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In host-parasite systems, the interplay of biotic and abiotic factors can alter the abundance and distribution of both host and parasite, profoundly impacting the evolution of both and the consequences for human and wildlife health. Understanding these dynamics holds particular significance in regions like Inner East Asia, where natural foci for plague (a flea borne illness caused by the bacterium, *Yersinia pestis*) continue to persist. However, limited sampling efforts for many mammalian species in this area highlight the need for further research to uncover the factors influencing host-parasite dynamics. The overarching goal of this work is to understand the historical (e.g., glacial cycles) and modern (host, climate, land use) factors that shape the diversity of mammals and their ectoparasites across Inner East Asia. To do this, this project aims to resolve phylogeography and response to past environmental change within the long-tailed ground squirrel *Urocitellus undulatus* and to describe how this and a suite of current host and climate factors shape the composition of its associated flea (Siphonaptera) community. Firstly, I examined the phylogeny and historical demography of *U. undulatus* across its range in Mongolia and Russia through a combined analysis of three mitochondrial DNA regions. The objective was to reveal the extent to which late-Pleistocene environmental changes have affected the genetic diversity within *U. undulatus* populations through time. Secondly, I examined the abundance and species richness of fleas on *U. undulatus* across Mongolia through a multi-level community analysis, with the goal of understanding how host-trait, climate, and land use factors influence flea community composition on host individuals. This work provides new biodiversity knowledge and a baseline for understanding host and parasite dynamics in a system that is especially important diseases such as plague.

GENES AND FLEAS (SIPHONAPTERA) OF THE LONG-TAILED GROUND SQUIRREL ACROSS INNER EAST ASIA

by

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Dr. Bryan McLean
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DEDICATION

This thesis is dedicated to my parents, Nelson Reyes and Debora Martins, who inspire me to pursue excellence and have instilled in me the courage to reach my goals, no matter the challenges.

APPROVAL PAGE

This thesis written by Amanda Ashley Reyes has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER I: INTRODUCTION 1

CHAPTER II: LONG-TAILED GROUND SQUIRREL (*UROCITELLUS UNDULATUS*) PHYLOGEOGRAPHY AND HISTORICAL BIOGEOGRAPHY 1

- INTRODUCTION 3
  - Significance Of Interior East Asia As A Study System 3
  - Late-Quaternary Environmental Change In Inner East Asia 4
  - Current Research On The Evolutionary History Of *Urocitellus undulatus* Throughout Inner East Asia 5
  - Current Knowledge Gaps In *Urocitellus undulatus* Phylogeography 7
  - Study Aims 8
- METHODS 9
  - Tissue Sampling And Museum Loan Requests 9
  - DNA Extraction And Polymerase Chain Reaction (PCR) Analysis 10
  - Mitogenome Annotation 12
  - Genetic Distances Calculation 13
  - Bayesian Phylogenetic Inference 13
  - Divergence Time Estimation 14
  - Bayesian Coalescent Skyline Analysis 14
- RESULTS 15
  - Pairwise Genetic Distances 15
  - Multi-Locus Phylogeography 17
  - Time-Calibrated Phylogeny 19
  - Coalescent Population Estimate 21
- DISCUSSION 22
  - Phylogeographic Clade Structure And Support 22
  - Impact Of Quaternary Climate Fluctuations On Divergence Dates And Demography 24
  - Evidence In Support Of A ‘Lowland Pleistocene Range Shift’ Hypothesis 26

CONCLUSION 28

CHAPTER III: COMMUNITY ECOLOGY OF FLEAS PARASITIZING THE LONG-TAILED GROUND SQUIRREL (*UROCITELLUS UNDULATUS*) 29

INTRODUCTION 29
Relationships And Impacts Of Parasite Dynamics 29
Drivers Of Flea Community Composition 30
Ecology Of Flea Communities In Mongolia 32
Study Aims 33
METHODS 35
Field Sampling And Archival 35
Flea Identification 37
Host Trait, Climate, And Grazing Variables 38
Flea Community Differences 39
Flea Abundance And Richness Regressions 40
Path Analysis Models 41
RESULTS 42
Flea Community Composition 42
Determinants Of Flea Abundance And Species Richness Of Hosts 49
Examining Direct And Indirect Effects Of Model Variables 53
DISCUSSION 56
Flea Community Structure 56
Dominance Of Fleas Belonging To The Genus *Citellophilus* 57
Comparing Importance Of Host Versus Climate Traits 58
Relevance Of Plague Within This Study System 61
CONCLUSION 63

REFERENCES 65

APPENDIX: SUPPLEMENTARY INFORMATION FOR CHAPTER III 83

LIST OF TABLES

Table II.1. Pairwise genetic distance matrix of ‘raw’ distances broken down by major clades for the cytochrome oxidase I mtDNA region sequences. 16

Table II.2. Pairwise genetic distance matrix of ‘raw’ distances broken down by major clades for cytochrome-b mtDNA region sequences. 16

Table II.3. Pairwise genetic distance matrix of ‘raw’ distances broken down by major clades for the control region mtDNA region sequences. 16

Table III.1. Summary of flea species collected from long-tailed ground squirrels (*U. undulatus*) across Mongolia, with including the total number of hosts sampled (“N”)................................. 43

Table III.2. Summary of the top-ranked models predicting flea abundance and richness on long-tailed ground squirrels. Statistically significant terms are in bold indicating p-values < 0.05.. ... 51

Table A1. List of all sampling localities for each of the nine sampled Mongolian aimags.......... 83

Table A2. Summary of the full models containing all fixed effects and interactions predicting flea abundance and richness on long-tailed ground squirrels. Statistically significant terms are in bold indicating p-values < 0.05. 84

Table A3. Summary of flea abundances and totals for each sampled Mongolian aimag. 85

LIST OF FIGURES

Figure II.1. Map of Inner East Asia and *U. undulatus* sampling sites for mitochondrial and mitogenome data. 10

Figure II.2. Multilocus phylogeny of *U. undulatus* and outgroups using Bayesian inference with Beast 2.0 supported by >50% posterior probability. Subspecies and geographic groups are color-coded and annotated, with asterisks (*) denoting Uvs samples.. 18

Figure II.3. Map of Mongolia with phylogeographic clades annotated by color, supported by a collapsed phylogenetic summary. 19

Figure II.4. Multilocus phylogeny of *U. urocitellus* samples and outgroups with divergence time estimations on a time scale of millions of years with shaded node bars displaying the 95% HPD CA-height confidence intervals. Subspecies groups are indicated at right. 20

Figure II.5. Coalescent Bayesian Skyline plot for *U. u. eversmanni* in Mongolia. The dark blue line is the median estimate of the estimated effective population size on a log scale. The light blue shaded region indicates the upper and lower bounds of the 95% HPD interval.. 21

Figure III.1. Figure III.1. Map of Mongolia displaying the range of *U. undulatus* (Cassola 2016) and sampling sites used in this study where individual hosts were screened for fleas. Host subspecies is annotated by color, with yellow displaying *U. u. undulatus* and orange displaying *U. u. eversmanni*. 36

Figure III.2. Barplot of flea intensity (abundances scaled by number of host specimens examined) for each of nine Mongolian aimags 44

Figure III.3. Barplot of total flea species counts collected for each of nine Mongolian aimags. . 45

Figure III.4. Barplot of mean flea species richness (richness scaled by the number of hosts screened) for each of nine Mongolian aimags.. 46

Figure III.5. Unweighted NMDS plot with observations grouped by aimag. Colors in the map at bottom represent sampled aimags and correspond to the NMDS plot 47

Figure III.6. Unweighted NMDS plot with observations grouped by subspecies. Colors in the map at bottom represent aimags where each subspecies was sampled. 48

Figure III.7. Effect plots visualizing major host-trait effects on flea abundance.. 52

Figure III.8. Effect plots visualizing major climate-trait effects against flea abundance and flea richness.. 52

Figure III.9. Path analysis diagram for the best fit flea abundance model. Directional arrows showcase the causal relationship between variables, with line thickness being proportional to coefficient strength. Red lines indicate a negative relationship while green indicates a positive relationship, and gray arrows are links that were non-significant. 54

Figure III.10. Path analysis diagram for the best fit flea species richness model. Directional arrows showcase the causal relationship between variables, with line thickness being proportional to coefficient strength. Red lines indicate a negative relationship while green indicates a positive relationship, and gray arrows are links that were non-significant................ 55

Figure A1. Weighted NMDS plots grouped by aimag (top) and host subspecies (bottom). 86

Figure A2. QQ plot residuals and residual vs. predicted plots for the optimal GLMM flea abundance model. 87

Figure A3. QQ plot residuals and residual vs. predicted plots for the optimal GLMM flea richness model. 88

CHAPTER I: INTRODUCTION

Historically, the region of Interior East Asia has played an instrumental role in the exchange of human cultures, ideas, and materials, as well as in the emergence and transmission of disease (Kim et al., 2017; Lynsteris 2019). Understanding historical biogeographical patterns in wildlife species that harbor and transmit pathogens is especially relevant in regions of emerging infectious disease such as this, as it sheds light onto how changes in past population demographics shape the distribution and diversification of host lineages. However, there has been limited sampling and genetic data derived from regions within central and eastern Asia which has hindered our ability to quantify and understand the extent of genetic differentiation in response to past climate changes in many mammalian species in the region (Simonov et al. 2017), including species that are key disease reservoirs.

Parasites can be found on nearly all free-living organisms and can impact hosts on both an individual and community-wide level by influencing health, behavior, population sizes, and community structure (Ostfeld et al., 2000; Stanko et al., 2002; Irvine 2006). The interactions between abiotic and biotic factors affecting parasites underscores the complexity of host-parasite systems and emphasizes the necessity of ecological models which quantify these effects. Fleas (Order Siphonaptera) are an important group of ectoparasites that are highly diverse on rodent species in the Palearctic (Medvedev 1996), and also the primary vector for plague. Burrow-dwelling mammals and their associated flea communities thus serve as an ideal study system for understanding of how the ecological interactions between host and parasite are influenced by biological and environmental change factors.

This aim of my first chapter is to conduct a comprehensive phylogenetic analysis of three mitochondrial DNA regions (cytochrome b, cytochrome oxidase I, and control region) to reveal relationships within the long-tailed ground squirrel (*Urocitellus undulatus*) across its range in Mongolia and Russia. This would provide further insight into the effects of late Pleistocene environmental changes on *U. undulatus* populations, and quantify the degree of genetic divergence and population connectivity through time to present day. Overall, this chapter aims to resolve intra-clade relationships within *U. undulatus*, a morphologically conserved species, to understand how late-Quaternary glaciation, permafrost expansion, and climate changes have shaped the modern phylogeographic structure of this species.

In my second chapter, I aim to characterize the community composition of fleas on *U. undulatus* individuals across steppe and grassland habitats in Mongolia. Using multi-year mammal and parasite inventory data from a series of collaborative biodiversity surveys, I will investigate how flea species richness and abundance at the component community level vary based on host-trait factors, climate conditions, and levels of ecological disturbance, such as grazing. This would contribute to our understanding of how host utilization and modern-day environmental changes influence flea distributions on a mammalian host which serves as a major disease reservoir in the region.

CHAPTER II: LONG-TAILED GROUND SQUIRREL (*UROCITELLUS UNDULATUS*) PHYLOGEOGRAPHY AND HISTORICAL BIOGEOGRAPHY

INTRODUCTION

SIGNIFICANCE OF INTERIOR EAST ASIA AS A STUDY SYSTEM

Interior East Asia is a key nexus for understanding human cultural exchange and the historic emergence of wildlife-borne diseases (Kim et al., 2017; Lynsteris 2019). The intricate web of historic Silk Road trade routes originating in this expansive region during the medieval period facilitated the exchange of ideas, technologies, and cultural practices among transcontinental lines (Lattimore et al., 1928; Mishra et al., 2020). Importantly, this also sparked a critical point for the emergence and dissemination of diseases, most notably plague, which is caused by the bacterium, *Yersinia pestis* (Benedictow 2013; Hymes 2015). The convergence of eastern and western populations and robust trade activities in this region played a crucial role in the transmission dynamics of pathogens, thereby influencing the trajectory of pandemics and global health patterns (Slavin 2023).

Historical biogeography is relevant in an era of emerging infectious disease because it provides insight into how past distributions and demography shapes geographic distributions and the diversification of host lineages. This provides a strong evolutionary foundation to predict the possible range dynamics of host species in changing climate conditions (Guo et al., 2005; Huntley et al., 2010; Monzón et al., 2011; Nicholson 1954). Unfortunately, a lack of extensive widespread field sampling and molecular investigations in many regions of Asia inhibit our ability to quantify genetic differentiation and link it to past geographic response (Simonov et al. 2017).

LATE-QUATERNARY ENVIRONMENTAL CHANGE IN INNER EAST ASIA

Much of innermost Asia is characterized by “cold desert” climates and physiographically complex landscapes with open, grass-dominated ecosystems and extensive mountain terrain (Dixon et al., 2014; Beck et al., 2018). The Mongolian Plateau (hereafter, MP), on which Mongolia and parts of adjacent countries sit, creates especially extreme climate contexts that include some of the world’s driest deserts and regions of high continentality (Bao et al., 2014). It is also a major latitudinal transition zone that, at its northern edge, contains boreal grasslands and woodlands (Bao et al., 2014). Like North America, landscapes of Inner Asia experienced a series of mountain glaciations in the Pleistocene and Holocene that led to a change in the landscape and climatic conditions (Simonov et al., 2017), although these changes were not as extreme as in North America. These factors led to changes in some species ranges, due to the formation and loss of refugia which promoted vicariant emergence of separate phylogenetic lineages (Simonov et al., 2017). The intricate paleoclimatic history of the region is postulated to play a substantial role in fostering genetic divergence among modern populations, with opportunities for population-scale genetic differentiation to occur through genetic drift or natural selection (Chen et al., 2022).

The extent and chronology of Pleistocene, Late Glacial, and early Holocene climatic fluctuations is still being refined for Mongolia and adjacent regions (Grunert et al., 2000). Two major mountain ranges - the Altai and Khangai Mountains - experienced Pleistocene glaciations featuring two to three main stages, including the Last Glacial Maximum (LGM) around 49,000-53,000 years ago (Florensov and Korzhnev 1982; Devjatkin and Murzaev 1989; Grunert et al., 2000; Hughes et al., 2013). During the LGM, a transition occurred to a drier and colder climate, reflected in low-stands in glacier and lake levels (Grunert et al., 2000). The subsequent Late

Glacial Interstadial (LGI), approximately 16,000-15,000 years ago, marked the first warming since the LGM, resulting in slow plant re-generation and a rise in lake levels (Grunert et al., 2000; Stuart and Lister et al., 2007). As the Holocene began, the region experienced elevated water tables, increased precipitation, and a rise in overall vegetation cover (Grunert et al., 2000). Around 4,000-2,500 years ago, the Neoglacial period introduced cool and moist conditions, leading to glacial readvances which influenced small-mammal biogeography (Grunert et al., 2000). In the later Holocene, lake levels decreased, precipitation reduced, and widespread desertification further shaped the landscape (Grunert et al., 2000). Additionally, human activities such as deforestation and grazing began to shape the Mongolian steppe during this epoch, and continue to do so substantially (Lehmkuhl et al., 2001).

CURRENT RESEARCH ON THE EVOLUTIONARY HISTORY OF *UROCITELLUS UNDULATUS* THROUGHOUT INNER EAST ASIA

Quaternary landscape changes likely played a crucial role in shaping distributions of steppe mammal species, many of which are colonial and also plague reservoirs such as marmots (*Marmota* spp.), ground squirrels (*Urocitellus* and *Spermophilus* spp.), and pikas (*Ochotona* spp.). For example, these taxa may have congregated within open, grass-dominated areas in lowland glacial refugia, creating ice-free contact zones that possibly heightened the opportunities for vector exchanges among hosts and disease transmission.

The long-tailed ground squirrel (*Urocitellus undulatus*) is an ideal model for studying the effects of Quaternary climate change on steppe mammals due to its abundant distribution and locally high population densities (Simonov et al., 2017). The species range extends across parts of Siberia, Mongolia, northwestern China, and easternmost Kazakhstan and Kyrgyzstan (Pavlov and Lissovsky 2012, Batsaikhan 2014, Cassola et al., 2016, McLean et al., 2018). Two distinct sub-species, the eastern *U. u. undulatus* and western *U. u. eversmanni,* are recognized and these share a contact zone near the center of the species' range in Mongolia (Pavlov and Lissovsky 2012; McLean et al., 2018). These taxa differ markedly in mitochondrial DNA sequence comparisons (Tsvirka et al., 2008; Ermakov et al., 2015; McLean et al., 2016; Simonov et al., 2017), suggesting a more complex history of diversification than revealed from morphological data alone.

The range of *U. undulatus* was glacially dynamic, with a large portion becoming free from extensive glaciation after the last glacial maximum (LGM; 30000–15000 years ago), allowing diverse ecosystems, from mountain steppes to forest tundra, to recolonize these territories (Simonov et al., 2017) and unique animal communities to dominate newly created mountain-steppe habitats. Previous paleontological excavations revealed late Pleistocene occurrences of long-tailed ground squirrels in caves (Denisova, Strashnaya, Kaminnaya, UstKanskaya) and non-glaciated areas of Northwestern and Central Altai floodplains (Simonov et al., 2017) on the eastern portion of its current range. However, while the eastern *undulatus* subspecies occupied the glaciated eastern and southeastern regions of the Altai (Simonov et al., 2017), the western *eversmanni* sub-species occurred in mostly non-glaciated areas during the LGM.

Current research supports the existence of multiple colonization routes following post-glacial retreat, with the Altai Mountains most likely serving as an impassible barrier due to its extensive range across the junction of Russia, China, Mongolia, and Kazakhstan (Tsvirka et al., 2008; Kapustina et al., 2011; Simonov et al., 2017). Two potential pathways have been proposed for the colonization of the Southeastern Altai region: one originating from northwest Mongolia around Lakes Uureg Nuur and Achit, near the Mongolian Altai and the southeast of the Russian

Altai, as this area is believed to have been unglaciated during the LGM; while the other route stems from the Tuva Depression along the Khemchik River valley in Southern Russia (Simonov et al., 2017). Genetic analyses favor the former route as more plausible, however, these conclusions, drawn from limited phylogeographic data, may not be universally applicable across the species' range due to restricted sampling and geographic coverage (Simonov et al., 2017).

Additional research has also supported the idea that, during the late Pleistocene, gene flow corridors among populations of *U. u. eversmanni* potentially existed in the "Great Lakes Depression" and the "Valley of the Govi Lakes," situated between the Mongolia Altai and Khangai mountain ranges (McLean et al., 2018). Population mixing during this period, possibly involving individuals from these lowland regions and contiguous areas of northwest Mongolia, is supported by evidence of late Pleistocene diversification, followed by postglacial isolation (Simonov et al., 2017; McLean et al., 2018). In this study, I aim to provide a more refined phylogeny and further test of this ‘Lowland Pleistocene Range Shift’ hypothesis, as these lowland regions likely hosted mesic environments that supported species growth and expansion, notably for *U. undulatus* which is a mesic-adapted species (McLean et al., 2018).

CURRENT KNOWLEDGE GAPS IN *UROCITELLUS UNDULATUS* PHYLOGEOGRAPHY

More detailed reconstructions of past and present population structure in the long-tailed ground squirrel (*U. undulatus*) throughout its entire range are critical, as this region is being impacted by major global change drivers such as climate change and overgrazing (McLean et al., 2018). Being able to diagnose this structure is especially important for known disease reservoirs like *U. undulatus*, a known reservoir for plague, as it may help refine understanding of how host-parasite associations themselves have evolved (Dubyanskiy and Yeszhanov et al., 2016). Due to the lack of range wide sequence datasets for many mammal species in this region, and the morphological conservatism within some steppe mammal lineages, there is potential for the presence of ‘cryptic lineages’ which cannot be easily distinguished through morphological characteristics alone (Kryštufek and Vohralik 2013). Recent phylogeographic studies on the western *U. u. eversmanni* lineage reveal geographically-associated clades along three major Mongolian mountain ranges: Khangai, Mongolian Altai, and Govi Altai (McLean et al., 2018; McLean et al., 2022); however, intra-clade relationships are still largely unresolved which hinders our understanding of exactly how Late Quaternary glaciation, permafrost expansion, and climate changes have shaped the phylogeographic structure of taxa in countries like Mongolia, Russia, and China.

