Predator to Prey to Poop: Bats as Microbial Hosts and Insectivorous Hunters

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Abstract

Bat fecal samples are a rich source of ecological data for bat biologists, entomologists, and microbiologists. Feces collected from individual bats can be used to profile the gut microbiome using microbial DNA and to understand bat foraging strategies using arthropod DNA. We used eDNA collected from bat fecal samples to better understand bats as predators in the context of their unique gut physiology. We used high throughput sequencing of the COI gene and 16S rRNA gene to determine the diet composition and gut microbiome composition of three bat species in Minnesota: *Eptesicus fuscus*, *Myotis lucifugus* and *M. septentrionalis.* In our analysis of insect prey, we found that *E. fuscus* consistently foraged for a higher diversity of beetle species compared to other insects. We found that the proportional frequency of tympanate samples from *M. septentrionalis* and *M. lucifugus* was similar, while *M. septentrionalis* consistently preyed more often upon non-flying species. We used the same set of COI sequences to determine presence of pest species, rare species, and insects not previously observed in Minnesota. We were able to combine precise arthropod identification and the foraging areas of individually sampled bats to observe possible range expansion of some insects. The taxonomic composition of the bat gut microbiome in all three species was found to be consistent with the composition of a mammalian small intestine. The gut community was dominated by microbes that subsist on mucins and simple sugars, mostly in the phyla *Proteobacteria* and *Firmicutes.* Lactic acid bacteria were proportionally more abundant than most other groups of bacteria across all host demographic variables measured. As high throughput sequencing costs continue to drop and bioinformatic techniques mature, studies such as this will become more valuable for evaluating ecological hypotheses in a holobiontic context.

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Chapter 1

The Gut Microbiome of Insectivorous Bats is a Small, Conserved Community

Introduction

The vertebrate intestinal tract is composed of many distinct microclimates that provide stable environments to a broad variety of resident microbes. While all vertebrate intestines host microbes, the warm and stable intestines of homeotherms host a richer diversity of microbes than ectotherms (Stevens and Hume, 2004; Colston and Jackson, 2016; Furness *et al.*, 2015). Rows of villi covered in microvilli enable nutrient and gas exchange with the bloodstream in both the upper and lower intestines. These deep crypts are colonized by microbes that increase in density in the lower intestines. The crypts of the small intestine are highly oxygenated and are the creation site of antimicrobial peptides (AMPs), which limit the growth of many microbes. The villi are covered in a thick layer of mucus that supports bacteria able to withstand AMPs. The large intestine has a layer of looser mucus that is more anaerobic and has fewer AMPs (Donaldson *et al.*, 2016), thus supporting a much larger community of microbes (Ley *et al.*, 2008b).

Bats only partially follow the general mammalian pattern. Bats have shorter, simpler digestive systems (Ishikawa *et al.*, 1985). Most bats do not have colons to support fermentative digestion, which changes the profile of bacteria expected to play roles in their microbiomes (Stevens and Hume, 2004). Colons have lower pH, lower flow rate, and lower oxygen levels relative to the small intestine. The microbes which colonize the small intestine are therefore more likely to be acidophilic, have attachment mechanisms to combat the flow of liquid through the intestines, and are more likely to be facultative anaerobes (Donaldson *et al.*, 2016). Simple sugars and bile salts are abundant in the small intestine, so microbes adapted to this environment often metabolize them. While the lack of a colon changes the microbiome in bat intestines, the bacterial communities in the small intestine may be similar to those of other mammals. The saliva and the stomach of insectivorous bats is less acidic than the stomach and the saliva of frugivorous bats, which may ameliorate the effect of a short tract length on acidity (Dumont, 1997).

Because of these differences the digestive tracts of bats support unique gut microbiomes compared to other mammals, in some cases showing more similarity with the gut microbiomes of birds (Song *et al.*, 2020). Few studies have been published to date on the gut microbiomes of these unique animals, though bats have recently been suggested as an ideal order to study the effect of diet on intestinal microbiota due to wide variation in diet within the order (Ingala *et al.*, 2019). Samples in other bat gut microbiome studies have been obtained from feces and the last one *cm* of the rectum (Phillips *et al.*, 2012; Baxter *et al.*, 2014; Ingala *et al.*, 2018; Xiao *et al.*, 2019). Six Phyllostomid bat species with different diets had only marginal differences between different regions of the digestive tract. The microbiomes of all six species were dominated by Proteobacteria, typical inhabitants of mammal small intestines (Carrillo-Araujo *et al.*, 2015).

In addition to structural differences in the bat's digestive physiology, adaptations for flight have consequences on the environments available to gut microbes. In both bats and birds, flight imposes restraints on body plan that extend from the structural to the cellular level (Zhang *et al.*, 2015). Volant animals typically have shorter intestinal tracts than similarly sized non-flying animals (Maina, 2000). Volant animals compensate for shorter intestinal tracts with increased digestive efficiency (Price *et al.*, 2015a). Birds and bats accomplish this in part by increasing the rate of passive paracellular absorption of nutrients in their intestines (Caviedes-Vidal *et al.*, 2007, 2008; Price *et al.*, 2015b). The epithelial cells in the small intestine are smaller but highly villous with porous tight junctions that allow for the diffusion of sugars into the blood stream (Stevens and Hume, 2004). Increased intestinal permeability is non-selective, and may allow the absorption of toxins in the diet or undesirable macromolecules from the microbiome (Chang *et al.*, 2004). Both bats and birds have reduced community diversity of microbes in their intestines, perhaps reflecting these increased constraints (Song *et al.*, 2020).

Changes to the circulatory and urinary systems associated with flight may also impact the gut microbiome environment. Oxygen carrying capacity of blood increases because of the high cellular respiration rates required for flight (Maina, 2000; Thomas and Suthers, 1972). To meet this demand, the blood of flying animals contains more hematocrit and blood cells have higher surface area to volume ratios (Neuweiler, 2000; Wołk and Ruprecht, 1988; Wimsatt, 1970). Bird and bat intestines still contain anoxic environments, but the oxygen gradient from the bloodstream to the lumen is steeper. Blood vessels in the bat are also narrower to accommodate small blood cells and high viscosity, further increasing the steepness of diffusion gradients (Pries *et al.*, 1992). Steep oxygen gradients may constrain the microbial populations that are able to colonize the intestinal mucus. Growth of aerotolerant taxa such as *Enterococcus*, *Lactobacilli* and Gammaproteobacteria could be enhanced (Espey, 2013; Albenberg *et al.*, 2014).

Oxygen levels and temperature are high throughout the body of the bat during flight. Unlike most mammals, bats also use daily torpor to conserve energy. While in torpor, internal body temperature is reduced to ambient levels and blood oxygen levels are dramatically lower (van Breukelen and Martin, 2015; Willis, 2017). Some small birds, such as hummingbirds and wrens, also use torpor to conserve energy (Romano *et al.*, 2019). Hematological parameters of bat blood do not change significantly during daily torpor (Kallen, 1960; Arad and Korine, 1993). Daily variation of temperature and oxygen on the gut microbiome of bats probably reduces the number of taxa of bacteria that can colonize the gut. Facultative anaerobes in the Proteobacteria and Actinobacteria phyla could be enriched at the expense of obligate anaerobes and aerobes alike (Albenberg *et al.*, 2014).

Because the gut environment of bats is different from that of other mammals, foundational data is critical to understanding the role of microbiota in the bat digestive system. One family of bats, the Vespertilionidae, includes almost 10% of extant mammal species (Stadelmann *et al.*, 2007). This study reports the taxonomic composition of the gut microbiome for three vespertilionid bat species, *Eptesicus fuscus*, *Myotis lucifugus* and *M. septentrionalis* based on 16s rRNA illumina sequencing. The major questions we asked were: (a) What is the taxonomic composition of the gut microbiome and how is it shaped by the evolutionary pressures that constrain other organs in the bat digestive system? (b) Does age, sex, reproductive state, or host species affect variation in the gut microbiome? (c) Does habitat influence the gut microbiome composition of bats?

Methods

Field Collection

Bat fecal sample collections were made at 25 locations across the forested region of Minnesota in June and July over the summers of 2015 to 2017. (Figure 1.1). Mist-nets were deployed at each location along forest roads, trails and streams that could act as flight corridors (Swingen *et al.*, 2018). After capture, the bats were placed in handling bags and transported to a central processing site. Feces found in the bags were collected in sterile microcentrifuge tubes and stored at -20°C. Each captured bat was classified to species and its sex, age, and reproductive status was determined by physical examination. We obtained 125 fecal samples from *Eptesicus fuscus*, 140 fecal samples from *Myotis lucifugus*, and 95 fecal samples from *M. septentrionalis.*

Lab Methods

DNA was extracted from individual fecal pellet samples using a ZYMO Quick-DNA Fecal/Soil Microbe Kit and the standard protocol. Extractions with DNA concentrations less than $5ng/\mu l$ were discarded. The University of Minnesota Genomics Center carried out library preparation and sequencing of the V4 region of 16S rRNA using a paired 2x250bp run on the Illumina Miseq Platform (Illumina, inc., San Diego, CA).

Bioinformatics Analysis

We used the mothur pipeline (Schloss *et al.*, 2009) to pair and align the 16S rRNA sequences against mothur's recreation of the SILVA SEED alignment database (v. 1.32). Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level using UCLUST and assigned taxonomy at a genus level using the full SILVA reference database (v 1.32). The resulting dataset of binned OTUs and samples was analyzed using the phyloseq and ALDEx2 packages in R (McMurdie and Holmes, 2013; Fernandes *et al.*, 2013). The analyses were repeated with OTUs taxonomically binned at phylum level to facilitate comparison to other published mammalian microbiomes. Samples with less than 1000 reads were dropped from the study.

Consensus sequences of OTU sequences that occurred more than one time in a sample were constructed using the Biopython platform (Cock *et al.*, 2009). PhyML was then used to infer a maximum likelihood phylogenetic tree using a $GTR+G+I$ substitution model (Guindon *et al.*, 2005).

Rarefaction curves of gain in unique taxa per subsampled read depth were computed using a custom R script that discarded OTU records with only one read per sample (See R workflows in Supplemental Documents). We considered confidence in the completeness of sampling roughly proportional to the slope of a rarefaction curve and selected samples that achieved zero-slope rarefaction curves for further analysis. Rarefaction curves where bat populations were subsampled instead of reads were then calculated to quantify the completeness of sampling at varied taxonomic depth. To quantify the completeness of sampling, the gain in taxa count from the 99^{th} to the 100^{th} percentile sampling rarefaction was bootstrapped 100 times. The geometric mean for each host species group was calculated to make comparisons that are unbiased by variation in sampling depth.

Alpha Diversity

Many frequently used metrics of alpha diversity assume even sample depth and a discrete distribution of values. To meet these statistical assumptions, we scaled the OTU counts in the dataset using counts of bootstrapped random samples drawn at the lowest library depth of 1,442 reads. Scaled richness of OTUs was measured using the modified OTU counts.

To accommodate the wide range of read depths in this dataset, we defined proportional OTU richness as OTU richness normalized to the read depth of each sample and the maximum normalized richness in the dataset. Proportional OTU richness ranges between values of zero and one. Differences in proportional OTU richness among groups were tested using ANOVAs and t-tests on the centered log ratio transform of the values relative to the geometric mean proportional richness. The statistical significance of differences between pairs were evaluated using post-hoc Tukey tests.

Evenness was calculated with a scaled Shannon diversity index and the scaled OTU count table. Shannon's diversity index measures how the OTU abundance within each sample differs from the uniform distribution. Evenness was scaled to the maximum possible evenness given the number of non-zero OTU counts in each sample. Welch's t-tests and ANOVAs were used to test differences in values. Significant differences between pairs were assessed using post-hoc Tukey tests.

High through-put sequencing data is inherently compositional, meaning that read depth alone does not contain useful information. The abundance of reads can only be interpreted relative to the total sequencing depth of the sample (Tsilimigras and Fodor, 2016). To treat this problem, OTU counts were normalized using a centered-logarithm ratio transform (CLR), following Gloor *et al.* (2017). CLR datasets are centered on the geometric mean of OTU abundances, which makes the proportional abundances stable when using subsets of the entire dataset and does not require even sampling depth. The original OTU counts with single record only OTUs were used in the transformation. Distances for beta diversity analysis were computed using the Aitchison difference, which is the Euclidean distance between CLR transformed points (Aitchison, 1982). Principle component analysis (PCA) was then used to visualize data and identify clusters based on explanatory categorical values.

Using a CLR required that zeros in the OTU database be replaced because the algorithm to calculate a CLR relies on logarithms. The R package 'zcompositions' was used to impute values for zeros using multiplicative centered replacement and transform the CLR counts into probabilities (Palarea-Albaladejo and Martin-Fernandez, 2015). OTU records with less than 100 total read counts across all samples were dropped before transformation to improve stability of the dataset.The R package 'ALDEx2' was also used to impute values for zero counts as part of a larger workflow identifying pairwise differences in host OTU abundances.

The CLR values computed using ALDEx2 were used to construct pairwise compositional biplots on subsets of taxa counts for each set of categorical variables (Gloor, 2015). Biplots were constructed for binned counts of taxa at levels between clustered OTUs and Phyla. Differences in abundance for each taxon were tested using t-tests with Benjamini-Hochberg corrected p-values and with standardized distributional effect size (roughly 71% the size of Cohen's d).

Beta Diversity

We tested for the influence of host species, age, sexual reproductive class, Ecological Land Classification (ELC) class, and National Landcover Database (NLCD) habitat type on diversity measurements and ordination of OTUs. Differences in OTU composition between sample types were also assessed on a frequency only basis using the Jaccard index (Real and Vargas, 1996) and with unweighted Unifrac distances

(Lozupone and Knight, 2005). Unifrac distances weight the phylogenetic distance of OTUs when calculating the dissimilarity of each sample. These distance matrices along with the Aitchison distance were used to create minimum spanning trees (MSTs) of pairwise distances between samples. Graph permutation tests, where the structure of an MST remains constant while labels are permuted, were used to assess clustering of samples (Friedman and Rafsky, 1979; Callahan *et al.*, 2016). Beta diversity analyses were repeated on a subset of the samples containing only adult bats and again on a subset containing only juvenile bats.

Gross habitat composition was classified using the Ecological Land Classification (ELC) Hierarchy (Hanson and Hargrave, 1996). ELC sections are defined by major climate zones, biomes, regional elevation, and distribution of plants. Subsections are further defined within sections using surface bedrock formations, local climate, topographic relief, and tree distribution. Fecal samples were from bats captured in four ELC sections: Minnesota and Northeast Iowa Morainal (n = 30), Northern Minnesota Drift and Lake Plains (n = 111), Northern Superior Uplands (n = 78), and Western Superior Uplands (n = 67) (Figure 1.1).

A finer scale of habitat classification was based on the 2016 National Land Cover Database (NLCD) (Figure 1.2). Available covertypes were estimated by extracting the NLCD raster values with a circular mask estimated using average flight speed and egestion time for each species. The flight speed for *M. septentrionalis* was estimated using data from *M. keeni* (Hayward and Davis, 1964). Nine land cover types that accounted for less than 10% of area were compressed to one category. The most abundant habitat type was used as a categorical variable in diversity analyses.

Taxonomic and Functional Composition

We used the taxonomic assignments of OTUs that consistently occurred with a high proportional abundance (the 20 most abundant) across all samples to define a core insectivorous bat gut microbiome. Other OTUs of interest were collected from OTUs that occurred frequently in only one explanatory categorical variable of interest. We used BLASTN to align each OTU consensus sequence to the GenBank *nr* and 16S rRNA database and compiled common phenotypic characteristics of results with a 98% identity match.

The Functional Annotation of Prokaryotic Taxa (FAPROTAX) database and mapping tool was used to identify probable metabolic functions of microbes from genera with cultured representative species (Liang *et al.*, 2020). The counts of identified functions were transformed with CLR in the same way as the OTU counts relative to each sample's read depth. Differences in proportional abundance of metabolic functions were assessed between the same set of explanatory categorical variables. Particular interest was paid to intracellular pathogens, invertebrate parasites, fermentation functions, and complex carbohydrate degradation functions.

Results

After removing non-16S sequences and low-quality reads, the average read length was 249 bp. The average read depth was 22,951 per sample for 332 successfully sequenced samples and 8,038,715 reads. Clustering sequences at 97% similarity resulted in 21,090 OTUs. In those 21,090 OTUs sampling depth was uneven, ranging from 18 to 70,213 with a standard deviation of 16,114. Rarefaction curves showed that communities within each fecal sample were not evenly sampled. In total 283 of 360 samples met criteria for analysis (Figures 1.3, 1.4 and Table 1.1).

Alpha Diversity

The average scaled richness across the dataset was 304 OTUs with a standard deviation of 249. Host species, age class, and ELC were significant factors in proportional OTU richness, while sex, reproductive class, and NLCD habitat type were not (Species: $F_{2,266} = 3.3, p = 0.04$; Age: $F_{1,266} = 18.5, p < 0.0001$; ELC: $F_{,2,3} = 4.3, p = 0.005$). *Myotis septentrionalis* bats had lower richness than *E. fuscus* by a mean of 5% proportional richness (Tukey-HSD, $p = 0.02$). The proportional richness of *M. lucifugus* microbiomes was not statistically different from the proportional richness of either *E. fuscus* microbiomes or *M. septentrionalis* microbiomes. Juvenile bat proportional OTU richness was 13% lower than that of adult bats (Tukey-HSD, $p < 0.0001$). The greatest pairwise difference in microbiomes between ELC types was 8.2% between samples from the Northern Minnesota Drift and Lake Plains (NMDLP) and samples from the Minnesota and Northeast Iowa Morainal (MNIM) group (Tukey HSD, $p = 0.06$). Some interactive effect between species and ELC was present ($F_{6,266} = 2.3, p = 0.03$).

The mean evenness across all samples was 2.4 nats with a variance of 0.78. Samples from different ELC and host species were significantly different in OTU evenness (Three Way ANOVA, Geography: $F_{3,279} = 3.01, p = 0.009$; Age: $F_{1,279} = 0.11, p = 0.74$; Species: $F_{2,279} = 9.8, p < 0.0001$). Although significantly different, the change in relative evenness was small, with all but one difference under 7 percent. In pairwise comparisons, only North Minnesota Drift and Lake Plains microbiomes were different from Northeast Iowa Morainal sections. *Myotis lucifugus* samples had significantly lower relative evenness than both *E. fuscus* and *M. septentrionalis* (Tukey Post-hoc tests, $p_{EPFU} = 0.0005, p_{MYSE} = 0.01$. The dominant vegetation class surrounding the sample area, sex, and sexual reproductive class did not effect OTU evenness.

Beta Diversity

The minimum spanning tree (MST) generated using binary Jaccard dissimilarity showed structure when labels of all explanatory variables were permuted (Friedman Rafsky Graph Tests, all $p < 0.01$). Only the age class of the bat had no effect on tree structure. Some subtrees were consistent for multiple independent explanatory variables, making it difficult to interpret the driver of difference (Figure 1.5).

An MST based on unweighted Unifrac distances also had significant structure (Friedman Rafsky Graph Tests, all $p < 0.05$), but for fewer explanatory variables. The species, geographic location, reproductive class, and sex of the bat were each clustered into subtrees. When proportional abundance was incorporated into the MST using Aitchison distance, tree structure appeared different, but still contained structure along the same categorical variables. (Figure 1.6)

The first three axes of a PCA based on proportional abundance accounted for 19.3% of variation at an OTU level. Visual exploration of the data did not reveal clustering of samples based on any explanatory variable, though the samples from juvenile bats appeared as a subset within the larger spread of samples from adult bats.

Pairwise comparisons of proportional OTU abundances within categorical variables in compositional biplots revealed no differences with effect magnitudes greater than one. Many taxa grouped at the OTU, genus, family, and order levels had proportional abundances which were significantly different between host species, age class, and geographic sampling location, but all differences in proportional abundance were within one order of magnitude.

Taxonomic Composition

The most proportionally abundant taxa in each sample were consistent across all categorical variables. All samples were dominated by the same three taxa at a phyla level: Firmicutes were most abundant, then Proteobacteria, and then Actinobacteria, comprising a mean 92% of relative abundance per sample ($s = 13.6\%$) (Figure 1.7) Chlamydiae members were present in some *M. lucifugus* samples at relative proportions up to 25% of reads. The full taxonomy of the top most common OTUs is summarized in Table 1.2.

