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Small mammal and ectoparasite community variation and abundance is important for monitoring the transmission rate of zoonotic diseases and informing conservation efforts that maintain host and parasite biodiversity in ecosystems facing global climate change. The purpose of this study was to identify the factors driving variation in small mammal and ectoparasite communities in the Southern Appalachian Mountains. I took an approach to sampling that allowed me to test predictions from island biogeography theory; namely, that host species richness varies with distance from the main Appalachian mountain range. I also examined how ectoparasite species richness varied with small mammal richness as well as ecological variables. Finally, I analyzed ectoparasite abundances at the community- and individual-host levels to understand how changes in host species richness may affect infestation rates. Comprehensive field surveys and ectoparasite screenings were performed across four field sites, two isolated from the Southern Appalachian Mountains and two along the Southern Appalachian Mountains. I found that these field sites were characterized by a mix of high and low elevation mammal species, and that community structure varied with degree of isolation for mammals, but not ectoparasites. Habitat type was a significant driver of species variation within and among sites. I found decreased abundances in ectoparasite compound communities when host species diversity was highest, which is consistent with predictions from a dilution effect. However, when evaluating abundances of individual ectoparasites, only one (*Leptotrombidium peromysci*) of four species displayed patterns consistent a dilution effect. My results provide new information on small mammal distributions and ectoparasite associations at disparate sites across the foothills of the Southern Appalachians but suggest that host-parasite associations and the intensity of infection are subject to additional environmental or ecological drivers beyond those investigated here.

SPATIAL VARIATION IN MAMMAL AND ECTOPARASITE COMMUNITIES IN THE FOOTHILLS ALONG THE SOUTHERN APPALCHIAN MOUNTAINS

by

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CHAPTER I: INTRODUCTION

Global temperatures have increased by 0.18°C (0.32°F) since 1981, and they are predicted to continue to increase at a rapid rate (Root et al. 2003, Dahlman 2020). Many biological systems and processes are already showing significant change in response to global warming (Moritz et al. 2008, Warren et al. 2010, Löffler et al. 2011). Parasites are major components of many ecological food webs (Lafferty et al. 2006), but the structure of host-parasite networks and the causes of their variation through space and time are understudied in most ecosystems. This lack of baseline data hinders our ability to determine global change effects on parasite biodiversity, as well as entire ecosystem processes. For example, temperature shifts are expected to alter the distribution and ecology of parasites, including their life cycles, transmission rates, phenology, and encounter rates with novel or naïve hosts (Poulin 2006, Polley and Thompson 2009, Polley et al. 2010). Therefore, expanded monitoring efforts are critical for obtaining more knowledge about parasites, their persistence on the landscape, and their ability to impact future wildlife dynamics under altered climate regimes (Brooks and Hoberg 2007, Polley and Thompson 2009).

A parasitic interaction is defined as an organism (a parasite) living off another organism (the host), harming it and possibly causing death (Poulin, 2007). Hosts typically provide the parasite with nutrients to be able to live and reproduce, either on the outside (ectoparasites) or inside (endoparasites) of the body (McDonald et al 1989). Importantly, some ectoparasites are vectors of pathogens that can cause zoonotic diseases (e.g., viruses, bacterial, parasites, fungi that spread between vertebrate animals to humans), indirectly transmitting these to terminal hosts (Brazier, 2018, World Health Organization, 2020). In the Southeastern U.S. alone, numerous parasite vectors occur including the Blacklegged or Deer tick (*Ixodes scapularis*), which is known for transmitting Lyme disease spirochete, *Borrelia burgdorferi*, a cause of potentially chronic health issues. Deer ticks contract *B. burgdorferi* through bloodmeals (horizontal transmission) and can subsequently pass it to hosts (Schwan and Piesman 2002, Kurokawa et al. 2020). Similarly, the

American dog tick (*Dermacentor variabilis*) uses horizonal transmission and a blood meal to transmit the causative agent of Rocky Mountain Spotted Fever, the bacterium *Rickettsia rickettsia*, a disease that is serious or deadly in humans if not treated early (CDC, 2020). American dog ticks are found throughout the Southeastern U.S. and are common in North Carolina, Tennessee, Missouri, Arkansas, and Oklahoma (CDC, 2020). Other ectoparasitic vectors include lice (Order Phthiraptera; Smith et al. 1997) and fleas (Order Siphonaptera; Bitam et al. 2010). For example, fleas play a role in transmission of *Bartonella* in rodents (Gutiérrez et al. 2015). Lice (*Ediculus humanus corporis*) can similarly transmit diseases including Epidemic Typhus, which is caused by the bacteria *Rickettsia powasekii* which it contracts from infected southern flying squirrels (*Glaucomys volans*). When asymptomatic and untreated, the bacterium *R. powasekii* can lead Brill-Zinsser disease in humans (CDC, 2020).

Drivers of Ectoparasite Distribution and Abundance

Parasite communities are typically characterized at 3 different levels: infracommunity (all of the parasites within a single individual host), component community (all of the parasites within a monospecific population of hosts), and compound community or supracommunity (all of the parasites within individuals of all host species in an ecosystem; Dove, 2006). Understanding how increasing temperature and disrupted ecological processes will affect the diversity and abundance of ectoparasites at each of these levels is critical for developing a better understanding on the potential contributors to zoonotic disease transmission, which remains a critical goal in biology (CDC, 2017). To accomplish this, it is important to quantify geographic distributions of individual parasite species that can spread these diseases and understand how composition of entire parasite communities is modulated by environmental variables as well as distributions of their hosts.

Two central factors for determining parasite occurrence and density (e.g., per host individual) are the identity and abundance of host species. For example, high host population densities can cause transmission rates of parasites to also increase (Krasnov et al. 2007). Conversely, the dilution effect predicts that pathogen transmission rates decrease with increasing host species richness (Gibson and Nguyen 2020). This may occur because a high proportion of host species may be suboptimal reservoirs for maintaining the disease agent (Schmidt and Ostfeld 2001, Civitello et al. 2015, Halliday et al. 2020). Thus, a stronger dilution effect is expected when there is an abundance of poorly competent reservoir species, effectively lowering the probability of pathogen transmission for any given bite from a vector (Schmidt and Ostfeld 2001, Johnson and Thieltges 2010). However, the dilution effect has inconsistent support to date, and within ectoparasite systems it has not been well studied. Krasnov et al. (2007) studied infestation rates of *Ixodid* ticks (*Ixodes rincinus* and *I. trianguliceps*) in small mammals and found a dilution effect in the generalist *I. ricinus* but not for the specialist *I. trianguliceps* (Krasnov et al. 2007). More broadly, Civitello et al. (2015) performed a metanalysis and provided evidence that host diversity can indeed inhibit parasite abundance per individual host, but also demonstrated variation in the magnitude of dilution when comparing across parasite type, life cycle, functional group, and level of specialization (Civitello et al. 2015).

Montane Ecosystems as Natural Experiments in Parasitology

Montane systems provide a potentially fruitful model for understanding linkages between host communities, parasite communities, and effects of global climate change. In temperate North America, many montane regions at southern latitudes contain boreal communities that are comprised of species often associated with higher latitudes (called “sky islands”). These communities can be shaped by complex processes of colonization and extinction, and their patterns of species diversity have sometimes been viewed in the context of island biogeography theory (MacArthur and Wilson 1967, Brown 1971). Sky islands are defined as “isolated

mountain surrounded by radically different environments" (Browne and Ferree 2007). Island biogeography theory predicts that diversity within "sky islands" reflects a balance between colonization and extinction. More isolated islands are predicted to have lower species richness than islands closer to the "mainland" due to lower colonization rates (MacArthur and Wilson 1967, Bierregaard and Zimmerman 1986).

Naturally occurring differences in habitat isolation levels may provide a "natural experiment" for studying how parasite diversity and infestation rates relate to the spatial and host setting in mountain systems. Indeed, the distribution and abundance of parasites and pathogens in montane systems is generally expected to mirror biogeographic patterns observed in hosts. A study by Williamson et al. (2018) focused on avian haemosporidian parasites and their variation with respect to species composition and abundance across eight sky islands in the southwestern United States, within a single host species (Audubon's Warbler; *Setophaga audubonii*). They found that parasite turnover was three-fold higher than bird community turnover and could be predicted by elevation, climate and host composition, implying that hosts as well as environment play a role in determining which parasite species will be present and abundant in a community (Williams et al. 2018).

Additional studies like the one above are critical as montane vertebrate communities shift and come into contact with those associated with lower elevations (Rickart 2001, Chen et al. 2011). At southern latitudes in particular, montane-associated boreal communities may be especially sensitive to global climate change because they often exist on the southern periphery of species' ranges, such as in the Southern Appalachians or ranges of the Great Basin or desert Southwest (Allen and Lendemer 2016). If these systems are operating in accordance with island biogeographic theory, one potential outcome is reduction in boreal habitat area and increase in isolation which can lead to reduced species richness via extinction (McCain and Grytnes 2010). Reduced species richness may occur due to a lack of gene flow, limited access to necessary

resources (Himes and Kenagy 2010), competition from lower-elevation species, and other altered landscape ecological processes. Therefore, focusing monitoring effort on intermediate elevations where high- and low-elevations communities may begin to interface with one another is an important component of monitoring efforts.

STUDY SYSTEM

The Southern Appalachians are an extremely biodiverse mountain range located in the southeastern United States (Simon et al. 2005), stretching across Tennessee, Virginia, North Carolina, South Carolina, and Georgia. This project focused on the Southern Appalachian Mountains of North Carolina and a small portion in northeastern Georgia; these mountains span a large elevational gradient up to the highest peak east of the Mississippi River (Mount Mitchell, 2037.3 m). North Carolina has additional high peaks such as Grandfather Mountain (1,818 m), Albert Mountain (1,592 m), and Looking Glass (1,210 m) that provide habitat for a diverse mix of plants and animals (Peakbagger, 2020).

For example, the Southern Appalachians are home to some of the most extensive broad-leaved deciduous forest in North America and nearly 2,000 plant species, 200 of which are endemics (Muir, 2018). The region also harbors more than 460 species of animals (although the actual number may be as high as 800; Muir (2018)). Importantly, this includes boreal species that are typical of the Northeastern United States and Canada, which exist only at high elevations in North Carolina. For mammals, these include the Northern flying squirrel (*Glaucomys sabrinus coloratus*; Loeb et al. 2000), Masked shrew (*Sorex cinereus*), Smoky shrew (*Sorex fumeus*), Water shrew (*Sorex palustris*), Rock shrew (*Sorex dispar*), Woodland jumping mouse (*Napaeozapus insignis*), Northern short-tailed shrew (*Blarina brevicauda*), and the Southern red-

backed vole (*Myodes gapperi*; Ford et al. 1999). As climates warm, these communities could come in contact with species typical of the foothills and Piedmont including the White-footed mouse (*Peromyscus leucopus)*, Southern short-tailed shrew *(Blarina carolinensis),* Meadow vole (*Microtus pennsylvanicus*), and Eastern woodrat (*Neotoma floridana*; LeGrand et al. 2021).

Insufficient research has been done in the Southern Appalachians and surrounding foothills to determine how changing climates may be altering small mammal and ectoparasite distributions and the structure of mammal-parasite networks (i.e., the sum of interactions between parasites and hosts). Many ectoparasites have been documented in the mountains of North Carolina to date, including focused studies on fur mites (*Myobia* spp., *Haemogamaus ambulans, Protomyobia* spp*., Xenoryctes nudus, and Myonyssus jamesoni*; Owen, 1984)*,* botflies *(Cuterebra fontinella*; e.g., Lackey et al. 1985), and numerous species of ticks, fleas, and lice (McCay and Durden, 1996; Madhav et al. 2004). However, these data are limited in both space and time, and have rarely been considered in both ecological and host community contexts. For example, previous studies in other montane systems suggest that mammal communities at higher and lower elevations are usually less diverse compared to the intermediate elevations where communities meet (McCain, 2003) and potentially share parasites and pathogens.