STUDY AIMS

The aim of this chapter is to perform a combined phylogenetic and historical demographic analysis of three mitochondrial regions (cytochrome b, cytochrome oxidase I, and control region) to reveal relationships among *U. undulatus* across its range and insight into the effects of late Pleistocene environmental changes within the species. Specific aims are:

(1) Bayesian phylogenetic reconstruction of three mitochondrial loci. **I hypothesized that there was higher population connectivity before the LGM, as glaciers and other unsuitable conditions pushed *U. undulatus* populations downslope into mesic basins, resulting in shallow divergences among modern populations.**

(2) Divergence time estimation in a Bayesian framework to infer the absolute ages of lineage divergences. **I hypothesized an early Pleistocene diversification event in the common ancestor of the two *U. undulatus* subspecies, driven by repeated glacial cycles as**

barrier events. Following from Aim 1, I also hypothesized recent (post-LGM) divergences in any subclades within *U. undulatus*.

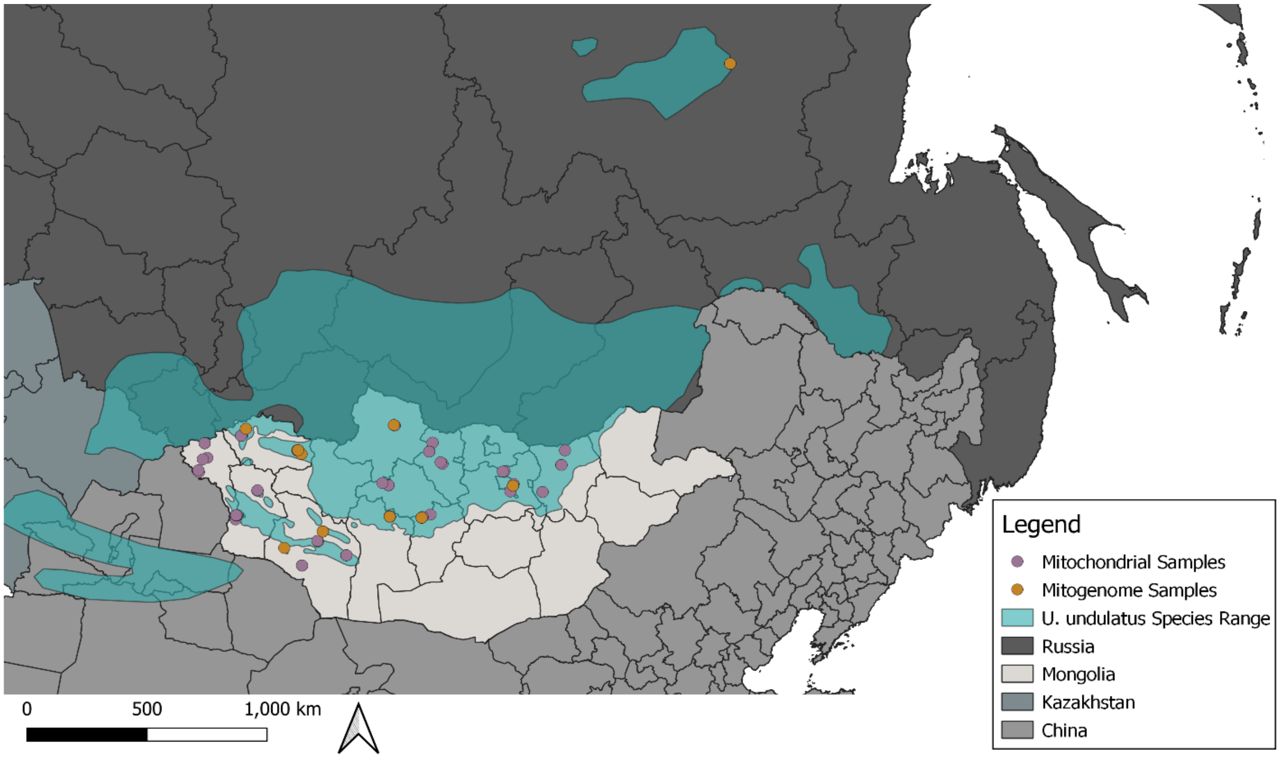
(3) Extrapolation of past population dynamics within *U. u. eversmanni* using a Bayesian Coalescent Skyline analysis. **I hypothesized that there would be a population increase event sometime during the Late Pleistocene (c. 129,000 - c. 11,700 years ago) due to high population connectivity throughout lowland refugia sites, followed by a population decrease due to increased isolation and habitat contraction as glaciers retreated with warming climates in the Holocene (c. 11,700 years ago – present day).**

METHODS

TISSUE SAMPLING AND MUSEUM LOAN REQUESTS

The *U. undulatus* specimens used in this study were collected over four joint U.S.-Mongolian expeditions from 1999-2022. From these expeditions, over 7,900 mammal specimens were collected across a wide range of Mongolian habitat types and provinces, and these mammal specimens were catalogued and housed at the Museum of Southwestern Biology (MSB) Division of Mammalogy. Preserved liver tissues were loaned for this study by the MSB Division of Genomic Resources from a total of 133 *U. undulatus* specimens. Additionally, whole mitogenome data from 13 additional *U. undulatus* specimens were processed and analyzed. In total, I sampled 146 *U. undulatus* individuals across 12 Mongolian aimags and 1 Russian oblast (**Figure II.1**).

Figure II.1. Map of Inner East Asia and *U. undulatus* sampling sites for mitochondrial and mitogenome data.



DNA EXTRACTION AND POLYMERASE CHAIN REACTION (PCR) ANALYSIS

A portion of the genetic sequences (129 COI sequences and 127 Cyt-b sequences) were obtained from McLean et al. 2018 via Genbank, while the remaining samples were processed according to the protocol described below. To obtain the genomic DNA, a salt-ethanol extraction was performed on the liver tissues. During this process, <20 mg of tissue was placed in 550 ul of tissue lysis buffer and 11 ul of 20 mg/ul proteinase K (Promega Corporation). The solution was then incubated at 55-56° Celsius for 24 hours. To precipitate proteins from the tissue solution, 350 ul of 5M sodium chloride (NaCl) was added and mixed well by inverting the tube 20-30 times. The resulting 911 ul solution was then centrifuged for 30 minutes at 14000 RPM and the supernatant transferred to each of two identically-labeled 1.5 ml microcentrifuge tubes, disposing of the pellet in the original tube containing proteins and other cellular debris. To precipitate the DNA, I added 900 ul of 100% absolute, cold ethanol to each tube and inverted each tube gently several times. The samples were then incubated overnight at -20° Celsius. To pellet the DNA, the samples were centrifuged once more for 30 minutes at 14000 RPM, the supernatant poured off, and the DNA pellet washed twice with 1000 ul of cold 70% ethanol to remove excess salts. This solution was then micro-centrifuged for 5 minutes at 6000 RPM each round. The ethanol rinse was then poured off from each sample, and the samples were placed in a 37°C incubator until dry. The DNA pellet was then suspended in 25 ul of elution buffer (Zymo Research). All samples were stored at -20°C, until used for PCR analyses.

Three mitochondrial genes were selected for and amplified using PCR. I used the primer pair CTRL-L (5' –CACYWTYAACWCCCAAAGCT – 3') and TDKD (5' – CCTGAAGTAGGAACCAGATG – 3') (Kocher et al., 1993) to amplify 530 base pairs of the mitochondrial DNA (mtDNA) control region (D-Loop). I used the primer pair MVZ05 (5' – CGAAGC TTGATATGAAAAACCATCGTTG – 3') and MVZ14 (5' – CTTGATATGAAAAACCATCG TTG – 3' (Smith and Patton 1993) to amplify all 1140 base pairs of the mitochondrial cytochrome b (Cyt-b) gene (McLean et al., 2018). As well as, the primer pair HCO2198 (5' -TAAACTTCAGGGTGACCAAAAAATCA – 3') and LCO1490 (5' - GGTC AACAAATCATAAAGATATTGG-3' (Folmer et al., 1994) to amplify 657 base pairs of the mtDNA Cytochrome c oxidase subunit 1 (COI) gene (Mclean et al., 2018). DNA amplification for all three regions occurred in 25 ul reactions, and PCR conditions for control region included 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 45 °C for 1 minute, and extension at 72 °C for 1 minute. The annealing temperatures for Cyt-b and COI were 52 °C and 48 °C, respectively (McLean et al., 2018). The final PCR product was Sanger sequenced at the Genomics Sciences Laboratory at NC State University on an Applied Biosystems 3730xl capillary sequencer and sequences were visualized, cleaned up, and aligned in Geneious (Geneious Prime 2023.2.1).

MITOGENOME ANNOTATION

To annotate the 13 raw mitogenome samples I utilized a Linux-based super computing system called the Longleaf Cluster, which is maintained through the University of North Carolina – Chapel Hill. The samples were first processed in the Phyluce software package (Faircloth 2016). To clean the raw, untrimmed data I used the illumiprocessor tool within Phyluce to trim each file of Illumina adapter contamination. A second round of trimming was performed using the program Fasp (Chen et al., 2023), to remove low quality base pairs at the start and end of each sequence. The output was two paired singleton files per sample, for a total of 26 reads. Each paired singleton was then combined using an auxiliary python script within the MITObim program. The 13 trimmed and combined fastq files were then processed in MITObim, a program for mitochondrial baiting and iterative mapping. During this process each mitochondrial genome was mapped against a *U. undulatus* reference genome (McLean et al., 2018) to reconstruct a full genome sequence for each of the 13 samples. I performed a gene annotation on each sample using MITOS 2 on the assembled genomes which extracted two of my three target mitochondrial genes, COI and Cyt-b (Bernt et al., 2013). To extract control region, I aligned the whole mitogenomes against a *U. undulatus* control region reference in Genious and clipped from 15,536 base pairs to 16,067 base pairs along the genome. These annotated data were then added to my PCR-extracted mitochondrial datasets.

GENETIC DISTANCES CALCULATION

To compute pairwise genetic distances among clades for each mitochondrial region (COI, Cyt-b, control region) I used the 'dist.dna' function within the ape package (Paradis and Schliep 2019) in R. I computed 'raw' distances which are the proportion of sites that differ between pairs of sequences. The resulting distance matrix between individual sequences for each mtDNA region were grouped by major phylogeographic clade, and the average for each pairwise clade combination was taken and visualized in a table.

BAYESIAN PHYLOGENETIC INFERENCE

Sequence data from all three mitochondrial regions in FASTA format were subjected to Bayesian phylogenetic analysis in Beast v2.0 (Drummond and Rambaut 2007). Four outgroup samples corresponding to close relatives (*U. parryii* and *U. columbianus*) were used as outgroups. Prior to analysis, I used jModeltest (Guindon and Gascuel 2003; Darriba et al., 2012). to statistically select the best-fit models of nucleotide substitution for each mtNDA region using a likelihood ratio test. The mtDNA datasets were analyzed using a concatenated approach, assuming similar evolutionary history among the three regions due to their belonging to the mitochondrial genome. Separate HKY substitution models with gamma-distributed site heterogeneity (unlinked substitution models) were used for all three mtDNA regions, with their respective proportion invariant and kappa values estimated via jModeltest. A strict clock model was used for each partition (unlinked clock model), and a linked Yule tree model. The Markov chain Monte Carlo (MCMC) was run for 100 million generations, sampling every 5,000 generations and assessing convergence by examining parameter trend plots and effective sample size (ESS) values (all >200) in TRACER 1.5 (Rambaut and Drummond 2007). The analyses were repeated at least four times with different random seeds to ensure that parameter estimates converged on similar values. The maximum clade credibility tree was generated after removing 10% of generations as burn-in with TreeAnnotator (Beast package). Resulting trees were visualized in TreeGraph2 (Stöver 2010).

DIVERGENCE TIME ESTIMATION

To incorporate temporal information into the phylogenetic relationships derived from my Bayesian tree, I performed a BEAST dating analysis assuming a molecular clock in the Cyt-b gene identical to the mutation rate of the closely related Arctic ground squirrel (*Urocitellus parryii*) at 1.52×10^{-8} per base pair per year (Eddingsaas et al., 2004). The clock rate was fixed for Cyt-b, while clock rates for COI and control region were estimated from the data. Convergence was confirmed with TRACER 1.5, with all ESS values surpassing 200 (Rambaut and Drummond 2007). The resulting tree topology, divergence dates (in millions of years), node heights, and posterior support values were summarized using TreeAnnotator and presented visually in Figtree v1.4.4 (Rambaut 2018).

BAYESIAN COALESCENT SKYLINE ANALYSIS

To assess historical population patterns within the *U. u. eversmanni* subspecies, which dominates the species range in Mongolia, a coalescent skyline analysis was conducted using the BDSKY package in BEAST, employing a non-parametric approach. Parameters were configured as described in 'Bayesian Phylogenetic Construction', with changes including setting the tree prior to 'Coalescent Bayesian Skyline', adjusting the dimensions of the bPopSizes and bGroupSizes parameters to 4, and setting the MCMC to 80 million generations. Population dynamics were reconstructed using both the *.log and *.trees files, where the former contained group size and population size information, and the latter provided coalescent event times. The

Bayesian Skyline Reconstruction analysis functionality within TRACER was then used to visualize and plot estimated effective population size over time.

RESULTS

PAIRWISE GENETIC DISTANCES

In analysis of pairwise genetic distances for each mitochondrial region of study, each value presented corresponds with the genetic distance between a pair of clades, indicating a degree of similarity between groups. Lower values indicate a closer genetic relationship between samples and, conversely, higher values signify greater genetic divergence. The ranges for COI, Cyt-b, and control region genetic distances were: 0% - 5%, 0% - 9%, and 1% - 13%, respectively (**Tables II.2, II.3, II.4**). These values indicate that higher nucleotide differences were observed among the control region samples, followed by Cyt-b, and then COI which had the lowest level of divergence.

Across all three mtDNA regions, the most notable pattern observed was the high level of dissimilarity between the *eversmanni* and *undulatus* clades. Specifically, for COI (3% - 4%), Cyt-b (6%), and control region (8% - 9%), the *undulatus* clades (Eastern and Russia) exhibited the greatest dissimilarity when compared to the *eversmanni* clades, encompassing the Govi-Altai, Khangai, Khovsgol, Uvs-East, Uvs-West, and Western clades, which corresponds with existing taxonomy. Comparisons within subspecies showed a high degree of similarity (≤ 3%), with the only pairwise combination within subspecies exceeding 3% being Uvs-West – Govi-Altai (4%) in the *eversmanni* clade.

Table II.1. Pairwise genetic distance matrix of 'raw' distances broken down by major clades for the cytochrome oxidase I mtDNA region sequences.

COI Pairwise Matrix by Phylogeographic Clade									
	Russia	**Eastern**	**Govi-Altai**	**Khangai**	**Khovsgol**	**Uvs-East**	**Uvs-West**	**Western**	**Outgroup**
Russia	0.00	-	-	-	-	-	-	-	-
Eastern	0.00	0.00	-	-	-	-	-	-	-
Govi-Altai	0.03	0.04	0.00	-	-	-	-	-	-
Khangai	0.03	0.04	0.00	0.00	-	-	-	-	-
Khovsgol	0.04	0.04	0.00	0.00	0.00	-	-	-	-
Uvs-East	0.03	0.04	0.00	0.00	0.00	0.00	-	-	-
Uvs-West	0.04	0.04	0.00	0.01	0.01	0.00	0.00	-	-
Western	0.03	0.04	0.00	0.00	0.00	0.00	0.01	0.00	-
Outgroup	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.03

Table II.2. Pairwise genetic distance matrix of 'raw' distances broken down by major clades for cytochrome-b mtDNA region sequences.

Cyt-b Pairwise Matrix by Phylogeographic Clade									
	Russia	**Eastern**	**Govi-Altai**	**Khangai**	**Khovsgol**	**Uvs-East**	**Uvs-West**	**Western**	**Outgroup**
Russia	0.00	-	-	-	-	-	-	-	-
Eastern	0.01	0.01	-	-	-	-	-	-	-
Govi-Altai	0.06	0.06	0.00	-	-	-	-	-	-
Khangai	0.06	0.06	0.01	0.00	-	-	-	-	-
Khovsgol	0.06	0.06	0.00	0.01	0.00	-	-	-	-
Uvs-East	0.06	0.06	0.01	0.01	0.01	0.00	-	-	-
Uvs-West	0.06	0.06	0.00	0.01	0.00	0.01	0.00	-	-
Western	0.06	0.06	0.01	0.01	0.01	0.01	0.01	0.00	-
Outgroup	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.05

Table II.3. Pairwise genetic distance matrix of 'raw' distances broken down by major clades for the control region mtDNA region sequences.

Ctrl Pairwise Matrix by Phylogeographic Clade									
	Russia	**Eastern**	**Govi-Altai**	**Khangai**	**Khovsgol**	**Uvs-East**	**Uvs-West**	**Western**	**Outgroup**
Russia	0.01	-	-	-	-	-	-	-	-
Eastern	0.02	0.01	-	-	-	-	-	-	-
Govi-Altai	0.09	0.09	0.01	-	-	-	-	-	-
Khangai	0.08	0.09	0.03	0.01	-	-	-	-	-
Khovsgol	0.09	0.09	0.03	0.03	0.02	-	-	-	-
Uvs-East	0.08	0.09	0.03	0.03	0.02	0.01	-	-	-
Uvs-West	0.08	0.08	0.04	0.03	0.03	0.03	0.01	-	-
Western	0.08	0.09	0.03	0.03	0.03	0.02	0.03	0.01	-
Outgroup	0.13	0.13	0.12	0.11	0.11	0.11	0.11	0.11	0.07

MULTI-LOCUS PHYLOGEOGRAPHY

The multi-locus phylogenetic inference in Beast 2.0 recovered two major clades corresponding to the two subspecies of *U. undulatus* (*U. u. undulatus* and *U. u. eversmanni*) (**Figure II.2**). Samples within the *undulatus* clade fell within eastern Mongolia, occurring throughout Hentei and Tov Aimags; as well as, Sakha (Northeastern) Russia. Samples from the *eversmanni* clade occur across a wide range within central and western Mongolia, encompassing the aimags of Bayan-Olgii, Uvs, Govi-Altai, Khovsgol, Arkhangai, Bayankhongor, Ovorkhangai, and Bulgan (**Figure II.3**). Within the *U. u. eversmanni* clade, six major sub-clades were supported encompassing the following regions: Chandmani and Taishir sums (towns) of Govi-Altai (Govi-Altai clade); Khovd, Bayan-Olgii, and the Altai and Khaliun sums of Govi-Altai (Western clade); the Turgen sum of Uvs (Western Uvs clade); the Ondorkhangai sum of Uvs (Eastern Uvs clade); Bayankhongor, Arkhangai, Bulgan, and Ovorkhangai (Khangai clade); and the Chandmani-Ondor sum of Khovsgol (Khovsgol clade). Additionally, two minor sub-clades comprising Uvs samples were recovered within the Khovsgol and Govi-Altai clades; both clusters containing samples from Ondorkhangai sum in Uvs.

Figure II.2. Multilocus phylogeny of *U. undulatus* and outgroups using Bayesian inference with Beast 2.0 supported by >50% posterior probability. Subspecies and geographic groups are color-coded and annotated, with asterisks (*) denoting Uvs samples.