Bacteroidetes was present at low levels. Bacteroidaceae had a mean proportional abundance of 0.3 ($s = 3.9$), indicating that it does not make up a major part of the microbiome. Lachnospiraceae had a mean relative abundance of 2.6 ($s = 4.1$) and Ruminococcaceae had a mean relative abundance of 2.1 ($s = 4.2$). Two members of Lachnospiraceae and one *Clostridium* were identified in the most common OTUs across all samples. *Bifidobacteriales* members were uncommon, occurring with a mean proportional abundance of -1.74($s = 2.9$). Other common orders of the large intestine mucosa were more abundant (means > 2.8). Micrococcales was the most abundant order in Actinobacteria.

The alignment tool in mothur was unable to assign a taxonomic identity to the most common OTU in the dataset beyond the order level (Lactobacillales). A BLASTN search with the consensus sequence against the 16S rRNA databases resulted in matches with 98.29% identity and 100% coverage for many species, all in the *Enterococcus* genus.

The most common OTU that was identified as part of *Candidatus Rhabdochlamydia* was composed of 777 sequence reads. The consensus sequence was 292 bp long with four ambiguous base calls. The consensus sequence had 97.6% identity with a *Candidatus Rhabdochlamydia* sequence associated with *Oedothorax gibbosus* (KF720713.1). It had 98.2% identity, but less coverage (96%) with a Rhabdochlamydiaceae bacterium isolate (MF620051.1)

Functional Phenotype of OTUs

Phenotypic function was assigned to 4678 OTUs based on the assigned genus (33% of identified genera). Of these functions, 60% were chemoheterotrophs, and 34% were aerobic chemoheterotrophs. Bats across all category types shared function groups. When labeled by species, sex, or geographic sampling location and projected onto an MST, grouping of like variables was statistically non-random (Friedman Rafsky Graph Tests, all $p < 0.05$). Subtrees of like variables were small and scattered throughout the tree, indicating a complex interaction of categorical variables (Figure 1.8).

Fermentation was identified as a phenotype for a mean of 18% of OTUs that could be assigned a function across all samples. Intracellular pathogens comprised 12% on average of functions, and nitrate reduction comprised 4.6%. Other identified functions with a mean $> 1\%$ presence included ureolysis, aromatic compound degradation, hydrocarbon degradation, aromatic hydrocarbon degradation, aliphatic non-methane hydrocarbon degradation, methanotrophy, and methanol oxidation.

Chitinolysis was detected in seven OTUs found across 37 samples. All but one OTU record in one bat was most closely related to the genus *Lysobacter*, a group of gliding bacteria that are common in freshwater and soil. Other complex sugar degradation phenotypes that might be expected in the large intestine of a mammal include xylan degraders and cellulose degraders. Xylanolysis was detected in *Microbulbifer*, present in only one *M. septentrionalis* sample. Cellulolysis was identified in 41 OTUs and 71 taxa. Most OTUs were most closely related to Acidothermus (Actinobacteria: Frankiales). Bacteroidetes and Proteobacteria OTUs were also identified with lower frequency.

Ureolysis was identified as a function in 154 OTUs distributed across 94% samples. Five genera accounted for 129 OTUs. *Methylobacterium* was most common, followed by *Massilia, Singulisphaera, Morganella*, and *Roseomonas*.

Nitrate reduction was identified as a function of 63 taxa distributed between 275 samples. *Stenotrophomonas* (Gammaproteobacteria: Xanthomonadales), comprising three OTUs, occurs in 80% of samples. Six OTUs are members of *Aeromonas*, only one of which is common in 45% of samples.

Invertebrate parasites were more than six times more common in *M. septentrionalis* than in either *M. lucifugus* or *E. fuscus*. The most common parasite was in the genus *Arsenophonus*, followed by *Pasteuria* and *Xenorhabdus*. Intracellular parasites were more common in *M. lucifugus* (7.8% identified functions) than they were in *E. fuscus* (0.7%) or *M. septentrionalis* (2%). OTUs related to 35 genera from the Chlamydiae and Proteobacteria phyla were present in 240 samples. The greatest number of intracellular parasite OTUs were members of *Rickettsia*, *Legionella*, *Candidatus Rhabdochlamydia*, unclassified Chlamydiales OTUs, and *Wolbachia*.

Discussion

The gut microbiome of *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* had relatively consistent differences from the typical mammalian gut microbiome. The core microbiome of these bats was dominated by members of the Proteobacteria, Firmicutes, and Actinobacteria phyla. In contrast, typical mammals have a gut microbiome dominated by a larger proportion of Bacteroidetes and have a lower proportion of Firmicutes taxa. These findings are consistent with the dominance of Proteobacteria and Firmicutes identified in Phyllostomid insectivorous bats (Carrillo-Araujo *et al.*, 2015; Song *et al.*, 2020). The presence of large amounts of intracellular pathogens (*Candidatus Rhabdochlamydiae*) in *M. lucifugus* is also a notable difference from the composition of a typical mammalian gut microbiome.

Mammals with simple, foregut, and hindgut digestion types harbor different microbial communities, but still typically share microbes from core taxa in the Firmicutes and Bacteroidetes phyla (Ley *et al.*, 2008a; Langer, 2002). The composition of the mammalian intestinal microbiome varies with large changes in diet, but typically contains a majority of bacteria from these two groups. Other phyla include Actinobacteria, Verrucomicrobia, Fusobacteria, Proteobacteria, Spirochaetes, and Fibrobacteres. Firmicutes in the gut microbiome are usually Bacilli or Clostridia, and Proteobacteria are either Betaproteobacteria or Gammaproteobacteria (Nishida and Ochman, 2018).

Microbes that ferment complex polysaccharides when found in the large intestines of most mammals are rare in the bat gut microbiome. The loose mucous layer of the large intestine in humans and mice is occupied by mucin degrading organisms such as *Bifidobacteria*, *Bacteroides*, and *Akkermansia*. The rarity of organisms typical of large intestines in the bat microbiome is not surprising because insectivorous bats do not have large intestines. While the fermentation function was detected using FAPROTAX, most associated OTUs were lactic acid bacteria, not complex polysaccharide fermenters typical of the large intestine (Park and Hall, 1951; Stevens, 1980; Brun *et al.*, 2019).

Human and murine gut microbiomes are the most well characterized of mammalian systems, and differences between regions of intestinal microbiomes are known from these species. The families most typical of the lumen in human and murine large intestine microbiomes are Prevotellaceae, Rikenellaceae, and Bacteroidaceae (Donaldson *et al.*, 2016). All three groups were detected in the bat gut microbiome, but Rikenellaceae and Prevotellaceae were relatively uncommon. Families typical of the large intestine mucosa and the caecums of mice were relatively more common. The presence of any mucous associated families suggests that the rapid transit time in the bat intestinal tract does not prevent colonization by some microbes typical of a large intestine.

In the typical mammalian digestive system, Lachnospiraceae and *Clostridium* are known as plant degraders that primarily digest pectin, cellulose, and starches. Only small amounts of pectin cellulose and starches are in the bat diet because the complex carbohydrates that bats ingest are limited to those found in insects. This suggests the functions of Lachnospiraceae, Ruminococcaceae, and Clostridiaceae in the gut microbiome could be different in bats. Chitobiose, an intermediate product in the digestion of chitin, is a possible substrate (Biddle *et al.*, 2013).

Microbes inhabiting the small intestines of mammals are often lactic acid bacteria or other bacteria that ferment simple sugars (Donaldson *et al.*, 2016). Adhesion to the mucosa or walls of the small intestine is necessary to sustain growth in a high flow environment. The most common fermenting families detected using FAPROTAX meet these requirements.

The most common OTU across all samples was a lactic acid producing bacteria in the *Enterococcus* genus. *Enterococcus* species have been identified as core members of the small intestine microflora of humans, where *E. faecium* and *E. faecalis* are well studied as antibiotic resistant species that often cause urinary tract infections (Tannock and Cook, 2002). In pigeons, *E. columbae* was identified as part of the permanent intestinal flora (Baele *et al.*, 2002). As a member of the class Lactobacillales and a facultative anaerobe, *Enterococcus* should be considered a typical member of a small intestine gut microbiome (Gu *et al.*, 2013; Donaldson *et al.*, 2016; Lebreton *et al.*, 2014). The dominance of this organism in fecal samples reflects the proportional importance of the small intestine in the bat digestive system.

The second most common OTU in the dataset, which aligned most closely with *Carnobacterium* in the mothur pipeline analysis, had a consensus sequence that also aligned well with *Enterococcus* species in a BLASTN search with greater than 97%

identity. Both genera are known lactic acid producers, though *Carnobacterium* is more typically known from the gut microbiomes of fish rather than mammals (Robertson *et al.*, 2000).

Other bacteria identified from the twenty most common OTU consensus sequences with $\geq 98.3\%$ identity included *Lactococcus lactis*, *Lactococcus garvieae*, a member of Enterobacterales, *Obesumbacterium proteus*, and *Dysgonomonas gadei*. These genera are common constituents of a mammalian small intestine, where some ferment simple sugars and most are bile resistant (Bolotin *et al.*, 2001; Prest *et al.*, 1994; Hernández *et al.*, 2015; Vendrell *et al.*, 2006). *Leuconostoc* was also identified, where *L. mesenteroides* was more than 97% identical to the consensus sequence. *Leuconostoc* is known from the intestinal microbiomes of fish, reptiles, and yaks, where cultured species produce bacteriocins with high tolerance to acids and bile salts, indicating that they are more likely present in more acidic and bile rich portions of the intestines (Wang *et al.*, 2018; Allameh *et al.*, 2012)

In both the large and small intestinal environments, the ability to subsist on host mucous is what allows bacteria to form persistent communities when the flow of nutrients from the host is inconsistent. In mammals this community is typically composed of species in the Actinobacteria phyla and is dominated by members of four orders: Corynebacteriales, Bifidobacteriales, Proprionibacteriales and Streptomycetales (Binda *et al.*, 2018). While the bat gut microbiome samples lacked Bifidobacteriales, the most well studied of these mucin degraders, Proprionibacteriales and Streptomycetales were present to fulfill this niche. *Aeromonas* has also been known to adhere to the gut mucous of fish, subsisting on mucins (Van der Marel *et al.*, 2008).

An insectivorous diet impacts the bat gut microbiome in several ways. Metabolism of trehalose, the sugar storage molecule of most insects, could be enriched in the microbiome. Many bacterial species can use trehalose as a carbon source, including those common to all vertebrate gut microbiomes like *Escherichia coli* and some species of *Bacillus* (Argüelles, 2000). While OTUs from these genera were present in this study, measuring the activity of the periplasmatic trehalase enzyme (TreA) would be necessary to detect active trehalose degradation in the gut microbiome.

Chitin degradation could also be enhanced in the gut microbiome of an insectivore. Known chitinase producing bacterial genera previously isolated from *M. septentrionalis* and *M. lucifugus* include members of *Bacillus*, *Enterobacter*, *Serratia*, *Hafnia*, and *Citrobacter* (Whitaker Jr *et al.*, 2004). All perform best in slightly acidic environments (pH = 5) and were grown in aerobic conditions. None of these genera were detected as dominant members of the gut microbiota for any bat species in this study. Facultatively anaerobic bacteria capable of degrading chitin were identified in a broader array of mammals including non-insectivores such as horses and rabbits (Šimnek *et al.*, 2001). The isolates were species of *Clostridium* and *Enterococcus*, both of which appear often in the abundant OTUs for each bat species. Chitinolysis was detected using FAPROTAX in the genus *Lysobacter*, which was present in only a small fraction of samples ($n = 37$). *Lysobacter* extracellular chitinase has been studied for its antifungal properties (Yasir *et al.*, 2009). While there is some evidence of chitin degradation being a possible function of the gut microbiome, rapid gut transit time makes it unlikely.

Insect related parasites and symbionts were present in the most common OTUs of the gut microbiome. The sixth most common OTU across all samples was most closely related to the genus *Rickettsiella*. *Rickettsiella* are found in vacuoles of cells of invertebrate and vertebrate hosts, though the genus is more typically associated with invertebrates (Cordaux *et al.*, 2007). They are common pathogens of insect larvae and other arthropods including arachnids and crustaceans. An OTU most closely related to an uncultured proteobacterium clone sequenced from the guts of termites and cockroaches was also present in many samples.

Candidatus Rhabdochlamydia is an insect associated genus, perhaps with roles as an endosymbiont. It is associated with the fat body of cockroaches as well as the ovaries. It occurred in co-culture with *Wolbachia* in wolf spiders, and may have a role in the bodies of ticks as well (Pillonel *et al.*, 2019; Vanthournout and Hendrickx, 2015). *Rhabdochlamydiae porcellonis* and *R. crassificans* are the most well known *Rhabdochlamydiae*, but this sequence is only 96.92% similar to it, indicating that cockroaches and pillbugs are likely not the source of this microbe. *Rhabdochlamydiae*-like organisms have been identified in the feces of *M. daubentonii* that are unconnected to insect sources (Hokynar *et al.*, 2017). *Rhabdochlamydiae* have been noted in other recent bat gut microbiome studies, suggesting this organism may play a larger as yet undescribed role in some bat gut microbiomes (Xiao *et al.*, 2019).

Several insect associated intracellular pathogens are present in the fecal samples, including *Wolbachia*, *Rickettsiella*, and *Rickettsia*. Only *Candidatus Rhabdochlamydia* occurs in high proportional abundance in some samples however, suggesting that it may be able to reproduce in settings outside of arthropod cells. Closely related genera have this capability. *Parachlamydia acanthamoebae* for example is a species associated with pneumonia and an internal parasite of Acanthamoeba, a free-living amoeba common in many aquatic environments (Greub *et al.*, 2005). The bacterium has been shown to replicate outside of Acanthamoeba, in the lungs of mice and humans (Greub, 2009).

A response to differences in local habitat could indicate a rapid response time of the microbiome to ingestion and a lack of a persistent gut community. Human host location has been demonstrated to have a strong effect on variability in the gut microbiome composition (He *et al.*, 2018). Often changes in geography are confounded with changes in diet and the microbial contents of drinking water (Jha *et al.*, 2018). In the case of bats, changes associated with NLCD likely reflected changes in insect prey availability (See Chapter 2). However across all analyses, NLCD had no significant effects on microbial diversity. The consistency of gut microbiome composition across bat species, sex, reproductive conditions, and geography suggests that the community is conserved.

Like passerine birds, bats have streamlined digestive organs that preclude the long fermentation times that characterize common functions of the gut microbiome of many vertebrates. However the gut microbiome function is not purely determined by the ability to extract more nutrients from the host's diet. The microbial community in bat feces reflects a gut microbiome dominated by niches of the small intestine. Taxa that can subsist in the intestinal mucus are present, indicating a persistent community able to survive short and long term changes in nutrient availability. Lactic acid bacteria dominate the taxonomic profiles of all bat microbiomes sampled and likely share similar roles in host immune systems as they do in other mammals. Our study indicates there is high potential for for microbes to have meaningful roles as part of atypical digestive systems.

Figures and Tables

Figures

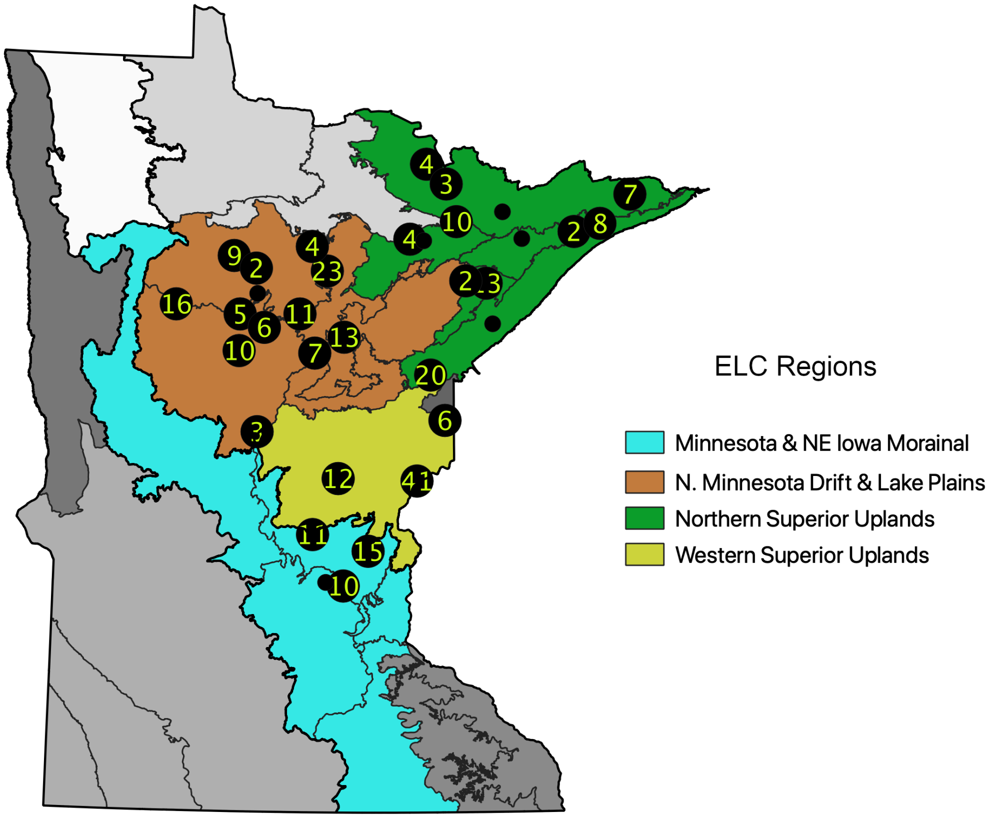

Figure 1.1: **Sampling Locations.** ELC Sections are in color: MNIM = teal, NMDLP = brown, NSUP= green, WSUP= gold.

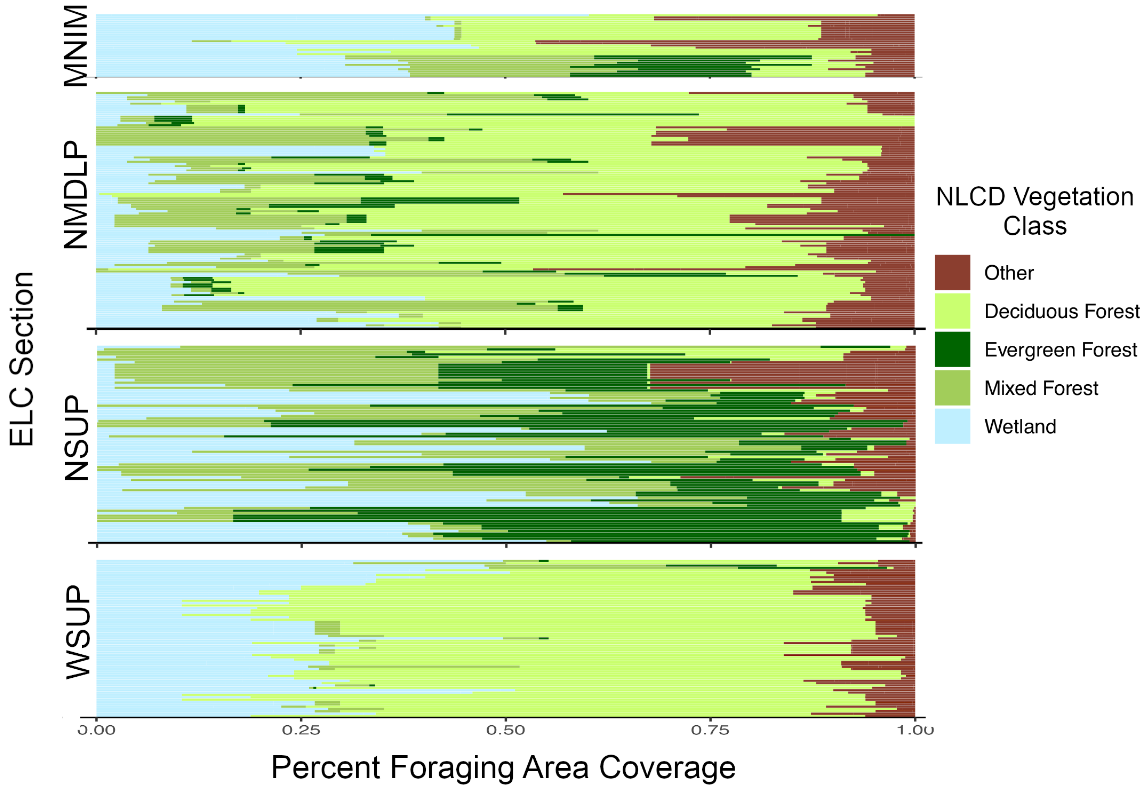

Figure 1.2: **NLCD landcover composition across ELC areas** Each bar represents one sample. MNIM = Minnesota and Northeast Iowa Morainal, NMDLP = Northern Minnesota Drift and Lake Plains, NSUP = Northern Superior Uplands, WSUP = Western Superior Uplands.