The purpose of my research was to provide more comprehensive knowledge on small mammal and ectoparasite communities at intermediate elevations in the foothills of the Southern Appalachians. Specifically, I wanted to evaluate how the degree of isolation of montane ecosystems affects the diversity and abundance of mammalian hosts and the ectoparasites they carry. To do that, I studied mammalian and ectoparasite diversity in two montane sites which are part of the main Appalachian mountain chain ("mainland" sites) and two isolated sites located further from the Appalachians. My specific aims were to:

(1) Evaluate the effect of montane habitat isolation on mammalian species richness. **I hypothesized that mammal species richness and community composition will vary**

with distance from the main Appalachian cordillera. Based on island biogeography theory, I specifically predicted a negative relationship between species richness and distance from the Appalachians. To test this, I performed field and laboratory research focused on characterizing variation in small mammal community richness using comprehensive field sampling and DNA barcoding.

(2) Evaluate the effect of montane habitat isolation on ectoparasite distributions. **I hypothesized that ectoparasite species richness and community composition will vary with distance from the Appalachian cordillera.** Based on island biogeography theory and the intimate host associations of many ectoparasites, I predict that more depauperate small mammal communities will harbor fewer ectoparasite species. To test this, I performed screens of all small mammals captured in the field to sample and identified entire ectoparasite communities to the species level.

(3) Evaluate the relationship between host species richness and ectoparasite abundances. **I hypothesize that ectoparasite abundances will be altered by variation in species richness of host communities.** Specifically, based on the dilution effect, I predict that sites with relatively low host species richness (potentially, those more isolated from the Appalachian cordillera) will have higher ectoparasite loads per individual mammal host, and vice versa.

CHAPTER II: METHODS

FIELD SAMPLING

My research focused largely on compound communities of ectoparasites. A total of four sites along the foothills of the Southern Appalachian Mountains (ranging in elevation from 275 to 952m) were chosen for field surveys; Chattahoochee National Forest (Georgia), Cold Mountain Game Land, South Mountains Game Land, and Green River Game Land (all North Carolina; Table 1, Figure 1). All sites lie between the Piedmont and the high peaks of the Southern Appalachian Mountains, but they are isolated to varying extents from the main cordillera. Each site was visited twice within the time frame of June-September 2020 for 6 nights total (3 nights each trip), except for Chattahoochee-Oconee National Forest which was visited once for 6 consecutive nights in August 2020.

At each site, small mammals and associated ectoparasites (ticks, lice, fleas, and mites) were sampled for between 2,721-2,850 total trap nights (Table 1). Trap nights were calculated as the total number of traps used per night multiplied by the total number of nights spent at a field site. I used two trap types in each habitat class at each site: Sherman live traps and pitfall traps (40 oz plastic cups buried flush with the ground). In addition, I used snap traps on a single transect at a single site (Chattahoochee). Traps were placed using a transect method in the two major habitat classes present in this system: mature forest and meadow. These habitats were trapped roughly in proportion to their abundance on the landscape, with a majority of traps at each site being within mature forest (the predominant habitat), One exception to this was at Cold Mountain Game Land, where presence of maintained meadow habitats led to 50 more traps being used in meadow than forest. Across all sites, there were 19 total transects within meadows (ranging from 30-60 traps per transect) and 28 total transects in forests (ranging from 20-80 traps per transect). Traps

were opened at dusk and closed at dawn to minimize the time captured individuals were in traps, to enhance animal well-being, and to maximize ectoparasite retention.

Captured small mammals were anesthetized using an isoflurane vapor method, then screened for ectoparasites (see below). Standard field measurements were taken on captured mammals (lengths and weight) as well as sex, external reproductive status, and age class (based on pelage and external traits). A portion of the small mammal specimens were preserved as voucher specimens with associated tissue samples, providing a clear record of the species caught and allowing future researchers the opportunity to verify my findings. To avoid replication in non-vouchered individuals, and to provide material for DNA barcoding, an ear punch was collected from the right ear and preserved in 95% ethanol. Recaptured individuals received a second ear punch in the left ear to avoid replicate ectoparasite screening. If an individual was trapped and had two ear punches it was released and not re-screened. Animals not collected as voucher specimens were released unharmed on the trap site at which they were caught. All small mammal capture and handling protocols followed guidelines of the American Society of Mammalogists Animal Care and Use Committee (Sikes et al. 2016) and were conducted under approved IACUC protocols (UNCG #20-008).

To screen anesthetized individuals for ectoparasites, I combed through the fur with a flea/louse comb to remove parasites. I also used the back of fine tipped forceps to dislodge ectoparasites that did not come off while being brushed. Parasites were collected into a white container for optimal contrast and visualization. All ectoparasite specimens collected off each individual were preserved as a single lot in a 75% ethanol and then sorted taxonomically by Order in the lab under a dissection microscope.

IDENTIFICATION OF HOST AND PARASITE SPECIES

To obtain species identifications for mammal taxa hard to distinguish in the field, I used DNA barcoding at a single mitochondrial (mtDNA) locus (Johns and Avise, 1998) – cytochrome b (cyt-b). *Peromyscus* and *Blarina* were the two focal genera for DNA barcoding, as each is potentially represented by two species at our mid-elevation field sites (*P. leucopus, P. maniculatus*; *B. brevicauda*, *B. carolinensis*). However, some individuals of additional species were also barcoded to confirm my field identifications.

To perform DNA extraction, an ear punch or a subsample of liver tissue (for voucher specimens only; roughly 10mg) was placed into an Eppendorf tube labeled with a unique UNCG tissue ID (GT number). DNA extractions were performed using a Purelink Genomic DNA kit (ThermoFisher Scientific) following manufacturer's instructions. Following DNA extraction, standard polymerase chain reaction (PCR) amplifications were done in 25µl volumes using a combination of the primers MSB05 and MSB14 (Hope et al. 2014) on a Veriti PCR thermocycler (Applied Biosystems). Cycling parameters were identical for all samples and consisted of the following: initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation (94°C for 15 seconds), annealing (51°C for 20 seconds), and extension (72°C for 1 minute); and a final extension step of 72°C for 5 minutes. PCR products were quantified fluorometrically using a Qubit 4.0.

In preparation for sequencing, two 96 well plates were filled with 10µl of PCR products and cleaned using EXOSAP-IT and sequenced in forward and reverse directions on an ABI 3730 sequencer at the NC State University Genomic Sciences Laboratory (Raleigh, NC, USA). Resulting chromatograms were manually edited and aligned using the software Genious 2021.0.3. The BLAST plugin in Genious was used to determine species identifications.

Specifically, determinations were based off the match that had the highest percent identity to samples in the NCBI database. I used a minimum percent identity threshold to determine species identification of 90%, although all full cyt-b sequences had between 97-99% similarity. Because smaller fragments led to lower percent similarities with GenBank entries, I also considered these and assigned identities in light of other mammal species barcoded from the same site. The smallest cyt-b fragment I sequenced was 574 bp and this had a 75% similarity to sequences in GenBank.

All ectoparasites collected as part of this work were identified to species and age class using field guides and expert taxonomist identifications (Dr. Lance Durden, Georgia Southern University).

COMMUNITY ANALYSES

I used a combination of community ecology and ecological network analyses to compare small mammal and ectoparasite diversity and abundance within and among the four sites. My analyses employed a mix of classical statistical models of community composition data, including restricted and unrestricted multivariate analysis of site-by-species abundance tables with spatial pattern analysis (Dray et al. 2012).

First, species richness was used to quantify the total number of the species in a community at each site, utilizing DNA barcode data when available (for mammals only). I also computed Bray-Curtis dissimilarities for mammal and ectoparasite communities, as the sample size was large enough to compare the four field sites and their relative abundance of ectoparasites (Ricotta and Podani 2017). Bray-Curtis dissimilarities are obtained by calculating a distance matrix, selecting

two reference points, and projecting all the samples onto the axis by their relationships based on the two reference points that were selected (Beals, 1984). The response data for both mammals and parasites were scaled total richness. For small mammal communities, this was the total number of species per site by the total number of individuals captured per site. For ectoparasite communities, this was total number of ectoparasite species scaled by total number of host individuals per site. I performed a nonmetric multidimensional scaling (NMDS) analysis on dissimilarity matrices to compare each community type in multivariate space. I also repeated all of these tests for ectoparasite component communities found on the best-represented host species (*P. leucopus*). All analyses were performed using the vegan package v. 2.57 (Oksanen et al. 2020) in R (R Core Team, 2013). I also used functions in ggplot2 v. 3.3.3 to visualize the data. Due to overlap on some plots, I used the geom_jitter plotting function to allow better visualization.

A series of more in-depth statistical tests was performed to test potential drivers of community diversity and structure. First, I tested whether small mammal and ectoparasite communities varied among mid-elevation sites. Second, I tested whether additional ecological characteristics influenced community structure; specifically, degree of isolation from the Appalachians (binary; mainland or isolated) and habitat type (meadow vs. forest). For each test, I used a PERMANOVA to test differences between group means accounting between the structure of data. The PERMANOVA was implemented in the function adonis2 in the vegan package v. 2.16 (Oksanen et al. 2020) in R.

NETWORK ANALYSES

Network ecology investigates the structure, function, and evolution of ecological systems at different scales with the use of metrics that quantify interaction networks. Network models can increase understanding of ecosystem-level phenomena by illuminating complex, emergent properties of networks such the role of a particular species or a trait in structuring communities and maintaining long-term species interactions (Lau et al. 2017). Previous studies have used similar methods to reveal important aspects of host-parasite community structure (Wells et al. 2011, Esser et al. 2016).

I first constructed a bipartite ecological network for all sites combined using the bipartite v. 2.16 package (Dormann et al. 2008) in R. This allowed me to visualize connections within the entire host-parasite assemblage and to quantify complexity of host-parasite associations. To construct this network, small mammal and ectoparasite associations were tabulated and represented as both presence/absence and as scaled abundances (described above). The former was the input for an unweighted analysis, and the latter was the input for a weighted analysis. Based on PERMANOVA results (see *Community analyses* above) and the differences observed among habitat types, I also performed these workflows to construct meadow and forest networks.

To further quantify network properties among ecological categories, I used the metrics of links per species, interaction strength and connectance (Dormann et al. 2009). Connectance is defined as the realized proportion of possible links, and links per species describes the average amount of ectoparasites associated with an individual host (Doormann et al. 2009). Finally, interaction strengths quantify the imbalance between the interaction strength of a species pair (Doormann et al. 2009). Connectance and links per species were calculated only from the unweighted data, and interaction strength was calculated from the weighted data.

Tests of Differences in Infestation Rate

To understand how site- and host-based metrics influenced ectoparasite loads on small mammals, a series of statistical tests was performed. Due to the data being non-normally distributed, a Kruskal-Wallis was used to test for effects of field site, degree of isolation, and habitat type (meadow and forest) on ectoparasite densities for the whole small mammal community, as well as every individual *P. leucopus*. Kruskal-Wallis tests evaluate whether the medians of two or more groups are different (Stephanie, 2016), and these tests were performed using the kruskal.test function in stats v. 3.6.1 (Wickham et al. 2020) in R. Subsequently, a pairwise Mann-Whitney U test was performed to identify statistically significant differences in ectoparasite loads. The Mann-Whitney U tests for whether two samples are likely to derive from some population (LaMorte, 2017), and I ran these tests using function wilcox.test in the R package stats version 3.6.1 (Wickham et al. 2020). To visualize differences in total ectoparasite load, I used boxplots of ectoparasite communities found on *P. leucopus* captured in each habitat type (meadow vs forest) within the four field sites. In this case, a single extreme outlier (87 ectoparasites on an individual) was removed to aid in visualization of more subtle differences.

Finally, as a more precise test of whether infestation rates decreased with increasing host species richness and were thus consistent with a dilution effect, I examined abundances of single ectoparasite species. To do this, I plotted abundances for four ectoparasite species that were present at each of the four sites against the small mammal species richness observed at a site-wise basis.

CHAPTER III: RESULTS

FIELD RESULTS AND TAXONOMIC IDENTIFICATION

Field data collection occurred between 15 June and 1 September 2020, including a total of 7 expeditions and 11,211 trapnights (Table 1). Ratios of traps in forest versus meadow habitats were 730/140 (Chattahoochee-Oconee National Forest), 450/500 (Cold Mountain Game Land, 730/560 (Green River Game Land), and 710/245 (South Mountain Game Land). Chattahoochee-Oconee National Forest had an average temperature of 29°C for the entire trapping period; the first portion of the trip was sunny ending with a significant amount of rainfall. Cold Mountain Game Land had average temperatures of 26°C in the June session and 28°C in the August session. The average temperatures for Green River Game Land were 25°C (with moderate rainfall) in the June session and 28°C in the August session. South Mountain Game Land average temperatures were 20°C (with substantial rainfall) in the June session, and 31°C for the August session (Table 1).