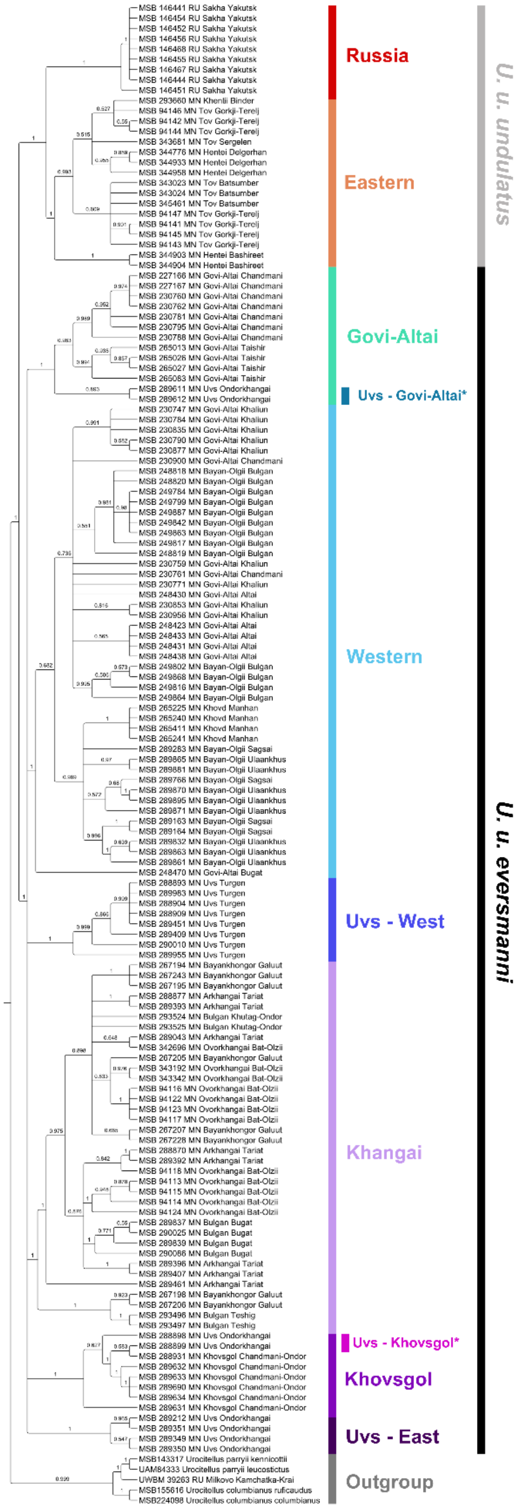
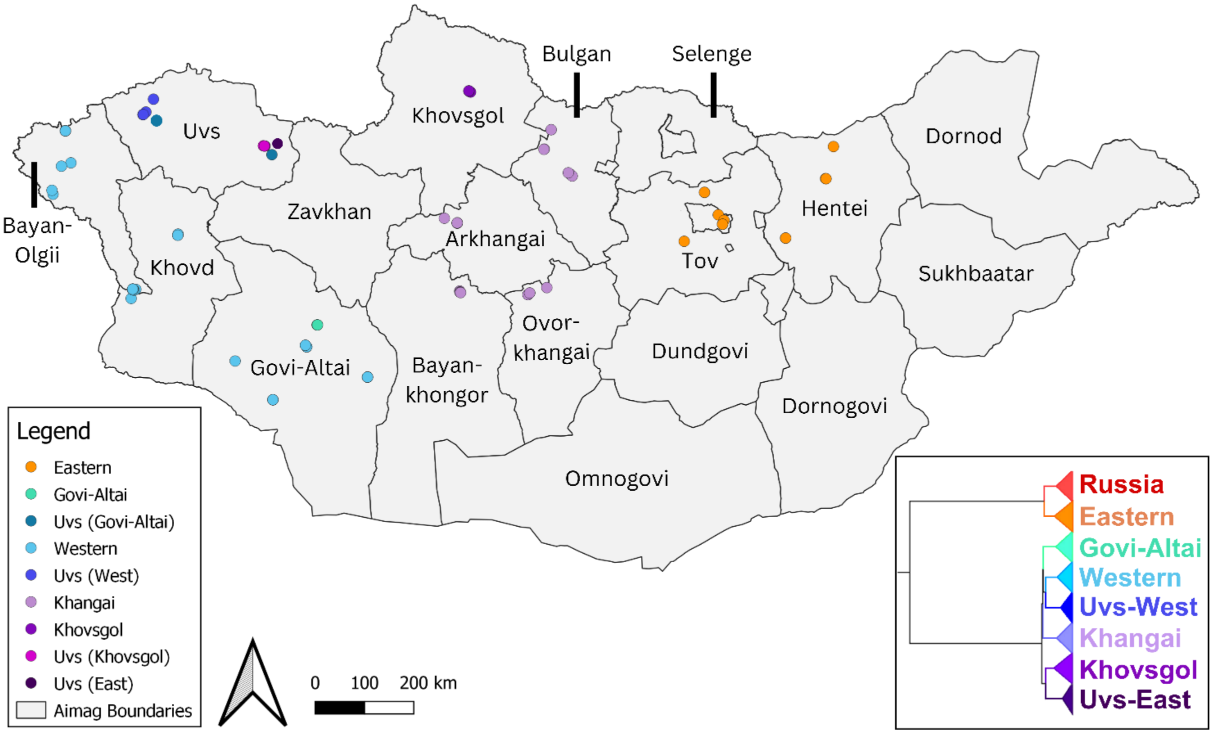


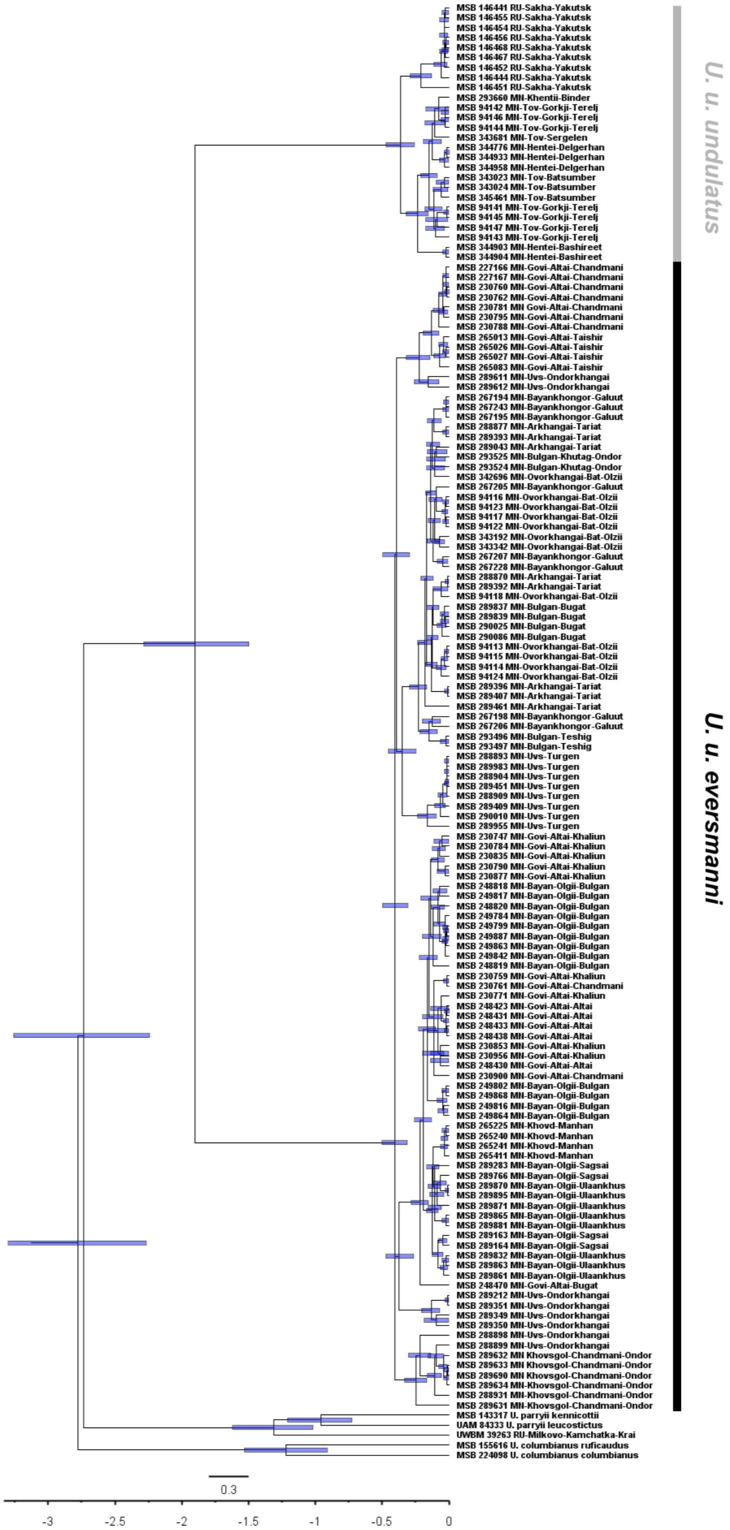
Figure II.3. Map of Mongolia with phylogeographic clades annotated by color, supported by a collapsed phylogenetic summary.



TIME-CALIBRATED PHYLOGENY

Using a 1.52% Ma−1 substitution rate for Cyt-b (Eddingsaas et al., 2004), and given the lack of a calibrated molecular clock for COI or control region, the basal split between *U. parryii*/*U. columbianus*/*U. undulatus* occurred approximately 2.75 mya with a deviation of 2.25 to 3.25 million years (**Figure II.4**). The most recent common ancestor of *U. u. undulatus* and *U. u. eversmanni* is estimated in the early to mid-Pleistocene at 1.8 mya, with a range deviation of 1.5 to 2.25 million years ago. Shallow genetic diversification within each subspecies of *U. undulatus* is suggested to have occurred roughly 0.4 mya, with a deviation of 0.25 to 0.5 million years ago.

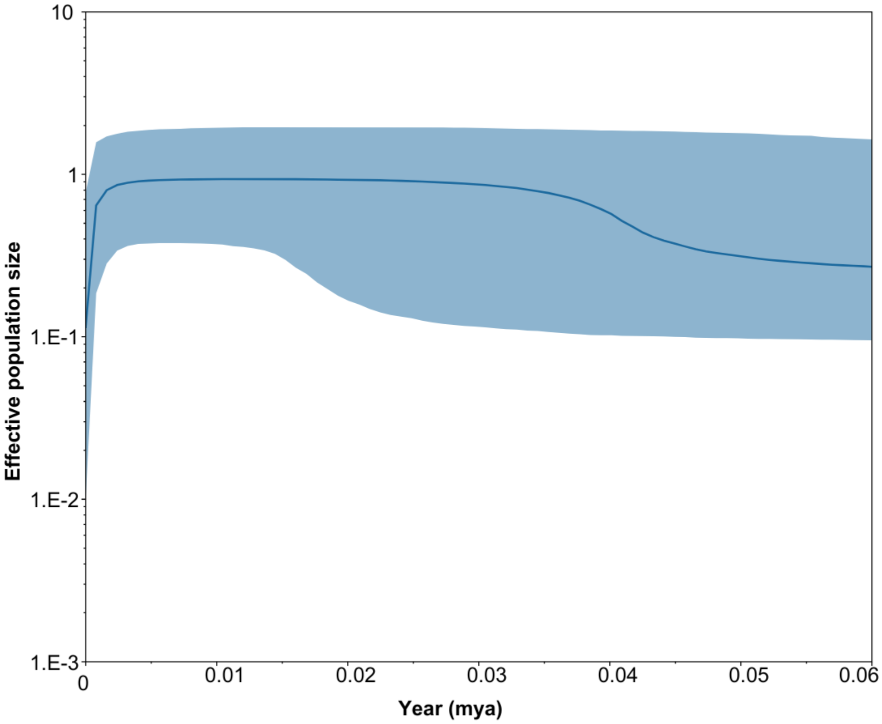
Figure II.4. Multilocus phylogeny of *U. urocitellus* samples and outgroups with divergence time estimations on a time scale of millions of years with shaded node bars displaying the 95% HPD CA-height confidence intervals. Subspecies groups are indicated at right.



COALESCENT POPULATION ESTIMATE

All ESS for skyline analysis parameters exceeded 200, indicating satisfactory tree convergence for the *U. u. eversmanni* samples. The analysis assumes no significant population structure and random sampling, which is generally consistent with our biodiversity survey and sampling approach. The Bayesian Skyline analysis plot shown here spans 60,000 years and reveals a relatively consistent population pattern throughout the Late Quaternary and Holocene periods for *U. u. eversmanni* ground squirrels throughout Mongolia (**Figure II.5**). The population density in the plot depicts two uniform periods where the population was estimated to be largely static from 5,000-35,000 years ago and 45-60,000 years ago. Notably, two population events stand out: a slight increase around 35-40,000 years ago followed by a decrease after 5,000 years ago, close to present day.

Figure II.5. Coalescent Bayesian Skyline plot for *U. u. eversmanni* in Mongolia. The dark blue line is the median estimate of the estimated effective population size on a log scale. The light blue shaded region indicates the upper and lower bounds of the 95% HPD interval.



DISCUSSION

PHYLOGEOGRAPHIC CLADE STRUCTURE AND SUPPORT

This study aimed to resolve unsettled phylogeographic relationships identified in the *U. undulatus* phylogeny by incorporating genetic data from the control region mtDNA region, joined with expanded sampling across Central and Eastern Mongolia. The mtDNA control region, newly used alongside Cyt-b and COI, exhibits expected high polymorphism, leading to elevated observed nucleotide diversity among individuals. This pattern was supported in long-tailed ground squirrels, as demonstrated by the pairwise distance analysis, where control region displayed the highest dissimilarity values when compared to Cyt-b and COI.

The Bayesian inferred phylogenetic analyses, alongside pairwise genetic distances, added further support to the current hypothesis which proposes that there are two main genetic lineages within *U. undulatus*: an eastern subspecies (*U. u. undulatus*) and a western subspecies (*U. u. eversmanni*) (Kryštufek and Vohralík 2013; Pavlinov and Lissovsky 2012; McLean et al., 2018). Our analysis identified eight major clades with posterior probability support levels >50: two within *U. u. undulatus* and six *within U. u. eversmanni*. Additionally, two minor sub-clades containing Uvs-based samples were also observed. The *U. undulatus* clades identified were the Eastern (Hentei and Tov aimags) and Russian clades (Sakha Yakutsk) which is consistent with the McLean et al. 2018 phylogeny. The *U. u. eversmanni* clades included the Govi-Altai (Chandmani and Taishir sums), Western (Govi Altai: Khaliun, Altai, Bugat; Bayan-Olgii and Khvod sums), Uvs-West (Turgen sum), Uvs-East (Ondorkhangai sum), Khangai (Bayankhongor, Arkhangai, Bulgan, Ovorkhangai sums), and Khovsgol (Chandmani-Ondor sum) clades. In addition, the Khovsgol and Govi-Altai clades both included samples from Uvs-Ondorkhangai. These genetic clades provide further support that genetic differentiation within *U. u. eversmanni* aligns with the three major Mongolian mountain systems (Govi-Altai, Khangai, and Khentii), and provide further evidence that historical (late-Quaternary) population mixing across lowland areas of western Mongolia occurred (McLean et al., 2018). This analysis achieved higher support for some inter-clade relationships in the *U. u. eversmanni* subspecies, but the placement of several subclades remained unresolved.

The mitochondrial lineages within *U. u. eversmanni*, which currently inhabit isolated mid- and high-elevation steppe habitats, have divergences which may be due to population mixing which occurred during the late Quaternary, as populations sought refugia in the lowland regions of Mongolia during periods of glacial readvancement (McLean et al., 2018). One potential example of this is the presence of Uvs samples from the Uvs-East clade within the Khovsgol and Govi-Altai clades, which suggests that Uvs may have served as a refugium during glacial re-expansion, which enabled genetic mixing between neighboring populations to occur. A novel observation enabled by my denser sampling is the division of the Uvs clade into a western and eastern partition, indicating that some isolation might be present even at this finer (i.e., within-aimag) scale currently. This division may be attributed to the Khan Khokii mountains, situated between the Turgen and Ondorkhangai sums, which emphasizes the influence of Mongolia's landscape on genetic differentiation, in conjunction with late-Quaternary climate changes. Similarly, the division of samples from Hentei-Batshireet and Hentei-Delgerkhaan into distinct sub-clades within the Eastern clade also suggests a possible reproductive isolation mechanism, likely influenced by a geographic barrier. Batshireet, situated in the northern Khentii mountains, and Delgerkhaan, positioned at a lower elevation in southeast Hentei, illustrate this geographical distinction.

IMPACT OF QUATERNARY CLIMATE FLUCTUATIONS ON DIVERGENCE DATES AND DEMOGRAPHY

The dating analysis revealed that *U. paryii/U. columbianus/U. undulatus* diverged around 2.75 million years ago (interval of 2.25 to 3.25 mya), aligning with previous research suggesting a divergence of 4.4 mya, which was associated with credible intervals from 2.9 to 6.3 mya (Harrison et al., 2003). The subspecies *U. u. undulatus* and *U. u. eversmanni* diverged around 1.8 million years ago, coinciding with the late onset of the Pleistocene epoch, spanning approximately 2.58 million to 11,700 years ago. The Pleistocene featured reoccurring glacial periods, characterized by significant ice sheet expansion through-out Mongolia’s montane regions (Lehmkuhl et al., 2011). The most recent of these climatic shifts likely influenced shallow genetic divergences within each subspecies, which are estimated to be centered around 400,000 years ago. Notably, during this mid-Pleistocene transition, glacial cycles transitioned from 41,000-year to asymmetric 100,000-year cycles, which intensified climatic variability (Watanabe et al., 2023). Within Mongolia, large valley and cirque glaciers were common across the Central and Eastern parts of the country, and these glaciers formed an extensive interconnected system within the Mongolian Altai and Khangai montane regions (Lehmkuhl et al., 2011; Rother et al., 2014). These montane glaciations may have induced the migration of long-tailed ground squirrels downslope, facilitating genetic exchange across Mongolian lowlands, a process that has likely been intermittent since the LGM considering additional periods of cooling throughout the Holocene, such as the Neoglacial period, which caused additional glacial readvancement within Mongolia. As long-tailed ground squirrel populations were driven downwards, they likely faced considerable environmental pressures, which impacted small-mammal biogeography as a whole in the region (Grunert et al., 2000; Hughes et al., 2013; Florensov and Korzhnev 1982; Devjatkin and Murzaev 1989).

My analysis of past population dynamics within the long-tailed ground squirrel revealed two major population events. Firstly, there was an estimated slight population increase around 35 to 40,000 years ago, during the Late Pleistocene, which was still characterized by alternating advance and retreat of glacial conditions (Lehmkuhl et al., 2011). Around 40,000 years ago this region was still in a glacial phase, however, population inter-mixing at lowland refugia sites possibly fueled this population increase (Lehmkuhl et al., 2011). Subsequently, a phase of relative population stability ensued from 35,000 to 5,000 years ago, demonstrating the species' resilience to the most extreme climatic fluctuations of the late glacial period. Secondly, around 5,000 years ago, coinciding with the onset of the Holocene, a population decline is estimated to have occurred. Despite minor climate shifts like the Neoglacial period, the Holocene generally experienced warmer conditions, with elevated water tables, and increased precipitation and vegetation cover (Grunert et al., 2000). As lake levels dropped in the late Holocene, temperatures decreased, precipitation reduced from 5,000 years ago, vegetation cover increased, and environmental change was further influenced by rising human activities across Central Asia (Grunert et al., 2000). As human impacts intensified during this period, forest clearing, along with steppe overgrazing notably altered the Mongolian landscape (Lehmkuhl et al., 2001). An explanation for this recent population decline, can be attributed to competition with livestock grazing, as well as increased human development. Especially, given studies which have indicated that grazing has degraded Mongolia's vegetation over the past few decades resulting in reduced vegetative species diversity in steppe habitats, with notable negative impacts on high mountain steppe habitats (Tuvshintogtokh and Ariungerel 2013; Munkhzul et al., 2021). This pattern has also been observed in various ground squirrel species which have had notable population declines, and in some cases, total colony extinctions due to increasing human pressures and climate change (Hofmann et al., 2003; Tukhbatullin et al., 2023). Additional studies and denser genomic sampling will be necessary to more confidently ascribe demographic declines to one or both of these factors.

