea samples and bat blood sample using the PureLink™ Genomic DNA Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

Species identification was performed by amplifying the mitochondrial cytochrome c oxidase subunit I (COI) gene using polymerase chain reaction (PCR). Amplification was carried out with the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAACA-3'), which target an approximately 700 bp fragment of the COI gene (Folmer et al., 1994; Phumee et al., 2025).

PCR amplification was performed in a total reaction volume of 20 µL containing 5 µL of 4× AllTaq Master Mix, 1 µL of each primer, 11 µL of nuclease-free water, and 2 µL of genomic DNA template. The thermocycling conditions were initial denaturation at 95 °C for 2 min; 40 cycles of denaturation at 95 °C for 5 s, annealing at 52 °C for 15 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 3 min. Amplified PCR products were separated on a 1.5% (w/v) agarose gel stained with SafeView™ DNA stain. Electrophoresis was performed at 110 V for 60 min, followed by visualization under ultraviolet illumination.

DNA sequencing for genotyping

PCR products that produced clear bands of the expected size were subjected to Sanger sequencing at MacroGen Europe BV (Amsterdam, the Netherlands). The PCR amplicons were purified and sequenced using a BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3730xl Genetic Analyzer (Applied Biosystems).

DNA sequence analysis and phylogenetic inference

Raw DNA sequences were edited and assembled into consensus sequences using Geneious Prime 2026.1. Sequence similarity was assessed via Basic Local Alignment Search Tool for Nucleotide (BLASTn) searches against the NCBI GenBank database. Multiple sequence alignments were performed in Molecular Evolutionary Genetics Analysis (MEGA) version X. Phylogenetic analyses were inferred via the maximum likelihood method with 1,000 bootstrap replicates under the

Kimura 2-parameter model to estimate sequence divergence for COI-based species identification (Kimura, 1980; Kumar et al., 2018).

RESULTS

Bat collection and identification

A total of 193 cave-dwelling Egyptian fruit bats (*Rousettus aegyptiacus*) were captured during the study (Figure 2): 75 from Kyerwa, 43 from Muleba, and 75 from Bukoba Rural, from which six fleas were recovered from five bats, two mites from two bats, and two ticks from two bats.. BLAST analysis revealed 99.46% nucleotide identity, 100% query coverage, and an E-value of

0.0 when compared with reference *R. aegyptiacus* sequences available in GenBank, providing strong molecular confirmation of the host species identity. The sequence generated in this study was deposited in GenBank under accession number PZ513219. The ectoparasites recovered from *R. aegyptiacus* belonged to two arthropod classes: Insecta, represented by fleas, and Arachnida, represented by mites and ticks.