Overall trap success (total number of captures divided by the total number of trapnights) was low, and variable among field sites. Trap success at Chattahoochee-Oconee National Forest was lowest, at just 0.9%. Cold Mountain, Green River, and South Mountains Game Lands had trap successes of 1.6%, 2.1%, and 2.7%, respectively. Combined trap success across all sites was 1.8%. Between the four sites, a total of 205 specimens were either captured and released, or preserved as voucher specimens. Eleven different small mammal species (Table 2) were collected in total. *P. leucopus* was the most abundant species captured from all four field sites (140 out of 205 captures). Ninety-six small mammal individuals were DNA-barcoded to confirm

field identifications; 36 were identified from full cyt-b sequences and the remainder with partial fragments (minimum size 574bp). The percent identities in BLAST searches ranged from 90% to 99% with the exception being the smallest fragment (547bp) which had a percent identity of 74%.

Twenty-two different species of ectoparasites (Table 3) were collected in total. The most abundant ectoparasites were Acariformes (mites) and Trombidiformes (chiggers) and the least abundant were Ixodida (ticks). All ectoparasites were identified to species and life stage with 100% identification success.

MAMMAL COMMUNITY COMPARISON

The composition of small mammal communities across the four field sites included a mix of high- and low-elevation species (Figure 2). Species characteristic of lower elevations were *O. nuttalli* (Keller et al. 2003) and *Sigmodon hispidus* (Dunnum et al. 2002, Webster et al. 2004, LeGrand et al. 2021) and those characteristic of higher elevations were *B. brevicauda* (Hess, 2016), *S. cinereus* (LeGrand et al. 2021), *S. cooperi* (Linzey, 1984), *N. insignis* (Harrington, 2004), and *Z. hudsonius* (Whitaker, 1972). *P. leucopus* and *S. hispidus* were the two most dominant species collected (166 combined individuals, 80% of the total captures).

The NMDS for small mammal communities (Figure 3a) displays variation among sites, but also within-site variation between two habitat types. Meadow habitats at South Mountain Game Land and Green River Game Land had the most similar small mammal communities; these general field sites were also the closest to one another spatially. The highest dissimilarity was found

between meadow habitats at Chattahoochee-Oconee and Cold Mountain; these were over double the dissimilarity of the former comparison. Considering within-site patterns, meadow and forest at Cold Mountain Game Land had the most similar mammal communities, while meadow and forest at South Mountains Game Land had the most dissimilar communities within sites. A PERMANOVA of the mammal community did not recover an effect of site on small mammal community composition. However, I did find an effect of both isolation ($P = 0.04$) and habitat type ($P < 0.01$) as drivers of the small mammal communities (Table 4).

ECTOPARASITE COMMUNITY COMPARISON

The twenty-two different ectoparasite species collected (Table 3) varied in abundance among sites (Figure 4). Ectoparasite community variation as visualized by NMDS (Figure 3b) revealed that, as with mammal communities, ectoparasite communities in meadow habitats at Chattahoochee-Oconee National Forest and Green River Game Land were the most dissimilar, with more than double the distance in community composition as compared to meadows at South Mountain Game Land and Green River Game Land. Conversely, forest habitats at Chattahoochee-Oconee and South Mountain were the most similar in ectoparasite community composition, which is opposite the trends for meadow habitats but similar to the small mammal community comparison (Figure 3a, b).

Structural differences in species composition among ectoparasite communities are as follows. The lice *Hoplopleura hesperomydis* and *H. hirsuta* were only collected from South Mountain and Green River Game Lands, and thus could be major contributors to the similarity between those two field sites (Figure 3b). *H. hirsuta* in particular is a specialist to *S. hispidus*, which was collected in abundance (Table 2) from both sites. Conversely, *Comatacarus americanus*

(Trombidiformes) was only collected from Chattahoochee National Forest and was one contributor to the dissimilarity of this site relative to South Mountain and Green River Game Lands (which lacked *C. americanus*). Further, the sucking louse *H. erratica* was only encountered on a single *T. striatus* collected from Cold Mountain Game Land, which may have contributed to the uniqueness of ectoparasite communities at this site.

Despite the above levels of variation, a PERMANOVA did not support field site as having a significant effect on the ectoparasite community data. Among ecological variables, isolation also had no effect on community composition, but habitat type trended towards an effect ($P = 0.09$; Table 5).

FOOTHILLS HOST-ECTOPARASITE NETWORK

My finding that there were no significant effects of site on mammal or ectoparasite communities supported use of a bipartite network analysis for the combined data, based on all small mammal species that hosted ectoparasites (9 of 11 total species captured). In the unweighted network analysis, input data are binary (0 for no association, 1 for association) and all links in the graph are the same width. This network showed that *P. leucopus* had the most diverse ectoparasite load, with 11 different ectoparasite species hosted (Db, Ol, Af, Ew, Is, Eu, Hhe, Lp, Ph, Dv, Gh; see Table 3 for species codes). Conversely, two hosts (*Z. hudsonius* and *T. striatus*) only had one ectoparasite species collected from them; *Z. hudsonius* hosted *Glycyphagus hypudaei* and *T. striatus* hosted *Hoplopleura erratica.* I calculated the indices of connectance and links per species from this unweighted network. Connectance among all hosts and ectoparasites was 0.18, meaning the realized proportion of associations was 0.18 ectoparasites cooccurring with the 9

different hosts. Links per species was calculated as 1.19, indicating an average number of 1.19 ectoparasite species associated with each individual host species.

Since habitat was a contributing factor for mammal community structure, and a marginal (although non-significant) effect for parasite communities, I constructed a similar unweighted network for each of the two habitat types (meadow and forest). In the meadow network, there were 6 small mammal hosts and 14 ectoparasite species. *P. leucopus* and *S. hispidus* were the most infected hosts in meadows; both had 6 different ectoparasites collected off of them (*P. leucopus* hosted Ph, Dv, Hhe, Lp, Ol, and Af; *S. hispidus* hosted Ol, Af Hhi, Cp, Pb, and Pg). *Z. hudsonius* and *B. brevicauda* were the least infected hosts with only 1 ectoparasite species (*Z. hudsonius* hosted *G. hypudaei*; *B. brevicauda* hosted *Doraptopsylla blarinae*). In the meadow network, connectance was 0.226 and links per species was 0.95.

Similar to the meadow, the forest network contained 6 small mammal hosts, but a larger diversity of ectoparasite species (17). *P. leucopus* was again the most infected host, having 10 different ectoparasite-host interactions, and *N. insignis and T. striatus* had the fewest ectoparasite-host interactions, at 1 per species. The 10 different ectoparasites found on *P. leucopus* were Db, Ph, Eb, Ew, Is, Hhi, Dv, Lp, Ol, Af, and Gh. *N. insignis* hosted 1 *G. hypudaei*, and *T. striatus* hosted only *H. erratica.* In the forest network, connectance was 0.245 and links per species was 1.09, both of which were higher than in the meadow network.

WEIGHTED HOST-PARASITE NETWORKS

To assess differences in intensity of ectoparasite-host interaction across sites, a weighted bipartite network analysis was also performed, wherein wider edges indicate stronger ectoparasite-host interaction compared to narrower edges (Figure 5a. The two species that had the highest diversity of ectoparasites were *P. leucopus* and *S. hispidus* (11 and 6 interactions, respectively). The strongest interaction (7) was between the southern bog lemming (*Synaptomys cooperi*) and the mite *Laelaps alaskensis* (La), but I note that only 1 *S. cooperi* was collected in my study. *B. brevicauda* and *O. nuttalli* also had relatively strong interactions; *B. brevicauda* was a host of 5 different ectoparasites but the strongest interactions were with *C. americanus* and *D. blarinae*. *O. nuttalli* had one less interaction than *B. brevicauda*, and its strongest interaction was with the mite *A. fahrenholzi*.

Like unweighted analyses above, I computed weighted networks for each habitat type (Figure 5b, c). The mammals *Z. hudsonius, S. cooperi*, and *S. hispidus* were only collected in the meadow habitat, where the latter two hosts had the strongest ectoparasite-host interactions; *S. cooperi* again displayed a strong association with *L. alaskensis*, and *S. hispidus* displayed a strong association with *A. fahrenholzi*. *S. hispidus* was also the only host to carry the sucking louse *H. hirsuta*. Within the meadow there was a relatively low interaction strength of -0.224.

Figure 5c displays the weighted network for the forest habitat. The ectoparasites *D. blarinae, A. fahrenholzi, G. hypudaei, L. peromysci,* and *O. leucopus* had at least 2 or more host interactions in this network. *P. leucopus, B. brevicauda, N. insignis,* and *T. striatus* had the strongest ectoparasite-host interactions. *P. leucopus* had 11 different ectoparasite interactions; the strongest was with *L. peromysci*. *B. brevicauda* had 5 ectoparasites interactions; the strongest were with *C. americanus* and *D. blarinae*. *N. insignis* and *T. striatus* (the latter was represented by a single host individual) had the two strongest ectoparasite-host interactions, with *G. hypudaei* and *H. erratica*, respectively. The strength of ectoparasite-host interactions was much higher in forests (0.104) than in meadows.

ECTOPARASITE LOADS OF SMALL MAMMALS

Infestation rate was first measured using abundances across the component parasite community; the unit of analysis was the total number of ectoparasites collected off all host individuals, scaled by the total number of small mammals captured at each site (or habitat type). A Kruskal-Wallis test revealed that site did not play a role in combined ectoparasite loads on the small mammal community ($P = 0.32$). The same test assessing whether ecological variables contributed to ectoparasite loads recovered no statistical evidence that degree of isolation contributed to ectoparasite abundance ($P = 0.44$), but again there was a marginally significant trend for habitat ($P = 0.09$; Table 6). A post-hoc, pairwise Mann-Whitney U test did not recover any differences among sites for scaled ectoparasite loads (Table 7).

As *P. leucopus* was by far the most abundant species collected, I performed the same workflow to assess how site, habitat type, and degree of isolation influenced ectoparasite load on this species (Table 6). For *P. leucopus* individuals there was a strong effect of site on parasite load ($P = 0.0003$). South Mountain Game Land had the highest ectoparasite abundance (239 total ectoparasites collected off *P. leucopus*) and Cold Mountain Game Land had the lowest (28 total ectoparasites). There was no statistical effect of isolation, but, unlike for all small mammals, there was a statistically significant effect for *P. leucopus* ($P = 0.04$). *P. leucopus* from forests had higher ectoparasite richness and abundance (17 ectoparasite species and 475 in total collected; mean of 4.20 ectoparasites per individual) compared to those in the meadows (14 ectoparasite species and 18 in total collected; mean of 0.67 ectoparasites per individual; Figure 6). However, I note a higher number of *P. leucopus* individuals were captured within the forest, which could contribute to these differences among habitat types.

To assess if abundances of individual ectoparasites varied with mammal species richness, four ectoparasite species that were each collected at all four field sites (*L. peromysci, G. hypudaei, O. leucopus, and A. fahrenholzi*) were analyzed using a Kruskal-Wallis test, both for the whole mammal community and those found on *P. leucopus* (Table 8). Site had an effect on abundance only of *L. peromysci* ($P << 0.01$). Degree of isolation contributed to the abundance of *G. hypudaei* ($P = 0.05$) and *A. fahrenholzi* ($P = 0.03$), but not *L. peromysci* or *O. leucopus*. Habitat affected the abundance for *L. peromysci* ($P = 0.01$) and *A. fahrenholzi* ($P = 0.01$). *O. leucopus* did not show any statistical evidence that field site, habitat type, or isolation was driving the abundance of this species. For the *P. leucopus* ectoparasite community, three species (*L. peromysci, G. hypudaei*, and *O. leucopus*) were collected from the four field sites and were analyzed here. Site impacted abundance of *L. peromysci* and *G. hypudaei* on white-footed mice (*L. peromysci*, $P << 0.01$; *G. hypudaei*, $P = 0.01$). Isolation only impacted abundance of *G. hypudaei* ($P = 0.05$). Again, *O. leucopus* had no statistical evidence for isolation, habitat, or field site in driving the abundance of this species.