EVIDENCE IN SUPPORT OF A 'LOWLAND PLEISTOCENE RANGE SHIFT' HYPOTHESIS

The 'Lowland Pleistocene Range Shift' hypothesis suggests that a series of repeated migrations of long-tailed ground squirrels into the Great Lakes Depression region of western Mongolia occurred during Pleistocene glaciation cycles, driven by environmental shifts. This was then followed by upslope movements as these ground squirrels dispersed from the refugia to more suitable montane habitats as a result of Holocene aridification (McLean et al., 2018). This theory is supported by the increased mtDNA diversity in *U. u. eversmanni* from the southern Altai Mountains (Simonov et al., 2017), indicating potential isolation in lowland glacial refugia. My study also further supports the hypothesis that one these refugia may have been located in 'Great Lakes' region of northwestern Mongolia (McLean et al., 2018). The Uvs Aimag, which encompasses the northern 'Great Lakes Depression' region, revealed genetic intermingling through our phylogenetic analyses, with Uvs samples found in Khovsgol and the Govi-Altai. Specifically, two Uvs-Ondorkhangai samples grouped with the main Khovsgol clade (0.62 posterior probability support), while two others grouped with a sub-clade sister to the Govi-Altai clade (0.80 posterior probability support).

Today, the lowland regions within the 'Great Lakes' area lie between Mongolia's two major mountain ranges, Govi-Altai and Khangai, consisting of mesic-steppe habitat and serving as a corridor for arid mammalian species (Batsaikhan et al., 2014). However, during the Pleistocene, this area likely comprised a mix of more mesic steppe and forest-steppe habitats (Grunert et al., 2000; Böhner and Lehmkuhl 2005). As Pleistocene glaciers advanced across Mongolia's mountain ranges, mesic fauna and vegetative species likely migrated downslope into the Great Lakes depression, as this area may have had suitable refugia for species like *U. undulatus,* shielding them from the expanding ice sheets (McLean et al., 2018). Additionally, lowered lake levels during Pleistocene glaciation events likely increased land exposure of the Uvs Nuur and Bayan Nuur lakes in northwestern Mongolia, potentially expanding the area available for colonization by downslope migrating species (Grunert et al., 2000). As the latter Holocene brought warmer temperatures and aridification, these montane habitats likely became more hospitable, potentially promoting range expansion for *U. undulatus* populations and leading to the phylogenetic divergences observed in *U. u. eversmanni* today. From the results of this study, there's evidence that some of these ground squirrels may have extended their range southward and eastward into the Govi-Altai and Khovsgol regions.

Moreover, similar east-west phyletic subspecies divergence occurred in the Mongolian Pallid ground squirrel (*Spermophilus pallidicauda*) and Mongolian marmot (*Marmota sibirica*) (Kapustina et al., 2015; Kapustina et al., 2023), supporting the idea that ecological barriers formed through past climatic changes separated populations during the late-Quaternary. Further investigation into the impact of landscape-scale features on long-tailed ground squirrels and similar species requires intensified field sampling efforts in proposed refugium sites like Uvs. This would aid to discern movement patterns and isolation events contributing to the genetic differentiation seen today, along with isolating new genetic markers to better differentiate nucleotide variations among subspecies of *U. undulatus*.

CONCLUSION

The lack of dense genetic sequencing data for many Inner East Asian species raises the potential for cryptic species and lineages to exist within the landscape, especially since many steppe mammals are often difficult to distinguish through morphological traits alone. This underscores the necessity for more comprehensive genetic analyses, which is particularly critical for disease reservoirs such as *U. undulatus,* a known plague reservoir. To further understanding of diversification within *U. undulatus*, a multilocus phylogenetic analysis was conducted. This provided further support of an east-west lineage split corresponding to two recognized subspecies (*U. u. undulatus* and *U. u. eversmanni*) and existence of eight major clades within *U. undulatus,* with more pronounced phylogenetic diversification seen in the *U. u. eversmanni* clade. Divergence dating revealed that an estimated split between the two subspecies occurred ~ 1.8 million years ago, with more recent bursts of diversification between clades occurring ~ 0.4 million years ago in the mid- to late Pleistocene. A reconstruction of historical demography via a skyline analysis showed relative population stability throughout the late-Quaternary, with an estimated decline in the recent Holocene, possibly due to climate or anthropogenic activities like livestock grazing and human development. These results support a Pleistocene downslope migration of *U. undulatus* to the 'Great Lakes Depression' area, particularly within the Uvs Aimag, which promoted genetic intermixing and population stability but was followed by subsequent upslope migration during Holocene aridification and origination of the more isolated phylogeographic clades present today. This work underscores the complexity of tracking species' historical movements across vast, complex landscapes and emphasizes the need for comprehensive datasets to parse the true history of steppe species.

CHAPTER III: COMMUNITY ECOLOGY OF FLEAS PARASITIZING THE LONG-TAILED GROUND SQUIRREL (*UROCITELLUS UNDULATUS*)

INTRODUCTION

RELATIONSHIPS AND IMPACTS OF PARASITE DYNAMICS

Parasitic species (helminths, arthropods, protozoa, bacteria, and viruses) likely comprise a large portion of the total percentage of Earth’s species currently unknown to science (Pedersen et al., 2007). Many of these groups have deep evolutionary histories of host association and are often regarded as integral ecosystem components, with potential involvement in up to 75% of food web linkages (Lafferty et al., 2008). Parasites are so ecologically pervasive that they can be found in almost every free-living species, with a range of influences on host health, behavior and population size, food web dynamics, and community structure (Stanko et al., 2002; Irvine 2006; Ostfeld et al., 2000), including spillover events where parasites colonize novel host species. However, many parasites have free-living phases and/or complex life cycles, making it difficult to predict the sensitivity of both prevalence and spillover potential to environment, host factors, or both (Rynkiewicz et al., 2015).

Many studies have attempted to identify the primary factors influencing the richness and abundance of parasite species on hosts, beyond just understanding a parasite's resource niche, primarily, the host it inhabits. For example, distinct species within a parasite community may exhibit unique responses to environmental gradients despite their shared reliance on the host as a critical element of their ecological niche (Maestri et al., 2017). In addition, parasites can be impacted by the intrinsic host factors of age, sex, body size and condition, host population density, as well as host migration and behavioral patterns (Shaw et al., 2018; Dáttilo et al., 2020; López-Pérez et al., 2022). This interplay of biotic and abiotic factors potentially influencing parasites speaks to the complex balance of many host-parasite systems and the need for ecological models which take all of these factors into account.

DRIVERS OF FLEA COMMUNITY COMPOSITION

Fleas (Order Siphonaptera) are obligate blood-feeding parasites of mammals and birds. Out of the approximately 2,600 known species of fleas, approximately 90% parasitize mammals (Bourne et al., 2018). Fleas are found on every continent but are especially diverse on rodents (Order Rodentia) in the Palearctic and Nearctic biogeographic realms (Medvedev 1996). The ecological interactions of fleas and mammals are ideal for studying the assembly of parasite communities, and the potential consequences of environmental change on these assemblages (Krasnov 2008). Critically, both host traits and environmental characteristics appear important in structuring flea communities (Krasnov 2008).

First, flea abundance and species richness are thought to be intricately linked to broad- and fine-scale environmental factors important for life history parameters of flea populations; this may result in changes in their interactions with host populations (Krasnov et al., 2019) and possibly influence epidemiological risks in wildlife, as well as livestock and pets (Hamidi and Beuno-Marí 2021). Climatic conditions such as temperature, precipitation, and humidity have been shown to play pivotal roles in flea abundance, with warmer, moister environments generally supporting accelerated flea development, and higher humidity levels contributing to their survival (Krasnov et al., 2001). Air temperature impacts both the larval (pre-imaginal) and adult stages of fleas which are susceptible to changes in the ambient air environment, with fleas exhibiting optimal development and reproduction rates within a temperature range of 20-30°C (Silverman et al., 1981; Krasnov et al., 2001). Moreover, humidity positively impacts flea survival, with higher humidity promoting egg hatchability and larval development (Cooke et al., 1988). Precipitation jointly plays a significant role, as it influences the availability of suitable breeding sites, although excess moisture may inhibit flea survival (Silverman et al., 1983).

Despite the role of climate on flea abundance and richness, effects are not consistent across flea species. Research suggests that flea abundance and richness sometimes decrease with higher winter precipitation (Poje et al., 2020); increase with cooler, wetter winters (Smith et al., 2021); and increase with areas of higher all-year and summer rainfall (Van Der Mescht and Matthee 2017), all depending on flea species and the geographic region of study. Additionally, finer-scale landscape features, such as vegetation density and land use patterns can shape the composition of flea assemblages in a given area, with evidence suggesting that flea abundance and richness can differ within a single host species inhabiting different habitat types (Brinkerhoff 2008). Microhabitats, such as nesting sites and burrows, further influence flea species richness by creating localized conditions suitable for specific flea species (Van der Mescht et al., 2016). Additionally, anthropogenic disturbances can alter host infestation rates, with increases in flea infestations noted in disturbed areas (Friggens and Beier 2010).

Second, beyond environmental factors, host traits play a crucial role in influencing flea community composition at the level of individual hosts, individual species, and ecosystems. At the finest scales, the traits of individual mammals influence the abundance and prevalence of fleas, including sex (Kiffner et al., 2014), age (Hawlena et al., 2007, Sackett 2018), size (Sackett 2018), body condition (Krasnov et al., 2005), reproductive status (Dlugosz et al., 2014), and genetic diversity (Krasnov et al., 2004). Sex-specific parasite susceptibility is particularly common in many systems, possibly because host behaviors contribute to the quantity of fleas on male and female hosts (Kiffner et al., 2014). Many studies report male-biased parasite loads, but this is not universal because the mechanisms of parasite acquisition by an individual host are species-specific and have evolved independently in different host-parasite systems (Zahn et al., 2004; Christe et al., 2007; Kiffner et al., 2014). Additionally, host mobility can have potential impacts on flea richness, as sedentary hosts may harbor different parasite species than more mobile counterparts (Boyer et al., 2010). The availability and diversity of host species in an ecosystem significantly impact the diversity of flea species present, with more diverse mammalian communities and a broader range of potential hosts fostering a more diverse array of flea species present in a population (Hammond et al., 2019). At broader scales, host attributes such as the phylogenetic diversity of host communities may be a driving factor for host choice in co-habiting fleas across ecosystems, with varying patterns of similarity based on continent (Krasnov et al., 2022).

The lack of generality in explaining patterns of parasite community heterogeneity demands new tests of the relative influence of host traits and environmental characteristics. Environmental factors may more strongly shape flea assemblages when compared against host species composition (Krasnov et al., 2015), for example, but general effects of host and climate factors differ between parasite taxa due to differing ecological mechanisms (Krasnov et al., 2010; Krasnov et al., 2015; Maestri et al., 2017, Surkova et al., 2018). To accomplish this, fine-scale investigations that can integrate traits of host individuals and detailed environmental data at the site of observation are critical, and would foster more targeted control strategies and comprehensive management of flea-related health risks in known disease foci.

ECOLOGY OF FLEA COMMUNITIES IN MONGOLIA

This study focuses on parsing the joint impacts of environmental variables, host traits, and land use characteristics on flea species richness and abundance in Mongolia, a country that remains a natural focus of plague (caused by *Y. pestis,* a gram-negative coccobacillus bacterium).

Plague is a disease with significant influence on humans and animals worldwide, killing nearly 25 million people in Europe in the 1300s (Thrupp 1966; Wall and Shearer 1997) and persisting in wildlife reservoirs due to *Y. pestis* maintaining a benign relationship with its rodent reservoirs between outbreak periods (Wimsatt and Biggins 2009). Natural plague foci now occur on all continents except Australia and Antarctica, including numerous countries in Central and East Asia (Balakhonov et al., 2014; Verzhutsky 2018). Burrow-dwelling mammals such as marmots, ground squirrels, and gerbils are often the principal reservoirs for bubonic plague, as well as rats, voles, and pika in the Eurasian foci, with their fleas being the plague vector (Gage and Kosoy 2005; Girard et al., 2004; Karimova and Neronov 2007).

Mongolia has a diverse flea fauna, having been described in part in connection with plague-focused studies (Shchekunova et al., 1964; Jun et al., 1993; Galdan et al., 2010), which suggest the total number of flea species currently present in Mongolia is approximately 90 (Smit 1967). Macroecological patterns of flea diversity in Mongolia have also been studied previously (Kiefer et al., 1984; Galdan et al., 2010; Maestri et al., 2017; Medvedev et al., 2019; Smit 1967), but there is still limited understanding of how individual hosts attributes and modern change-related factors contribute to patterns of flea richness and abundance across varying habitat and climate types within Mongolia. To do this, models that move beyond population means to understand the variance in flea richness and abundance and in detailed climate contexts are necessary.

STUDY AIMS

The purpose of this study is to characterize the species richness and abundance of fleas on the long-tailed ground squirrel (*Urocitellus undulatus)* across steppe and grassland habitats of Mongolia. The long-tailed ground squirrel is a quintessential component of open and relatively mesic ecosystems in northern and western Mongolia (Pavlinov and Lissovsky 2012). This study makes use of multiple years of mammal and parasite inventory across the country conducted as part of international collaborative biodiversity surveys. Here, I aim to describe how the richness and abundance of fleas on *U. undulatus* individuals vary with environmental characteristics, host traits, and levels of ecological disturbance across Mongolia. This research is focused at the component community level, including all infrapopulations of fleas associated with a subset of a host species within a subset of the abiotic environment (Bush et al., 1997). My specific objectives are to:

(1) Evaluate the effect of host-trait components on fleas. **I hypothesized that flea abundance and richness would be related to host sex (higher values for both in males), positively correlated with body size, and negatively correlated with host body condition.**

(2) Evaluate the effect of climate components on fleas. **I hypothesized a positive correlation between a) flea abundance and richness and b) annual winter precipitation, soil moisture, and annual minimum temperature, and a negative correlation between flea abundance and richess and the Palmer Drought Severity Index (PDSI).**

(3) Evaluate the anthropogenic effect of grazing on fleas**. I hypothesized that flea abundance and richness would be negatively correlated with grazing intensity.**

METHODS

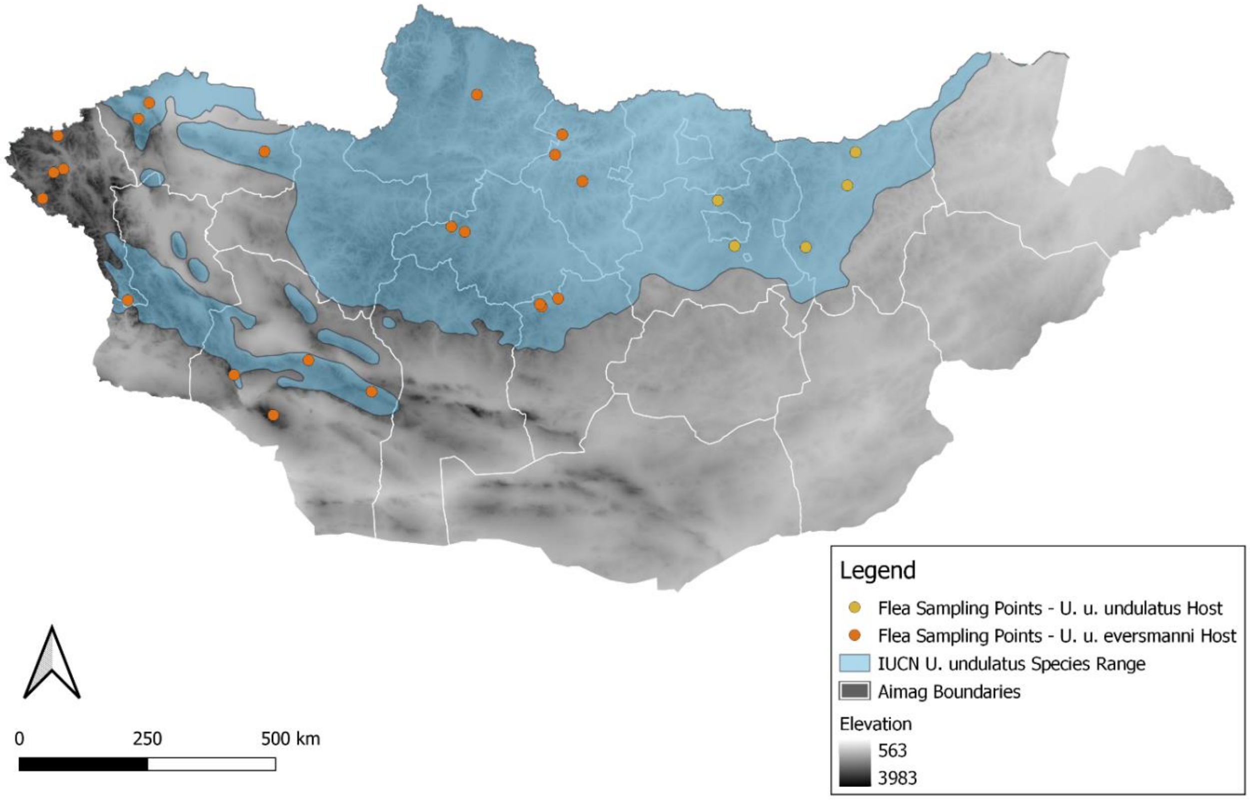
FIELD SAMPLING AND ARCHIVAL

Flea specimens were collected as part of three collaborative, multi-year U.S. – Mongolia survey projects, which occurred from 2009 to 2012 (Mongolian Vertebrate Parasite Project, NSF DEB 0717214); 2015 to 2016 (Collaborative Integrative Inventories of Biomes of the Arctic, NSF DEB 1258010); and in 2022 (Steppe Parasite Networks, NSF DEB 2228385). Ectoparasite examinations employed two general approaches, one before and during 2012 and another during and after 2015. For the 2009-2012 expeditions, a chloroform-inhalation euthanasia method was used whereby each individual animal was placed in a clean plastic bag and 5-10 ml of chloroform was used to euthanize the mammal. Ectoparasite screens were done by gentle brushing within the bag, removing any ectoparasites present, and preserving bag contents in ethanol for analysis at a later time. For the 2015-2022 expeditions, a more thorough ectoparasite examination was conducted where all euthanized mammals were taken out of the bag and the entire body brushed with a fine-tipped comb against the fur grain, with particular focus on the back, stomach, legs, and face/ears. All ectoparasites detected in bag contents or remaining on the body were preserved for archival and future analysis. This variance in flea detection methodology between expeditions was accounted for in subsequent analyses. All wild mammals screened during these expeditions were handled under approved IACUC protocols held by the Museum of Southwestern Biology (MSB; University of New Mexico) and following the American Society of Mammologists guidelines for the care and use of wild mammals in research (Sikes et al., 2016).

Fleas analyzed as part of this study are archived at either the MSB Division of Parasitology (2015-2022) or the Harold W. Manter Lab of Parasitology at the University of

Nebraska (2009-2012). In total, 27 flea individuals across 12 *U. undulatus* specimens (2009-2012; Manter Lab of Parasitology), 103 fleas representing 29 *U. undulatus* specimens (2015-2016; MSB Division of Parasitology), and 132 fleas representing 23 *U. undulatus* specimens (2022; MSB Division of Parasitology) were loaned and used. The 262 flea samples span 9 Mongolian aimags (provinces) with a minimum of two sampling localities per aimag (**Table A1**), providing coverage throughout the species range of *U. undulatus* in Mongolia (**Figure III.1**).