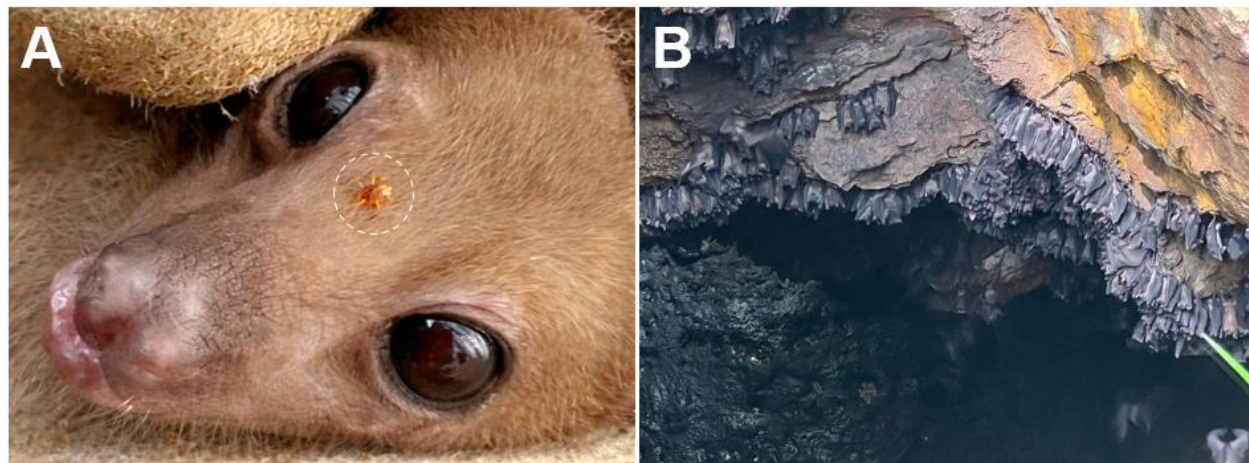


Figure 2. Egyptian fruit bat and its cave-roosting habitat. (A) An adult *R. aegyptiacus* carrying an ectoparasite during sample collection. (B) Bushungwizi Cave in Katangalala Village, Bukoba Rural District, one of the sampled roosting sites supporting a large colony of *R. aegyptiacus*.

Morphological identification of bat flea

The collected flea specimens belonged to the order Siphonaptera and family Ischnopsyllidae. Morphological identification was based on diagnostic characteristics, including a short head

and a short pronotum separated from the pronotal ctenidium (Figure 3). Based on these features, the specimens were identified as *Thaumapsylla breviceps* (Amarga et al., 2017; Amarga & Hastriter, 2023; Hastriter & Bush, 2013).

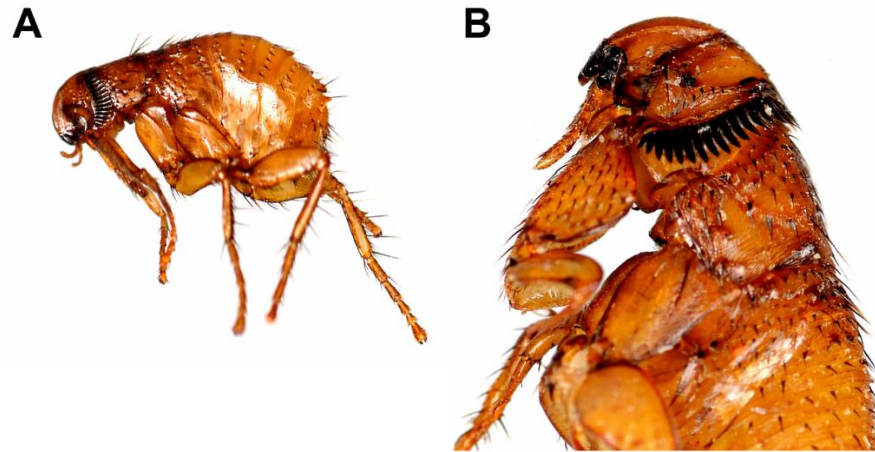


Figure 3: Morphological characteristics of the bat flea *T. breviceps* collected from *R. aegyptiacus* in the Kagera Region, Tanzania. Micrographs were captured at 40x magnification using a stereoscopic microscope; A) Whole-body morphology; B) Head and thoracic characteristics used for morphological identification.

Molecular and phylogenetic analysis of bat flea

All three pooled bat flea samples yielded successful PCR amplification of the cytochrome oxidase subunit I (COI) gene, producing amplicons of the expected size (~700 bp). The generated sequences were compared with reference sequences available in GenBank (Table 1). BLASTn analysis of representative samples (Kagera/Tan/F02/2025,

Kagera/Tan/F03/2025 and Kagera/Tan/F04/2025) revealed 100% nucleotide identity with *T. breviceps* sequences deposited in GenBank (accession number PP973737). Phylogenetic analysis showed that all sequences generated from bat fleas collected in the Kagera Region clustered with a *T. breviceps* isolate previously reported from China (Figure 4).