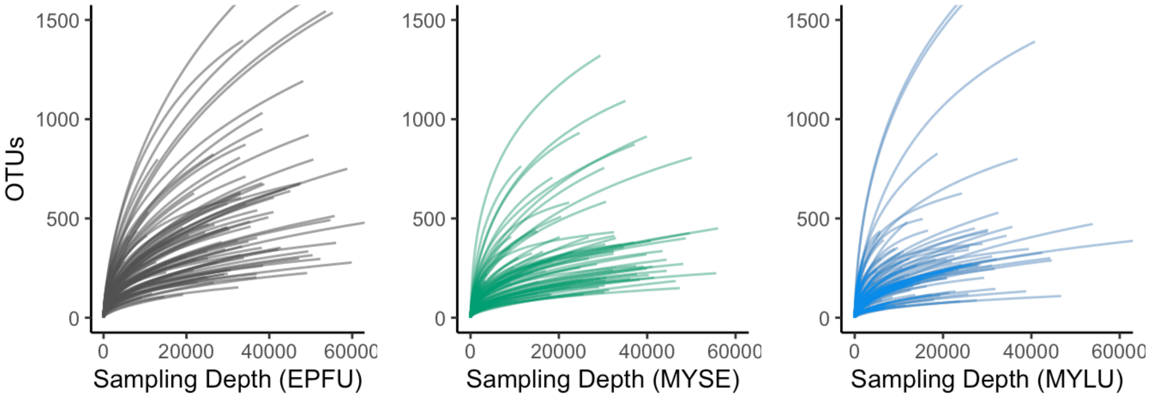

Figure 1.3: **Sequencing rarefaction curves by host species.** Supplemental probably. EPFU=*Eptesicus fuscus*, MYLU=*Myotis lucifugus*, and MYSE=*M. septentrionalis*.

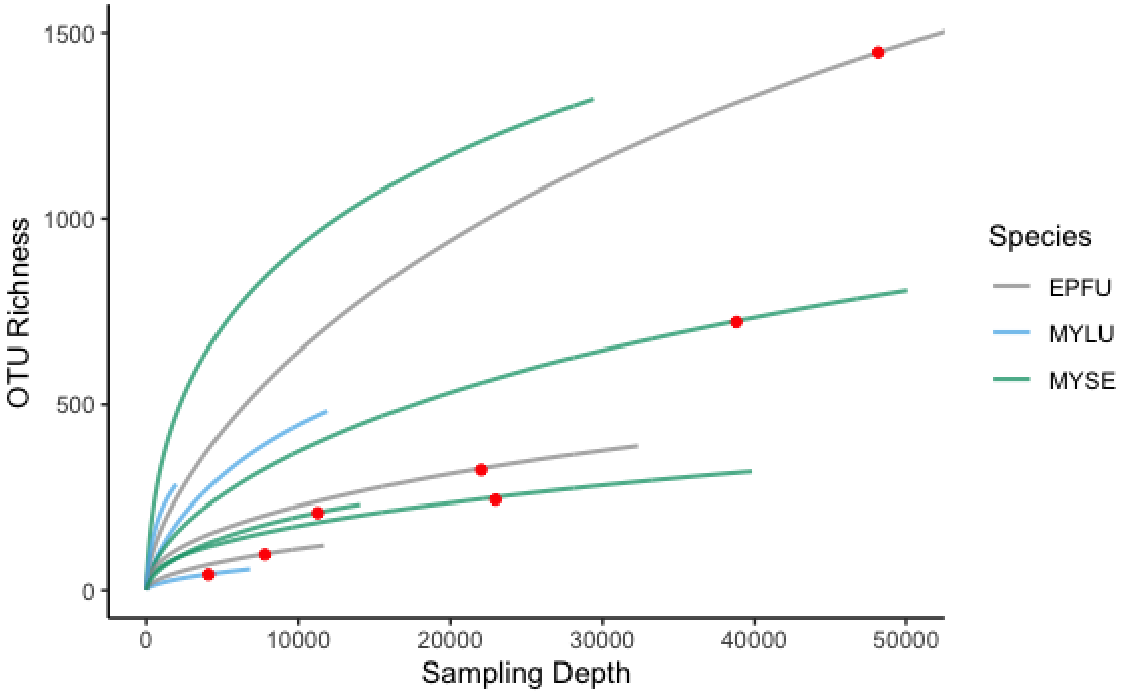

Figure 1.4: **Rarefaction curves for 10 random samples.** Only the samples which had rarefaction curves with slopes <= 0.01 were used in analysis. Slope of 0.01 is marked with a red dot.

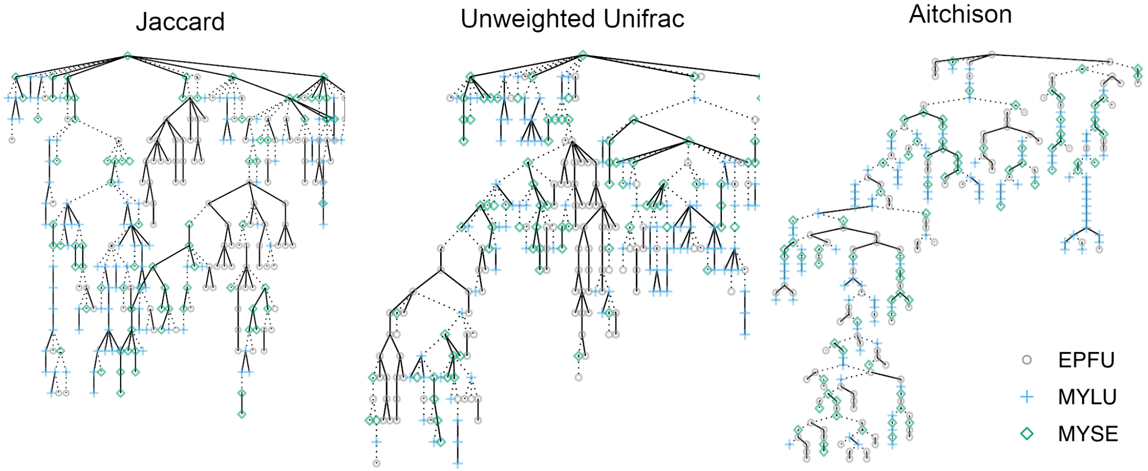

Figure 1.5: **The structure of MSTs using three distance metrics, colorized by host species.** Right to left: Jaccard index, unweighted Unifrac distance, Aitchison distance. Small subtrees form significant structure in all three trees. Solid lines connect like species, dashed lines connect others.

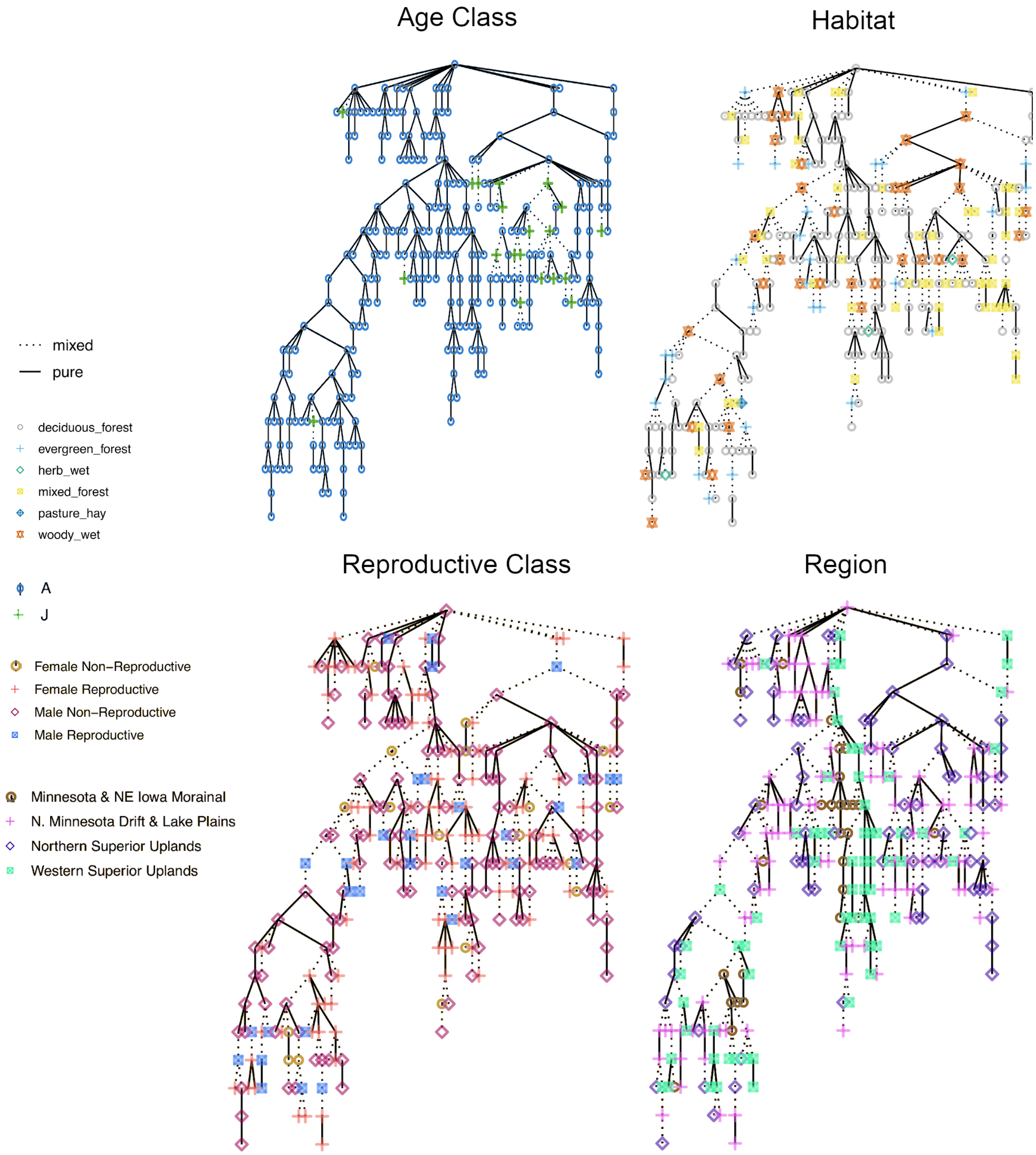

Figure 1.6: **The structure of uweighted Unifrac MST, colorized by categorical variables.** Solid lines connect like species, dashed lines connect others.

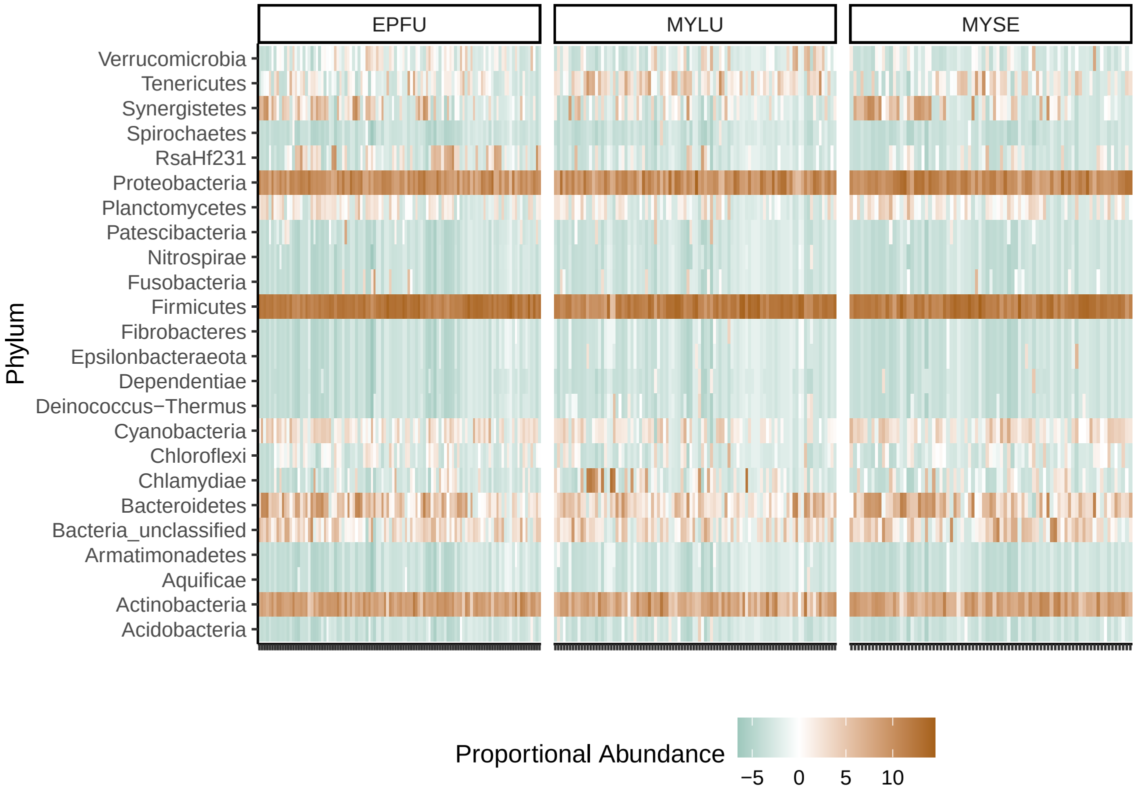

Figure 1.7: **Proportional abundance of OTUs binned by phylum.** Host species host similar proportions of taxa at a phylum level. Proportional abundance is in natural logarithm fold units relative to the geometric mean of each sample. *Myotis* species hosts Chlamydiae OTUs with greater abundance than *Eptesicus fuscus* does.

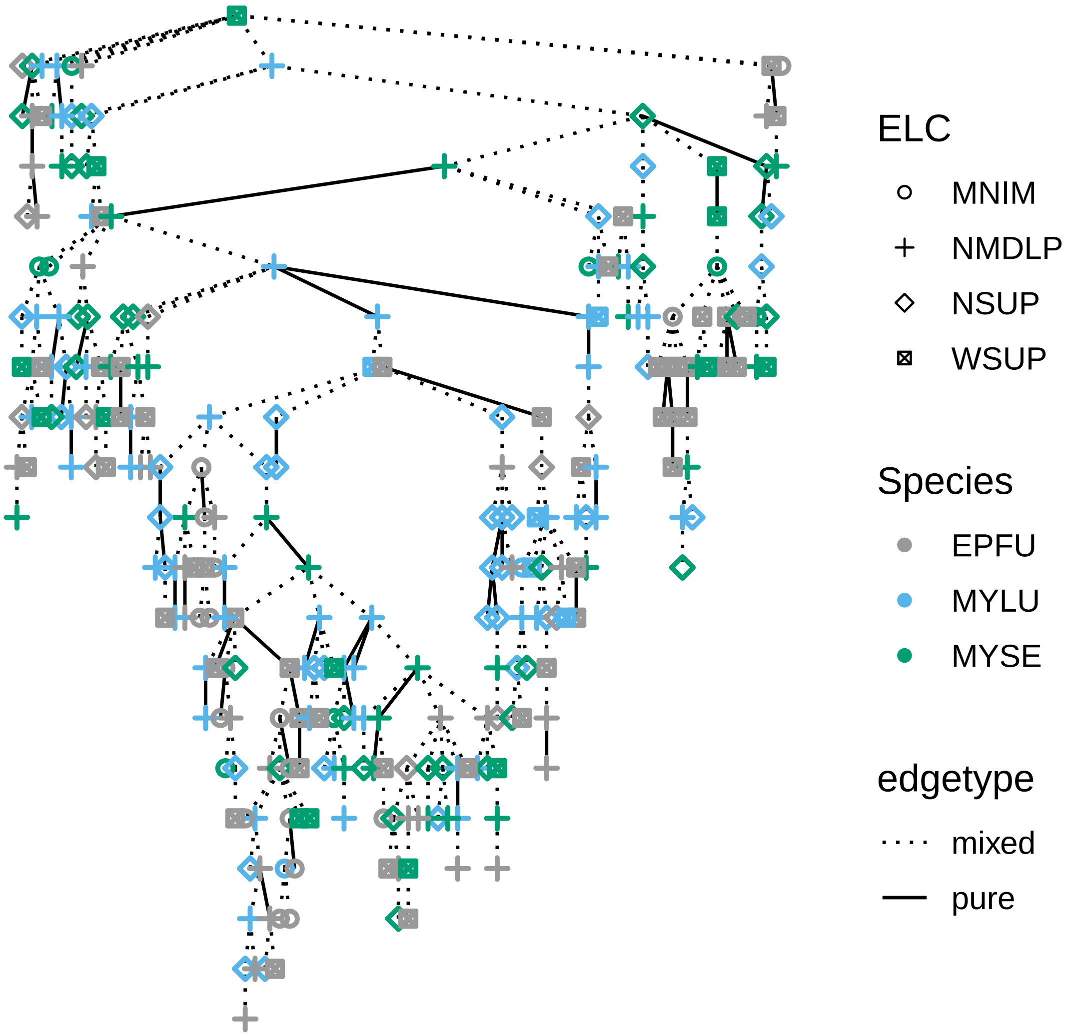

Figure 1.8: **MST of FAPROTAX assigned functions.** Aitchsion distance was used as a distance metric to construct and test the MST. Both host species and ELC labels create significantly non random subtrees.

Tables

Table 1.1: **Age, reproductive class, and species of bats used in diversity analysis.**

Species	**Age Class**	**Sex**	**Reproductive Class**	***n***
Eptesicus fuscus	A	F	R	34
n=108			NR	4
		M	R	19
			NR	48
	J	M	–	3
Myotis lucifugus	A	F	R	17
n=96			NR	3
		M	R	9
			NR	53
	J	F	–	7
		M	–	7
Myotis septentrionalis	A	F	R	42
n=79			NR	4
		M	R	4
			NR	26
	J		–	3

Table 1.2: **Unique OTUs from the top 20 ranking mean centered log ratio abundances for three bats.** Mean and SD represent proportional abundance values for all OTUs. Percentages are relative to identified OTUs, not averaged relative abundance.

Phylum	**Class**	**Order**	**Family**	**Genus**	**Mean ± SD**
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Unclassified	12.3 ± 2.6
46%	42%	30%	Carnobacteriaceae	Carnobacterium	7.8 ± 3.9
			Streptococcaceae	Lactococcus	6.2 ± 4.4
			Leuconostocaceae	Leuconostoc	4.2 ± 4.4
		Bacillales	Staphylococcaceae	Staphylococcus	3.5 ± 3.4
		Unclassified	Unclassified	Unclassified	7.7 ± 3.4
	Clostridia	Clostridiales	Lachnospiraceae	Unclassified	3.9 ± 4.4
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Unclassified 1	7.7 ± 3.4
39%	31%	22%	22%	Unclassified 2	6.5 ± 3.6
				Unclassified 3	3.9 ± 4.2
				Hafnia-Obesumbacterium	4.1 ± 4.2
		Betaproteobacteriales	Burkholderiaceae	Unclassified	4.6 ± 2.2
		Diplorickettsiales	Diplorickettsiaceae	Rickettsiella	3.7 ± 4.0
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Unclassified	4.6 ± 4.7
	8%	Rhizobiales	Beijerinckiaceae	Methylobacterium	3.6 ± 2.9
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Unclassified	3.6 ± 3.1
20%	20%	7%	7%	Unclassified	3.5 ± 3.1
		Corynebacteriales	Mycobacteriaceae	Mycobacterium	5.4 ± 2.8
		Pseudonocardiales	Pseudonocardiaceae	Actinomycetospora	4.1 ± 2.8
		Streptomycetales	Streptomycetaceae	Unclassified	3.7 ± 3.5

Chapter 2

Bat Fecal Samples as a Source of Terrestrial Insect eDNA

Introduction

Bat fecal samples are a rich source of ecological data for both bat biologists and entomologists. Feces collected from individual bats and guano deposits beneath colonies or summer roosts have been used to profile the gut microbiome community using the 16S rRNA gene sequenced from microbial DNA (Phillips *et al.*, 2012; Baxter *et al.*, 2014; Ingala *et al.*, 2018; Xiao *et al.*, 2019; Song *et al.*, 2020). The mitochondrial cytochrome-x oxidase I (COI) gene in arthropod DNA can be used to better understand the foraging habits of bats from fecal samples (Clare *et al.*, 2014a, 2009, 2011, 2014b; Dodd *et al.*, 2012; Weinkauf *et al.*, 2018) (See also Chapter One). Insight on the habitats and geographic distribution of arthropods can be gained if the arthropod DNA is treated as environmental DNA (eDNA).