As a formal test of whether ectoparasite infestation rates were consistent with a dilution effect, I plotted infestation rates (total number of each species per individual host) against host species richness on a site-wise basis. I did this only for the four ectoparasite species mentioned above, each of which were found at all four field sites. At the community level, the chigger *L. peromysci* was the only ectoparasite species that showed the expected pattern, whereby infestation rate decreased as species richness increased, which is thus consistent a dilution effect in this species (Figure 7). However, within *P. leucopus*, none of the three ectoparasite species analyzed displayed a trend in host abundance impacting the infestation rate across the four field sites (Figure 8).

CHAPTER IV: DISCUSSION

SMALL MAMMAL COMMUNITIES AND ISLAND BIOGEOGRAPHY THEORY

Small mammal communities that I sampled along the foothills of the Southern Appalachian Mountains differed within field sites (i.e., between habitat classes), but not significantly so among sites. Even after accounting for these habitat effects, however, there was a weak but significant effect of degree of isolation on species richness. This supports the hypothesis that mammal communities fundamentally differ in the Southern Appalachians with distance from the highest peaks. Unfortunately, my data did not allow an explicit test of island biogeographic expectations that formed my initial hypothesis (decreasing species diversity with increasing isolation), specifically due to low trap success and the likelihood that some species were present, but not encountered, in my live-trapping surveys. However, my data were still potentially consistent with theoretical expectations, as explained below.

Eleven different mammal species were captured across sites, representing a mix of high and low elevation species coexisting at intermediate elevations. When *D. virginiana* (which is ubiquitous but was only detected at a single site in this study) is excluded from the comparison, just 5 mammal species were captured at isolated sites. Conversely, the least-isolated site (Cold Mountain; Figure 1) had the highest small mammal diversity (7 species). Island biogeography theory predicts increased dispersal and colonization with decreasing isolation (Cook et al. 2002), which could explain these patterns, especially the highest diversity being observed at Cold Mountain. Conversely, one complicating factor is that surveys at the other site not considered to be isolated (Chattahoochee-Oconee) resulted in 5 species, identical to isolated sites but with a slightly different species composition.

Although island biogeography theory has been applied to a variety of sky island systems in North America, it is possible that the Southern Appalachians lack the extreme degrees of isolation that is a fundamental assumption of the theory. An alternative (but not mutually exclusive) scenario is that all sites surveyed here exist along a single broad elevational gradient, and that patterns of species diversity in the region are driven by a phenomenon similar to the mid-domain effect. The mid-domain effect is a hypothesis to describe why species diversity is higher at intermediate elevations compared to the high and low elevation communities (McCain, 2003). Rickart (2001) also found support for this theory in the intermontane West, and the communities I sampled were likewise a combination of low elevation species such as *O. nuttalli* (Keller et al. 2003) and *S. hispidus* (Dunnum et al. 2002), and high elevation species such as *B. brevicauda* (Hess, 2016), *S. cooperi* (Linzey, 1984), *N. insignis*, and *Z. hudsonius* (Whitaker, 1972).

One factor that prevents parsing the true drivers of mammal diversity in this system is the lack of comprehensive, site-based field sampling along individual mountain ranges. This would help disentangle potential effects of elevation and isolation in structuring small mammal communities. Still, regardless of specific drivers of richness, my data support the hypothesis that small mammal community structure varies with distance from the main Appalachian cordillera. As climate changes reshape these elevation communities, there is potential for ectoparasites to colonize new hosts and disrupt modern host-parasite networks – specifically, the system-wide interactions among hosts and their parasites (Bellay et al. 2018).

FURTHER NOTES ON SMALL MAMMAL COMMUNITIES

To provide additional support for patterns of small mammal distributions found here, a comprehensive literature comparison was done to contextualize results with historic mammal records in North Carolina and Georgia. Among lower-elevation species, I found *S. hispidus* in abundance at the lower elevation (~250 m) sites (Green River Game Land and South Mountain Game Land), which matches prior records indicating this species has an elevation range of roughly 0-1,130 m, but is scarce above 400 m (Meikle and Powers 2011, LeGrand et al. 2021). *P. leucopus* was also found in abundance at all sites and, while not necessarily restricted to low elevations (range of 0-1,800 m), it is more commonly found at lower elevations (LeGrand et al. 2021). Regarding higher-elevation species, I found *B. brevicauda* primarily at the higher elevation (782-952 m) sites (Chattahoochee-Oconee National Forest and Cold Mountain Game Land). This species has an elevational range of roughly 450-1,770 m (Ballenger, 2011, LeGrand et al. 2021). Similar to *B brevicauda, S. cooperi* and *S. cinereus* have higher elevation ranges (*S. cinereus*, 500-1,861 m; *S. cooperi*, 0-1,818 m; LeGrand et al. 2021) and were only collected from the highest-elevation (~782 m) site (Cold Mountain Game Land).

Although my data offer a view of community structure in the most common small mammals along the Southern Appalachian Mountains, as stated above they are unlikely to represent a comprehensive picture of mid-elevation communities due to the roughly 2% trap success I observed. This could have been due to the almost exclusive use of Sherman live traps, which was necessary so that individuals could be screened alive for ectoparasites. Nevertheless, my data do offer baseline information on some small mammals that are particularly poorly studied in North Carolina; specifically, the southern bog lemming (*S. cooperi*) and meadow jumping mouse (*Z. hudsonius*).

In my literature search, I found just 130 museum records of *S. cooperi* and 53 records of *Z. hudsonius* combined from North Carolina (out of 14,849 small mammal records from 39 different museum collections). Little is known about the current distributions and ecology of either species in our state. Recent records have shown that the range of *S. cooperi* may have shifted into North Carolina from Tennessee, along higher elevations of the Southern Appalachian Mountains (Campbell et al. 2010). According to Laerm et al. (1995), in Tennessee and South Carolina, *Z. hudsonius* is regarded as a species of special concern but has insufficient records available. The putative rarity of each species on the landscape may be due to competition by other small mammals (*Z. hudsonius* by *N. insignis*, *S. cooperi* by *M. pennsylvanicus*; Linzey, 1984). For example, *M. pennsylvanicus* has been found in Haywood County (Lee et al. 1982), and *M. pennsylvanicus and S. cooperi* have similar habitat preferences with the former outcompeting the latter (Linzey 1984, Krupa and Haskins 1996). LeGrand et al. (2021) lists records from 5 different counties in North Carolina that contain 1-3 *Z. hudsonius* records each (Henderson, Madison, Watauga, Alleghany, and Wake Counties). Here, I collected 3 *Z. hudsonius* from Cold Mountain Game Land in Haywood County, the latter of which had no prior county-level records. One reason for this may be competition with *N. insignis*. Although *Z. hudsonius* is found mostly in moist meadows and sometimes forest edges, and *N. insignis* is found in cool moist forests, they may overlap in edge habitats where competition for habitats and food causes low abundance of *Z. hudsonius* (Webster et al. 2004, LeGrand et al. 2021).

ECTOPARASITE COMMUNITY COMPOSITION

Ectoparasite communities displayed some variation among the four field sites and between habitat classes (meadow and forest), but a PERMANOVA recovered neither geographic (site) nor ecological (degree of isolation, habitat type) as a significant driver of composition. Still, as in mammal communities, habitat classes were a marginally significant driver ($P = 0.09$). The

isolated sites Green River Game Land and South Mountain Game Land were most similar to one another, and these were most divergent from meadow habitats at non-isolated sites (Cold Mountain Game Land and Chattahoochee National Forest; Figure 3b). Six ectoparasite species were found at Chattahoochee and not at Green River (*Asiochirus blarina, Comatacarus americanus, Doratopsylla blarinae, Echinonyssus blarinae, Haemogamasus ambulans, Peromyscopsylla hesperomys*). Nine ectoparasite species were found at Green River but not at Chattahoochee (*Ctenophthalmus pseudagyrtes, D. variabilis, Echinonyssus utahensis, Epitedia wenmanni, Hoplopleura hesperomydis, I. scapularis, Listrophorus mexicanus, Prolistrophorus bakeri, Polygenis gwyn*i). Green River Game Land had fleas, ticks, mites, and lice collected whereas Chattahoochee National Forest only had chiggers, mites, and fleas. Of the ectoparasites that were collected, 6 are known diseases vectors: *I. scapularis* (Lyme disease: *B. burgdorferi;* Burgdorfer et al 1985), *D. variabilis* (RMSF: *R. rickettsii*), *P. gwyni* (Myrune typhus: *Rickettsia typh*i; Durden et al. 2005), *Orchopeas leucopus* (*Rickettsia-felis*: cat-flea typhus; Fedele et al. 2020), *C. pseudagyrtes* (Bartonella : Carrion's disease, cat scratch disease, trench fever; Reeves et al. 2007), and *H. ambulans* (Hantavirus, Tularemia: *Francisella tularensis*; Valiente Moro et al. 2005).

As stated above, habitat likely played a role in affecting the variation in the ectoparasite community composition in addition to the small mammal community. Different habitats harbored various species, probably depending on the resources available, habitat preference, and other biotic and abiotic factors (Stevens and O'Connor 2006). A possible explanation for why there is a difference between the meadow and forest ectoparasite communities is the host community composition. Indeed, networks also showed important variation in structure and connectance properties. Both Green River and South Mountain Game Lands had a high abundance of *S. hispidus* within the meadow habitat, and this species had a high parasite load (8.81), which may have driven the similarity between these habitats. In comparison, forest

habitats provide many species with canopy cover and resource availability, and the may not be as resource-limited or patchy compared to a meadow habitat (Carey and Harington 2000).

ECTOPARASITE COMMUNITIES IN CONTEXT

Variation among habitats had a stronger contribution to small mammal and ectoparasite communities than site identity. Forest habitats displayed the highest number of both mammal and ectoparasite species. Forest ecosystems at the four field sites were primary Oak, Dry-Oak, and Cove forests, with herbaceous understory of rhododendron (*Rhododendron catawbiense*) and mountain laurel (*Kalmia latifolia*; NCWRC, 2015). Conversely, maintained ecosystems such as meadows showed a negative impact on species variation, including a negative effect on ectoparasite loads. All three Game Lands meadows were maintained as grassy areas for target game species (NCWRC, 2015); moreover, they tended to be surrounded by forest and isolated which likely creates low accessibility for meadow-dwelling species to colonize. Meadows were also usually small in areal extent; this could generate interspecific competition for space and resources in a given patch (Balčiauskas et al. 2019). Mammals caught at both habitat types were *P. leucopus, B. brevicauda*, and *N. insignis.* In the meadow these 3 species display less ectoparasite host interactions but the interactions they had were stronger in the meadow vs the forest. In the forest there was higher host species abundance overall, thus allowing more generalist ectoparasites to interact with different hosts. Within the forest habitat, *P. leucopus* ectoparasite load were not diluted by the high species richness. Similar to *P. leucopus, B. brevicauda*, had a lower parasite load in the meadow vs the forest. *B. brevicauda* only had interaction with *D. blarinae* in the meadow. The lower infestation on *B. brevicauda* in the meadow can potentially be explained by the high *S. hispidus* abundance and its specialist *H. hirsuta.* Infestation of ectoparasites have a higher chance of being diluted is there are more specialist in the community (Agosta et al. 2010).

Different habitat types also led to altered ectoparasite-host interactions (Figure 5a). A weighted bipartite network analysis describes the strengths of interactions from the 8 mammal species that had ectoparasite infections. *P. leucopus* had the most interactions with the 22 ectoparasite species collected, although this may in part be due to the abundance of *P. leucopus* in the study (Table 3). The other species with the most ectoparasite interactions were *B. brevicauda* and *S. hispidus*. Interestingly, however, these mammals with highest infection rates also differ in their elevational ranges, *B. brevicauda* being found in higher elevations, *S. hispidus* in lower to middle elevations, and *P. leucopus* being in both (LeGrand et al. 2021). While *P. leucopus* and *B. brevicauda* were most abundant in forest habitats, *S. hispidus* and *S. cooperi* were only collected from meadows. Both had multiple ectoparasite interactions, on *S. hispidus,* Phthiraptera (*H. hirsuta*), Siphonaptera (*C. pseudagyrtes, O. leucopus, Polygenis gwyni*), and Acariformes (*A. Fahrenholzi, Prolistrophorus bakeri* were collected and *S. cooperi* had Acariformes (*A. Fahrenholzi, L. mexicanus, L. alaskensis*). These patterns suggest that each mammal species brings their ectoparasites to this mid-elevation interaction, likewise, causing mixing of ectoparasite communities at the mid-elevation.