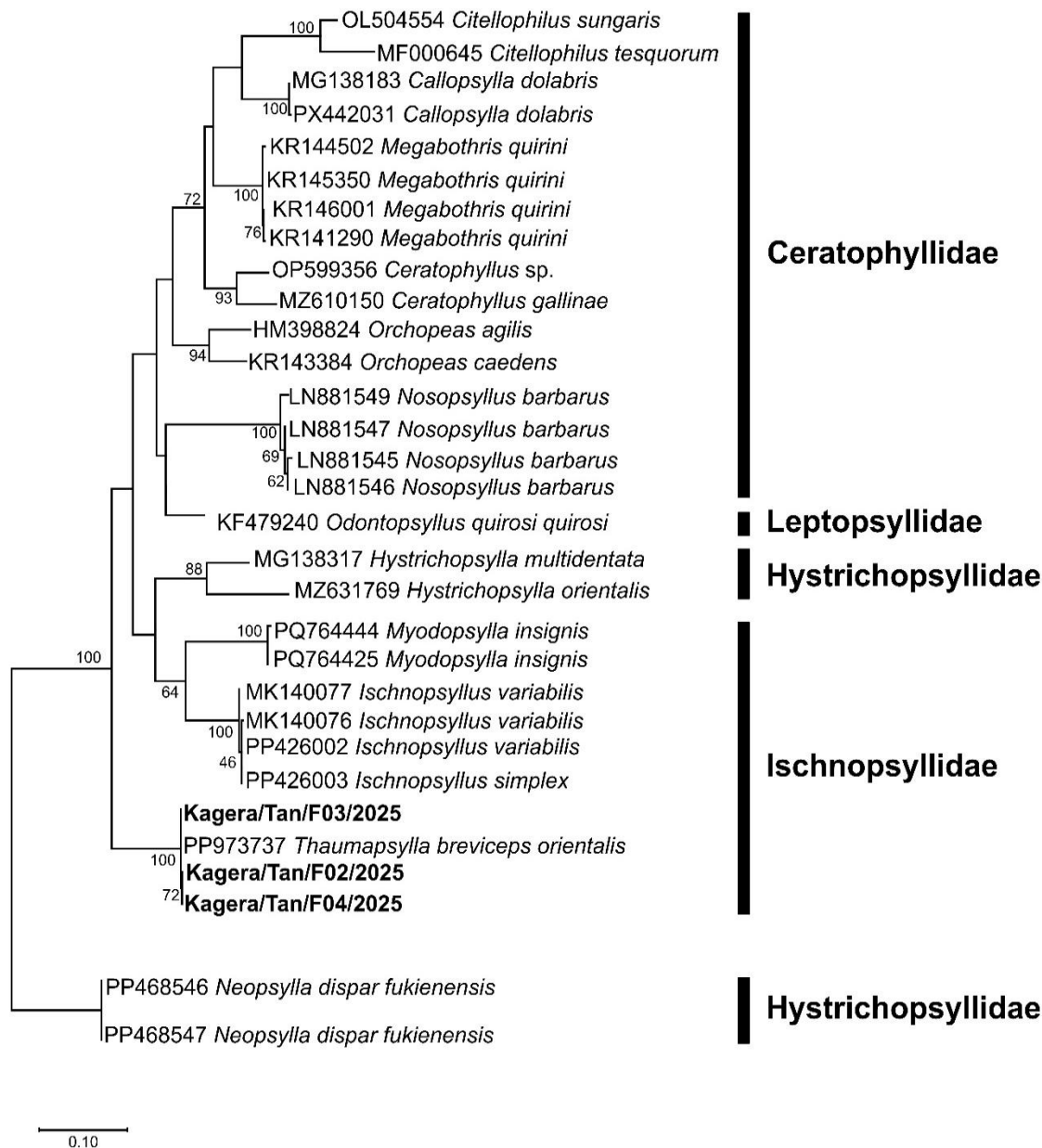


Figure 4: Maximum-likelihood phylogenetic tree of bat fleas inferred from mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. Branches are annotated with maximum likelihood bootstrap values. Sequences generated in this study are shown in **bold**.

Table 1. GenBank accession numbers, associated species, and country of origin for reference sequences utilized in this study