The DNA of prey in fecal matter can yield taxonomically precise information about the diet of the predator. In the last 20 years, DNA barcode studies of bat feces have greatly expanded what had been learned about bat diets from pellet and stomach dissection studies (Jinbo *et al.*, 2011; Coissac *et al.*, 2012). For example, most pellet dissection analyses have supported the hypothesis that *Eptesicus fuscus* (the big brown bat) specializes on Coleoptera (beetles) both in terms of frequency and percent volume consumption (Whitaker Jr, 1972; Brigham and Saunders, 1990; Whitaker Jr, 1995; Hamilton and Barclay, 1998; Agosta and Morton, 2003; Feldhamer *et al.*, 2009; Carter *et al.*, 2003; Thomas *et al.*, 2012). Only one published pellet dissection study of *E. fuscus* listed another order, Trichoptera, as the majority component of the diet. In that study of a maternity colony foraging near a river, aquatic beetles were also common (Verts *et al.*, 1999; Whitaker Jr, 1972). Molecular diet studies of *E. fuscus* also support Coleoptera as a major component of species identified in diet, along with a near equal number of Lepidoptera species (butterflies and moths) (Long *et al.*, 2013; Clare *et al.*, 2014b). Excluding the wings, which bats often discard before ingestion, Lepidopteran taxa have softer bodies than Coleoptera that are less likely to survive digestive transit intact enough to be dissected out of a dry pellet (Hickey *et al.*, 1996). Smaller fragments of arthropods and softer bodied, more decomposed insects are more likely to be detected when DNA is extracted from bulk fecal matter rather than dissected insect parts (Jusino *et al.*, 2019). The expansion of the big brown bat diet to orders other than Coleoptera shows how DNA analyses of fecal samples can demonstrate the degree to which insectivorous bats are opportunistic foragers.

The COI gene has regions of high variability and conservation, making it suitable for alignment and identification. Using a primer set specific to regions of variability within the phylum Arthropoda and a reference database of COI sequences, it is possible to achieve species level identification using a small amount of starting DNA (Hebert *et al.*, 2003b,a; Jusino *et al.*, 2019). The Barcode of Life Database (BOLD) curates a large collection of vouchered specimens and accompanying COI sequences ideal for this purpose (Ratnasingham and Hebert, 2007; Dodd *et al.*, 2012). In published molecular barcode studies of *Myotis lucifugus* (the little brown bat) fecal samples, 149 arthropod species in 56 families and eight taxonomic orders have been identified (Clare *et al.*, 2014a, 2011; Shively *et al.*, 2017). In contrast, only one insect taxon, *Diabrotica undecimpunctata* (potato beetle), was identified to species in four published pellet or stomach dissection studies of the little brown bat (Anthony and Kunz, 1977; Feldhamer *et al.*, 2009; Whitaker Jr and Lawhead, 1992; Whitaker Jr, 1972). Similarly, the molecular diet studies have identified 84 more families than the 10 families identified through pellet dissection.

The precision of DNA-barcodes in taxonomic identification offers the opportunity to test hypotheses of bat foraging strategies and diet in new ways. Traits that reduce predation of an insect species can not be assumed to be present in related insect species, and sometimes appear in unrelated species. For example, tympanate insects with sensitivity to the 20—100kHz frequency range that bats use for echolocation are not exclusively Arctiid moths. Other tympanate insects include green lace wings in the family Chrysopidae, several Sphingidae hawkmoths, and many insects in the Orthoptera order can also detect bat echolocation pulse frequencies (Miller and Surlykke, 2001). Similarly, economic pests are typically known to species. For example *Lymnatria dispar dispar* (gypsy moth) is a destructive pest in the otherwise innocuous moth family Erebidae. Caterpillars of most other species in the same family only consume decaying vegetation or fungi instead of leaves (Arnett Jr and others, 1985). The species level composition of bat diet is important for evaluating the ecosystem service values that bats provide.

Insectivorous bats have a high economic benefit as pest control agents. In 2011, the contribution that bats made in North America to agricultural crop systems was estimated as $3.7 billion a year (Boyles *et al.*, 2011). *Tadarida brasiliensis* (Mexican free-tailed bat), a common North American species, contributed 2—29% of the $6 million value of the cotton crop in Texas by reducing insect populations eating cotton plants (Cleveland *et al.*, 2006). Traditional pellet dissection studies in combination with qPCR based studies have quantified the impact of bat predation on many pest species, including *Spodoptera exigua* (beet armyworm), *Acrobasis nuxvorella* (pecannut casebearer moth), and *Cydia caryana* (hickory shuckworm moth) (Kunz et al. 2011; Lee and McCracken 2005; Cleveland et al. 2006; Federico et al. 2008). *Helicoverpa zea* (Corn earworm moths) are major agricultural pests of cornfields. A fecal pellet using PCR found that *H. zea* made up a significant portion of the diet of Mexican free-tailed bats (McCracken *et al.*, 2012).

Bats provide ecosystem services on agricultural landscapes, and also impact populations of insects that vector diseases, including mosquitos. Not all mosquitos are disease vectors, and when quantifying the impact of bat predation, identification of mosquitos to species is critical. Acoustic studies and other observational studies confirm that both *M. lucifugus* and *M. septentrionalis* feed on mosquitos (Rydell *et al.*, 2002; Reiskind and Wund, 2009). Two molecular diet studies to date have focused on the frequency of mosquito consumption by bats. Australian species of bats consumed mosquitos with relatively lower frequency than they consumed other insects, though smaller bats tended to consume more mosquitos (Gonsalves *et al.*, 2013). More locally, Wisconsin area *M. lucifugus* and *E. fuscus* bats consumed mosquitos belonging to 17 taxonomic BINs and 16 species, and mosquitos were present in 72% of fecal samples (Wray *et al.*, 2018). For comparison, estimates of the number of mosquito species in Minnesota range between 31 in a recent survey and 45 from historical records (Barr, 1958; Kinsley *et al.*, 2016)

These and other studies have been effective at connecting bat diet to the activity of known pests, but they are limited by design to confirming the presence of only a few species. Environmental DNA (eDNA) is extracted from an environmental sample without prior isolation of target organisms (Deiner *et al.*, 2017). Using high throughput sequencing on DNA samples rather than qPCR enables detection of pest insect species without targeting them explicitly. Usually eDNA is extracted from samples of water and used in aquatic systems to survey for possible invasive invertebrate species (Garlapati *et al.*, 2019). Passive sampling using eDNA barcoding is being used to assess biodiversity, detect invasive species, and to monitor threatened species in many aquatic systems (Lacoursière-Roussel *et al.*, 2018). Terrestrial systems can also be monitored using eDNA from soil, though the technique is less established than other passive monitoring techniques (Leempoel *et al.*, 2020). Saliva on browsed twigs, wildflower petals, bulk scat, and the contents of pit fall traps are examples of more exotic sources of eDNA (Nichols *et al.*, 2012; van der Heyde *et al.*, 2020; Thomsen and Sigsgaard, 2019).

Bats collect arthropod DNA from the environment where they forage, offering a broad sample of environments that are difficult to access directly through transects or observation plots. Light traps are typically used to sample nocturnal insects, but are less effective in urban and suburban areas with high ambient light levels or on full moon nights (Aagaard *et al.*, 2017). Alternatively, malaise traps can be used but are sensitive to placement and cannot be placed over water (Matthews and Matthews, 2017). Insects harvested from light or malaise traps can be physically analysed or DNA can be extracted and used for barcode identification (Young *et al.*, 2020).

Barcode identification of eDNA collected from bat fecal samples can be used to identify cryptic, difficult to collect arthropod species across a large study area. Beyond monitoring pests and key threatened species, these identifications can also update the known geographic ranges of insects based on the foraging area where the fecal sample from the bat was collected(Piper *et al.*, 2019). Insect populations are declining in many parts of the world, and changes in insect distributions are expected as global climate changes (Stange and Ayres, 2010; Goulson, 2019). Globally, insects in the Lepidoptera, Hymenoptera, Odonata, Plecoptera, Trichoptera, Ephemeroptera, and Coleoptera orders are being extirpated, but on the local level, population trends are unknown for many taxa (Sánchez-Bayo and Wyckhuys, 2019). Broad sampling of insects in the midwest is needed to establish baseline diversity before decline or change can be addressed (Didham *et al.*, 2020). Bats collect samples for many of these insect groups, especially Lepidoptera and Ephemeroptera (Whitaker Jr, 1972; Clare *et al.*, 2014a,b; Dodd *et al.*, 2015).

We used eDNA collected from bat fecal samples to better understand both predator and prey. We used high throughput sequencing of the COI gene to determine the diet composition of three bat species in Minnesota. We evaluate how consistently *E. fuscus* forages for beetles compared to other insects, and we compare the proportional frequency of tympanate and nonvolant prey species in fecal samples from *Myotis septentrionalis* and *M. lucifugus.* We then use the same set of sequences to determine presence of pest species, rare species, and insects not previously known from Minnesota.

Methods

Field

Bat fecal sample collections were made at 25 locations across the forested region of Minnesota in June and July over the summers of 2015 to 2017 (Figure A1). Mistnets were deployed at each location along forest roads, trails and streams that could act as flight corridors (Swingen *et al.*, 2018). After capture, the bats were placed in handling bags and transported to a central processing site. Feces found in the bags were collected in sterile microcentrifuge tubes and stored at -20°C. Each captured bat was classified to species and its sex, age, and reproductive status was determined by physical examination. We obtained 125 fecal samples from *Eptesicus fuscus*, 140 fecal samples from *Myotis lucifugus*, and 95 fecal samples from *M. septentrionalis.*

Lab

DNA was extracted from individual fecal pellet samples using a ZYMO Quick-DNA Fecal/Soil Microbe Kit and the standard protocol. Extractions with DNA concentrations less than $5ng/\mu l$ were discarded. To target the arthropod prey in fecal pellets, we selected a 180 bp region of the mitochondrial cytochrome oxidase 1 gene (COI) using primers LCO1-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and CO1-CFMRa (5'-GGWACTAATCAATTTCCAAATCC-3'). Primers were selected on the basis of their taxonomic coverage and performance on low quality DNA from feces as reviewed in Jusino *et al.* (2019). We used 25μl reactions with 12.5μl Master Mix 7.3μl nuclease-free water, 1.5μl forward primer, 1.5μl reverse primer, and 0.2μl bovine serum albumin. The integrity of PCR product was evaluated using gel electrophoresis before sending the PCR product for sequencing at the University of Minnesota Genomics Center on the MiSeq Illumina platform (Illumina, inc., San Diego, CA).

Sequence Alignment and Identification

I adapted the MiSeq SOP mothur pipeline (Schloss *et al.*, 2009) to pair, align, and identify identify COI sequences with the Barcode of Life reference database (BOLD) (Ratnasingham and Hebert, 2007). MAFFT was used to create a reference alignment and taxonomy database of all arthropod COI sequences from BOLD (Katoh *et al.*, 2009). After removing chimeric sequences, non-target sequences, and low quality reads, reads were clustered into amplicon sequence variants (ASVs) at a 98.8% similarity level using UCLUST, corresponding to 2 bp differences in a 180 bp target sequence. A high similarity level was selected to accommodate the wide range of within species variability of the marker in different arthropod orders.

The mothur implementation of the RDP classifier was used to assign taxonomy to each ASV in a Bayesian framework. Taxonomic assignments were made when IDs had 80% or greater bootstrapped support. Sequences were assigned taxonomy at the species level, and the barcode index numbers (BINs) associated with the reference sequences were used in diversity analyses. Taxonomy was also assigned by performing a BLASTn search of the ASV consensus sequences against the Genbank and BOLD databases to evaluate the precision and coverage of the RDP classification method on COI sequences.

Diversity Analysis

Bootstrapped rarefaction curves implemented in R were used to select the most appropriate taxonomic levels for analysis and to qualify the sampling completeness of each sample and sample group (See R workflows in Supplemental Documents). Low count ASVs likely represent variation in BIN sequences that are captured in BINs with higher counts, or are spurious identifications. We set a threshold of five records for ASVs to be used in analysis based on the read counts in sequencing blanks. Fecal samples from 28 bats with BIN counts greater than 1.5 times the interquartile range were considered outliers and removed from the study.

Richness was calculated at the BIN, species, genus, family, and order level. The Simpson index was used to calculate unevenness of predation within major insect orders based on the number of unique BINs in each sample. Higher Simpson diversity was considered an indicator of a more generalist diet. A decomposition of Simpson diversity at the BIN level was used to test hypotheses of specialization on subgroups of arthropods. Wilcox ranked sum tests with Benjamin-Hochberg corrected p-values were used to assess the significance of difference between the medians of sample groups.

For arthropod species identified in bat fecal samples, functional ecological traits were retrieved from iNaturalist, Bug Guide, and the Encyclopedia of Life (Nugent, 2018; Bartlett, 2003; Parr *et al.*, 2014). These traits were recorded from verified records for each species. Key traits included in the search were habitat used by each insect species, flight ability, and whether the species was tympanate. Not all traits were available for every arthropod species. Data and some traits of rare species were assumed to be consistent with closely-related species or were extrapolated from higher levels of taxonomic classification where possible.

BIN counts weighted by frequency of occurrence within sample categories were compiled using functional ecological trait categories from the insect database. Statistical significance of differences were assessed using paired t-tests between bat species. Species that would only be incidentally eaten were not included in the diet diversity analysis. For example, several orders of insect parasites were detected. Springtails (Subclass Collembolla) were also not included in diet analysis.

Pest insects were generally defined as those that feed on plants and have had a historically noticeable impact on forest health. A list of pest insects was compiled from the Forest Pests of North America and a regional publication from the United States Forest Service (Forest Pests of North America, No Date; Service, 2020 accessed July 2020). The present and historical geographic ranges of pest insects were determined using the USGS Biodiversity Serving Our Nation geodatabase (Survey, 2015 accessed July 2020). This database compiles records from museum and university insect collections and online citizen science organizations. The list of identified species from all samples was compared to the list of pest insects and their recorded geographic locations to identify possible range expansions.

To evaluate the use of bat fecal samples as a source of eDNA, we examined the species detected in three environmentally sensitive insect orders in greater detail. Ephemeroptera, Plecoptera, and Trichoptera are often used as environmental indicator species and have well documented ranges compared to many other insect orders. We compared the geographic locations where the insects in each sample were collected to the known range of each insect species. Ranges were determined using the BISON database and a checklist published online by the University of Minnesota Cedar Creek Ecosystem Science Reserve and compiled from pinned insects in the Bell Museum collection 1985–1986 (Haarstad, 2002 accessed July 2020).

To test possible autocorrelation due to uneven geographic sampling of bat species, sample sets collected on the same night and same location (net events) were compared. To compare diet overlap within bat species, we analyzed only net events where three or more bats of the same species were captured. Pairs of different species were compared within all net events to assess difference in species richness of beetles, tympanate insects, and flightless arthropods. The significance of results were assessed using paired t-tests and ANOVAs.

Results

Sequencing Results and Alignment

A total of 9,052,673 reads were produced from the sequencing run. After removing chimeric sequences, non-target sequences, and low-quality reads, 6,019,611 reads remained with an average read depth per sample of 16,611. Reads were clustered into 286,693 ASVs with 98.8% similarity. Using the RDP classification method, 90% of all ASVs could be assigned to an arthropod order, 54% could be assigned to a family, 38% could be assigned a BIN, and 25% were assigned a species identification.

After filtering outlier samples and low count ASVs, 5,661 ASVs representing 931 unique BINs and 585 unique species with full taxonomic records were identified. An additional 275 unique records were identified but could not be assigned a consensus species, although 91% of these unidentifiable records could be assigned a genus.

A BLASTn search of the consensus sequence for each ASV resulted in 2,450 matches (8.5%), of which 1,442 had a percent identity score greater than or equal to 98%. ASVs were mapped to 396 unique species, and 41 ASVs were identifiable only to the order or family level. The RDP classification method and BLAST method identified the same species in 289 cases. When the RDP classification and BLAST methods differed in identification, there were inconsistencies in the name format of NCBI submissions. Taxonomy names were often listed under BOLD BIN numbers, or the name format included both BOLD BIN number and taxonomy name. There were few genuine differences in species level identification. Alpha and beta diversity analyses were performed on BINs to reflect the underlying format of the BOLD reference taxonomy database. In most cases a BIN contains one arthropod species, but some BINs contain groupings of arthropods with low variation in the COI region, which means that alpha and beta diversity measures would be higher than reported.

Records with complete BIN or species level identifications comprised 1,213 unique species and 1,117 BINs, containing a mean of 15.8 ASVs ($sd = 64$) per BIN (Table A1, Table A2). BLAST identified a more precise taxonomy 25% of the time when there was a mismatch, and never differed from the RDP classification method in actual species identification. In two cases, ASVs were identified as bat DNA. Non-prey species detected included mites and springtails from the Poruromorpha, Mesostigmata, Sarcoptiformes, Symphypleona, and Trombidiformes orders.

At maximum read depth, samples gained a mean of 0.042 unique ASVs (variance 0.007) in a bootstrapped rarefaction analysis. *Myotis lucifugus* samples were the least exhaustively sampled and gained a mean of 0.056 ASVs at maximum depth with higher variance (0.0145). *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* sample groups respectively had minimum rarefaction slopes that were 95%, 97%, and 100% above 0.01, indicating that most rarefaction curves did not reach asymptotes.

Diversity

The mean BIN richness per sample was 19 ($sd = 11.5$), and the mean species richness was 17 ($sd = 10$). When only records with full taxonomic identification were included, the mean BIN richness was 14.0 ($sd = 8.8$) and species richness was 10.3 ($sd = 6.5$) (Figure 2.1). Each bat fecal sample contained DNA from arthropods in a mean of 5 ($sd = 2.1$) different orders. Bats captured in July consumed a significantly higher diversity of arthropods than those captured in June across all levels of taxonomic classification ($6650.5 \leq W \leq 6886, p < 0.05$). Over 25% of bat fecal samples contained DNA from more than six arthropod orders, 55% of bat fecal samples contained DNA from three to six arthropod orders, and 20% of bat fecal samples contained DNA from fewer than three arthropod orders. Taxonomic richness of arthropods did not differ significantly between bat species.

The mean Simpson diversity level was 0.60 (sd = 0.12) at the BIN level, and trended upward to a mean of 0.68 (sd = 0.15) at the family level. Differences between bat species were small, with *M. lucifugus* fecal samples having lower Simpson diversity than *E. fuscus* and *M. septentrionalis* by a mean of 0.1.

Order Level Composition

Most identified species were in the orders Diptera, Lepidoptera, Coleoptera, and Ephemeroptera (Figure A2). Dipteran BINs were present in 88%, Lepidoptera BINs were present in 70%, Ephemeroptera BINs were present in 63%, and Coleoptera BINs were present in 59% of all fecal samples. Trichoptera were present in 38% of all fecal samples, comprising 45 BINs. Several non-flying Arthropods were detected, mostly from the Araneae order (34 BINs). Insects from the Megaloptera (2 BINs) and Hemiptera (30 BINs) orders were present in greater than 15% of all fecal samples, and Neuroptera (9 BINs) were present in 10%. Hymenoptera (18 BINs) were present in 6% of all fecal samples. All other identified orders were present in less than 3% of all fecal samples, including 17 total BINs from the Psocodea, Odonata, Blattodea, Plecoptera, Isopoda, Poduromorpha and Julida orders (Figure 2.2).

Bats consumed beetles from 28 families, but over 63% of prey BINs were from six families in Coleoptera. Elateridae (12 BINs) and Scarabaeidae (15 BINs) both were families with high BIN richness across all three bat species. Greater than 26% of samples contained at least one BIN belonging to Elateridae and Scarabidae. Cerambycidae (18 BINs), Carabidae 259 BINs), Dytiscidae (11 BINs), and Hydrophilidae (10 BINs) were present in more than 30% of *E. fuscus* samples, but were present in less than 5% of *M. lucifugus* samples. Cerambycidae and Pyrochroidae BINs were present in more than 13% of *M. septentrionalis* samples, and the 17 other beetle families were present in less than 4% of fecal samples. (Figure 2.3).