To account for the effect of habitat type on network structure, I calculated weighted and unweighted host-ectoparasite interactions from the meadow and forest habitat, across all sites (Figure 5). The meadow habitat had a lower overall connectance (realized proportion of possible links) of 0.226 compared to the forest of 0.246, indicating a lower proportion of host-ectoparasite interactions in the meadow systems. Also, the meadow had 0.95 links per species compared to the forest of 1.09, indicating that forest-dwelling mammals have more interactions with different ectoparasites, which likely leads to their higher ectoparasite loads. Interaction strength can be measured on a scale of -1 to 1, with zero indicating highly symmetric interaction strength and values close to 1 or −1 indicating high asymmetry (Vazquez et al. 2007). A negative interaction strength means that there is strong effect from interaction partners but does not exert a strong

reciprocal effect (Vazquez et al. 2007). From the meadow interaction strength was -0.224, this meant that there were overall lower ectoparasite-host interactions and the forest had 0.104, which had a higher ectoparasite-host interaction. However, I note that interaction strength can be impacted by the abundance load on an individual and by the scaled amount of host that were collected of that species. For *T. striatus* and *S. cooperi*, the high interaction strength comes from the fact that only 1 host individual was encountered.

Finally, to investigate factors important for driving ectoparasite abundance, I analyzed the latter data with respect to the same variables as for community structure (site, habitat, and degree of isolation). I limited my analysis to 4 ectoparasite species (*L. peromysci, G. hypudaei, O. leucopus*, and *A. fahrenholzi*) found at all field sites, and found statistical evidence for site playing a role in the abundance of *L. peromysci* ($P << 0.01$). I found statistical evidence for habitat playing a role in the abundance of *L. peromysci* as well as *A. fahrenholzi* ($P = 0.01$). Further, I found that degree of isolation influenced the abundance of *A. fahrenholzi* and *G. hypudaei* ($P <= 0.05$). Some of these same trends were recovered for the ectoparasite communities found only on the white-footed mouse (*P. leucopus*). From these tests alone, it is difficult to know if these specific ecological variables are the cause of changing abundances, or it host-mediated variables are also involved. However, they do suggest high individuality in how ectoparasite transmission and infestation vary among habitats.

ECTOPARASITE LOADS AND HOST SPECIES DIVERSITY

Abundance and species composition of host communities are crucial factors in driving the abundance of ectoparasites (Johnson and Thieltges 2010). The dilution effect describes a

negative relationship between the parasite infestation parameter and host abundance and diversity of an individual parasite species (Johnson and Thieltges 2010), and it has been widely observed among parasite and pathogen species (Civatello et al. 2015). In that scenario, life stages of the ectoparasite may become crippled due to use of suboptimal hosts, leading to infection rates being diluted in a community with high host diversity (Pfäffle et al. 2015). Conversely, such a finding could result when host species densities are high (irrespective of host identity), which should permit increased transmission rates of many ectoparasites.

Across field sites, South Mountain Game Land had the highest scaled abundance of ectoparasites on individual mammals (N = 5.3) and the lowest number of mammal species (N = 5) collected. This site-wide pattern is consistent with predictions from the dilution effect. However, this pattern was not observed in each habitat. At South Mountains, forest habitats had 4 small mammal species collected and several *P. leucopus* with high ectoparasite loads (one individual had ~37 *L. peromysci*). Conversely, meadows at South Mountain were dominated by the cotton rat (*S. hispidus*; 74% of captures) and while this species had the highest ectoparasite load, one ectoparasite (the louse *Hoplopleura hirsuta*) was especially common. *H. hirsuta* is a specialist for *S. hispidus* (Agosta et al. 2010) and is able to take advantage of the high abundance of its preferred host species. Opposite of South Mountains Game Land, Cold Mountain Game Land had the lowest abundance of total ectoparasites (N =1.5 individuals per host individual) and the highest small mammal diversity (N = 7; Table 2). This site-wide pattern is also consistent with a dilution effect. However, a comparison of the raw data indicates that at least one of the highest scaled ectoparasite loads at Cold Mountain was in *S. cooperi*, of which only a single individual was collected, which could easily bias my results.

The dilution effect is still debated in the literature due to the lack of research done (Tetlock et al. 1996). To achieve a more precise test of whether mammal species diversity impacted species-

specific infestation, I analyzed four individual ectoparasite species that each occurred across all four sites (*Leptotrombidium peromysci, Glycyphagus hypudaei, Orchopeas leucopus,* and *Androlaelaps fahrenholzi*). If ectoparasite loads are influenced by a dilution effect, I expected to see lower abundance within each ectoparasite species in scenarios of highest host species diversity, and vice versa. However, I found little evidence for such an effect (Figure 7). The sole exception was the chigger *L. peromysci*, which displayed the lowest infestation rates in conditions of highest mammal diversity (Green River and Cold Mountain), consistent with the dilution effect. As ectoparasite samples themselves were biased towards *P. leucopus* (the most common capture) as a host, I analyzed infestation rates on this single host to understand if similar patterns emerged. Indeed, patterns of infestation within this host species were more consistent with the dilution effect, generally being lower when host diversity at sites was higher, and vice versa. The effect was weak, but present, in *G. hypudaei* and *L. peromysci.* Future work on additional parasite species will be critical to understand the extent to which dilution may indeed be present.

High host species diversity and abundance dilutes the infection rate by increasing opportunities for infection of species that are not optimal reservoir hosts, potentially also preventing completion of the full life cycle and transmission of bacteria to the host (Khalil et al. 2016). However, the dilution effect also assumes parasites are not extreme host specialists. As previously mentioned, Krasnov et al. (2007) studied the generalist *I. rincinus* and specialist *I. trianguliceps* and displayed how ectoparasites that are generalist have a higher chance of prevalence compared to specialist. Specifically, extreme specialists have stronger constraints on their ability to exploit competent hosts and are less likely to demonstrate the dilution effect (Kransnov et al. 2007). Another study done by Krasnov et al. (2002) supports the argument that the dilution effect requires parasites not to be extreme host specialists. Those authors found that, in the specialist fleas *Xenopsylla dipodilli* and *Nosopsyllus iranus theodori* on the desert rodent species *Gerbillus dasyurus*, infestations increased as favorable host density increased (Krasnov

et al. 2002). Civatello et al. (2015) also found that parasite dynamics may be driven by the particular host species present rather than the diversity and undisturbed habitats having higher densities of parasite or vectors compared to disturbed sites (Civatello et al. 2015).

PEROMYSCUS LEUCOPUS BIAS

Across all sites, *P. leucopus* was the most commonly captured mammal (Table 2), and it had the highest amount of different ectoparasite interactions as well (N = 11), suggesting (as above for ectoparasite loads) that there may be sample bias in my community data. *P. leucopus* also had more interactions within the forest (11) than in meadow (6). Similarly, Mize et al. (2011) studied habitat correlations with the spatial distribution ectoparasites on *P. leucopus* in southern Michigan and found that habitat should be included in one of the drivers of assessments in spatial distribution of ectoparasites with *P. leucopus* (Mize et al. 2011). Those authors found that ticks, in particular *I. scapularis* and *D. variabilis,* were more abundant in recently disturbed habitats. This agrees in part with my data; for *I. scapularis* and *D. variabilis*, 67% of individuals were collected from a recently maintained meadow habitat, compared to 33% was from an old-growth forest.

While *P. leucopus* had the highest amount of ectoparasite interactions, many ectoparasites were generalists and found to be associated with other small mammals as well. This may be why in the forest I found the highest number of ectoparasite-host interactions. For example, *O. leucopus* was found on *P. leucopus*, but also *P. maniculatus* and *O. nuttalli*. *O. leucopus* in other studies has shown to favor for mice as a host, this can explain why it was found on *P. leucopus, P. maniculatus*, and *O. nuttalli* (Veitch et al. 2020*).* Also, *G. hypudaei* was collected from *P.*

leucopus, P. maniculatus, and *S. hispidus*. Thus, while my data are biased towards *P. leucopus,* the fact that many ectoparasites I found are generalists makes it reasonable to believe that it is not driving all patterns reported here (Agosta et al. 2010). In general, there is a pressing need to understand how ectoparasite abundance and variation vary not only among ecological factors (e.g., Table 8), but also whether an ectoparasite has a generalist or specialist life history.

CONCLUSION

Overall, my results provide new information for patterns of parasite occurrence on Southern Appalachians mammal communities. For small mammals themselves, species diversity varied only slightly with distance from the Appalachian cordillera, providing minimal support for an island biogeographic model in diverse Southern Appalachians ecosystems. Conversely, degree of isolation as well as habitat type each contributed significantly to variation in small mammal community structure. Ectoparasite communities on small mammals were less sensitive to the above predictors than hosts, largely varying between forest and meadow habitats. Host-parasite networks were more diverse and more complex in forests relative to meadows. However, patterns of ectoparasite infestation rates on small mammals were complex and hard to interpret with respect to host diversity and composition. At the community level, infestation rates were highest when mammal species richness was lowest, and vice versa, providing preliminary evidence for a dilution effect. Yet, infestation rates within the best-sampled ectoparasite species, were only consistent with a dilution effect in one case, although these trends were still supported when data were limited to just the best sampled host species (*P. leucopus*). This study was conducted at multiple field sites where many biotic and abiotic factors likely impacted data collection, but it remains novel in that few comprehensive surveys like mine have been reported from North Carolina to date. Similar work spanning the field to laboratory will be critical for anticipating response of mid-elevation vertebrate communities in our region to climate and land

use change, as well as host turnover and changing ectoparasite community composition and abundance.

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APPENDIX A: FIGURES AND TABLES

Site Name	Distance classification	Elevational Range Trapped	County	Land Ownership	Average Temp.	Total Trapnights
Chattahoochee Oconee National Forest (CH)	Not isolated	425 - 782m	Rabun (Georgia)	U.S. National Forest Service	29°C	2,850
Cold Mountain Game Land (CM)	Not isolated	886 - 952m	Haywood	NC Wildlife Recourses Commission	27°C	2,790
South Mountains Game Land (SM)	Isolated	199 - 505m	Rutherford + Cleveland	NC Wildlife Research Commission	26°C	2,721
Green River Game Land (GR)	Isolated	275 - 490m	Henderson + Polk	NC Wildlife Research Commission	25°C	2,850

Table 1. Characteristics of field sites in this study. Several trap lines were established in each site spanning elevations as well as habitat types (meadow, mature forest). Field sites were classified on their degree of isolation from the Southern Appalachian Mountains (isolated or not isolated). Temperatures are the average from all sessions at a field site.

Field sites	Chattahoochee			Cold Mountain			Green River			South Mountain			Total Ind.	% Tot Capt.
Host	Meadow	Forest	All	Meadow	Forest	All	Meadow	Forest	All	Meadow	Forest	All		
Blarina brevicauda	3	4	7	1	7	8	0	2	2	0	2	2	19	9 %
Sorex cinereus	0	0	0	0	1	1	0	0	0	0	0	0	1	1%
Zapus hudsonius	0	0	0	2	1	3	0	0	0	0	0	0	3	1.5%
Napaeozapus insignis	0	1	1	1	2	3	1	0	1	1	0	1	6	3%
Peromyscus leucopus	2	14	16	6	21	27	12	36	48	7	42	49	140	68%
Peromyscus maniculatus	0	1	1	0	2	2	0	0	0	0	0	0	3	1.5%
Ochrotomys nuttalli	0	2	2	0	0	0	0	1	1	0	1	1	4	2%
Sigmodon hispidus	0	0	0	0	0	0	6	0	6	20	0	20	0	13%
Synaptomys cooperi	0	0	0	1	0	1	0	0	0	0	0	0	1	1%
Tamias striatus	0	0	0	0	1	1	0	0	0	0	0	0	1	1%
Didelphis virginiana	0	0	0	0	0	0	0	1	1	0	0	0	1	1%
Total Ind.	5	22	27	11	35	46	19	40	59	28	45	73	205	
% Tot. Captures			13%			22%			29%			36%		

Table 2. Small mammal occurrences within sites, and habitat types within sites. The total number of individuals captured, and the percent of total captures is listed for each species (right columns). The total number of individuals captured, and percent of total captures is also listed for each site (bottom row). In each case, the percent of total captures represents the total number of individuals scaled by the total number of trap nights (see Table 1).