Species	Country	Source	Accession number
<i>Thaumapsylla breviceps</i>	Tanzania	This study	PZ620356
<i>Thaumapsylla breviceps</i>	Tanzania	This study	PZ620357
<i>Thaumapsylla breviceps</i>	Tanzania	This study	PZ620358
<i>Thaumapsylla breviceps orientalis</i>	China	Genbank	PP973737
<i>Orchopeas agilis</i>	Canada	Genbank	HM398824
<i>Orchopeas caedens</i>	Canada	Genbank	KR143384
<i>Odontopsyllus quirosi quirosi</i>	Spain	Genbank	KF479240
<i>Megabothris quirini</i>	Canada	Genbank	KR141290
<i>Megabothris quirini</i>	Canada	Genbank	KR144502
<i>Megabothris quirini</i>	Canada	Genbank	KR145350
<i>Megabothris quirini</i>	Canada	Genbank	KR146001
<i>Nosopsyllus barbarus</i>	Spain	Genbank	LN881545
<i>Nosopsyllus barbarus</i>	Spain	Genbank	LN881546
<i>Nosopsyllus barbarus</i>	Spain	Genbank	LN881547
<i>Nosopsyllus barbarus</i>	Spain	Genbank	LN881549
<i>Citellophilus tesquorum</i>	China	Genbank	MF000645
<i>Callopsylla dolabris</i>	China	Genbank	MG138183
<i>Hystrihopsylla multidentata</i>	China	Genbank	MG138317
<i>Ischnopsyllus variabilis</i>	Netherlands	Genbank	MK140076
<i>Ischnopsyllus variabilis</i>	Netherlands	Genbank	MK140077
<i>Ceratophyllus gallinae</i>	Finland	Genbank	MZ610150
<i>Hystrihopsylla orientalis</i>	Finland	Genbank	MZ631769
<i>Citellophilus sungaris</i>	Russia	Genbank	OL504554
<i>Ceratophyllus</i> sp	Canada	Genbank	OP599356
<i>Ischnopsyllus variabilis</i>	Lithuania	Genbank	PP426002
<i>Ischnopsyllus simplex</i>	Lithuania	Genbank	PP426003
<i>Neopsylla dispar fukienensis</i>	China	Genbank	PP468546
<i>Myodopsylla insignis</i>	Canada	Genbank	PQ764425
<i>Myodopsylla insignis</i>	Canada	Genbank	PQ764444
<i>Callopsylla dolabris</i>	China	Genbank	PX442031

Morphological identification of other bat ectoparasites

Mite

The collected mite specimens were identified as belonging to the family Spinturnicidae. Morphological identification was based on diagnostic characteristics, including a markedly

enlarged first pair of legs, large plate-like distal setae on tarsus I, four pairs of setae surrounding the dorsal shield, and a broadened tritosternum on the ventral surface (**Figure 5**). Based on these morphological characteristics, the specimens were identified as *Meristaspis* sp (Delfinado & Baker, 1963).

A



B



Figure 5: Morphological characteristics of *Meristaspis* sp. collected from *Rousettus aegyptiacus* in the Kagera Region, Tanzania. Micrographs were captured at 40x magnification using a stereoscopic microscope; A) Ventral view showing the sternal plate, the first two pairs of legs, and the anal groove; B) Dorsal view showing the dorsal shield.

Tick

The collected tick specimens were identified as belonging to the class Arachnida, order Ixodida, and family Ixodidae. Morphological identification was based on diagnostic characteristics, including

mouthparts with palpi and chelicerae extending beyond the base of the capitulum and palpi approximately twice as long as wide (**Figure 6**). Based on these morphological characteristics, the specimens were identified as *Amblyomma* sp (Nicholson et al., 2019)