Most Lepidoptera that bats consumed belonged to the Tortricidae family, though moths from 32 families contributed to overall Lepidopteran diversity. Of 258 identified moth BINs, 88 were tortricid moths and 52% of all samples contained at least one tortricid moth BIN. The other most frequently consumed Lepidoptera families were Blastobasidae, with 22% of all samples containing at least one BIN from Blastobasidae, and Gelechiidae with 20% of samples containing at least one BIN. All other Lepidopteran families were present in less than 9% of fecal samples. Overall family consumption patterns were similar between bat species groups, with both *Myotis* species consuming moths more frequently than *E. fuscus*. (Figure 2.4).

In total, BINs from 34 families of Diptera were identified. Bats from all species frequently consumed craneflies in the Limoniidae family, with 49 BINs identified and 67% of all fecal samples containing at least one Limoniidae BIN. Mosquitos (Culicidae) were present in 59% of all fecal samples, comprising 23 BINs. Of 385 Diptera BINs, 152 were chironomid midges. Chironomidae were present in 48% of all fecal samples, and were present in 77% of *M. lucifugus* fecal samples. Craneflies in the Tipulidae family were present in 29% of fecal samples and consumed consistently among bat species. Six BINs in the Chaoboridae family were identified in 29% of *M. lucifugus* fecal samples, in contrast Chaoboridae BINS were found in < 6% of the *M. septentrionalis* and *E. fuscus* fecal samples. Other families of flies were present in less than 10% of all fecal samples (Figure 2.5).

Most Ephemeroptera were in either Caenidae or Heptageniidae, regardless of the bat species. While fewer Ephemeroptera BINs were identified in the study than other orders (26 BINs), those BINs were present across many samples. At least one of the four Caenidae BINs was present in 42% of all samples. Heptageniidae comprised 10 BINs and was similarly abundant across many samples (43%). All other Ephemeroptera families were present in 6% or fewer samples.

Bats consumed Trichoptera from seven families, with 10 BINs in Hydropsychidae, 15 BINS in Leptoceridae, 9 BINS in Limnephilidae, and 7 BINS in Phryganeidae. Hydropsychidae BINs were the most common caddisflies identified in all bat samples, with 15% containing at least one BIN. Phryganeidae BINs were present in 14% of samples, Limenphilidae were present in 14%, Leptoceridae were present in 7%, and the single Dipseudopsidae BIN identified was present in 3% of all samples. The remaining three BINs from two Trichoptera families were present in less than 1% of all samples.

Myotis septentrionalis and *M. lucifugus* both consumed a broader array of spider families than *E. fuscus*. Seven spider families were found in *M. lucifugus* and *M. septentrionalis* samples, while four families were identified in *E. fuscus* samples. In *E. fuscus* samples, spiders in the Araneidae family (orb weavers, 10 BINs) and

Tetragnathidae family (long-jawed orb weavers, 6 BINs) were present in 7% of samples. These families were similarly common in *M. lucifugus* samples (8%) and much more common in *M. septentrionalis* samples ($>$ 30%). Spider families were present in 19.8% of *M. lucifugus* samples, 7.5% of *E. fuscus* samples, and 51% of *M. septentrionalis* samples.

Three or more *E. fuscus* were captured at 16 sites and shared a mean 24.9% ($sd = 10.8\%$) of BINs with other *E. fuscus* foraging in the same location and on the same night. BIN overlap ranged from three to 39 BINs, accounting for at maximum 42.8% of BIN detections at one site. Between three and five *M. septentrionalis* were captured at nine net events and shared a mean 20% ($sd = 11.4\%$) of BINs. BIN overlap ranged from 15 to three, with a maximum of 42.9% overlap. In contrast, *M. lucifugus* less often shared BINs when foraging at the same locations. Between three and 10 *M. lucifugus* were captured at 16 net events, sharing a mean 14.2% ($sd = 7.0\%$) of BINs. BIN overlap ranged from zero to 16 with a maximum of 25.4% overlap. Intraspecific foraging overlap differed significantly between bat species (ANOVA, $F_{2,32} = 4.089, p = 0.03$). *Myotis lucifugus* shared BINs with 10.7% lower frequency than *E. fuscus* (Tukey HSD, $p = 0.02$).

Eptesicus fuscus captured in the same location on the same night as *Myotis* species shared a mean 28.9% ($sd = 12.7\%, n = 3$) of the arthropod species detected in *M. lucifugus* samples and 28.0% ($n = 1$) of those in *M. septentrionalis* samples. *Myotis lucifugus* samples shared a similar proportion of arthropod species detected in *E. fuscus* samples, but *M. septentrionalis* shared fewer arthropod species in common, with 18.9% at one site. Samples from *M. lucifugus* at the same location and date as *M. septentrionalis* shared 26.2% ($sd = 11.2\%, n = 4$) of the same arthropod species, while *M. septentrionalis* samples shared 35.2% ($sd = 15.8\%, n = 4$) species with *M. lucifugus* samples.

Beetle Specialists

Eptesicus fuscus consumed a greater diversity of beetle BINs than *M. lucifugus* in tests of 84 pairs of bats from 17 netting sites on 18 dates where both species of bats were captured. On average, *M. lucifugus* samples contained 0.64 BINs and *E. fuscus* samples contained 3.27 BINs. Beetle BINs made up a mean 34.1% ($sd = 26.9$) of all BINs identified in *E. fuscus* samples, while in *M. lucifugus* samples, they accounted for 26.9% ($sd = 9.8\%$). The difference in beetle diversity between *E. fuscus* and *M. lucifugus* pairs was significant both in terms of BIN frequency and percentage (paired t-test, Frequency: $t_{83} = 6.48, p < 0.01$, Proportion: $t_{83} = 9.34, p < 0.001$). *Eptesicus fuscus* consumed beetle BINs that overlapped with the beetle BINs that *M. lucifugus* consumed 15.4% of the time.

There were 58 unique *M. septentrionalis* and *E. fuscus* pairs from 10 netting sites and 11 dates. *E. fuscus* samples contained on average 4.9 Coleoptera BINs, differing significantly by 3 BINs from *M. septentrionalis* samples which contained on average 1.8 Coleoptera BINs (paired t-test, $t_{57} = 4.9, p < 0.01$). Coleoptera BINs comprised a mean 39.4% ($sd = 14.3\%$) of BINs per *E. fuscus* samples and 26.3% ($sd = 13.3\%$) of *M. septentrionalis* samples. Coleoptera BINs in *Eptesicus fuscus* samples at each net event accounted for a greater proportion of taxonomic BINs by 19.4% (paired t-test, $t_{57} = 7.4, p < 0.01$) *Eptesicus fuscus* captured with *M. septentrionalis* shared 10.8% of the same BINs.

Myotis septentrionalis and *M. lucifugus* pairs occurred together in 16 netting sites and 16 dates. Both *Myotis* species consumed a similar breadth of beetle BINs relative to BINs from other orders (paired t-test, $t_{110} = 0.03, p = 0.51$), differing by an average of 0.3%. Coleoptera BINs in accounted for 3.9% ($sd_{mylu} = 12.6\%, sd_{myse} = 6.3\%$) of all taxonomic BINs in both *Myotis* species. Between 110 unique pairs, the mean absolute difference in Coleoptera BINs was 0.26, where both species had means less than one BIN ($t_{110} = -2.3, p = 0.01$).

Eptesicus fuscus samples had a higher Simpson diversity value of Coleoptera BINs than both *Myotis* species by a mean of 0.06, but differed significantly in diversity only from *M. lucifugus* (Wilcox ranked sum test, $W = 144420, p = 0.028$). While Coleoptera BIN Simpson diversity was higher for *E. fuscus* samples than other bats, within *E. fuscus* samples, Coleoptera BINs were not always the major component of Simpson diversity. In 50% of samples (53), Diptera BINs were the highest contributor to Simpson diversity, with a mean value of 0.09 (sd=0.06).

Tympanate insects

The three bat species consumed 73 species of tympanate insects, representing 9.0% of all prey species identified. Most tympanate insects were from the order Lepidoptera, but Chrysopidae in the order Neuroptera and one Orthopteran species were also present. Across all three bat species, 37% of samples contained at least one tympanate insect, and *M. septentrionalis* most consistently consumed tympanate insects (43% of samples). Most eared moths were part of the Geometridae family (22), followed by Noctuidae (14), Erebidae (10), Crambidae (8), and Pyralidae (5). Moths in the families Drepanidae, Notodontidae, and Sphingidae were also present. (Table 2.1)

Myotis lucifugus ($n = 101$ fecal samples) consumed 428 species of arthropods, and *M septentrionalis* ($n = 77$ fecal samples) consumed 418 species of arthropods. Of the species of arthropods that *M. lucifugus* consumed, 33 (7.7%) were tympanate. The proportions of families detected reflect those of the overall dataset, where most species were Geometrid moths, followed by Noctuidae, Crambidae, Sphingidae, Notodontidae, and Pyralidae. *Myotis septentrionalis* consumed 32 species of tympanate insects (7.7%). Most moths were in the Geometridae or Noctuidae family, as in *M. lucifugus*. Only 4 species of Crambidae were detected and Chrysopid lacewings were detected only in *M. lucifugus* samples.

Pachysphinx modesta (Sphingidae) was the most frequently consumed tympanate insect for both *E. fuscus* and *M. lucifugus*, though it was present in only 5.6% and 7.9% of fecal samples. *Myotis septentrionalis* samples more frequently contained *Hydriomena divisaria* (Dashed Hydriomena, Geometridae), with 6.4% of samples containing one of 15 *H. divisaria* BINs.

Myotis septentrionalis more consistently consumed a broader variety of tympanate insects. Geometrid moths and sphinx moths were both consumed frequently, as in *M. lucifugus*, but Noctuid moths and members of Erebidae were also consumed. There was no significant difference between the proportion or absolute frequency of tympanate BINs consumed by *M. lucifugus* and *M. septentrionalis* (paired t-test, Frequency: $t_{30} = 0, p = 0.5$; Proportion: $t_{30} = -0.59, p = 0.28$). At each of the 31 sites where any tympanate insects were consumed, both *Myotis* species consumed a mean 0.74 tympanate insect BINs, accounting for 3.7% ($sd = 4.5\%$) of all taxonomic BINs in *M. lucifugus* and 4.5% ($sd = 4.4\%$) of taxonomic BINs in *M. septentrionalis*.

Non-flying Prey

While most arthropods eaten by bats can fly, 34 species were detected that either can not fly (i.e. arachnids) or fly infrequently and poorly (aphids). These arthropods were from four orders, with most of these species in the order Araneae (spiders) (Table 2.2).

Non flying prey were present in 8% of *Eptesicus fuscus* samples and represented nine different species. *Myotis lucifugus* consumed 16 non-volant arthropod species, and *M. septentrionalis* consumed 27 non-volant arthropod species Both *Myotis* species consumed spiders, bark lice, and adelgids, although at different frequencies. At least one species of non-flying prey was present in 52% of all *M. septentrionalis* samples and in 22% of all *M. lucifugus* samples. *Theridion murarium* (Fence Long-legged Cobweaver) was the most common non-flying arthropod in both *M. lucifugus* and *M. septentrionalis* samples ($n_{mylu} = 7, n_{myse} = 13$).

Myotis lucifugus consumed spiders from eight families with a range of web building habits. Web-building spiders from Araneidae, Tetragnathidae, Clubionidae, and Theridiidae were the most frequent, with 10, 10, eight, and nine species, respectively. In contrast, only three species of non-web making spiders such as those in the Phylodromidae family were present. *Myotis septentrionalis* consumed spiders from seven families. There were greater than nine individual records each of spiders from Araneidae, Clubionidae, Tetragnathidae, Theridiidae, and Philodromidae (Figure 2.6). Dictynidae (cribellate spiders) were present in five *M. septentrionalis* samples and were not detected in *M. lucifugus* samples.

At 67 net events where both *M. septentrionalis* and *M. lucifugus* were captured and at least one non-flying arthropod BIN was detected, *M. septentrionalis* consumed a mean 1.4 ($sd = 0.95$) flightless BINs while *M. lucifugus* consumed 0.55 BINs ($sd = 0.68$) on average. The small difference of 0.82 BINs was consistent and significant (paired t-test, $t_{66} = -4.99, p < 0.01$). Flightless arthropod BINs accounted for 8.6% ($sd = 5.7\%$) of all BINs in *M. septentrionalis* and 2.9% ($sd = 3.9\%$) in *M. lucifugus* at paired sites. *Myotis* samples differed in flightless BIN proportion by 5.7% (paired t-test, $t_{65} = -6.1, p < 0.01$).

Insect Pests

Considering all fecal samples, 25 forest pest species in three insect orders were identified (Table 2.3). Long horned beetles and weevils made up the Coleopteran insects, one spittlebug was a member of Hemiptera, and the remainder were small phytophagous moths. *Malacosoma disstria* (forest tent caterpillar) was detected in 53 samples, and *Zeiraphera canadensis* (spruce bud moth) was detected twice, but *Lymantria dispar dispar* was not detected. Nine pest species identified from fecal samples were not listed as present in Minnesota in the BISON database (Figure 2.9).

Cydia piperana (ponderosa pine seedworm moth) was identified using the RDP classification method in eight samples as part of the BIN BOLD:AAC6105, which contains both *C. pipereana* and *C. toreuta* (33 members). Each sample contained a unique ASV assigned to the same BIN. Two ASVs were 98.3% identical to COI sequences of *C. toreuta* in a BLASTn search of NCBI records, and three were less than 98% identical to any one *Cydia* sequence. A manual search using the BOLD systems identification engine and the ASV consensus sequences showed all eight ASVs to be greater than 98% identical and in two cases greater than 99% identical to *C. piperana* sequences. The geographic records of *C. piperana* in the BOLD database are centered in the American Southwest, while *C. toreuta* are present in Michigan and bordering regions of Ontario. The BISON database has one observation of *C. toreuta* in southern Wisconsin (2016) and also has one observation in eastern North Dakota (2015). The easternmost observation of *C. piperana* in the BISON database is in Colorado (2010).

Mosquitos

Bats consumed mosquitos from 23 BINs in the mosquito family Culicidae. Species classifications were assigned to 17 ASVs. Two species shared multiple BIN assignments, and many mosquitos were identified as part of BINs that share multiple species. Most identified mosquitos were part of the genus *Aedes* (seven species), and others were part of *Culiseta, Anopheles, Coquillettidia, Culex*, and *Ochlerotatus*. Six species identified in this study were detected in a 2018 survey of mosquitos in Wisconsin, and 10 out of the 31 were identified in a 2016 Minnesota survey (Kinsley *et al.*, 2016; Wray *et al.*, 2018). Five species known to act as vectors of human disease were identified. Mosquito species that vector Eastern Equine Encephalitis (*Aedes canadensis, Culiseta melanura, Coquillettidia perturbans*) were detected in 8.5% of fecal samples, and mosquito species that vector Western Equine Encephalitis or Tahyna (*Aedes dorsalis, A. vexans*) were present in 1.1% of fecal samples.

Insect Distributions

We identified 27 Ephemeroptera species from nine families (Table A1). Of the nine species not listed as present in Minnesota in BISON, four are identified only as BINs in the BOLD database. Species members of the BIN BOLD:AAA7515 include *Caenis youngi, C. amica, C. punctata* and several unnamed sequences. BOLD:AAK5110 contains only one named species, *Paraleptophlebia volitans* and several other unnamed sequences. Unlike the complex of *Caenis* species, there are no records of *P. volitans* in the Midwest in either the BOLD or BISON databases. *Paraleptophlebia volitans* was also detected in this study. *Isonychia* sp.DHF1 is known from only one record in South Carolina in the BOLD database. The closest taxonomic relative is *Isonychia tusculanensis*, which has two records in the BOLD database, both in eastern North America. Geographic records of *Procleon* sp.JMW4 are mostly centered in Canada; the closest taxonomic relative is *Cloeon simile*.

Caenis punctata and *Caenis diminuta* were not listed as present in Minnesota in the BISON database but are present in the Bell Museum collections (Haarstad 2002, BISON 2020) (Figure A3). *Maccaffertium pulchellum* is known from Iowa, Michigan, and Wisconsin, and there are records of *Eurylophella temporalis* from southern Wisconsin in the BISON database, though its distribution appears to be centered in Illinois.(Figure A4)

Four BINs in the Plecoptera order were identified, all in the Perlidae family. *Perlinella ephyra, Agnetina flavescens*, and *Perlesta decipiens* have Minnesota records in the BISON database as recently as 2012 (Figure A5). One additional record could be identified only to a BIN level. BOLD:AEA6705 is a large complex of species, containing *P. lagoi, P. decipiens, P. placida, P. xube*, and *Tallaperla maria. Perlesta decipiens* is the only one of these species known to be present in Minnesota, in the southern most regions (Figure A6). Plecoptera BINs were detected in five bat fecal samples distributed across the two major forested provinces of Minnesota.

We identified 59 unique Trichoptera BINs, 12 of which could not be identified to Species. Of those with species names, 12 did not have Minnesota geographic records in the BISON database. Seven of these species had geographic records in adjacent states in the BISON database (Figure A7 and Figure A8). *Agrypnia improba* and *Ceraclea mentiea* were last detected in Michigan before 1985, and *Hagenella candensis* was detected in Wisconsin in 1936. More recently, *Molanna ulmerina, Hydropsyche scalaris, Triaenodes nox*, and *Ceraclea transversa* were recorded in Wisconsin. Bell museum collections include state records of *A. improba, A. macdunnoughi, Ayncharus mutatus, A. montanus, Triaenodes nox, C. transversus, C. mentiea*, and *Hagenella* *scalaris*, which are not listed in Minnesota in the BISON database. *Triaenodes connatus* has records in the BISON database near Lake Erie and northern Indiana, with no known records in the Midwest. *Lenarchus crassus* is a Canadian species with a wide continental distribution.

Records with only BIN level identifications were members of the Hydropsychidae, Lepidostomatidae, Leptoceridae, Limnephilidae, Molannidae, and Sericostomatidae families. Most BINs contained species detected at greater taxonomic resolution, with a few exceptions. BOLD:AAA7041 is a complex of *Limnephilus* species that were not detected individually in this study. Component species *L. sericeus, L. abbreviates*, and *L. fagus* are caddisflies with broad distributions across the northern Midwest and southern Canada. BOLD:AAD9749 contains two species, *Agarodes distinctus* with records in the Northern United States, and *A. libalis* in the south.

Discussion

Comparison to Previous Results

Dissection studies of cave bat fecal samples identified eleven orders of insects in bat fecal samples. Six additional orders were identified in this study, though most identified arthropods were part of the eleven orders identified in dissection studies. Hemipteran insects appear to be over-represented in dissections, while soft-bodied insects from Ephemeroptera and Trichoptera appear to be under-represented, sometimes dramatically so (Figure 2.7).

Two previously published DNA-barcode based studies of *E. fuscus* diet did not reach consensus on prey signature (Figure 2.8). One study was consistent with the fecal sample dissections, with Coleoptera comprising more than 75% of ASV species classifications (Long *et al.*, 2013). The other identified a nearly equal number of ASV species classifications from Coleoptera, Diptera, and Lepidoptera, which is not consistent with the hypothesis that *E. fuscus* is a beetle specialist (Clare *et al.*, 2014b). Our analysis of *E. fuscus* fecal samples also showed a more general diet, although the most frequently encountered BINs were in Coleoptera. Lepidoptera and Diptera BINs both accounted for a proportion of taxonomic richness similar to that of Coleoptera BINs in more than 50% of samples. All three studies used different primer sets and referenced the same COI barcode database. However, the reference database is a continuously updated product that undoubtedly has improved in its coverage over time (Ratnasingham and Hebert, 2007).

Three DNA-barcode studies have been published to date on the prey composition of *M. lucifugus* diets. All concluded that *M. lucifugus* is a generalist predator. Classified ASVs or OTUs belonged in similar proportions to Lepidoptera, Coleoptera, Diptera, and Ephemeroptera, with minor contributions from other orders, including non-flying prey. The results of this study are similar on an order level, though the proportion of species classified as Diptera are relatively high compared to previous results. The detection of Diptera in fecal samples has increased in molecular analyses compared to fecal pellet dissections, most likely because most Dipteran species are small and composed of softer tissues than other insect orders

Only one molecular diet study of *M. septentrionalis* has been published (Dodd *et al.*, 2012), which found that most ASVs were classified as Lepidoptera families. That finding differed greatly from the results of pellet dissection studies. Most fecal pellet dissection studies found order level composition of prey similar to that of *M. lucifugus*, with Lepidoptera, Coleoptera, Hemiptera, and Diptera occurring in similar proportions. The results in this study are more similar to those dissection study results, although as in the case of *M. lucifugus*, more Diptera and Ephemeroptera species were detected.