Figure III.1. Map of Mongolia displaying the range of *U. undulatus* (Cassola 2016) and sampling sites used in this study where individual hosts were screened for fleas. Host subspecies is annotated by color, with yellow displaying *U. u. undulatus* and orange displaying *U. u. eversmanni*.



FLEA IDENTIFICATION

All fleas were identified to species level using a combination of morphological characterizations and DNA barcoding at the cytochrome c oxidase subunit 1 (COI) gene. Prior to DNA barcoding, I performed DNA extractions on all fleas. I began by making a small abdominal incision, ensuring that the entire cleared exoskeletons could be used for further long-term preservation and/or mounting. Specifically, this method involves perforating the upper abdomen of flea specimens with a 0.115 mm insect pin and ultra-fine scalpel to create two small puncture openings. Each flea was put through a modified salt-ethanol extraction where 200 ul of tissue lysis buffer and 10 ul of proteinase K (20 mg/ul) was added to each vial and incubated at 55-56° Celsius for 5 days. After lysis digestion, each cleared flea exoskeleton was placed in a new Eppendorf tube with 95% ethanol solution and stored in -20° Celsius. One microliter of 5 µg/µL RNase A was then added to the original supernatant and mixed thoroughly. To precipitate the DNA, 200 µL of isopropanol was added and the tube inverted 30-40 times. The sample was then incubated overnight at -20° Celsius. To pellet the DNA, samples were centrifuged for 30 minutes at 14000 RPM. The supernatant was then removed and 500 ul of cold 70% ethanol was added to remove excess salts from the DNA pellet. This solution was micro centrifuged for 5 minutes at 6000 RPM, and any excess ethanol was gently poured out. The wash step was repeated for a second time and all vials were dried in an incubator at 37° Celsius. The dried DNA pellet was then suspended in 25 ul of DNA elution buffer and incubated at 55° Celsius for 1 hour. All final samples were stored at -20° Celsius until proceeding to PCR amplification.

For PCR assays, I amplified 657 base pairs of the COI gene using the primer pair LCO1490 (5’ – GGTCAACAAATCATAAAGATATTGG –3’) and HCO2198 (5’ – TAAACTTCAGGGTGACCAAAAAATCA – 3’; Folmer et al., 1994). All DNA reactions were

25 ul and the thermocycler settings were set at 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds, and extension at 72 °C for 1 minute. The resulting PCR product was sent to the Genomics Sciences Laboratory at NC State University and all flea DNA samples underwent the same sequencing conditions as the *U. undulatus* DNA analyzed in Chapter 1. The COI sequences were then visualized, cleaned and aligned in the bioinformatics software, Geneious (Geneious Prime 2023.2.1). All sequences were processed in BLAST (Altschul et al., 1990) and matches were recorded for all sequences with a BLAST match of $\geq$90% similarity. Cleared exoskeletons were later identified based on external and genital morphology by Dr. Lance Durden (Georgia Southern University).

HOST TRAIT, CLIMATE, AND GRAZING VARIABLES

Flea species identifications were incorporated into a community (host-flea) database, which also included four host traits: sex, body size (captured as head-body length; i.e., total length minus tail length), body condition (residuals from a regression of weight on head-body length, Labocha et al., 2014), host specimen freshness at the time of screening, and host subspecific identity. Host freshness was represented as a binary value based on whether specimens were freshly deceased or not when examined, combining information on trap type (kill traps or not), condition of host internal tissues upon necropsy, and documentation of whether the host was dead or alive upon examination (if available). The subspecies identities include the two subspecies of *U. undulatus* found within Mongolia; the western lineage *U. u. eversmanni* and the eastern lineage *U. u undulatus*. Host trait data was sourced from the Arctos online database, a public collection-management site where Museum of Southwestern Biology collections are hosted digitally (Cook 2023).

To test possible climate drivers of flea communities, I compiled monthly-scale climate data based on exact coordinates of host captures, including minimum temperature of coldest month (MTC), maximum temperature of warmest month (MTW), soil moisture (SM), annual winter precipitation (AWP), annual summer precipitation (ASP), average annual precipitation (AAP), previous-year precipitation (PYP) and Palmer severity drought index (PDSI) for each flea specimen. High-resolution raster files for my climate variables were acquired from the TerraClimate database at a monthly temporal resolution for each collection year at a ~4-km (1/24th degree) spatial resolution (Abatzoglou et al., 2018). A correlation test was conducted using the 'cor' function in R to identify any correlation between climate variables. Climate pairs with scores <0.6 and variables relevant to flea biology, based on prior knowledge, were retained. The climate variables included in the models were MTC, AWP, SM, and PDSI. Finally, to test one major axis of land use pressure, I used grazing percentage estimates by obtaining raster shapefiles for the grazing pressures of Mongolia from Chen et al. (2019). This is an estimate of percent of net primary productivity consumed by livestock grazing, and thus grazing intensity. To extract grazing percentage and climate values for each set of host coordinates, I used the ‘point sampling’ tool in QGIS (QGIS 3.20.2).

FLEA COMMUNITY DIFFERENCES

To visualize broad-scale flea community differences, I performed a non-metric multidimensional scaling (NMDS) analysis on both weighted (relative flea species abundance) and unweighted (presence-absence) community matrices. Stress values from each NMDS were recorded, and a Shepard's test was conducted to assess the goodness of fit for each point. Community ordinations were visualized in an ordiplot with each site color-coded using the *ordispider()* function in vegan v. 2.5-6 (Oksanen et al., 2020) and using the ggplot2 (Wickham

2016) package in R (R Core Team 2021). I utilized two major groupings for visualizations: Mongolian aimags and host subspecies.

Next, to assess whether there were significant differences in flea community composition at broad spatial scales (among aimags) and levels of host evolutionary uniqueness (between subspecies), I performed a PERMANOVA test on my weighted and unweighted NMDS configurations using the *adonis2* function in vegan. For the PERMANOVA, I compared the variance in flea community composition between groups to the variance within groups based on a dissimilarity matrix. All tests were implemented in the vegan package (Oksanen et al., 2020) in R.

FLEA ABUNDANCE AND RICHNESS REGRESSIONS

To explore the biological and ecological factors impacting flea communities present on long-tailed ground squirrels, I conducted a combined regression analysis that included predictors of host trait, climatic and grazing variables. I employed generalized linear mixed models (GLMM) using the glmmTMB R package (*glmmTMB()* function; Brooks et al., 2022) to analyze both flea richness and abundance. For the flea abundance dataset, I included not only counts of fleas used in the analyses above, but also known zeros for ground squirrels that were screened and were negative for ectoparasites, as recorded in original field datasheets and on the Arctos database.

Two separate, fully specified models were fitted to the response variables of flea abundance and richness, encompassing all fixed effects, two sex-associated interactions of host traits (host body condition * host sex, host body size * host sex), two interactions for climate variables (soil moisture * annual winter precipitation and soil moisture * PDSI), and a random effect representing the three expeditions, across which screening procedures and detection probabilities slightly varied (coded “Project”; see Field Sampling and Archival above). In both models, continuous fixed effects were scaled to have mean 0 and s.d. 1 to help balance the impact of all model variables on the distance calculation. A negative binomial distribution was used to model error distributions and zero-inflation in both richness and abundance. Additionally, a controlled optimization parameter using the Broyden-Fletcher-Goldfarb-Shanno (BFGS) method was used to improve model convergence. Given the large number of fixed effects and our desire to isolate only the most important drivers of flea community structure, we also used stepwise selection to find “optimal” reduced models with rankings based on Akaike’s Information Criterion (AIC) (Voeten 2023), using the Buildmer package in R. Effect plots of the linear regression of each fixed effect on my predictor variables were visualized using the ‘jtools’ R package (Long 2022).

To test for zero-inflation and overdispersion within raw data and model residuals, I used the DHARMa R package (Hartig 2016) to check if the residual variance was smaller or larger than expected for the fitted model using scaled (quantile) residuals. These residuals were visualized in a QQ plot and a residual vs. predicted plot to provide further explanation of the results.

PATH ANALYSIS MODELS

A path analysis was used to examine variable relationships allowing for the simultaneous analysis of direct and indirect effects of the GLMM analyzing effects of host, climate, and grazing variables on flea abundance and richness. Four path diagrams were constructed in the software AMOS (Arbuckle 2006), with two full models linking eight observed variables; three host (host sex, body size, host body condition), four climatic (minimum temperature, winter precipitation, soil moisture, PDSI Drought Index), and one grazing (grazing percentage) to respective dependent variables (flea abundance and flea richness). Two reduced models were also calculated where the climate variables of winter precipitation and PDSI drought index were dropped from both models due to their non-significance in the full models. Correlations were included between all pairs of exogenous variables, except for host sex which was not correlated with anything. Model fit estimates were calculated using the discrepancy divided by degree of freedom (CMIN/DF) and the goodness of fit index (GFI). Two final path diagrams illustrating the best fit models were chosen to show variable factor loadings which indicate the significance of each variable in influencing flea abundance and richness, alongside other observed variables.

RESULTS

FLEA COMMUNITY COMPOSITION

We screened 125 host individuals and detected 262 fleas comprising 12 species found on 60 unique long-tailed ground squirrels across five years: 2010, 2011, 2015, 2016, 2022. One species (*Citellophilus sungaris*) constituted the majority (69.8%) of the collected fleas, followed by *Citellophilus altaicus* (14.5%; **Table III.1**). All other flea species each comprised $\leq$3 % of the species found.

To standardize flea abundances, a flea intensity metric was calculated by scaling total abundance values to the number of hosts examined across each aimag. Across the nine sampled aimags, Hentei and Tov had the highest mean flea abundances (4.80), followed by Ovorkhangai (3.53) and Arkhangai (3.50). Khovsgol had the lowest mean flea abundance (0.17) with only one flea specimen collected across six unique host individuals (**Figure III.2**). For total flea species richness, Bayan-Olgii had the highest species count with six unique flea species found, followed by Hentei and Ovorkhangai with five and four unique flea species, respectively **(Figure III.3**). Mean flea species richness was also determined by averaging the number of unique flea species across all hosts examined within each aimag. Hentei exhibited the highest mean flea species richness (0.50), followed by Tov (0.40) and Arkhangai (0.33). Conversely, Govi-Altai had the lowest mean flea species richness (0.09; **Figure III.4**).

Table III.1. Summary of flea species collected from long-tailed ground squirrels (*U. undulatus*) across Mongolia, with including the total number of hosts sampled ("N").

Flea Species	N	# of Infested Hosts	# of Fleas Collected	Mean Intensity	Relative Abundance
Amphalius runatus	60	1	1	1.00	0.02
Citellophilus altaicus	60	8	38	4.75	0.63
Citellophilus sungaris	60	40	183	4.58	3.05
Frontopsylla elata	60	5	5	1.00	0.08
Frontopsylla hetera	60	4	7	1.75	0.12
Megabothris rectangulatus	60	1	1	1.00	0.02
Neopsylla pleskei	60	6	8	1.33	0.13
Neopsylla teratura	60	1	1	1.00	0.02
Neopyslla mana	60	1	1	1.00	0.02
Oropsylla silantiewi	60	4	9	2.25	0.15
Paradoxopsyllus microphthalmus	60	1	1	1.00	0.02
Rhadinopsylla li	60	5	7	1.40	0.12

Figure III.2. Barplot of flea intensity (abundances scaled by number of host specimens examined) for each of nine Mongolian aimags.

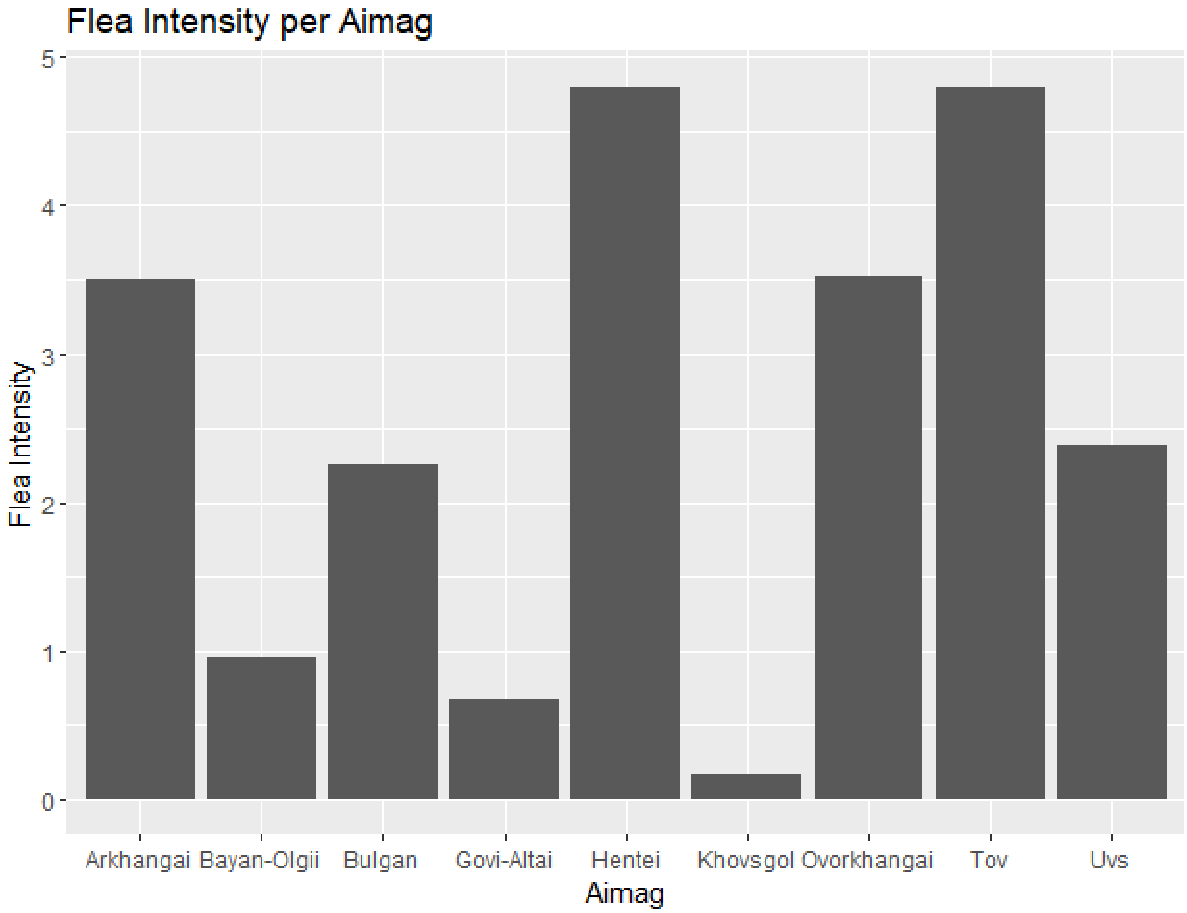


Figure III.3. Barplot of total flea species counts collected for each of nine Mongolian aimags.

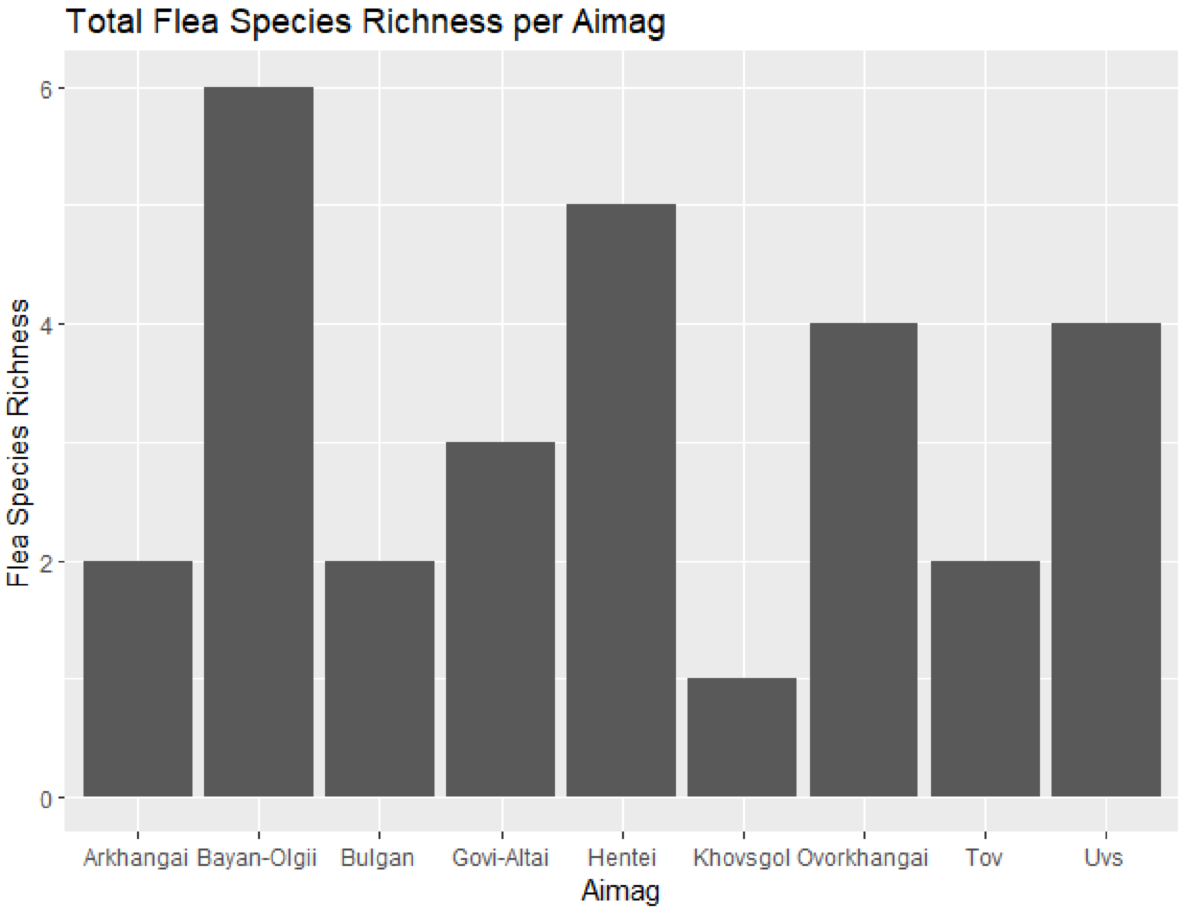
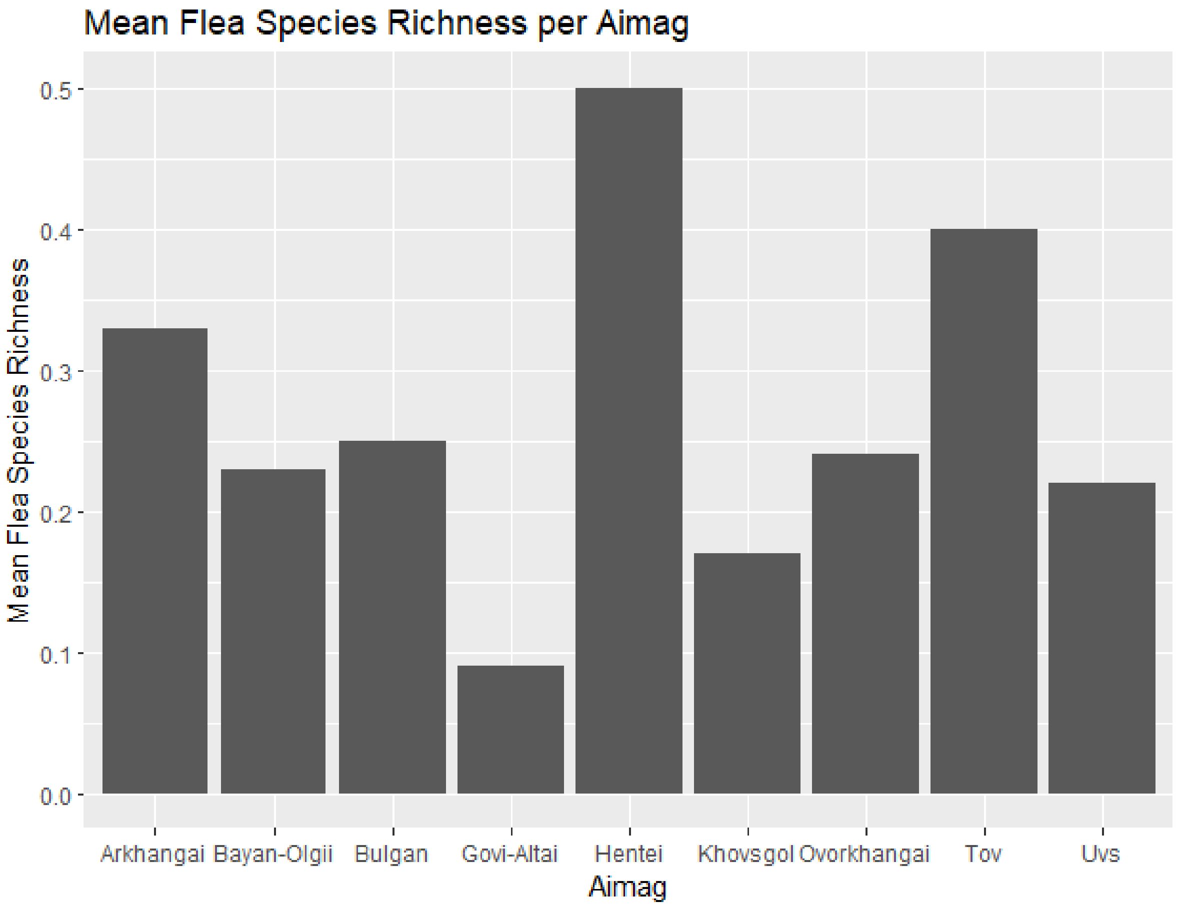


Figure III.4. Barplot of mean flea species richness (richness scaled by the number of hosts screened) for each of nine Mongolian aimags.