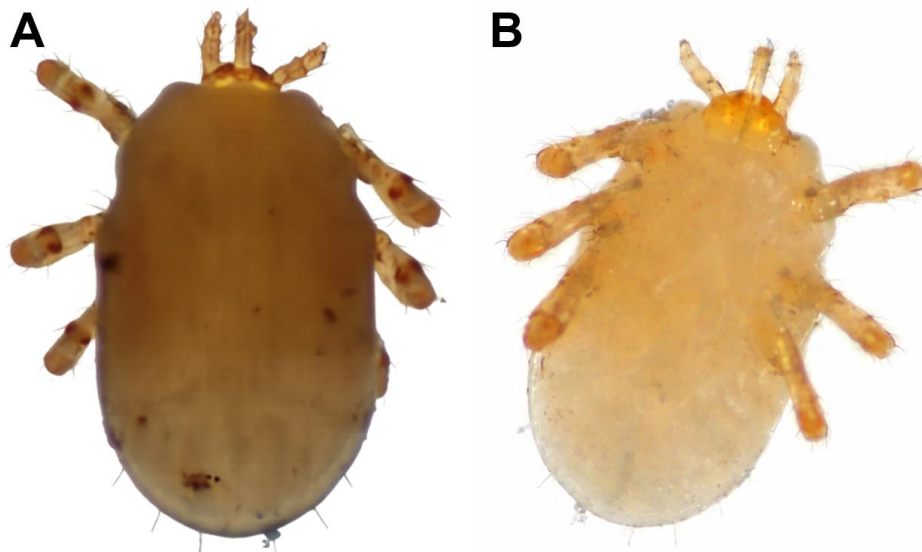


Figure 6: Micrograph for ticks observed at 40x magnification using Stereo Microscope, collected from bats in Kagera region; A) Dorsal view; B) Ventral view of the tick.

DISCUSSION

To the best of our knowledge, the detection of *T. breviceps*, *Amblyomma* sp., and *Meristaspis* sp. represents the first documented occurrence of these ectoparasites on cave-dwelling *R. aegyptiacus* in the Kagera Region of Tanzania. Fleas, ticks, and mites form part of the diverse assemblage of haematophagous arthropods associated with bats and play important ecological roles within host-parasite systems.

Both morphological and molecular methods confirmed the presence of *T. breviceps* on *R. aegyptiacus* in Kagera, consistent with the flea's well-documented association with fruit bat hosts (Pawęska et al., 2024). *T. breviceps* is widely distributed across the Afrotropical, Oriental, and Australasian regions, with records from sub-Saharan Africa, India, mainland Southeast Asia, the Philippines, and Indonesia (Amarga & Hastriter, 2023; Hastriter & Bush, 2013). Across its distribution, the species exhibits a strong association with cave-roosting *R. aegyptiacus* and other members of the family Pteropodidae, reflecting a close ecological relationship with fruit bat hosts (Pu et al., 2025). This host specialization suggests that the geographic distribution of *T.*

breviceps is largely influenced by the mobility, colonial roosting behavior, and dispersal capacity of its hosts rather than by geographic isolation alone. Consequently, its occurrence in Kagera is consistent with both its established host associations and known biogeographic patterns across tropical regions.

Recovery of larval *Amblyomma* sp. is consistent with previous reports indicating that immature hard ticks may parasitize bats opportunistically, particularly in caves or roosts shared with other vertebrates (Lourenço et al., 2023). In Africa and other tropical regions, species of *Amblyomma* are usually found on terrestrial mammals, reptiles, and other ground-dwelling vertebrates (Guglielmone et al., 2014; Lourenço et al., 2023; Nicholson et al., 2019). These ticks follow a three-host life cycle where each stage feeds on a separate animal and molts on the ground. The constant need for a new host, combined with the unselective feeding habits of larvae, increases the likelihood of accidental contact with atypical hosts (Sonenshine & Roe, 2014). Thus, the presence of these larvae on *R. aegyptiacus* likely represents an accidental infestation acquired from the cave environment or

co-occurring species, rather than a specialized relationship. This interpretation is supported by the exclusive recovery of the larval stage; the absence of nymphs or adults argues against a stable bat-tick association. However, morphological identification was limited to the genus level, restricting our ability to distinguish a bat-associated lineage from an opportunistic infestation. Molecular characterization is therefore needed to resolve the taxonomic identity of these ticks and clarify their ecological relationships.

The detection of *Meristaspis* sp. on *R. aegyptiacus* in the Kagera Region expands current knowledge of bat-associated spinturnicid mites in Tanzania. Members of the family Spinturnicidae are obligate, permanent ectoparasites that spend their entire life cycle on bats and are well adapted to the stable environmental conditions and close host contact typical of densely populated cave roosts (Bruyndonckx et al., 2009; Morales-Malacara, 2022). The occurrence of *Meristaspis* in the present study is consistent with reports from other regions. For example, *Meristaspis calcarata* has been reported from *Cynopterus* fruit bats in Indonesia and the Philippines (Amarga, 2023), while *Meristaspis lateralis* has been recorded on *R. aegyptiacus* in Egypt (Negm & Fakeer, 2014). Together, these findings support a strong ecological association between *Meristaspis* mites and pteropodid bats, an association likely facilitated by the bats' colonial behaviour and cave-roosting habits.