***Myotis* species foraging differences**

Tympanate insects were not abundant in the diet of bat species we studied. Tympanate insects were identified in less than 14% of fecal samples, with proportions similar for the gleaning species, *M. septentrionalis*, and the non-gleaning species *E. fuscus.* In this sense, the allotonic frequency hypothesis does not appear to be borne out, but we do not know the availability of tympanate insects relative to non-tympanate insects. However, it is clear that tympanate insects were not a large proportion of diet diversity.

Non-flying arthropods such as spiders do appear in the diet of both *Myotis* species more often than non-flying arthropods appear in the diet of *E. fuscus.* No arachnids fly, but arachnids could be consumed in aerial attacks if suspended in a web or drifting in air. Most arachnids identified in fecal samples were in the orb weaving spider families, Araneae and Tetragnathidae. A smaller proportion of spiders were in families that do not construct webs (Lycosidae) or which only rarely create webs. Those families occurred more frequently in *M. lucifugus* diets than in the diet of *M. septentrionalis*. Previous diet studies support the hypothesis that *M. lucifugus* may also glean insects from leaves in Northern parts of its range where aerial insect prey is more limited (Ratcliffe and Dawson, 2003). Previous diet studies support the hypothesis that *M. lucifugus* may also glean insects from leaves in Northern parts of its range where aerial insect prey is more limited. These results suggest that the gleaning trait is flexible and within the range of behavior for all three species of bat.

E. Fuscus as a Beetle Specialist

Eptesicus fuscus consumed a broader variety of beetle species with more consistency across samples than either *Myotis* species did. Morphological specialization and differences in foraging habitat have both been suggested as rationales for an observed beetle preference in *E. fuscus* relative to other bats (Agosta and Morton, 2003; Ober and Hayes, 2008). *Eptesicus fuscus* are larger than *Myotis* species present in Minnesota and have robust jaws more adept at piercing the carapaces of beetles (Kurta and Baker, 1990). *Eptesicus fuscus* may forage in a broader range of habitats than *M. septentrionalis* and *M. lucifugus* (Furlonger *et al.*, 1987), therefore encountering more beetles. However, we observed *E. fuscus* to consume more beetle species than *Myotis* bats did when captured in the same areas. Differences in beetle family predation suggest a possible third explanation.

Relative to terrestrial beetle species, aquatic beetles more often occurred in the diet of *E. fuscus* than in either *Myotis* species. Aquatic insects might be expected to be more common in *M. lucifugus* diets given the aerial hawking foraging style of the species. All species of bats consumed aquatic beetles in the Hydrophilinidae and Dytiscidae families, and *E. fuscus* additionally consumed aquatic beetles in the Gyrinidae family. All three insect families have adult forms that forage and live on the surfaces of both still water and flowing rivers. The adult beetles disperse by flight but fly noisily, seemingly making them easy targets for bats (Jackson, 1956).

Gyrinidae are uncommon components of aquatic lizard and fish diets because they produce an unpalatable pygidial secretion that both aids in swimming and acts as a chemical defense (Vulinec and Miller, 1989; Benfield, 1972; Ivarsson *et al.*, 1996). As Hydradephageous beetles, Dytiscidae share these pygidial glands and secretions but produce a less toxic chemical. Some subfamilies of Dytiscid beetles have prothoracic defensive glands that serve as chemical defense, including Colymbetinae and Dytiscinae (Dettner, 1985). *Eptesicus fuscus* consumed Dytiscid beetles from these and additional families, while *Myotis* bats consumed only Dytiscid beetles from families that do not produce chemical defense. Hydrophilid beetles, more present in *Myotis* samples than other aquatic beetles, are more distantly related to Hydradephageous beetles and lack complex defensive glands (Dettner, 2019).

In addition to consuming unpalatable aquatic beetles, *E. fuscus* also consumed Silphid beetles whereas *Myotis* species did not. Silphid beetles produce toxic terpenes as defensive mechanisms (Eisner *et al.*, 1986). Bats may reject insects such as Lampyridae (Fireflies) that contain toxic substances and learn to avoid them based on visual signals (Moosman Jr *et al.*, 2009; Leavell *et al.*, 2018). Variation in the toxicity of chemical defense that insects produce can modify the insect's palatability. Arctiid moths are more often avoided by *E. fuscus* when they have fed on cardiac glycoside containing plants than when they have fed on plants containing toxic biogenic amines (Hristov and Conner, 2005). Possibly, *E. fuscus* is relatively less sensitive to the toxins that Hydradephageous beetles and some Silphid beetles produce than *Myotis* species.

While *E. fuscus* appears to have consumed beetles more often than other bat species in this study, the hypothesis that beetles make up a large portion of their diet is not supported in terms of taxonomic richness. While they are able to exploit beetles as a food source, Diptera made up a near equal proportion of most samples. However, because there is little data on biomass of different insect species, and DNA analysis gives us presence but not relative abundance, we do not know the relative caloric value of Diptera vs. Coleoptera in the diet of *E. fuscus.*

Insects of Economic Significance

While bats do consume forest pests in both the Lepidoptera and Coleoptera insect families, their greater impact on pest insects could be predation on mosquitos and biting midges. Bats consumed mosquitos from at least 33% of the species native to Minnesota and many species of midges. This study is limited to taxonomic identification and can not quantify the impact of bat predation on mosquito and midge populations.

Defining a forest pest is somewhat subjective. Here pests were defined by their previous designation as a pest in published scientific literature and many phytophagous insects were ignored. Often insects do not become pests until after some disruption like the removal of a predator species or its introduction into a new environment. Bats consume many herbivorous insects that are not known to damage crops, spread disease, or emerge in great numbers. Where bats feed on these insects heavily, it is possible that they contribute to preventing those insect species from becoming pest species. Quantifying the impact of bat predation on pests is important given the 90% decline in *Myotis* populations impacted by White Nose Syndrome (Kramer *et al.*, 2019; Pettit and O'Keefe, 2017; Reynolds *et al.*, 2016).

Pest and Rare Species Detection

Plecoptera, Ephemeroptera, and Trichoptera have high degrees of endemism due to the strict ecological requirements of their nymphs and their limited dispersal habits (Zwick, 2000; Arimoro and Muller, 2010; Landeiro *et al.*, 2012). In the case of Plecoptera, long-lived species are likely to experience the greatest impact from climate change, whereas short-lived genera like *Perlesta* are expected to expand in range (Sánchez-Bayo and Wyckhuys, 2019) All Plecoptera that bats consumed in this study were members of the short-lived Perlidae family. While two species are known to be present in Minnesota, our records are to the north of their known occurrence in the state.

Four species of Ephemeroptera and one Trichoptera species were detected outside of their known ranges in the state. These species did not share a common range and represented both species at the northern edge of their ranges and species at the southern edge of their ranges. Insects known to act as pests outside of the Midwest were also detected at low levels. Detecting the movement of both sensitive species and those with the potential to become harmful pests contributes valuable information on the complex response of invertebrates to climate change.

While *Choristoneura conflicatana* (leaf-rolling moth) and*Dioryctria reniculelloides* (spruce coneworm moth) are not listed as present in Minnesota, many records of these species exist in adjacent states and they are likely undersampled in Minnesota. *Anelaphus villosus* (twig borer), *Hylobius pales* and *Pachylobius picivorus* (pine weevils), *Catastega aceriella* (trumet skelotinizer moth), *Caloptilia alnivorella* (alder leaf miner), and *Zeiraphera canadenis* (spruce bud moth) have known ranges more distant from north and central Minnesota.

Summary

We found the sympatric bat species *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* have distinct dietary habits in terms of beetle consumption and non-flying insect consumption. Bat fecal samples are a promising source of eDNA. Incorporating eDNA analysis of bat fecal samples into insect surveys and environmental monitoring projects may allow for the more systematic detection of changes to insect populations. We identified more than 900 arthropod BINs and species using bat fecal samples, and we measured taxonomic richness levels within many samples that were higher than those previously reported. Using both the BOLD COI reference dataset and the RDP classification method, we were able to systematically assign species level taxonomy to ASVs. We were able to combine precise arthropod identification and the foraging areas of individually sampled bats to observe possible range expansion of some insects. As sequencing costs continue to drop and bioinformatic techniques mature, this technology will only become more valuable in addressing ecological questions.

Figures and Tables

Figures

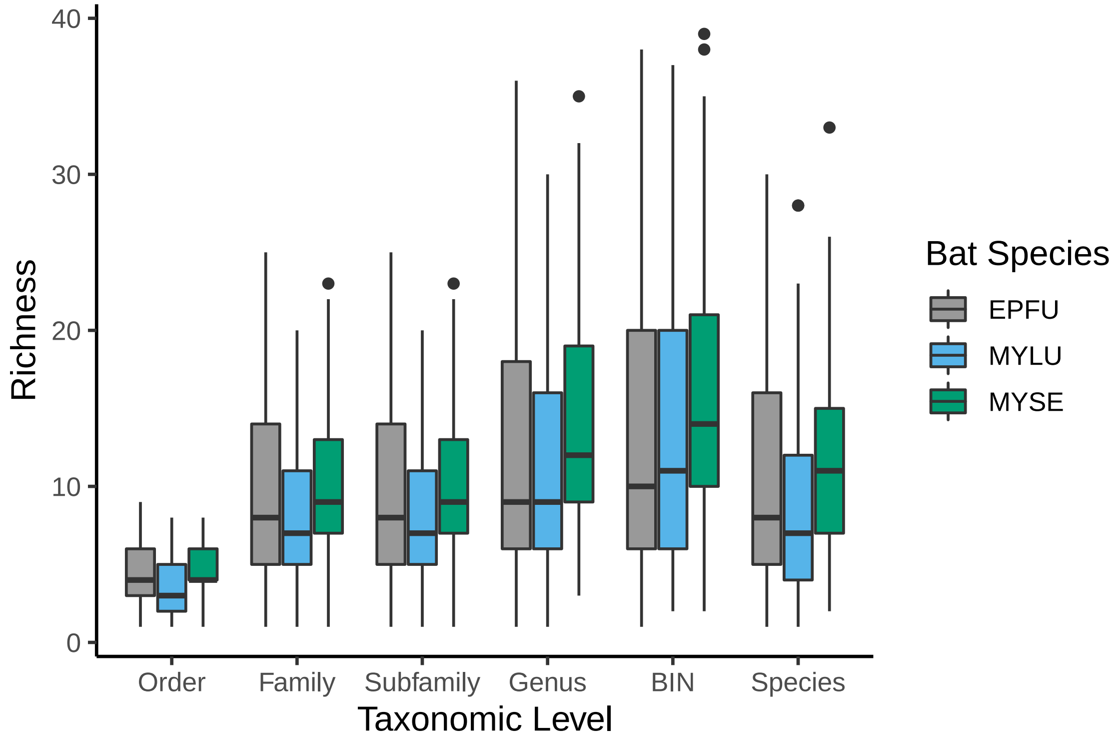

Figure 2.1: **Taxa richness of insect prey in fecal samples from each bat species.** Richness represents the count of unique identifications at each taxonomic level in each bat fecal sample. EPFU= *Eptesicus fuscus*, MYLU = *Myotis lucifugus*, MYSE = *Myotis septentrionalis*.

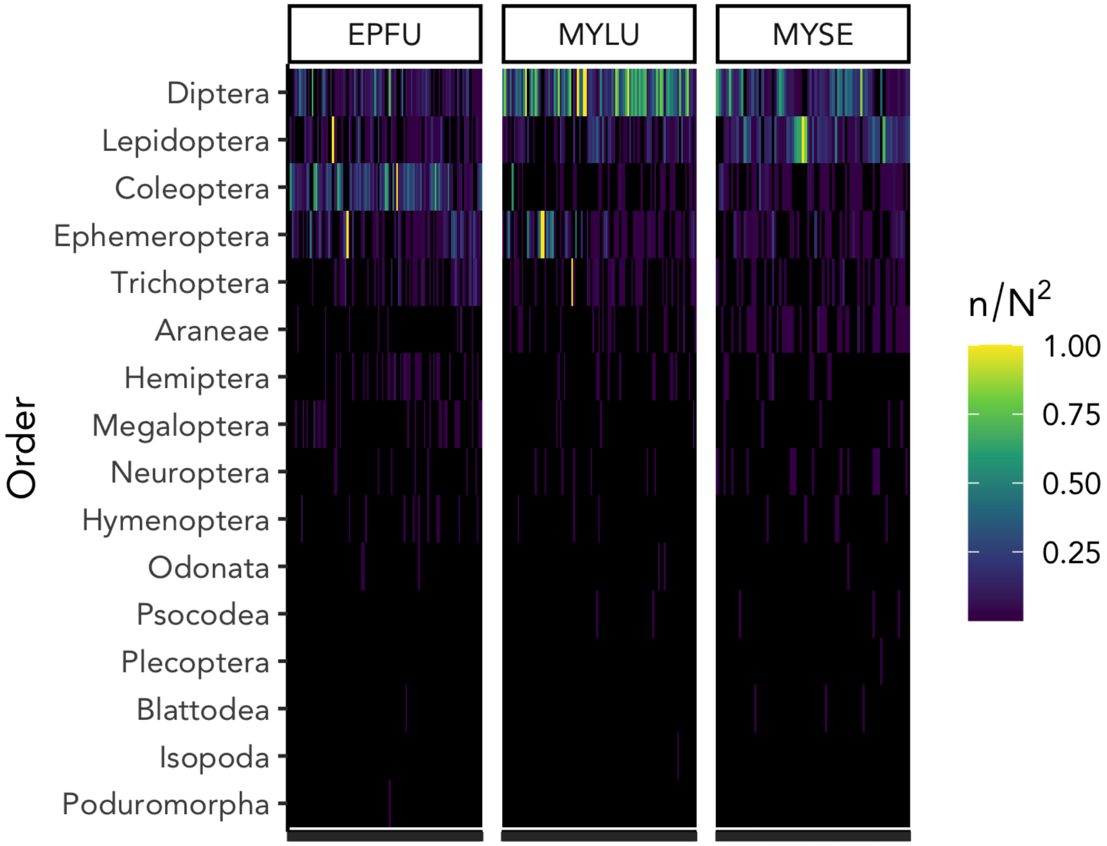

Figure 2.2: **Simpson diversity decomposition of arthropod orders.** Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order contributes most to the unevenness of diversity. BINs in the order Diptera had a high likelihood of occurring more than once in a sample in all bat species. BINs in the order Coleoptera were likely to be encountered more than once in *E. fucsus* samples.

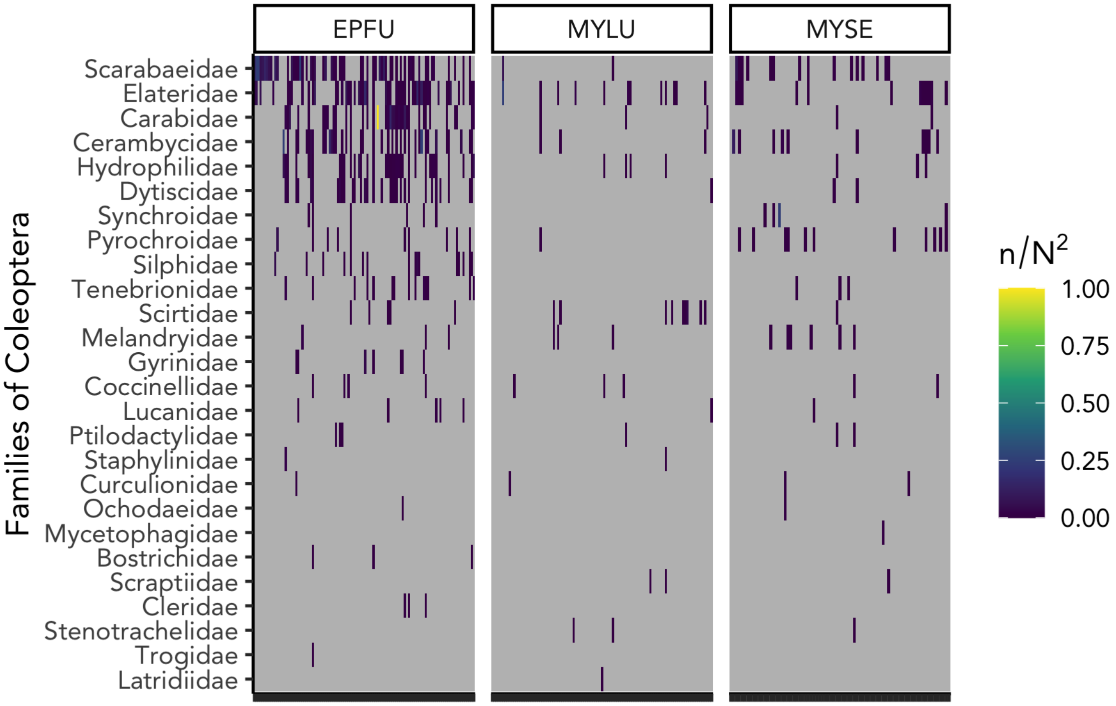

Figure 2.3: **Simpson diversity decomposition of beetle families.**. Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order contributes most to the unevenness of diversity. Beetles were identified in samples from all three bat types. With the exception of Carabid beetles (scarab beetles and june bugs), there was no strong pattern of family level specialization.

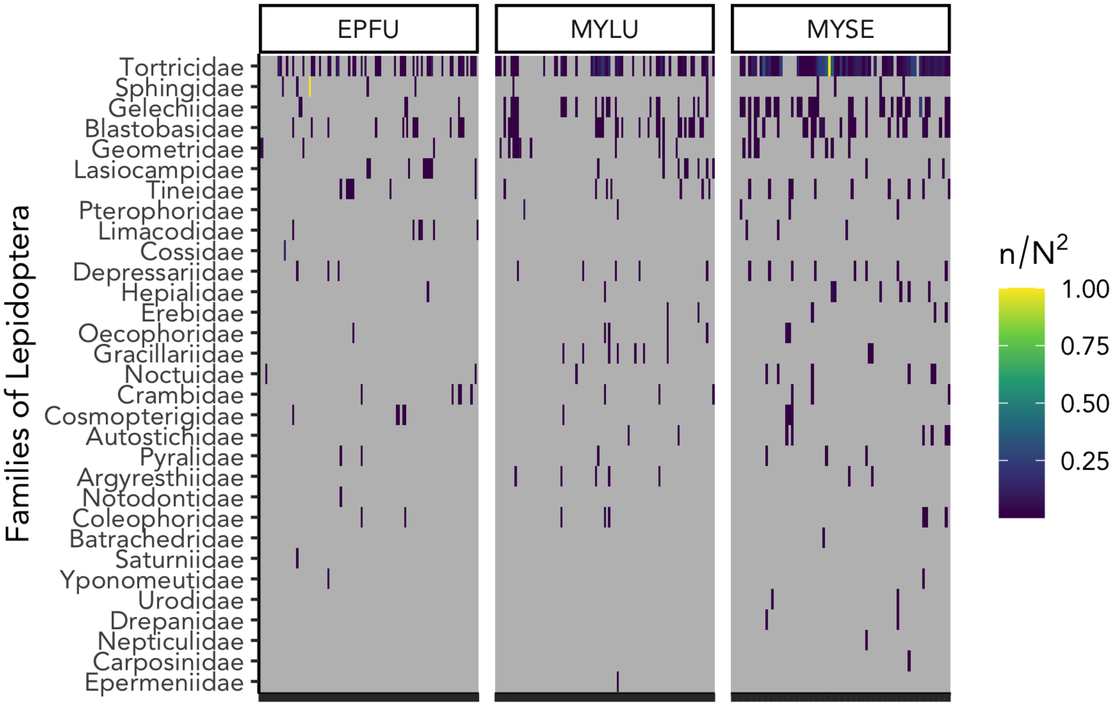

Figure 2.4: **Simpson diversity decomposition of moth families.**. Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order contributes most to the unevenness of diversity. Tortricid moths were the most commonly encountered family in all bat species but in most cases did not have high likelihoods of being encountered in a sample more than once.