Two NMDS scenarios were separately fit using abundance-weighted and presence-absence data, with aimag and subspecies as grouping parameters. The stress of each model had a stress value below 0.05, indicating a good fit. Plots of the top two NMDS axes (**Figure III.5, III.6, and Figure A1**) revealed slight overlap of data points among eight of the nine aimags, with the Uvs aimag showing clear separation from the primary aggregation. In NMDS plots with hosts grouped by subspecies, flea communities show high overlap with the *U. u. undulatus* subspecies (which has a more northern distribution and smaller range within Mongolia) falling mostly within fleas for the *U. u. eversmanni* subspecies.

Figure III.5. Unweighted NMDS plot with observations grouped by aimag. Colors in the map at bottom represent sampled aimags and correspond to the NMDS plot.

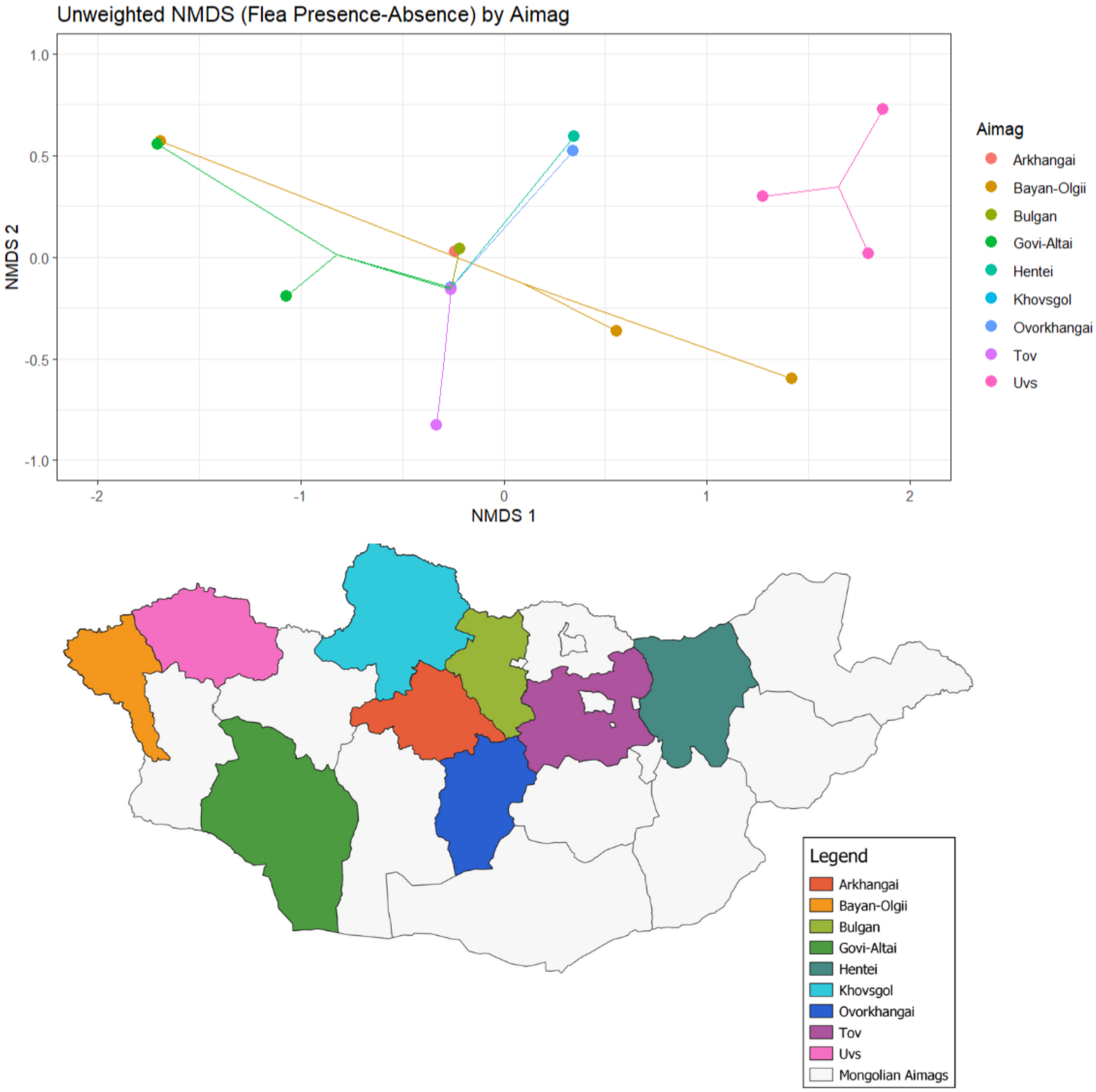
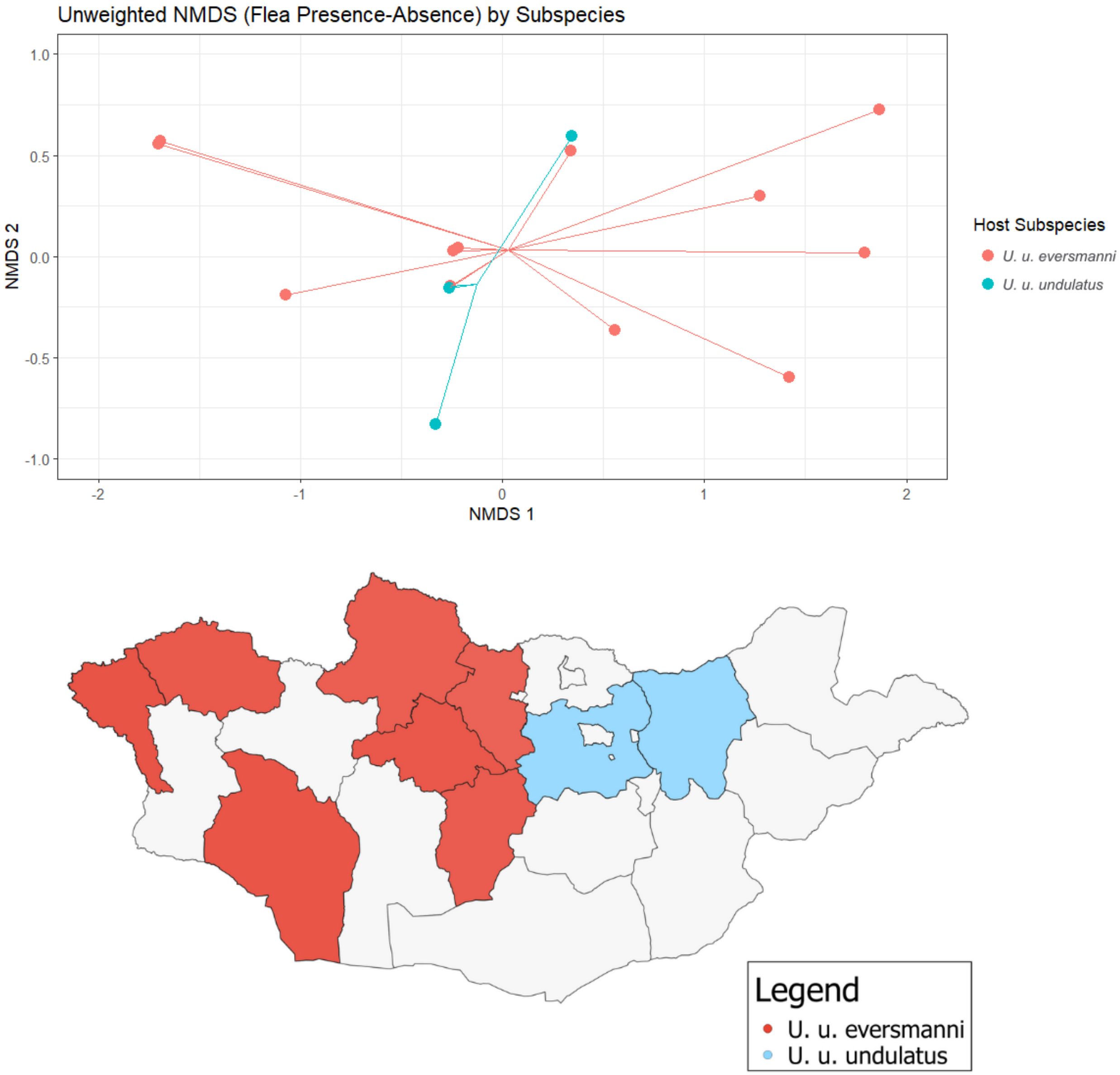


Figure III.6. Unweighted NMDS plot with observations grouped by subspecies. Colors in the map at bottom represent aimags where each subspecies was sampled.



The PERMANOVA used to assess differences among community groupings rejected the null hypothesis of no difference in flea presence/absence (permutations=5000, R^2= 0.60211, P=0.0089), supporting flea community differences among aimags. However, PERMANOVAs failed to reject the null hypothesis of no difference in flea presence/absence among host subspecies at the site level (permutations=5000, R^2= 0.04457, P= 0.4111). Similarly, for the community-weighted analyses, the null hypothesis was rejected when examining differences among aimags (permutations=5000, R^2= 0.65901, P=0.006199), but failed to be rejected when examining differences among subspecies (permutations=5000, R^2= 0.0531, P=0.3195).

DETERMINANTS OF FLEA ABUNDANCE AND SPECIES RICHNESS OF HOSTS

Two optimal "reduced" GLMMs out-ranked the full models which included all fixed effects and associated interactions (**Table A2**) and were employed to investigate the influence of host-related and climate factors on flea richness and abundance (**Table III.2**). Results from the regression analysis unveiled four significant host-trait determinants of flea abundance including host body size, host body condition, host sex, and host subspecies, along with the interaction of host body condition * host sex. Both host body size and body condition (**Figure III.7**) negatively correlated with flea abundance, with larger hosts and higher body conditions significantly decreasing flea abundance (estimate coefficients: -0.40 and -0.79, respectively). Conversely, host sex demonstrated a positive correlation, suggesting that male hosts have significantly increased in expected flea abundance (estimate coefficient: 0.85; **Figure III.7**). Host subspecies also exhibited a significant positive effect, indicating that *U. u. undulatus* have significantly flea abundances (estimate coefficient: 1.16). There was also a significant combined effect between body condition and host sex on flea abundance, suggesting host sex-specific changes in flea loads throughout host lifespan.

Climatic variables also significantly influenced flea abundance, with minimum temperature, the PDSI Drought index, and the interaction between soil moisture * PDSI all showing notable effects. Minimum temperature displayed a positive effect, suggesting that as temperatures become warmer, flea richness increases (estimate coefficient: 0.55; **Figure III.8**).

The PDSI Drought index displayed negative effects, indicating that less intense drought levels (wetter conditions) are associated with decreases in flea abundance (estimate coefficient: -0.34, respectively; **Figure III.8**). The combined effect of soil moisture and the PDSI drought index was significant, indicating that the impact of soil moisture on flea abundance varies depending on the level of the PDSI drought index, and vice versa.

The parallel analysis of flea species richness revealed several significant determinants, with two shared variables with the flea abundance model (**Table III.2).** Only one host trait (subspecies) significantly influenced flea richness, with *U. u. undulatus* hosts displaying a positive correlation (estimated coefficient: 0.74), consistent with the flea abundance model. Three climatic factors (minimum temperature, soil moisture, and winter precipitation) significantly impacted flea richness. Minimum temperature displayed a positive estimate (coefficient: 0.49), suggesting that as temperatures become warmer, flea richness increases (**Figure III.8**). In contrast, soil moisture, exhibited a negative estimate (coefficient: -0.43), suggesting that, on average, an increase in soil moisture is associated with a decrease in flea richness (**Figure III.8**). Finally, winter precipitation had a positive estimate (coefficient: 0.28), suggesting that an increase in winter precipitation is associated with an increase in flea richness.

Notably, grazing pressure was found to be non-significant in its effects and was excluded from both optimal models. Within the reduced models, host sex and host body condition were found to be non-significant in their effects on flea richness, while host freshness and soil moisture alone was non-significant in its effects on flea abundance. Additionally, a dharma residual test was performed to assess zero-inflation and over-dispersion in the dataset. The resulting QQ plot residuals showed a non-significant deviation, passing the Kolmogorov-

Smirnov (KS), dispersion, and outlier tests, affirming both model's validity and reliability in explaining the observed flea patterns (**Figures A2 and A3**).

Table III.2. Summary of the top-ranked models predicting flea abundance and richness on long-tailed ground squirrels. Statistically significant terms are in bold indicating p-values < 0.05.

Flea Abundance	Estimate ± S.E.	P-Value	Flea Richness	Estimate ± S.E.	P-Value
FIXED EFFECTS			**FIXED EFFECTS**		
Host Body Size	**-0.40 ± 0.16**	**0.01**	Host Body Condition	-0.07 ± 0.12	0.55
Host Body Condition	**-0.79 ± 0.26**	**0.002**	Host Sex (Male)	0.35 ± 0.25	0.16
Host Sex (Male)	**0.85 ± 0.29**	**0.003**	Host Subspecies (*Undulatus*)	**0.74 ± 0.27**	**0.007**
Host Subspecies (*Undulatus*)	**1.16 ± 0.40**	**0.003**	Minimum Temperature	**0.49 ± 0.13**	**0.0002**
Host Freshness	-0.68 ± 0.47	0.14	Soil Moisture	**-0.43 ± 0.21**	**0.03**
Minimum Temperature	**0.55 ± 0.15**	**.0003**	Winter Precipitation	**0.28 ± 0.14**	**0.04**
Soil Moisture	-0.40 ± 0.22	0.07			
PDSI Drought Index	**-0.34 ± 0.17**	**0.04**			
INTERACTIONS					
Host Body Condition*Host Sex (male)	**0.98 ± 0.37**	**0.007**			
Soil Moisture*PDSI Drought Index	**0.80 ± 0.33**	**0.01**			

Figure III.7. Effect plots visualizing major host-trait effects on flea abundance.

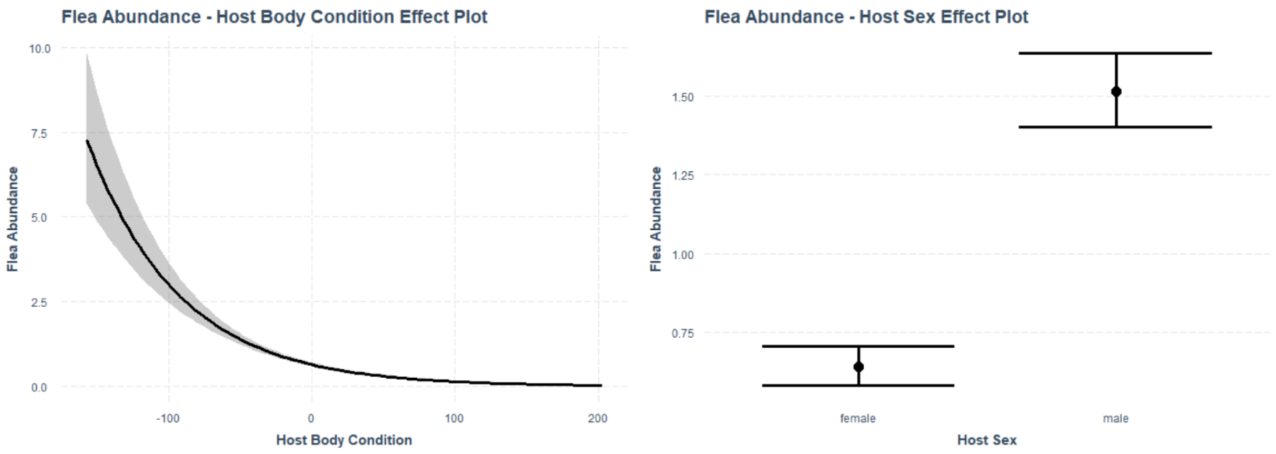
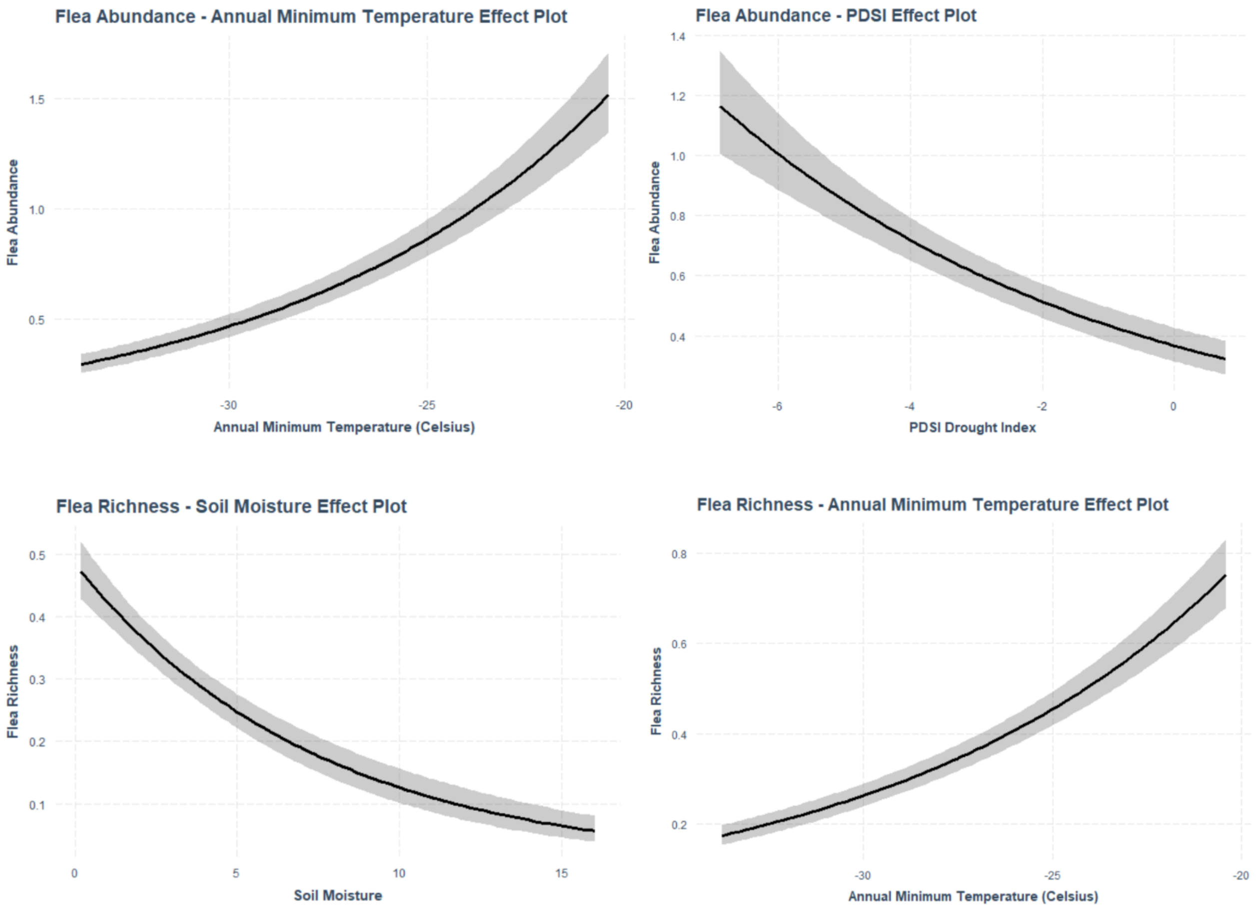


Figure III.8. Effect plots visualizing major climate-trait effects against flea abundance and flea richness.