Order	Species	Green River		Cold Mountain		South Mountain		Chattahoochee	
		Forest	Meadow	Forest	Meadow	Forest	Meadow	Forest	Meadow
Trombidi-formes	*Comatacarus americanus (Ca)*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	18 (0.82)	0 (0)
	Leptotrombidium peromysci (Lp)	5 (0.13)	0 (0)	7 (0.2)	3 (0.27)	203 (4.51)	3 (0.11)	134 (6.09)	0 (0)
Acari-formes	*Androlaelaps fahrenholzi (Af)*	9 (0.23)	41 (2.16)	2 (0.06)	2 (0.18)	4 (0.09)	31 (31)	1 (0.05)	0 (0)
	Laelaps alaskensis (La)	0 (0)	0 (0)	0 (0)	7 (0.64)	0 (0)	0 (0)	0 (0)	0 (0)
	Listrophorus mexicanus (Lm)	1 (0.03)	0 (0)	1 (0.03)	4 (0.36)	0 (0)	0 (0)	0 (0)	0 (0)
	Haemogamasus ambulans (Ha)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.05)	0 (0)
	Prolistrophorus bakeri (Pb)	0 (0)	4 (0.21)	0 (0)	0 (0)	0 (0)	66 (1.47)	0 (0)	0 (0)
	Echinonyssus utahensis (Eu)	1 (0.03)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Asiochirus blarina (Ab)	0 (0)	0 (0)	0 0)	0 (0)	3 (0.07)	0 (0)	2 (0.09)	0 (0)
	Echinonyssus blarinae (Eb)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.07)	0 (0)	1 (0.05)	0 (0)
	Glycyphagus hypudaei (Gh)	24 (0.6)	0 (0)	9 (0.26)	1 (0.09)	6 (0.13)	0 (0)	27 (1.23)	0 (0)
Ixodida	*Ixodes scapularis (Is)*	1 (0.03)	1 (0.05)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0|)	0 (0)
	Dermacentor variabilis (Dv)	0 (0)	3 (0.16)	1 (0.03)	0 (0)	1 (1)	1 (0.02)	0 (0)	0 (0)
Phthir-aptera	*Hoplopleura erratica (He)*	0 (0)	0 (0)	0 (0)	6 (0.55)	0 (0)	0 (0)	0 (0)	0 (0)
	Hoplopleura hesperomydis (Hhe)	3 (0.08)	1 (0.05)	0 (0)	0 (0)	8 (8)	0 (0)	0 (0)	0 (0)
	Hoplopleura hirsuta (Hhi)	0 (0)	36 (1.89)	0 (0)	0 (0)	0 (0)	37 (37)	0 (0)	0 (0)
Siphon-aptera	*Polygenis gwyni (Pg)*	0 (0)	9 (0.47)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Ctenophthalmus pseudagyrtes (Cp)	0 (0)	3 (0.16)	1 (0.03)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
	Epitedia wenmanni (Ew)	2 (0.05)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)
	Doratopsylla blarinae (Db)	0 (0)	0 (0)	7 (0.2)	0 (0)	6 (0.13)	0 (0)	7 (0.32)	1 (0.2)
	Peromyscopsylla hesperomys (Ph)	0 (0)	0 (0)	4 (0.11)	2 (0.18)	0 (0)	0 (0)	6 (0.27)	0 (0)
	Orchopeas leucopus (Ol)	5 (0.13)	4 (0.21)	10 (0.29)	0 (0)	10 (0.22)	2 (2)	7 (0.32)	0 (0)

Table 3 . Ectoparasite occurrences within sites and habitat types. For each ectoparasite x site combination (i.e., each cell), top and bottom values represent the total number of individuals collected and the ratio of total number collected to total infected hosts at each site, respectively.

	Df	Sums of Sqs	Mean Sqs	F.Model	R2	Pr(>F)
By field site						
Field Site	3	0.37	0.12	1.54	0.29	0.22
By ecological properties						
Habitat Type	1	0.64	0.64	8.60	0.51	0.002
Isolation	1	0.24	0.24	3.26	0.19	0.04

Table 4 Permutational Multivariate Analysis of Variance tests comparing small mammal community variation across three different variables (field site, isolation, and habitat type). The raw data were total number of each small mammal species scaled by total number of individuals captured per site.

	Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
By field site						
Field Site	3	1.26	0.42	1.19	0.44	0.23
By ecological properties						
Habitat Type	1	0.52	0.52	1.48	0.18	0.09
Isolation	1	0.45	0.45	1.19	0.16	0.31

Table 5 Permutational Multivariate Analysis of Variance tests comparing ectoparasite community variation on white-footed mice (*Peromyscus leucopus*) across three different variables (field site, isolation, and habitat type). The raw data were total number of each ectoparasite species scaled by total number of host individuals captured per site.

	chi-squared	df	p-value
Whole mammal community			
Field site	3.51	3	0.32
Habitat type	2.77	1	0.09
Isolation	0.60	1	0.44
***P. leucopus* community**			
Field site	18.75	3	0.0003
Habitat type	4.33	1	0.04
Isolation	1.17	1	0.28

Table 6 Results of Kruskal-Wallis tests comparing ectoparasite load of all the individual small mammals trapped across field sites, habitat types (meadow vs forest), and degrees of isolation. The top panel lists results for every individual ectoparasite species pooled across whole small mammal communities, and the bottom lists results for only ectoparasites collected from individual *P. leucopus.*

	w	p	95 % confidence interval	sample estimate (difference in location)
Whole mammal community				
Habitat type	418	0.09	-2.10, $6.75e^{-05}$-	-0.99
Isolation	3	0.69	-0.05, 0.34	0.15
***P. leucopus* community**				
Habitat type	2150.5	0.04	$1.84e^{-05}$, $9.99e^{-01}$	<< 0.01
Isolation	2398.5	0.28	$-9.99e^{-01}$ $2.62e^{-05}$	$-2.48e^{-05}$

Table 7 Mann-Whitney U Wallis tests comparing ectoparasite load of all the individual small mammals trapped across the two habitat types (meadow vs forest), and degrees of isolation .The top panel lists results for every individual ectoparasite species pooled across whole small mammal communities, and the bottom lists results for only ectoparasites collected from individual *P. leucopus.*

	chi-squared	df	p-value
Whole mammal community			
Leptotrombidium peromysci			
Habitat type	10.19	1	0.01
Field site	34.54	3	<< 0.01
Isolation	0.01	1	0.93
Orchopeas leucopus			
Habitat	1.69	1	0.19
Field site	096	3	0.81
Isolation	0.51	1	0.48
Androlaelaps fahrenholzi			
Habitat	9.81	1	0.01
Field site	5.33	3	0.15
Isolation	4.89	1	0.03
Glycyphagus hypudaei			
Habitat	2.70	1	0.10
Field site	6.52	3	0.09
Isolation	4.01	1	0.05
***P. leucopus* community**			
Leptotrombidium peromysci			
Habitat type	2.58	1	0.11
Field site	42.24	3	<< 0.01
Isolation	0.08	1	0.78
Orchopeas leucopus			
Habitat	0.001	1	0.8
Field site	1.47	3	0.69
Isolation	0.65	1	0.42
Glycyphagus hypudaei			
Habitat	1.96	1	0.16
Field site	11.69	3	0.01
Isolation	3.91	1	0.05

Table 8 Results of Kruskal-Wallis tests comparing abundances for the four most-commonly sampled ectoparasite species. The top panel lists results for every individual ectoparasite species pooled across whole small mammal communities, and the bottom lists results for only ectoparasites collected from individual *P. leucopus.*

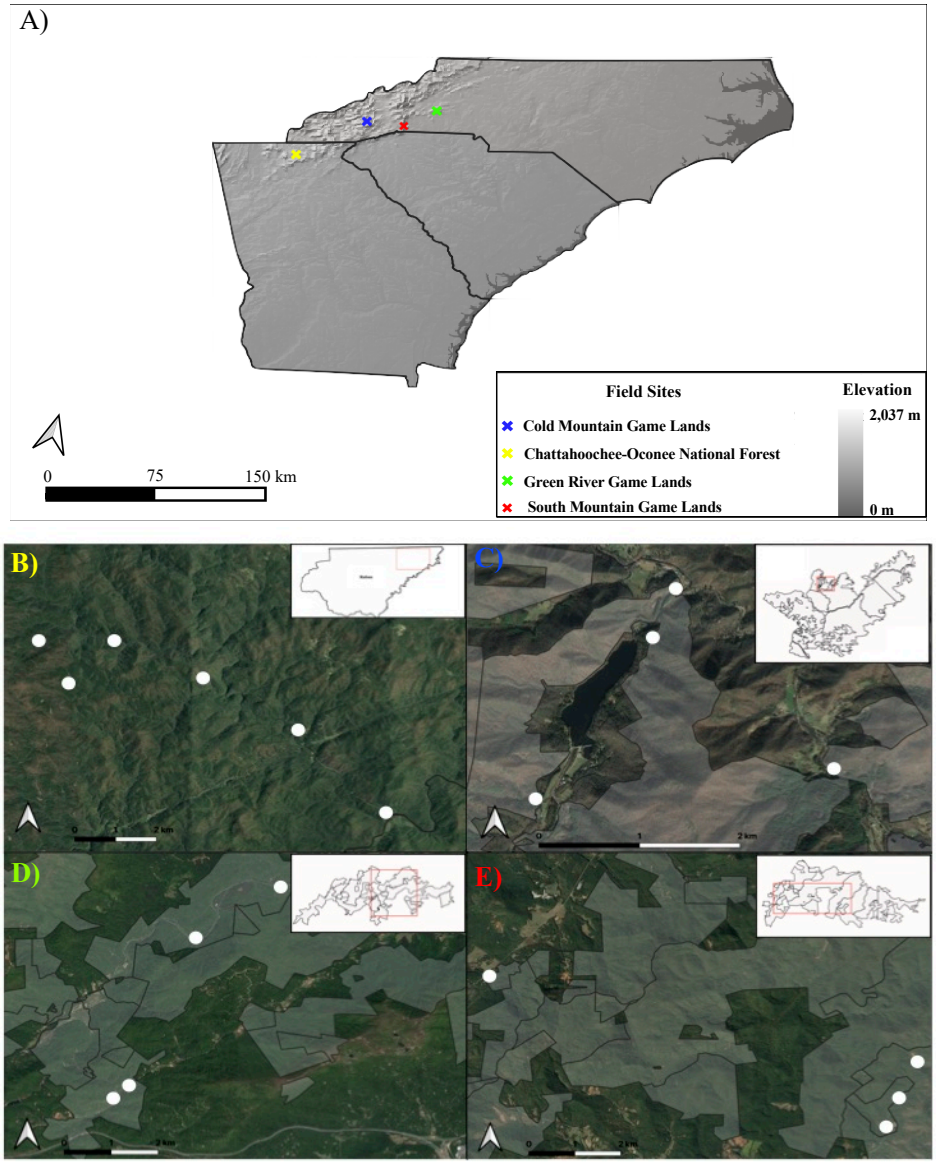


Figure 1.Map of field sampling sites. An elevation gradient map representing the location of each of the sampling sites located in North Carolina and Georgia, in the foothills of the Southern Appalachian Mountains.