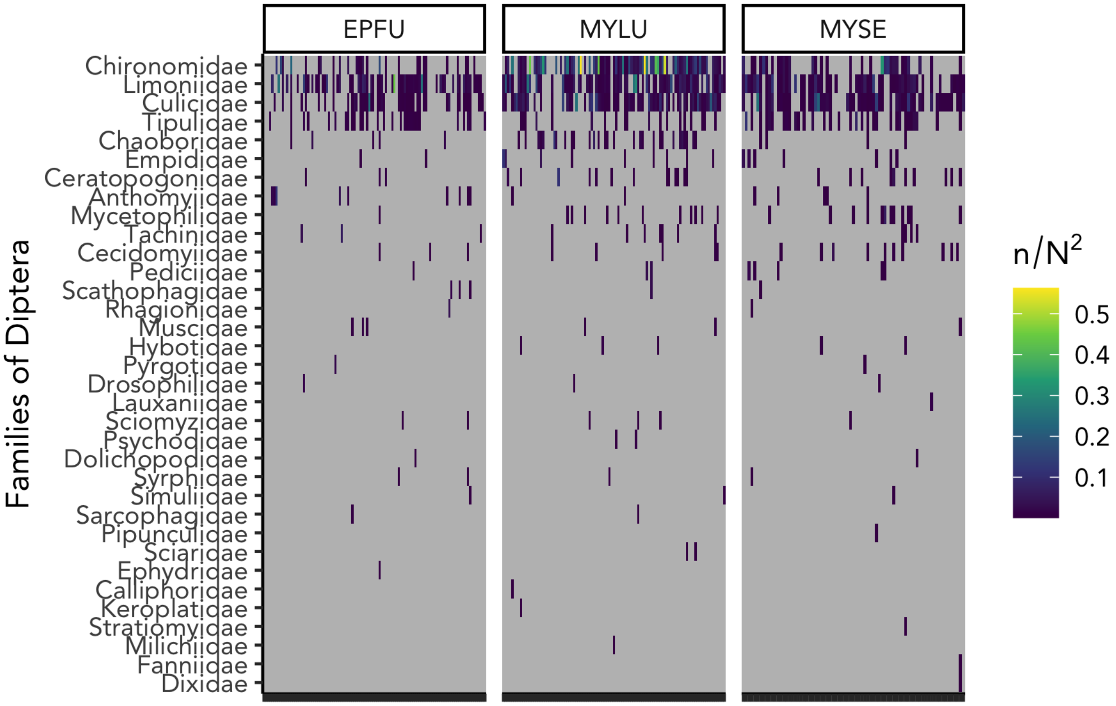

Figure 2.5: **Simpson diversity decomposition of fly and mosquito families.**. Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order contributes most to the unevenness of diversity. Chaoboridae BINs were more often encountered in *M. lucifugus* samples. When bats consumed *Chaoboridae* insects, they were likely to consume additional BINs from the same family.

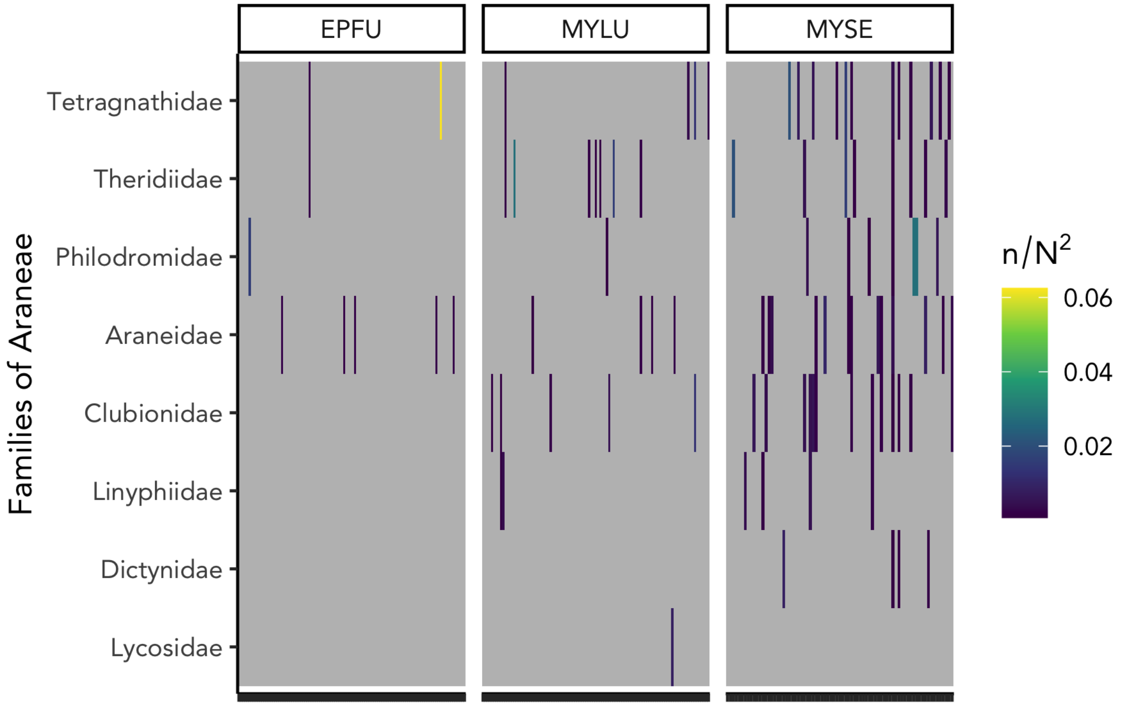

Figure 2.6: **Simpson diversity decomposition of spider families.**. Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order contributes most to the unevenness of diversity. *Myotis* species preyed on spiders more often than *E. fuscus*.

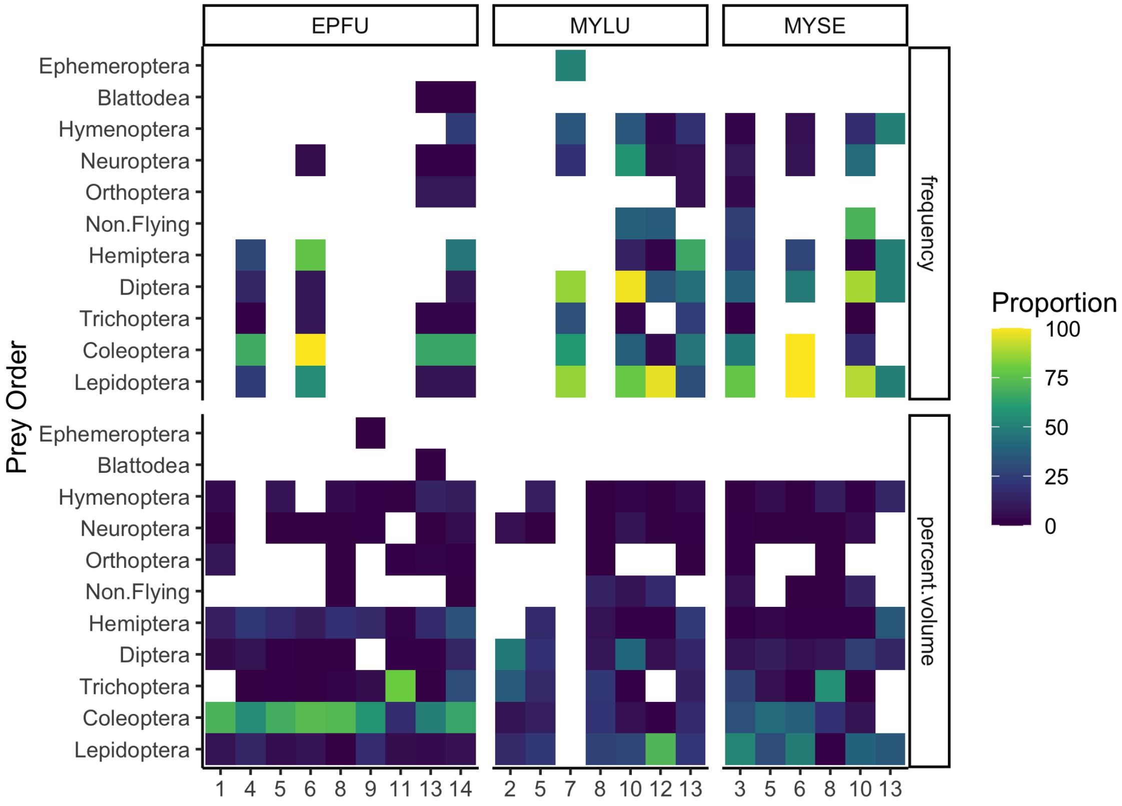

Figure 2.7: **Composition of insect prey detected in pellet dissection studies.** Frequency and percent volume values are presented if reported. Data sources include [1]Agosta and Morton (2003), [2]Belwood and Fenton (1976), [3] Brack Jr and Whitaker Jr (2001), [4] Brigham and Saunders (1990), [5] Thomas *et al.* (2012), [6] Dodd *et al.* (2015), [7] Anthony and Kunz (1977), [8] Feldhamer *et al.* (2009), [9] Hamilton and Barclay (1998), [10] Kaupas and Barclay (2017), [11] Verts *et al.* (1999), [12] Whitaker Jr and Lawhead (1992), [13] Whitaker Jr (1972), and [14] Whitaker Jr (1995).

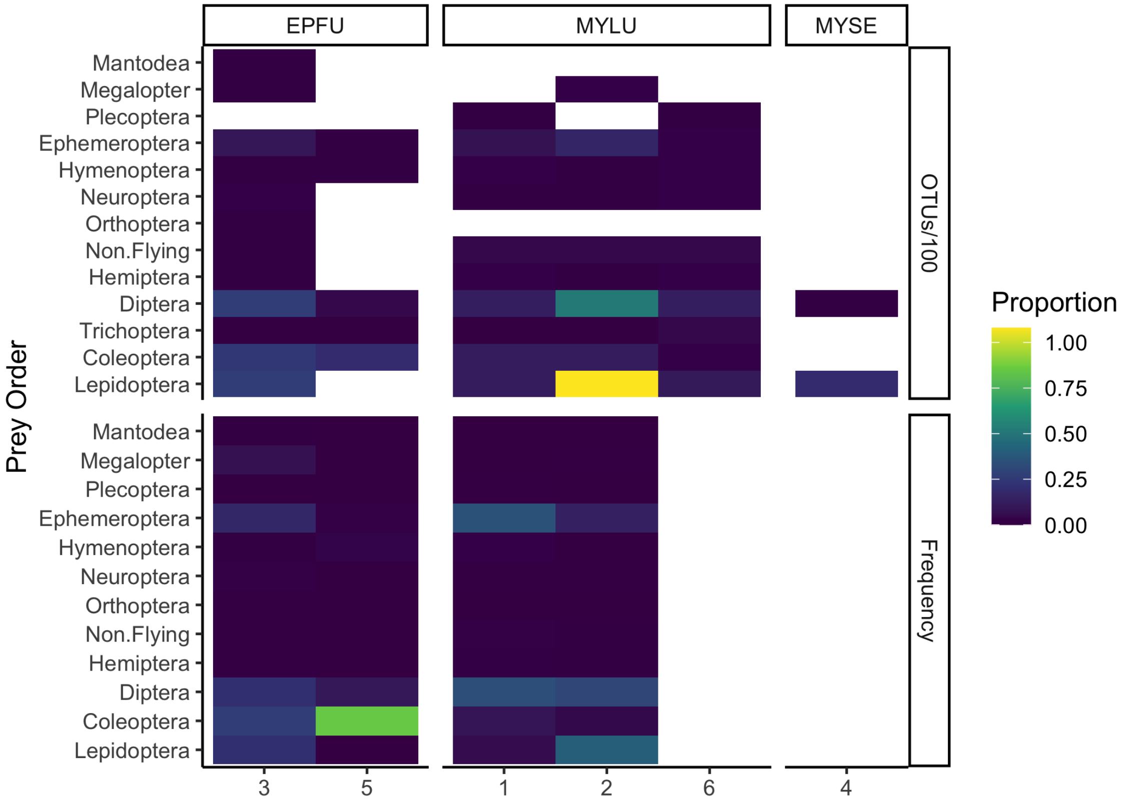

Figure 2.8: **Composition of insect prey detected in molecular prey studies.**. The number of unique OTUs reported in each study per 100 OTUs appears in the first row, and the frequency of their occurrence appears in the second. Data sources include [1] Clare *et al.* (2011), [2] Clare *et al.* (2014a), [3] Clare *et al.* (2014b), [4] Dodd *et al.* (2012), [5] Long *et al.* (2013), and [6] Shively *et al.* (2017).

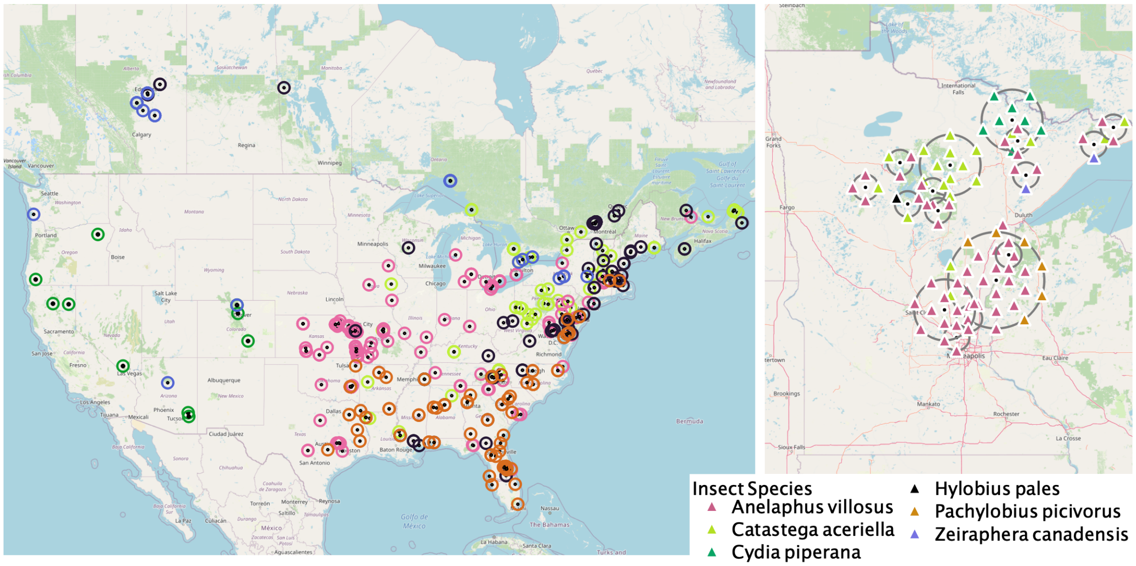

Figure 2.9: **Potential pest species previously unrecorded in Minnesota.** Locations of insect observations and specimen collections from BISON are shown across the contiguous United States on the left. Net locations of samples from this study containing the pest insects are pictured at right. *Anelaphus villosus*, *Catastega aceriella*, and *Pachylobius picivorus* have distributions with most species occurring in the Southeastern or Eastern coast of the United States. *Cydia piperana* and *Zeiraphera canadensis* however have western distributions.

Tables

Table 2.1: **Tympanate Insect Species.** Sample frequencies of tympanate insect species appear in sample columns: *(E) for Eptesicus fuscus*, *(U) for Myotis lucifugus*, and *M for M. septentrionalis*.

Arthropod Taxonomy					**Frequency**			
Order	**Family**	**Genus**	**Species**	**Common Name**	***E***	***U***	***M***	**Total**
Lepidoptera					17	14	22	53
	Crambidae	*Chrysoteuchia*	*topiarius*	Cranberry Girdler	1			1
		Crambus	*albellus*	Small White Grass-Veneer	3		1	4
			saltuellus	Pasture Grass-Veneer	1			1
		Eudonia	*alpina*	Highland Gray			1	1
		Petrophila	*canadensis*			1		1
		Scoparia	*biplagialis*	Double-striped Scoparia	1	2		3
	Drepanidae	*Pseudothyatira*	*cymatophoroides*	Tufted Thyatrid			1	1
	Erebidae	*Hypenodes*	*palustris*	Owlet Moth			1	1
			sombrus	Owlet Moth			1	1
		Idia	*rotundalis*				1	1
		Zale	*duplicata*	Pine False Looper			1	1
	Geometridae	*Campaea*	*perlata*	Pale Beauty		1		1
		Dysstroma	*truncata*	Marbled Carpet Moth			2	2
		Ectropis	*crepuscularia*	Saddleback Looper		1	1	2
		Eufidonia	*convergaria*	Pine Powder Moth			1	1
		Hydriomena	*divisaria*	Dashed Hydriomena	2	2		4
		Iridopsis	*vellivolata*	Pale-winged Gray		1		1
		Lobophora	*nivigerata*	Powdered Bigwing Moth		1		1
		Lomographa	*vestaliata*	White Spring Moth	1			1
		Nematocampa	*resistaria*	Filament Bearer			1	1
		Orthonama	*obstipata*	Gem Moth		1		1
		Xanthorhoe	*lacustrata*	Toothed Brown Carpet		1		1
	Noctuidae	*Acronicta*	*fragilis*				1	1
			grisea	Gray Dagger		1	1	2
			dactylina	Fingered Dagger	1		2	3
			innotata	Unmarked Dagger			1	1
		Euplexia	*benesimilis*	American Angle Shades			1	1
		Feralia	*comstocki*	Comstock's Sallow	1			1
		Lacinipolia	*lorea*	Bridled Arches Moth			1	1
	Notodontidae	*Gluphisia*	*septentrionis*	Common Glupisia Moth	1			1
	Pyralidae	*Ephestiodes*	*erythrella*	Snout Moth			1	1
		Meroptera	*pravella*	Lesser Aspen Webworm	1			1
			abditiva	Snout Moth		1		1
		Pyralis	*farinalis*	Meal Moth	1			1
	Sphingidae	*Ceratomia*	*undulosa*	Waved Sphinx	1			1
		Pachysphinx	*modesta*		2		1	3
		Smerinthus	*cerisyi*	One-eyed sphinx moth		1	1	2
Neuroptera						1		1
	Chrysopidae	*Meleoma*	*signoretti*	Green Lacewing		1		1

Table 2.2: **Non-flying Prey Species.** Frequencies of prey occurrence appear for each species of bat: *(E)* for *Eptesicus fuscus*, *(U)* for *Myotis lucifugus*, and *M* for *M. septentrionalis*.

Arthropod Taxonomy				**Frequency**				
Order	**Family**	**Genus**	**Species**	**Common Name**	***E***	***U***	***M***	**Total**
(A) Araneae	Araneidae	*Araneus*	*bicentenarius*	Giant Lichen Orbweaver			1	1
			nordmanni	Nordmann's Orbweaver			1	1
		Eustala	*emertoni*	Orb Weaver	1	2	5	8
			cepina	Riparian Duncecap Orbweaver		1		1
			anastera	Humpbacked Orbweaver	3		1	4
			rosae	Orbeweaver			2	2
		Larinioides	*patagiatus*	Ornamental Orbweaver	1	1	3	5
		Neoscona	*arabesca*	Arabesque Orbweaver			2	2
	Clubionidae	*Clubiona*	*moesta*	Leaf Curling Sac Spider			1	1
			canadensis	Leaf Curling Sac Spider		3	8	11
	Dictynidae	*Dictyna*	*brevitarsa*	Mesh Web Weaver			1	1
		Emblyna	*sublata*	Mesh Web Weaver			3	3
	Linyphiidae	*Grammonota*	*angusta*	Dwarf Spider		1	1	2
		Pocadicnemis	*americana*	Sheet Weaver			1	1
	Philodromidae	*Philodromus*	*rufus*	Running Crab Spider	1	1	2	4
			praelustris	Running Crab Spider			1	1
			vulgaris	Longlegged Crab Spider			1	1
			peninsulanus	Running Crab Spider			2	2
			keyserlingi	Running Crab Spider			1	1
	Tetragnathidae	*Leucauge*	*venusta*	Orchard Orbweaver			1	1
		Tetragnatha	*shoshone*	Long-Jawed Orbweaver	1	2	4	7
			viridis	Green Long-Jawed Orbweaver	1	1	1	3
			caudata	Long-Jawed Orbweaver		1		1
	Theridiidae	*Dipoena*	*nigra*	Cobweb Spider			1	1
		Theridion	*murarium*	Fence Long-Legged Cobweaver	1	6	7	14
			differens	Cobweb Spider		1		1
(I) Hemiptera	Adelgidae	*Adelges*	*lariciatus*	Adelgid		1		1
	Aphididae	*Eriosoma*	*americanum*	Woolly Elm Aphid	1	1	1	3
	Clastopteridae	*Clastoptera*	*obtusa*	Alder Spittlebug	1	1		2
(I) Psocodea	Amphipsocidae	*Polypsocus*	*corruptus*	Hairy-Winged Bark Louse			1	1
	Caeciliusidae	*Valenzuela*	*flavidus*	Lizard Bark Lice			1	1
	Psocidae	*Amphigerontia*	*bifasciata*	Bark Lice		1		1
		Metylophorus	*novaescotiae*	Common Bark Lice			1	1
		Trichadenotecnum	*majus*	Common Bark Louse		1		1

Table 2.3: **Detected Pest Species.** Pest species detected as part of bat diet appear with BIN frequency for each bat species sample category.