EXAMINING DIRECT AND INDIRECT EFFECTS OF MODEL VARIABLES

The full flea abundance and reduced flea richness models were the best fitting, with the CMIN/DF values ≤ 3 (1.05 for flea abundance, 1.42 for flea richness), indicating an acceptable fit. Both models also had GFI values ≥ 0.95 (0.98 for flea abundance and flea richness), indicating an excellent fit. The models indicated that the combined effect of host, climate, and grazing factors explained comparable amounts of variation in flea abundance (R^2=0.193) and in flea richness (R^2=0.20).

The flea abundance model (**Figure III.9**) revealed direct significant impacts of host sex, host body size, minimum temperature, and soil moisture on flea abundance. Additionally, grazing pressure exerted a significant indirect effect (-0.30) by negatively influencing host body size. Negative correlations were observed between soil moisture and host body size, indicating that wetter soil conditions and larger hosts were associated with decreased flea abundances. Female hosts also exhibited a negative correlation (-0.30), indicating lower flea abundances compared to males. Conversely, minimum temperature showed a positive correlation, suggesting that higher temperatures were associated with increased flea abundances.

The flea species richness model (**Figure III.10**) indicated direct impacts of minimum temperature, soil moisture, and grazing percentage, with grazing also exerting a negatively correlated (-0.3) indirect effect on host body size. Negative correlations were observed between grazing percentage and soil moisture (-0.20 and -0.24, respectively), suggesting that higher grazing pressure and wetter soil conditions were associated with decreased flea species richness. Conversely, minimum temperature showed a positive correlation, indicating that warmer temperatures were associated with increased flea species richness. Host body size was found to have no direct effects on flea species richness. Additionally, host body condition, winter precipitation, and the PDSI drought index were non-significant in their direct effects on both flea richness and flea abundance.

Figure III.9. Path analysis diagram for the best fit flea abundance model. Directional arrows showcase the causal relationship between variables, with line thickness being proportional to coefficient strength. Red lines indicate a negative relationship while green indicates a positive relationship, and gray arrows are links that were non-significant.

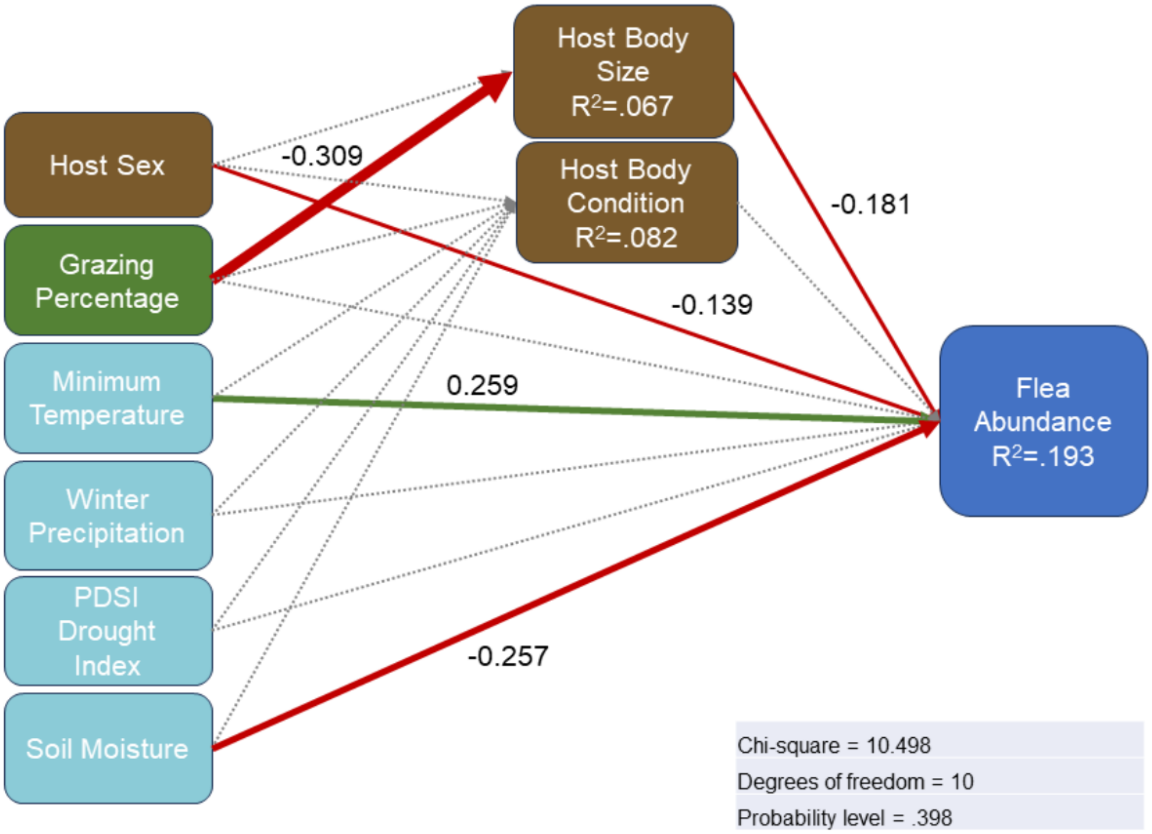
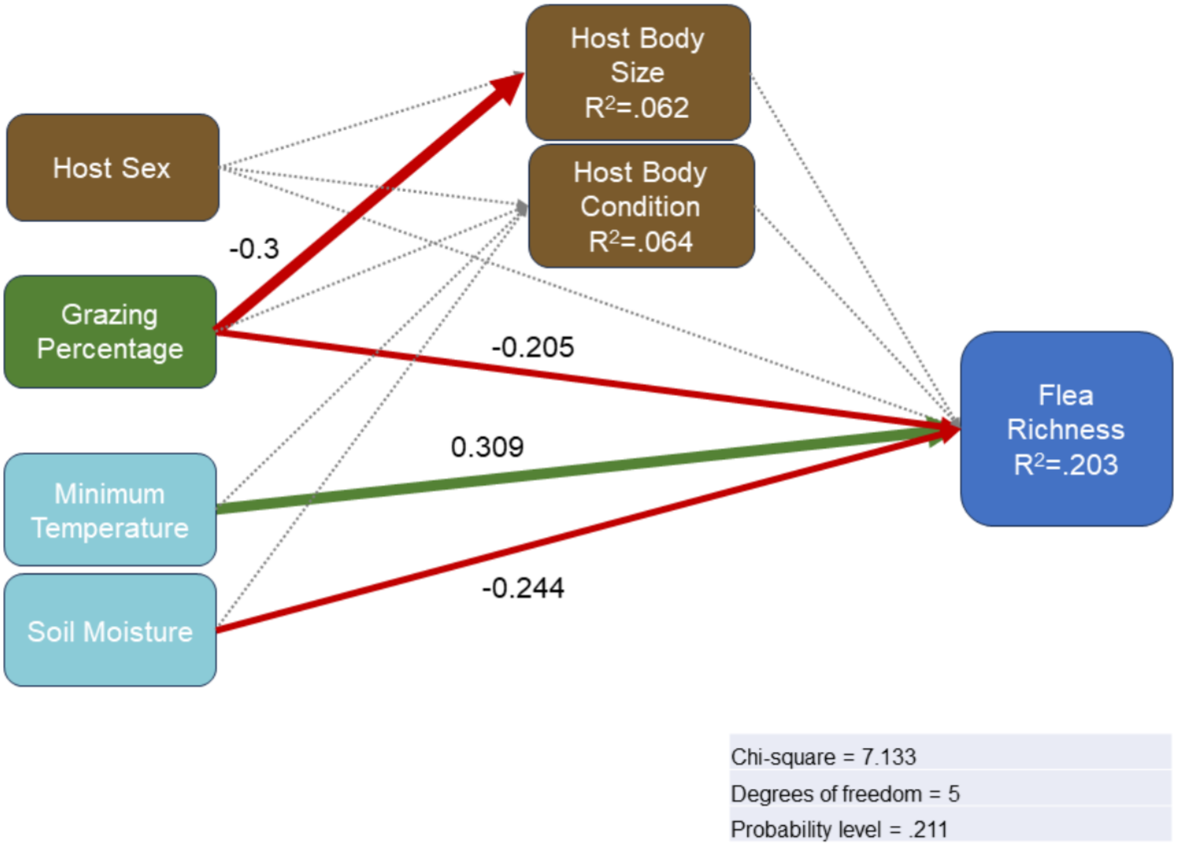


Figure III.10. Path analysis diagram for the best fit flea species richness model. Directional arrows showcase the causal relationship between variables, with line thickness being proportional to coefficient strength. Red lines indicate a negative relationship while green indicates a positive relationship, and gray arrows are links that were non-significant.



Chi-square = 7.133

Degrees of freedom = 5

Probability level = .211

DISCUSSION

FLEA COMMUNITY STRUCTURE

Flea community composition showed a degree of variation in both abundance values and in species richness across the sampling range throughout Mongolia, with a PERMANOVA recovering that geography (aimags) was a significant driver of relative flea abundance (weighted) and flea presence-absence (unweighted). Twelve unique flea species were collected across nine aimags, but their prevalence and abundance were highly concentrated between two species, *Citellophilus sungaris* and *Citellophilus altaicus*. Uvs aimag was recovered as having the most distinct flea composition primarily due to it was the only aimag in which *Citellophilus altaicus* was present, in addition to the presence of three less-common flea species *Frontopsylla elata*, *Oropsylla silantiewi,* and *Rhadinopsylla li* (**Table A3**). Arkhangai, Bayan-Olgii, Bulgan, Govi-Altai, Hentei, Ovorkhangai, and Tov were dominated by *Citellophilus sungaris*, with varying presence of other rare flea species, typically averaging one to four flea individuals per rarer species. For Khovsgol, only one *Citellophilus sungaris* flea was retrieved, but this aimag also contained the minimum number of screened ground squirrels (6), so further field sampling efforts in this area and across eastern Mongolia would help to delineate further differences in flea community composition bridging the western and eastern range of the long-tailed ground squirrel.

Ten out of twelve flea species collected were classified as either 'lesser common' or ‘rare’ based on their distribution. Fleas found in two to four aimags were labeled as 'lesser common', while those present in only one aimag were classified as rare. Lesser common flea species found to occur on long-tailed ground squirrels were *Frontopsylla elata* (three aimags)*, Frontopsylla hetera* (two aimags)*, Neopsylla pleskei* (three aimags)*, Oropsylla silantiewi* (four aimags), and

Rhadinopsylla li (three aimags). Flea species classified as rare were *Amphalius runatus*, *Megabothris rectangulatus*, *Neopsylla teratura*, *Neopsylla mana, and Paradoxopsyllus microphthalmos.* Geographically, *Amphalius runatus, Neopsylla mana, and Paradoxopsyllus microphthalmos* only occurred on ground squirrels in western Mongolia (Uvs, Bayan-Olgii, Govi-Altai), *Megabothris rectangulatus* only in central Mongolia (Khovsgol, Arkhangai, Bulgan, Ovorkhangai, Tov), and *Neopsylla teratura* only in eastern Mongolia (Hentei).

Mongolia includes six habitat zones consisting of high mountain alpine, mountain taiga, mountain forest steppe, rolling steppe, and semi-desert and desert zones (Harris et al., 2024). Additionally, Mongolia has high variability in latitude with three major mountain ranges, the Mongolian Altai, the Khangai Mountains, and the Khentii Mountains which are located in the western, central, and northeastern parts of Mongolia, respectively (Harris et al., 2024). Within the context of my study area, western Mongolia features high mountain and forest steppe in the north, transitioning to desert steppe further south, while central and eastern Mongolia mainly consists of meadow and forest grassland steppe (Wang et al., 2022). Not surprisingly given this, flea abundance and richness varied among aimags, with specific flea species found exclusively in western, central, or eastern Mongolia, likely influenced by habitat preferences and associated climate variations, as well as other abiotic and biotic factors.

DOMINANCE OF FLEAS BELONGING TO THE GENUS *CITELLOPHILUS*

The flea species richness present on the long-tailed ground squirrel was predominantly comprised (>80%) of two species belonging to the genus *Citellophilus*, *Citellophilus altaicus* and *Citellophilus sungaris*. These particular flea species infest several other ground squirrel species (*Spermophilus* spp.) inhabiting the steppe and mountain regions across Central Asia, Southern Siberia, Mongolia, and the northern regions of China (Medvedev et al., 2019). While previously noted as two subspecies of *Citellophilus tesquorum*, the now distinct species of *C. altaicus* and *C. sungaris*; are the two most common *Citellophilus* species present in Mongolia. This study further supports that *C. altaicus* occurs in Eastern Mongolia and *C. sungaris* occurs in Central and North-Western Mongolia (Ilinsky et al., 2022). There was no clear host preference observed in *C. sungaris* as they were found on both subspecies, while *C. altaicus* was only found on *U. u. undulatus*, likely due to *C. altaicus's* northeastern range boundary (Illinksy et al., 2022). Notably, no ground squirrels were found hosting both *Citellophilus* species, suggesting that competitive exclusion may be influencing distribution patterns among these flea species. One explanation as to why there was no geographical overlap between *C. altaicus* and *C. sungaris* across the sampled ground-squirrels is that recent genetic differences and a degree of reproductive isolation between these flea species have been revealed (Ilinsky et al., 2022). In Mongolia, the Altai, Khangai, and Khentii mountain regions act as significant geographical barriers, potentially affecting the distribution of flea species on long-tailed ground squirrels.

COMPARING IMPORTANCE OF HOST VERSUS CLIMATE TRAITS

This work revealed that flea abundance and flea species richness are influenced by a mixture of host-trait, climate, and grazing factors to varying degrees. Flea abundances are correlated strongly with host traits such as body size, body condition, and sex, as well as environmental variables including minimum temperature, PDSI drought index, and soil moisture. Similarly, flea species richness is primarily associated with climate factors such as minimum temperature, winter precipitation, soil moisture, and grazing percentage. These findings align with prior research indicating the significant impact of host traits on flea abundances (Kowalksi et al., 2015, Surkova et al., 2018; Hammond et al., 2019; Hamidi et al., 2021; Smith et al., 2021), as well as the influence of ecological conditions on both flea abundances and species diversity among mammalian hosts (Kranov et al., 2006; Krasnov et al., 2010; Hubbart et al., 2011; Eads et al., 2016; Poje et al., 2020).

Among the host traits examined, flea abundance was significantly influenced by, while flea species richness showed less sensitivity to, these factors. Regarding a host’s body condition and size, the data support the "poorly fed hypothesis", which states that parasites prefer juvenile hosts (Hawlena et al., 2005). Both host body size and body condition were found to be negatively correlated with flea abundance, indicating that larger, healthier hosts tended to have lower flea abundances. This could be attributed to behavioral adaptations such as grooming, which may be more prevalent in older, larger individuals, aiding in parasite avoidance or removal (Hart et al., 2011). Conversely, there was no discernible effect of host size or host body condition on flea species richness. Notably, host sex significantly influenced flea abundance, with male hosts having more fleas on average than females, consistent with established patterns of parasitism across vertebrates, including rodents (Morand et al., 2004). However, host sex did not affect flea species richness in this study. Additionally, host subspecies was included in the GLMM analysis as an auxiliary variable to account for its potential effects, revealing its significance in both the flea abundance and species richness models. *U. u. undulatus* individuals exhibited higher flea abundances and species richness, which may be attributed to environmental preferences rather than biological differences, given the morphological similarity between host subspecies. This pattern may stem from habitat variability, with *U. u. undulatus* inhabiting the uniform steppe of eastern Mongolia, while *U. u. eversmanni* persists in western Mongolia which comprises a mix of high mountain forest, mesic grassland, and arid steppe.

This study also uncovered significant effects of climate on flea abundance and flea species richness on hosts. Theory suggests that abiotic environmental factors (i.e., temperature, moisture, seasonality, and environmental stability) and interspecific competition drive parasite dynamics (Wimsatt and Biggins 2009; Lindsay and Galloway 1998). Flea species richness and abundance exhibited a positive correlation with minimum temperature, indicating that warmer temperatures corresponded to higher flea diversity and abundances on hosts. Conversely, flea species richness showed positive correlations with winter precipitation, but negative correlations with soil moisture, suggesting that increased winter precipitation is associated greater flea diversity while higher soil saturation reduces flea species richness on hosts. While the GLMM results showed no significant correlation between soil moisture and flea abundance, the path analysis model identified a significant negative effect of soil moisture on flea abundance. Consistent with other research, higher temperatures were found to be associated with increased flea abundance, whereas higher winter precipitation was found to reduce flea abundances and species richness in black-tailed prairie dogs (Poje et al., 2020). One possible explanation for the negative effect of high soil moisture on flea abundances is that moisture levels above a 20-50% saturation point have been found to inhibit flea larval development (Silverman et al., 1983). Furthermore, the PDSI drought index exhibited a significant negative correlation with flea abundance, suggesting that wetter conditions are linked to lower flea loads, potentially indicating that moderate aridity may be beneficial to fleas in this study system. These findings highlight the importance of understanding physiological tolerances among flea species and how fluctuations in temperature and moisture can shape flea distribution patterns on the long-tailed ground squirrel.

Although grazing percentage did not directly affect flea abundance and species richness in the GLMM analysis, the path analysis model revealed a significant impact of grazing percentage on flea species richness and an indirect effect on flea abundance mediated by host body size. Specifically, grazing percentage exhibited a negative correlation with flea species richness, indicating that increased grazing pressure led to reduced flea species diversity on hosts. This pattern aligns with research suggesting that the composition of flea assemblages is influenced by environmental properties (Krasnov et al., 1997). Specifically, variations in environmental conditions between habitats can alter ectoparasite distributions within the same host species (Krasnov et al., 1998), which suggests that habitat changes induced by grazing, such as biomass reduction, may influence flea species richness by potentially favoring species more resilient to habitat changes.