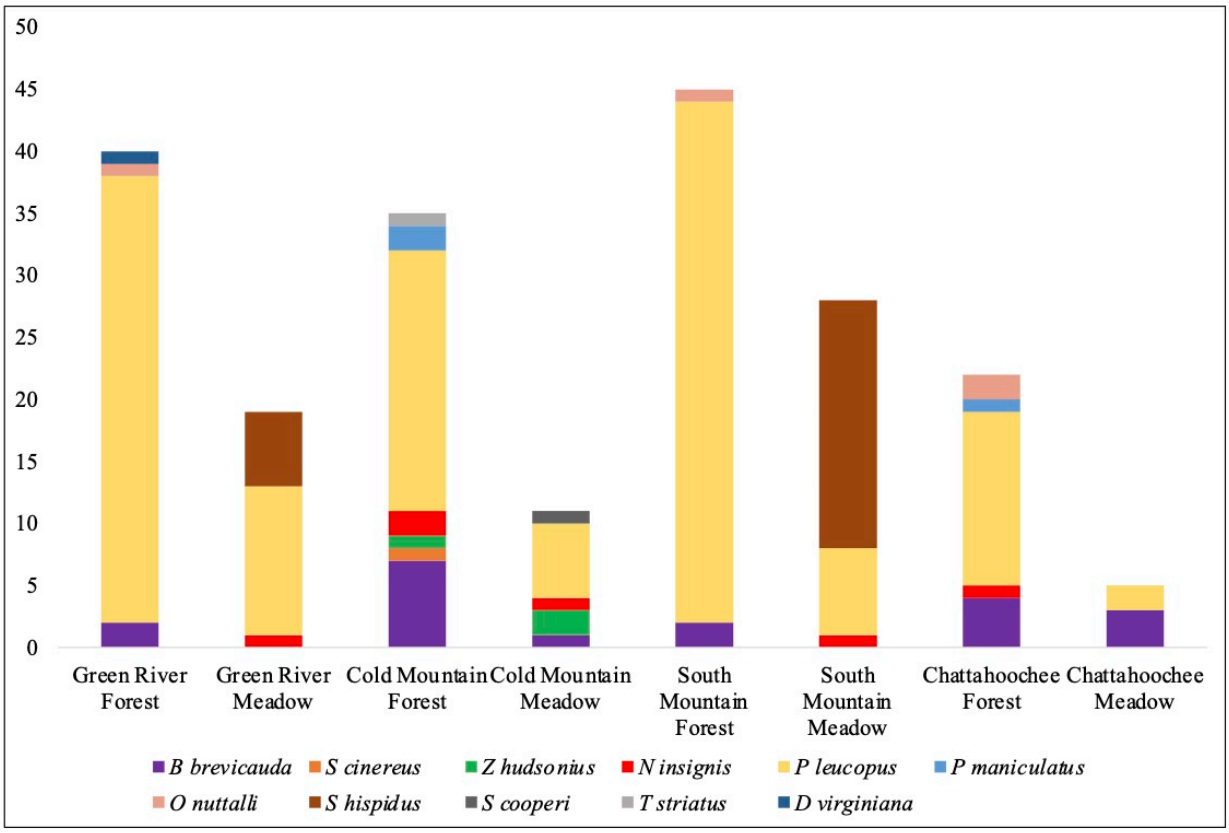


Figure 2. Small mammal community composition among sites and habitats. The Y-axis displays the total number of individuals per species captured, and colors represent the 11 different small mammal species.

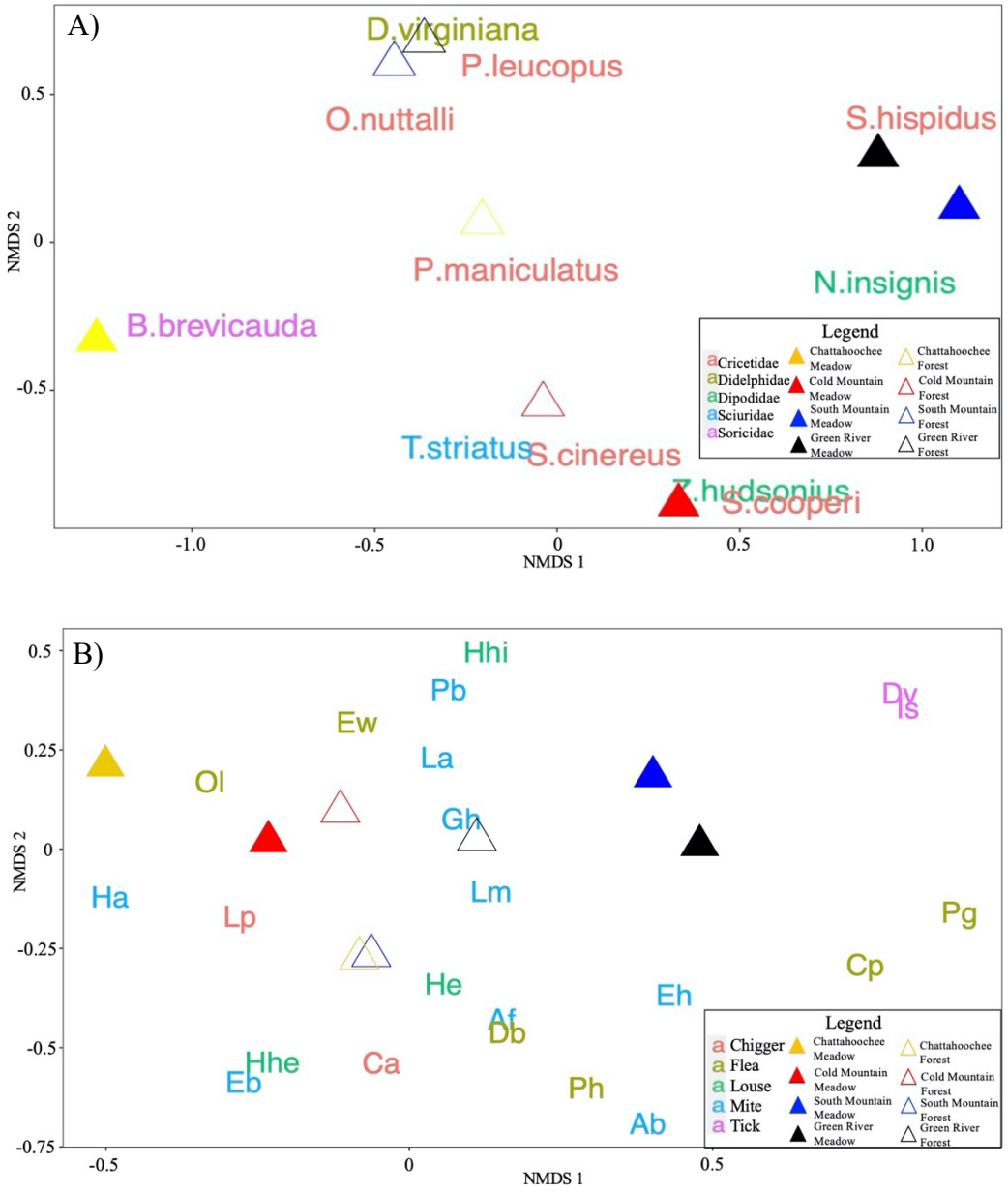


Figure 3 Non-metric multidimensional scaling (NMDS) plots for A) small mammal and B) ectoparasite communities. In each plot, field sites are coded as points taxonomic identities for species contributing to variation are listed as letters. In each plot, taxa are color-coded by taxonomic group (Table 3). Habitat classes (meadow and forest) are represented by open triangles (forest) and closed triangles (meadow) triangles and colored by field sites.

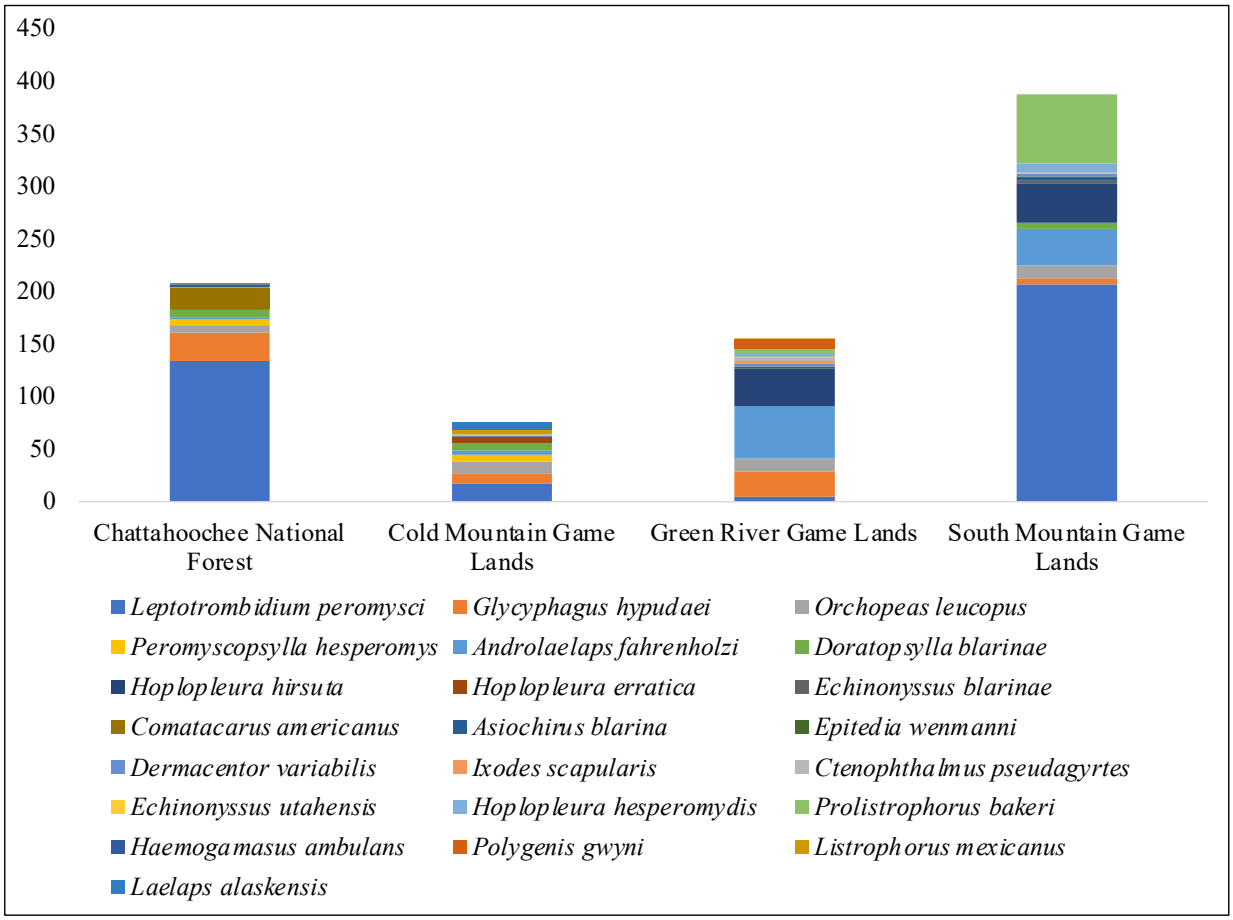


Figure 4 Ectoparasite community composition among sites and habitats. The Y-axis displays the total number of individuals per species captured, and colors represent the 22 different ectoparasite species.