Ecologically, these ectoparasites play important roles in shaping host-parasite interactions, influencing bat colony health, and contributing to

the micro-ecology of cave roosts (Fagundes et al., 2017; Szentiványi et al., 2019). Their presence reflects the suitability of cave environment as stable habitats for both bats and their associated ectoparasites, highlighting ecological relationships that develop within these systems. From a public health perspective, bat ectoparasites are increasingly recognized as potential vectors or reservoirs of pathogens. Bat fleas have been confirmed to harbor *Bartonella* spp., highlighting their potential role in the maintenance and transmission of these bacteria (Sakalauskas et al., 2024). Likewise, ixodid ticks, including *Amblyomma* sp., are well-established vectors of bacterial, protozoan, and viral pathogens in terrestrial vertebrates and may serve as incidental carriers when associated with bats (Guglielmone et al., 2014; Maqbool et al., 2022). Although mites such as *Meristaspis* are not currently recognized as important vectors of zoonotic pathogens, their permanent association with bats warrants further investigation into their potential role in maintaining or mechanically transmitting microorganisms.

In this context, molecular screening of both ectoparasites and their bat hosts would be valuable for assessing pathogen carriage and improving understanding of the role of cave ecosystems in pathogen maintenance and disease ecology. Integrating parasitological surveys with molecular pathogen detection, ecological studies, and long-term surveillance will be essential for determining whether these arthropods function solely as host-associated ectoparasites or also contribute to broader eco-epidemiological networks in the region.

CONCLUSION

To the best of our knowledge, this study presents the first documented record of *T. breviceps*, *Amblyomma* sp., and *Meristaspis* sp. infesting cave-dwelling *R. aegyptiacus* in the Kagera Region of Tanzania. Morphological and molecular analyses confirmed the identity of *T. breviceps*, while morphological examination identified *Amblyomma* sp. and *Meristaspis* sp. These findings expand the current knowledge of bat ectoparasite diversity in Tanzania and provide baseline information on their

host associations and distribution. Future studies integrating molecular identification, pathogen screening, and ecological investigations will be important for improving our understanding of the diversity, ecology, and potential public health significance of bat ectoparasites in Tanzania and across East Africa.

Study limitations

This study had several limitations that should be considered when interpreting the findings. Caves were purposively selected based on their known use as roosting sites for *R. aegyptiacus*, limiting the generalizability of the findings to other bat roosts and the wider Kagera Region. Ectoparasites were collected during physical examination using fine-tipped forceps, which may have resulted in under-collection, particularly of parasites hidden in the fur or attached to less accessible body areas, or those that detached before examination.

The relatively short sampling period also limited temporal coverage, and seasonal or environmental variation in ectoparasite occurrence may therefore not have been captured. Molecular characterization

was restricted to flea specimens because of the small numbers of mites and ticks recovered; consequently, identification of these taxa relied on morphological characteristics, which may introduce some taxonomic uncertainty.

Overall, the findings should be considered baseline information on ectoparasites associated with *R. aegyptiacus* at the selected sites rather than a comprehensive assessment of ectoparasite diversity across Kagera Region. Future studies incorporating systematic collection methods, sampling across different seasons, and molecular characterization of a wider range of ectoparasite taxa would provide a more comprehensive understanding of these associations.

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Author contributions

H.E.M. and E.K. conceived and designed the research study. E.G.K., E.N., M.M. and D.F. collected the samples. H.E.M. performed the sample and data analyses and wrote the draft of the manuscript. E.G.K., E.N., S.K., and P.W. reviewed the final draft. E.G.K., and E.N. supervised the

work. E.G.K. solicited funding for this study. All the authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Institutional Review Board Statement

Research clearance for sampling Egyptian fruit bats in the Kagera region was granted by the Joint Management Research Committee (JMRC) of the Tanzania Wildlife Research Institute (TAWIRI), Reference Number: AB.235/325/01” A”/50 of 12 February 2025; and the Tanzania Commission for Science and Technology (COSTECH), Reference Number: CST00001167-2025-2025-00290, issued on 19 March 2025.

Data availability statement

The nucleotide sequences generated in this study have been deposited in the GenBank database under accession numbers PZ620356, PZ620357 and PZ620358. Additional data are available from the corresponding author upon reasonable request.

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