Overall, it is important to note that these results only explained approximately 20% of the observed variation in flea abundance and species richness. This underscores the need for additional research to explore finer-scale factors especially for abiotic variables, such as vegetation monitoring and soil moisture conditions within long-tailed ground squirrel burrows. Furthermore, population size estimates or other abundance data for both ground squirrels and neighboring rodent hosts may aid in explaining flea abundance patterns and determining the likelihood of certain flea species to host-switch. A deeper understanding of these variables, among others, may provide insights into the factors that predominantly influence flea community composition among long-tailed ground squirrels across Mongolia.

RELEVANCE OF PLAGUE WITHIN THIS STUDY SYSTEM

Plague is widespread among rural Mongolia's wild rodent populations, with 37 natural plague foci consolidated into eight clusters across 17 of 21 Mongolian aimags (Platonov et al., 2015, Medvedev et al., 2020). These foci encompass 28.3% of Mongolia's landmass, with 47.1% show high activity, primarily in the western regions (Galdan et al., 2010). Specifically, plague dynamics within the *Marmota sibirica* foci in Mongolia have been widely studied, and give insight into what geographic regions are prone to plague outbreaks (Baigalmaa et al., 2016, Suntsov et al., 2021, Suntsov et al., 2022, Lei Xu et al., 2023). Our analysis of flea community composition on the long-tailed ground squirrel has revealed a few overlapping areas where high flea intensities, total flea species richness, and mean flea richness specific to the long-tailed ground squirrel also co-occur with incidences of plague. Bayan-Olgii had the highest number of unique flea species (six), while Hentei, Tov, and Arkhangai ranked high in flea intensity and mean flea species richness per host, respectively. These four aimags have consistently reported plague cases from 1998 to 2015 within the *M. sibirica* foci (Xu et al., 2023), emphasizing the need for landscape-scale studies on plague transmission dynamics involving *U. undulatus*, which may serve as a significant vector for plague in Mongolia akin to marmots.

Among the twelve flea species collected in this study, ten are associated with plague and can be categorized as primary vectors (e.g., *Oropsylla silantiewi, Citellophilus altaicus, C. sungaris*), accessory vectors (e.g., *Amphalius runatus*, *Frontopsylla hetera*, *Neopsylla mana, Rhadinopsylla li,*), or sporadic carriers (e.g., *Neopsylla pleskei*, *Neopsylla teratura*) of plague in Mongolia. *Oropsylla silantiewi*, commonly known as the marmot flea, is a significant vector, with over 3000 plague cultures isolated since 1897 (Medvedev et al., 2020a; Galdan et al., 2010). *Citellophilus altaicus* and *C. sungaris* act as plague carriers throughout the Palearctic region, predominantly associated with burrow-dwelling rodents, with long-tailed ground squirrels serving as their primary host in Mongolia (Wagner 1898; Medvedev et al., 2019). Moreover, studies have shown that the plague bacterium can persist in *Citellophilus* fleas for extended durations, as fleas in a simulated burrow retained the pathogen for up to 102 days, demonstrating their crucial role in plague transmission among long-tailed ground-squirrel communities (Evseeva and Firsov 1932).

Accessory vectors like *Amphalius runatus, Rhadinopsylla li, Frontopsylla hetera, and Neopsylla mana* also play a role in plague transmission, despite fewer reported cases. While *Amphalius runatus* significantly contributes to pathogen transmission in all three foci of the Gorno-Altai natural plague foci, its extent only reaches into the northern part of the Bayan-Olgii aimag (Korzun et al., 2014). *Frontopsylla hetera*, due to its high abundance in many Central-Asian mammal communities, also significantly contributes to pathogen spread as these fleas can carry the plague microbe for up to two months, suggesting prolonged infectivity (Medvedev at al., 2021a). *Neopsylla mana* similarly has a long-retention rate and can retain the pathogen for up to 380 days under quasi-natural conditions (Medvedev et al., 2021b). *Rhadinopsylla* species have also have been implicated in plague transmission in Mongolia, with 14 strains isolated, five of which came from long-tailed ground squirrels, underscoring their potential as vectors (Mededev et al., 2020b). Sporadic carriers like *Neopsylla pleskei* and *N. teratura* minimally contribute to plague transmission, with *N. pleskei* showing limited association, with a single positive specimen found in 2006 in Tuva (Medvedev et al., 2021b). While *N. teratura's* role in plague is rare in Mongolia, sporadic plague infections in China and Kyrgyzstan suggest its potential as a pathogen reservoir (Medvedev et al., 2021b).

CONCLUSION

These findings elucidate the diverse abiotic and biotic factors influencing flea community patterns on the long-tailed ground squirrel. Firstly, my flea community analysis revealed geographic variation in flea abundance and species richness across Mongolian aimags. Secondly, regression analyses underscored the significance of both host and climate traits in shaping flea abundance, with environmental factors primarily driving flea species richness. Thirdly, path analyses demonstrated that the variables considered account for ~ 20% of the variation in flea abundance and species richness among ground squirrel hosts. This underscores the importance of multiple axes of the ecological niche specific to each flea species in shaping community patterns, emphasizing the necessity for further research into the biology and ecological requirements of individual flea species, particularly those acting as disease vectors. Given the high prevalence of the collected fleas being associated with plague, especially with the identification of three significant plague vectors, a need exists to continue investigating patterns in flea community composition alongside relevant abiotic and biotic factors, especially since pathogen transmission relies on various factors including host preference, climate variables (i.e., temperature and humidity), flea phenology, and the feeding behavior of infected fleas (McLean et al. 2019; Medvedev et al., 2019). Further research on additional variables such as host population sizes may help to better understand and predict flea turnover and movement within and between mammal species in Mongolia, particularly in the context of known disease reservoirs such as the long-tailed ground squirrel.

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Table A1. List of all sampling localities for each of the nine sampled Mongolian aimags.

Aimag	Locality	Latitude	Longitude
Arkhangai	Zurkh Mountain	48.132	100.098
Arkhangai	Nariinii Gol, north side of Tenkiin Tsagaan Lake, Tariat Sum	48.227	99.746
Bayan-Olgii	Bulgan Soum	46.676	91.403
Bayan-Olgii	Huljaa River Valley	49.409	89.088
Bayan-Olgii	Songinot Gol	48.280	88.907
Bayan-Olgii	~35 km NE of Khar Nuur, Sagsai Sum	48.844	89.357
Bayan-Olgii	~18 km NE of Khar Nuur, Sagsai Sum	48.760	89.107
Bulgan	Chachirt River Valley	48.952	103.279
Bulgan	Hantai Mountains, north side of Burgastai River	49.793	102.801
Bulgan	Suujiyn Bulagiyn well	49.444	102.579
Govi-Altai	Altai Soum; Aj Bogd Uul, Bayan Gol.	44.833	95.272
Govi-Altai	Bugat Soum.	45.508	94.241
Govi-Altai	Gichgene Nuruu, Davaany Ovor, Emeeltseg Nuruu	45.303	97.706
Govi-Altai	Khar Azarga Nuruu, Khaalga Gol.	45.830	96.093
Hentei	Batsumber Sum, Sugnugur River	48.425	106.834
Hentei	Delgerhan Mnt. Shariin Gol	47.431	108.979
Hentei	Balj and Khumul river junction, Binder Sum	48.958	110.623
Khovsgol	Heegtsar River Valley,~ 20 km N of Chandmani-Ondor Sum	50.737	100.620
Khovsgol	Heegtsar River Valley, Chandmani-Ondor Sum	50.554	100.492
Ovorkhangai	Bat-Olzii Sum, 42 km WNW of Ulaan Tsutgalan, Orhon River Valley	46.905	102.483
Ovorkhangai	Bat-Olzii Sum, 7 km WSW of Ulaan Tsutgalan, Orhon River Valley	46.776	102.052
Ovorkhangai	Ulaan Tsutgalan, Orhon River Valley	46.817	102.010
Tov	Batshireet Sum	48.403	110.276
Tov	Bogd Uul Mtn. Shajin Huran	47.598	107.147
Uvs	5 km NW of Turgen Sum	50.171	91.453
Uvs	Han Huhnii Mountain, Baruun Turuun River Valley	49.478	94.691
Uvs	Juveriin Am, Juveriin Gol	49.872	91.206

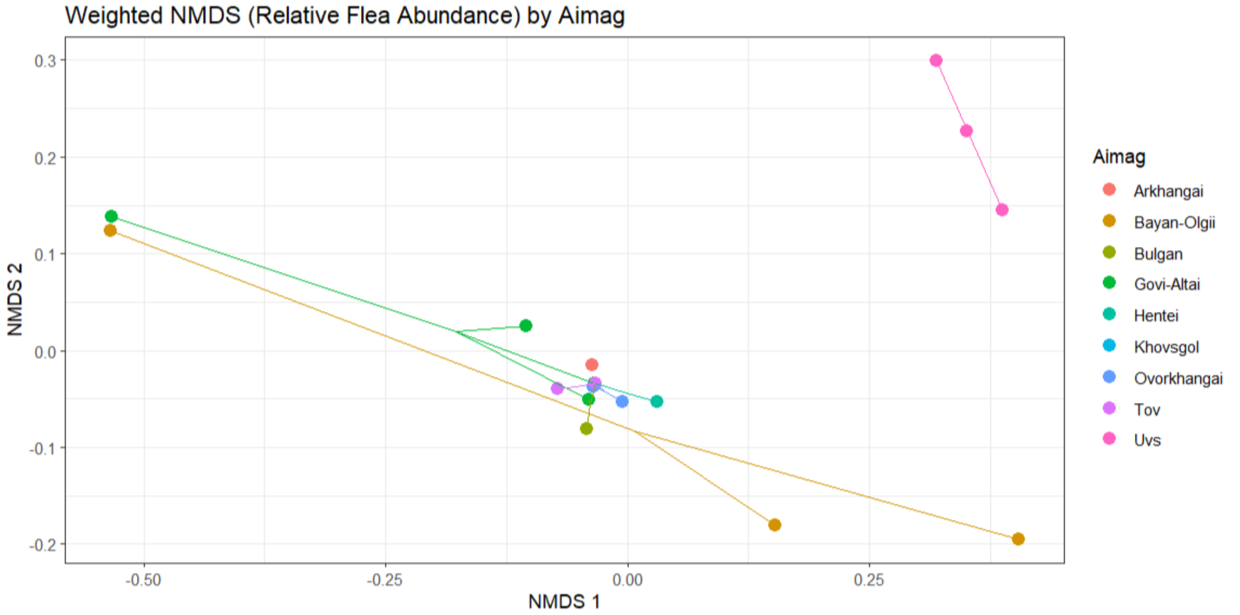
Table A2. Summary of the full models containing all fixed effects and interactions predicting flea abundance and richness on long-tailed ground squirrels. Statistically significant terms are in bold indicating p-values < 0.05.

	Flea Abundance Estimate ± S.E.	**Flea Abundance P-Value**	**Flea Richness Estimate ± S.E.**	**Flea Richness P-Value**
FIXED EFFECTS				
Host Body Size	**-0.58 ± 0.29**	**0.04**	-0.34 ± 0.23	0.13
Host Body Condition	**-0.74 ± 0.26**	**0.005**	-0.36 ± 0.21	0.09
Host Sex (Male)	**0.85 ± 0.29**	**0.004**	0.41 ± 0.27	0.12
Host Subspecies (*Undulatus*)	**1.44 ± 0.50**	**0.004**	**1.11 ± 0.43**	**0.009**
Host Freshness	-0.67 ± 0.49	0.17	-0.15 ± 0.40	0.70
Minimum Temperature	**0.63 ± 0.17**	**0.0003**	**0.54 ± 0.16**	**0.001**
Soil Moisture	-0.47 ± 0.37	0.20	-0.20 ± 0.32	0.54
PDSI Drought Index	**-0.43 ± 0.19**	**0.02**	-0.23 ± 0.18	0.20
Winter Precipitation	0.19 ± 0.20	0.32	0.26 ± 0.17	0.13
Grazing Percentage	0.25 ± 0.29	0.39	0.28 ± 0.31	0.36
INTERACTIONS				
Host Body Condition*Host Sex (male)	**0.93 ± 0.37**	**0.01**	0.45 ± 0.29	0.11
Host Size*Host Sex (Male)	0.28 ± 0.34	0.41	0.36 ± 0.28	0.20
Soil Moisture*PDSI Drought Index	**0.74 ± 0.36**	**0.04**	0.42 ± 0.29	0.15
Soil Moisture*Winter Precipitation	0.01 ± 0.20	0.93	-0.04 ± 0.18	0.80

Table A3. Summary of flea abundances and totals for each sampled Mongolian aimag.

Flea Species	Arkhangai	Bayan-Olgii	Bulgan	Govi-Altai	Hentei	Khovsgol	Ovor-khangai	Tov	Uvs
Amphalius runatus	0	1	0	0	0	0	0	0	0
Citellophilus altaicus	0	0	0	0	0	0	0	0	38
Citellophilus sungaris	20	11	17	19	38	1	54	23	0
Frontopsylla elata	0	2	0	0	0	0	1	0	2
Frontopsylla hetera	0	5	0	2	0	0	0	0	0
Megabothris rectangulatus	0	0	0	0	0	0	0	1	0
Neopsylla pleskei	0	0	1	0	4	0	3	0	0
Neopsylla teratura	0	0	0	0	1	0	0	0	0
Neopyslla mana	0	0	0	1	0	0	0	0	0
Oropsylla silantiewi	1	0	0	0	4	0	2	0	2
Paradoxopsyllus microphthalmus	0	1	0	0	0	0	0	0	0
Rhadinopsylla li	0	5	0	0	1	0	0	0	1
Total Flea Species	**2**	**6**	**2**	**3**	**5**	**1**	**4**	**2**	**4**
Total Flea Individuals	**21**	**25**	**18**	**22**	**48**	**1**	**60**	**24**	**43**

Figure A1. Weighted NMDS plots grouped by aimag (top) and host subspecies (bottom).



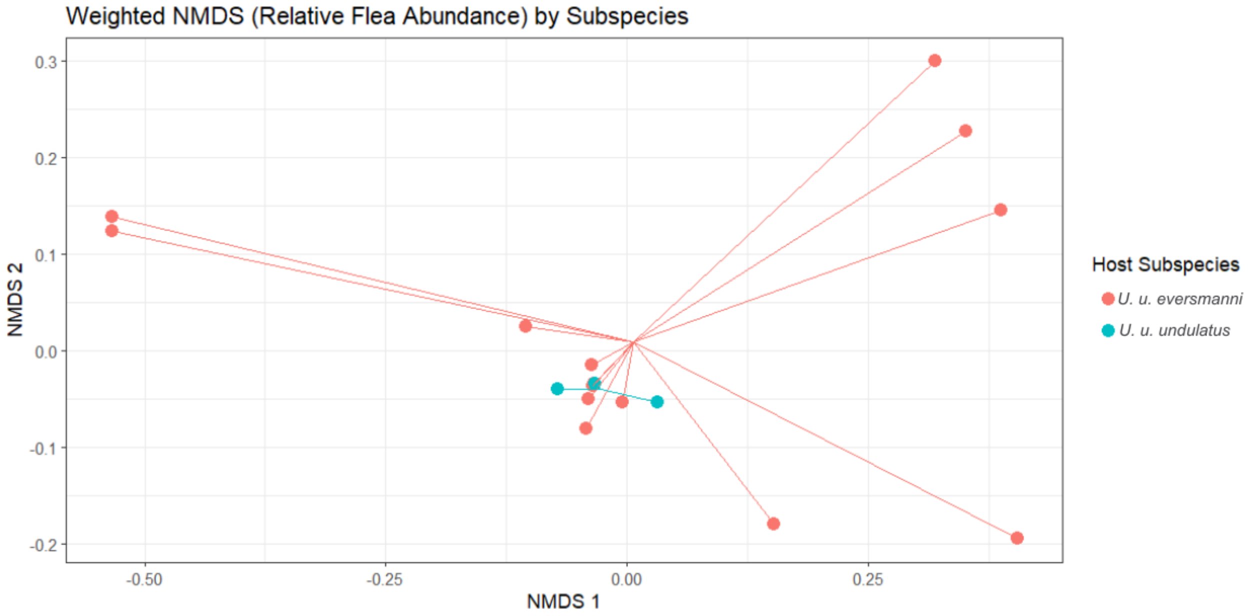


Figure A2. QQ plot residuals and residual vs. predicted plots for the optimal GLMM flea abundance model.

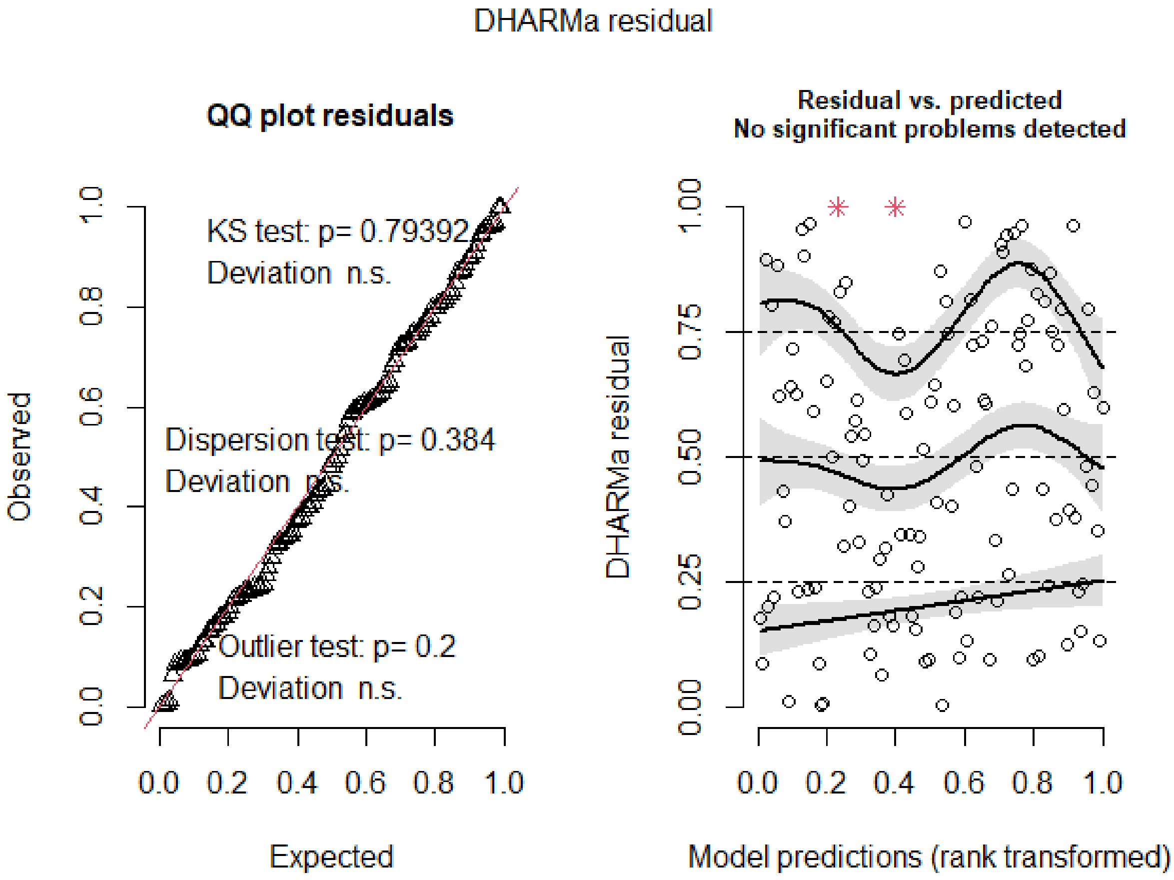


Figure A3. QQ plot residuals and residual vs. predicted plots for the optimal GLMM flea richness model.