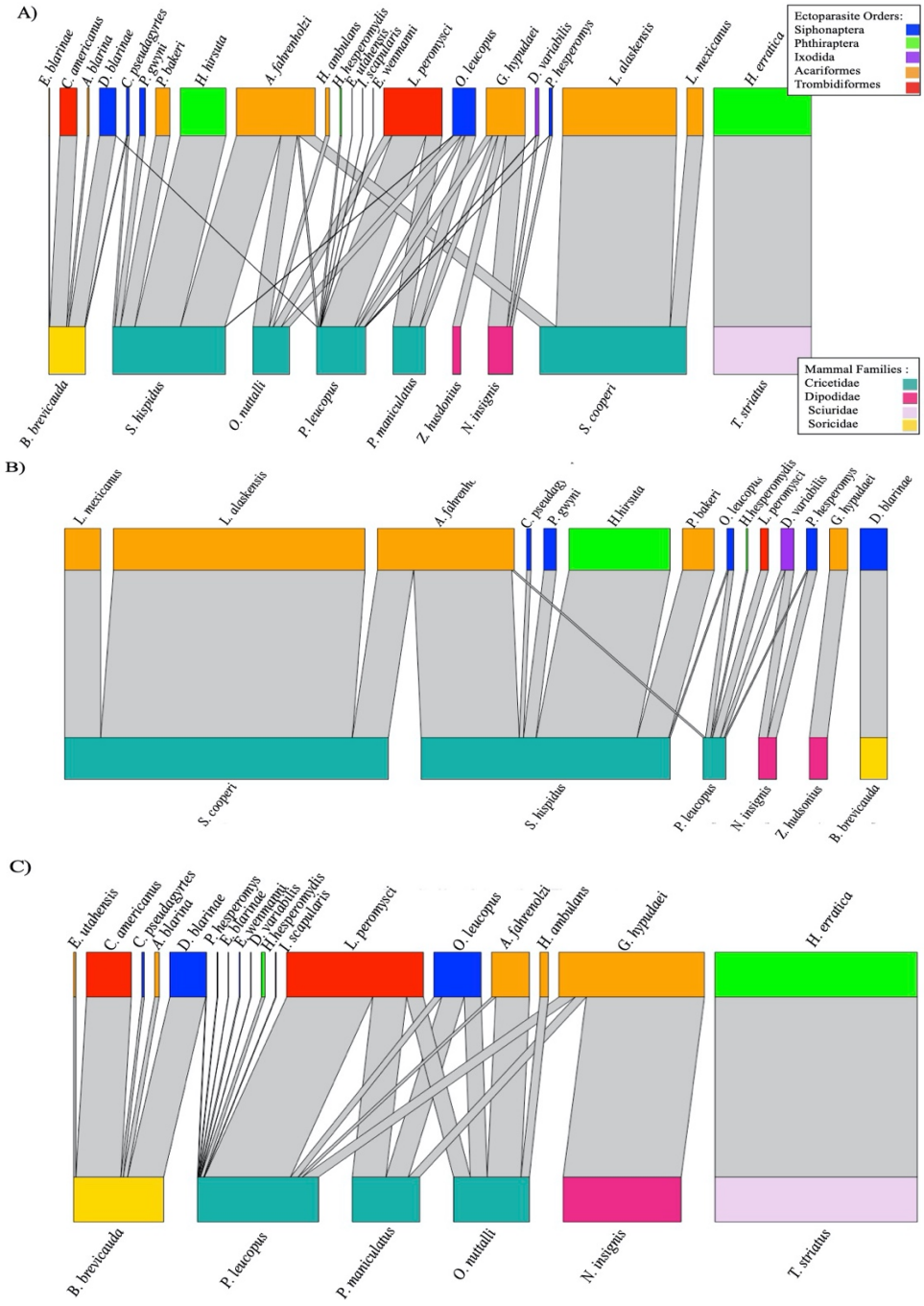


Figure 5. Weighted mammal-ectoparasite interaction networks, with link widths representing scaled ectoparasite loads on small mammal species. Colors on each network represent the different orders of ectoparasites (top) and small mammal families (bottom). A) Mid-elevation mammal-parasite network (all field sites combined); B) network for meadow habitats across all field sites; C) network for forest habitats across all field sites.

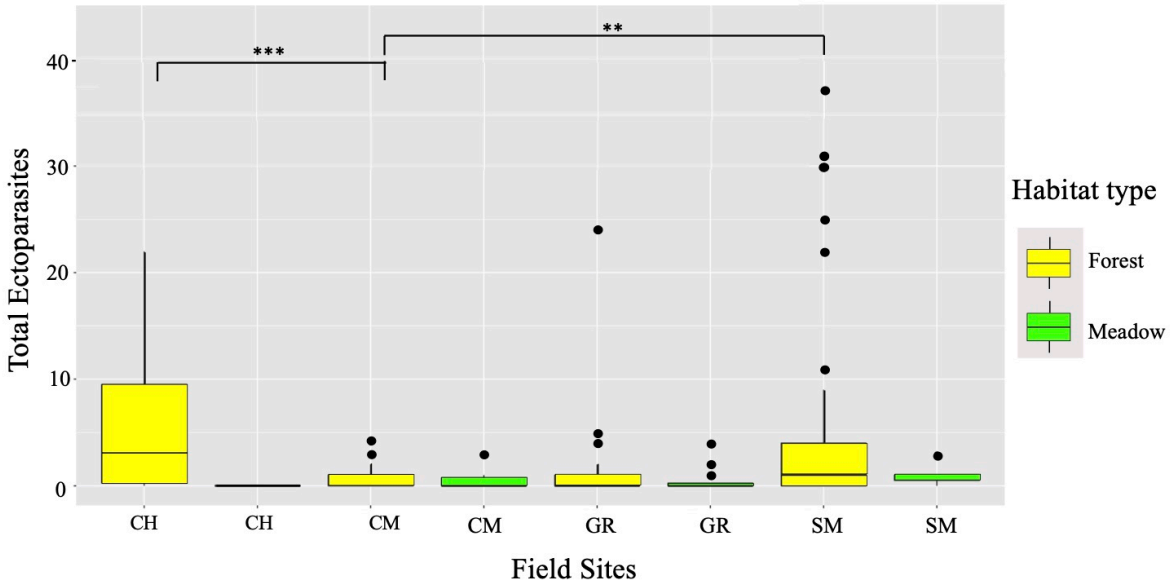


Figure 6. Total ectoparasite loads (i.e., number of ectoparasite individuals) found on white-footed mice (*Peromyscus leucopus*) in each site x habitat combination. Habitat types are colored identically for each site. One outlier (an individual with ~87 *Leptotrombidium peromysci*) collected from the Chattahoochee forest habitat was removed for better visualization. Sites on the X-axis are labeled using two-letter codes. Asterisks represent the significance between field sites based off of a pairwise Mann-Whitney U test.

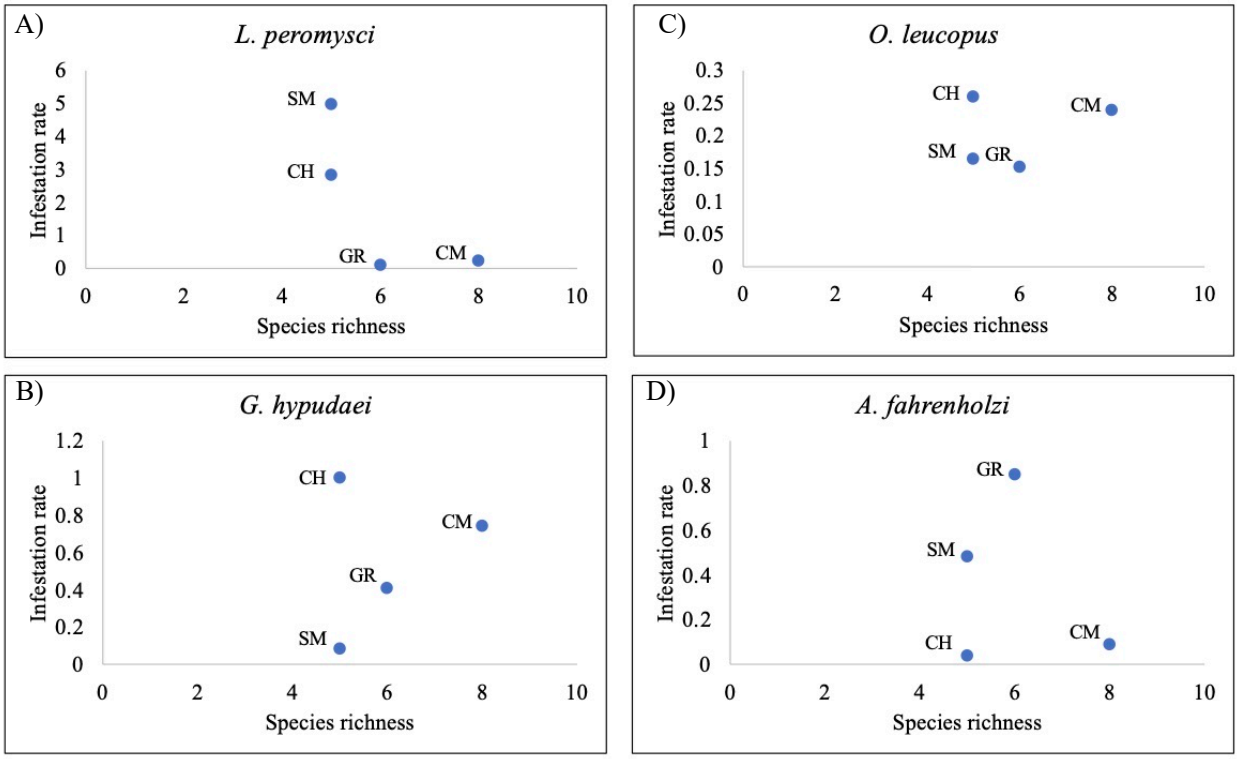
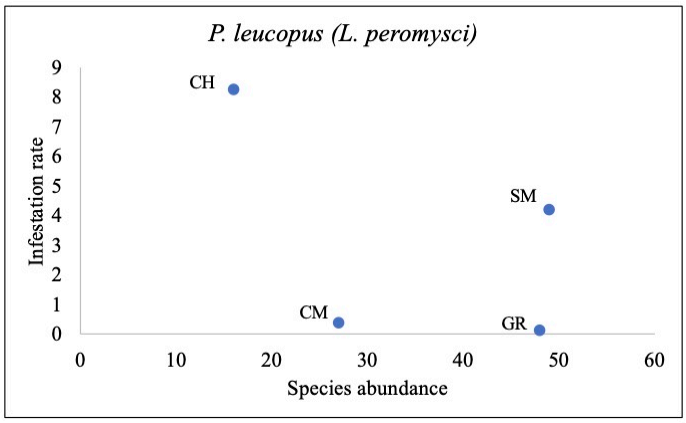
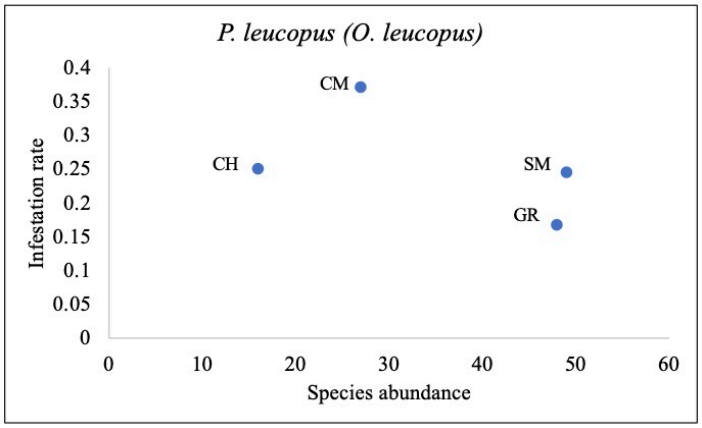


Figure 7 Abundances (individuals per host) plotted against total host species richness for the four ectoparasites collected at all field sites. The four figures A-D) represent *Androlaelaps fahrenholzi, Glycyphagus hypudaei, Orchopeas leucopus*, and *Leptotrombidium peromysci*, respectively. Field sites are labeled with two-letter codes.





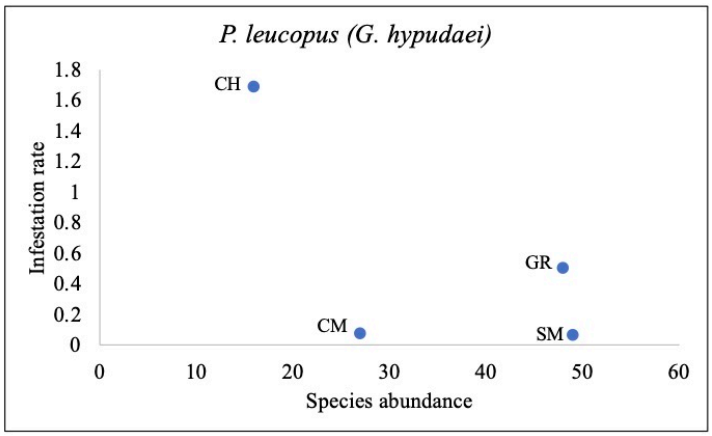


Figure 8 Abundances (individuals per host) plotted against total host species richness for the four ectoparasites collected on white-footed mice (*Peromyscus leucopus*) at all field sites. The three figures A-C) represent *Leptotrombidium peromysci*, *Orchopeas leucopus*, and *Glycyphagus hypudaei*, respectively. Field sites are labeled with two-letter codes.