Order	**Family**	**Genus and Species**	**CommonName**	**Geography**	**BIN**
Coleoptera	Cerambycidae	*Anelaphus villosus*	Twig Pruner	Southeast US	BOLD:AAZ1211
		Enaphalodes rufulus	Red Oak Borer	MN	BOLD:AAD5015
		Saperda calcarata	Poplar Borer	MN	BOLD:AAD7174
	Curculionidae	*Hylobius pales*	Pales Weevil	Northeast US	BOLD:AAG5243
		Pachylobius picivorus	Pitch-Eating Weevil	Southeast US	BOLD:AAG5216
		Phyllobius oblongus	European Snout Beetle	MN	BOLD:AAF9187
Hemiptera	Aphrophoridae	*Aphrophora saratogensis*	Saratoga Spittlebug	MN	BOLD:ACF3839
Lepidoptera	Cossidae	*Acossus centerensis*	Poplar Carpenterworm	MN	BOLD:AAB5740
		Prionoxystus macmurtrei	Little Carpenterworm	MN	BOLD:AAD9795
	Erebidae	*Halysidota tessellaris*	Pale Tussock Moth	MN	BOLD:AAA3425
	Geometridae	*Ectropis crepuscularia*	Saddlebacked Looper	MN	BOLD:AAA2073
				MN	BOLD:AAA2074
	Gracillariidae	*Caloptilia alnivorella*	Alder Leafminer	Not Present	BOLD:AAB7940
	Lasiocampidae	*Malacosoma disstria*	Forest Tent Caterpillar	MN	BOLD:AAA4130
	Noctuidae	*Acronicta dactylina*	Fingered Dagger Moth	MN	BOLD:AAA2802
		Orthosia hibisci	Speckled Green Fruitworm Moth	MN	BOLD:AAA4128
	Notodontidae	*Heterocampa guttivitta*	Saddled Prominent	MN	BOLD:AAA3773
	Pyralidae	*Dioryctria reniculelloides*	Spruce Coneworm	MN	BOLD:AAA5470
	Tortricidae	*Archips argyrospila*	Fruittree Leafroller	MN	BOLD:AAA7035
		Catastega aceriella	Maple Trumpet Skeletonizer	Northeast US	BOLD:AAA7894
		Choristoneura conflictana	Large Aspen Tortrix	MN	BOLD:AAA3301
		Cydia piperana	Ponderosa Pine Seed Moth	Southwest US	BOLD:AAC6105
		Proteoteras aesculana	Maple Twig Borer	MN	BOLD:AAA6740
		Retinia albicapitana	Northern Pitch Twig Moth	MN	BOLD:AAA6914
		Zeiraphera canadensis	Spruce Bud Moth	Canada	BOLD:AAB0484
	Yponomeutidae	*Zelleria haimbachi*	Pine Needle Sheathminer	MN	BOLD:AAB0436

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Appendix: Supplementary Figures and Tables

Figures

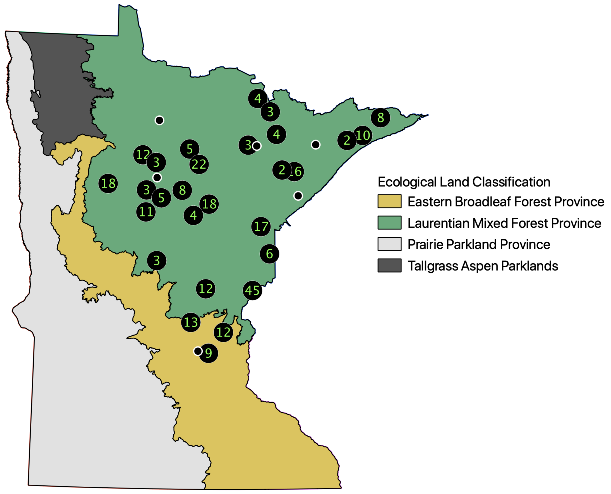


Figure A1: **Sampling Locations.** Sampling occurred in the forest regions of Minnesota. Most sampling occurred in the Laurentian Mixed Forest Province, which features more coniferous and mixed deciduous/coniferous forests than the Eastern Broadleaf Forest.

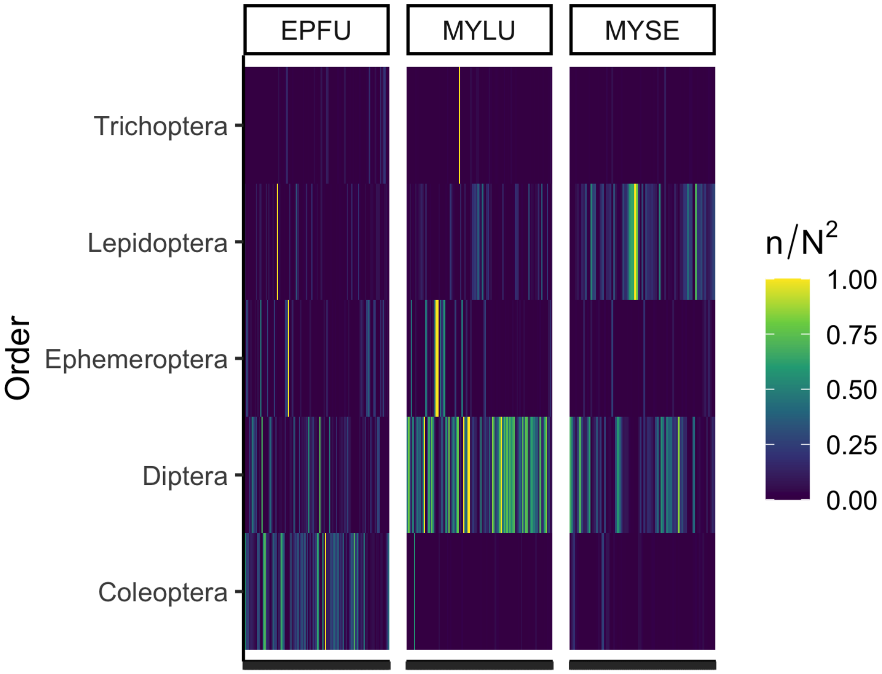
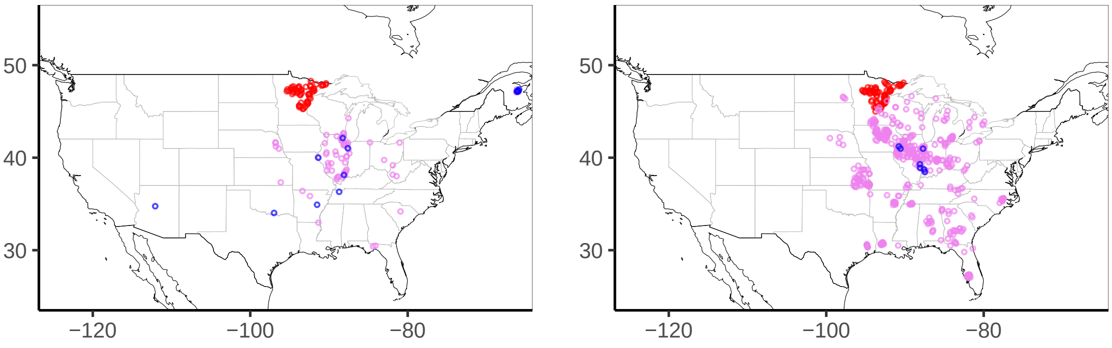
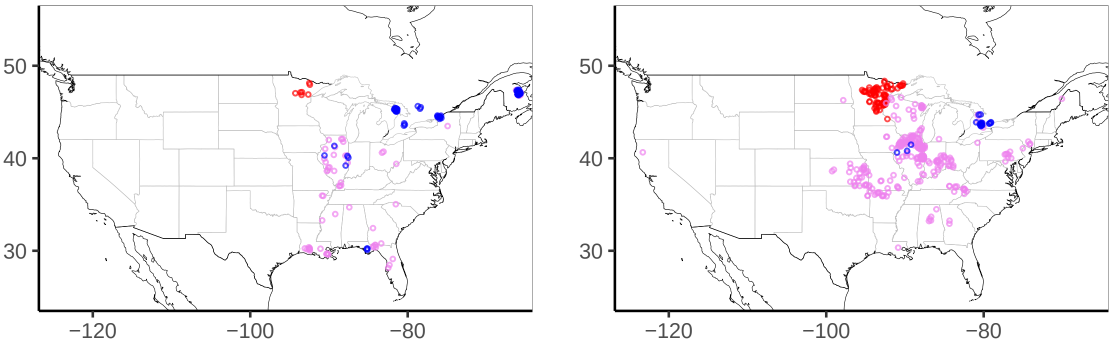


Figure A2: **Simpson diversity decomposition of major orders.** Simpson diversity is the sum of the likelihood of encountering each member of the composition twice. The decomposition of values reveals which order component contributes most to the unevenness of diversity. In these samples, BINs in the order Diptera had a high likelihood of occurring more than once in a sample in all bat species. BINs in the order Coleoptera were likely to be encountered more than once in *E. fuscus* samples.



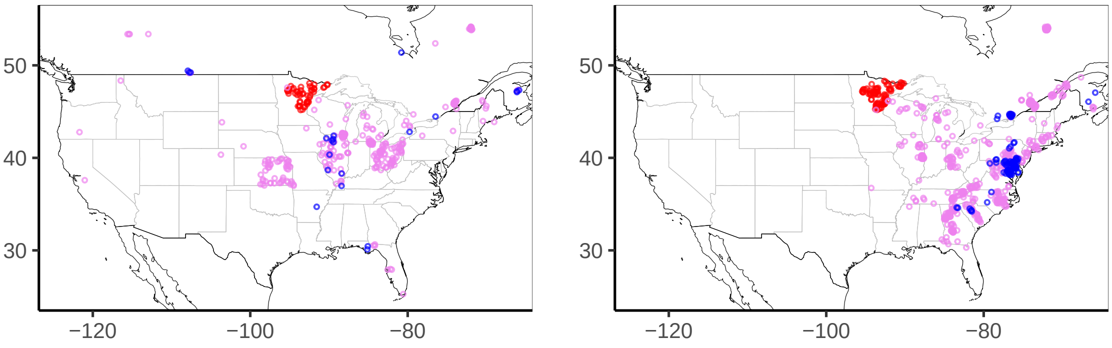
(a) *Caenis punctata*

(b) *Maccaffertium exiguum*



(c) *Caenis diminuta*

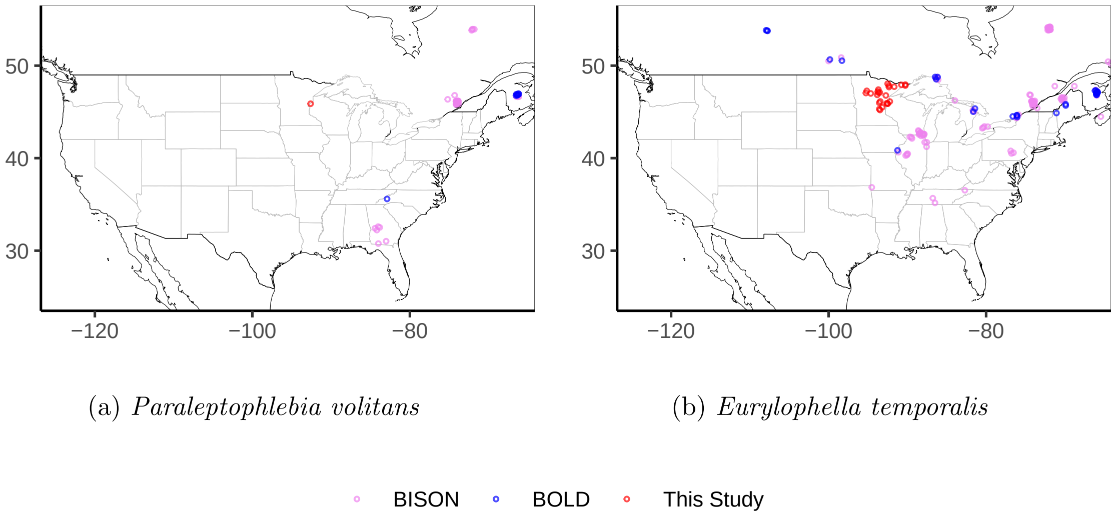
(d) *Maccaffertium pulchellum*



(e) *Caenis amica*

(f) *Maccaffertium modestum*

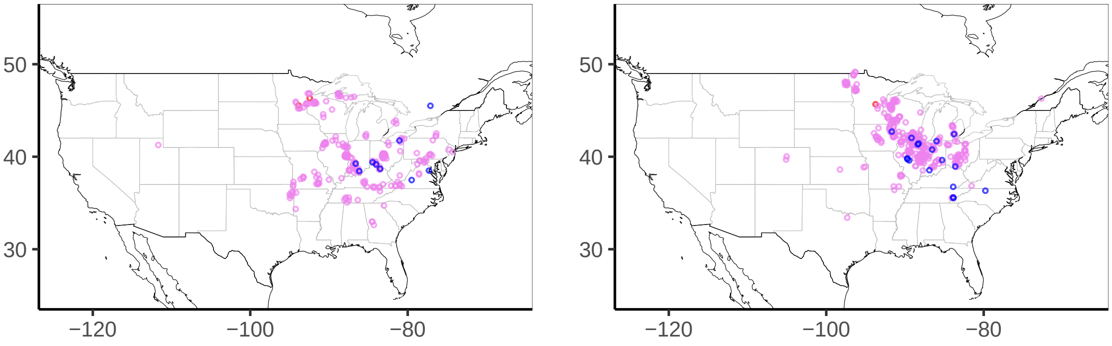
Figure A3: **Heptageniidae and Caenidae mayfly species observations.** *Caenis* (Caenidae) species appear in the first column followed by *Maccaffertium* (Heptageniidae) in the second. Minnesota is on the western edge of observations for three *Maccaffertium* species *(B, D, F)*. Observations in this study of *Caenis punctata (A)* and *Caenis diminuta (C)* appear at the northernmost edge of observations for these species in the BISON database. Sparse observations of *Caenis amica (E)* in Minnesota occur in the BISON database.



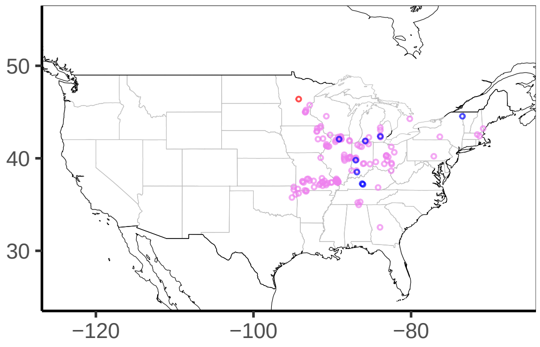
(a) *Paraleptophlebia volitans*

(b) *Eurylophella temporalis*

Figure A4: **Leptophlebiidae and Ephemerellidae mayfly species observations.** *Paraleptophlebia volitans (a)* (Leptophlebiidae) appears distant from observations in the BOLD and BISON databases. *Eurlophella temporalis (b)* (Ephemereliidae) is known from adjacent areas of the midwestern United States.



(a) *Agnetina flavescens* (b) *Perlesta decipiens*



(c) *Perlinella ephyre*

○ BISON ○ BOLD ○ This Study

Figure A5: **Plecoptera observations.** *Agnetina flavescens (a)*, *Perlesta decipiens (b)*, and *Perlinella ephyre (c)* have similar spatial distributions, where any observations in Minnesota are near the northwestern edge of the range. The BOLD database has a broader geographic range of samples for these species.

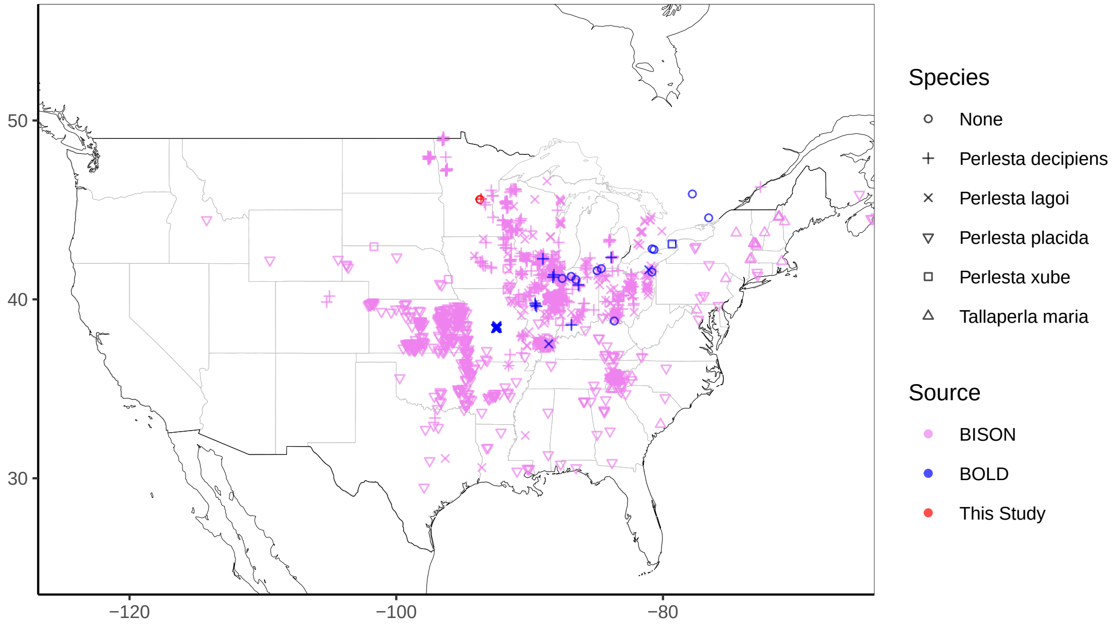
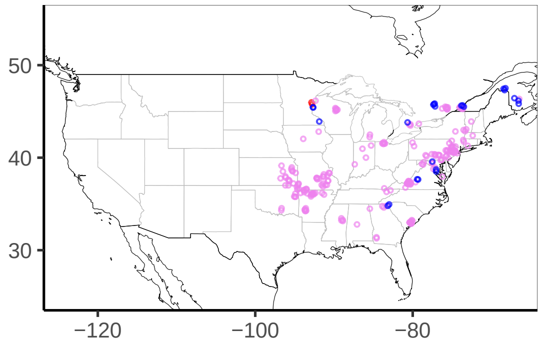
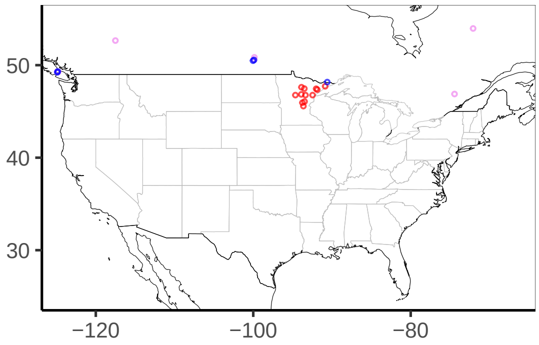


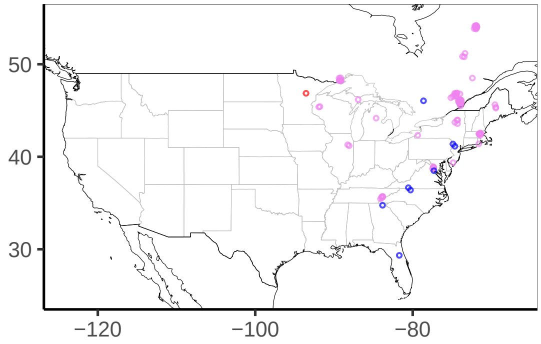
Figure A6: **Geographic distribution of BIN BOLD:AEA6705.** BIN BOLD:AEA6705 contains COI sequences from six Plecoptera species, none of which except *Perlesta decipiens* is well represented in MN.



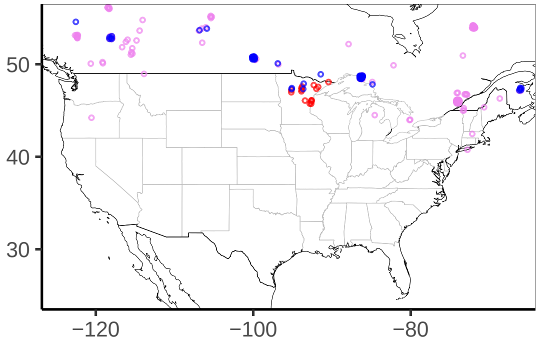
(a) *Hydropsyche scalaris*



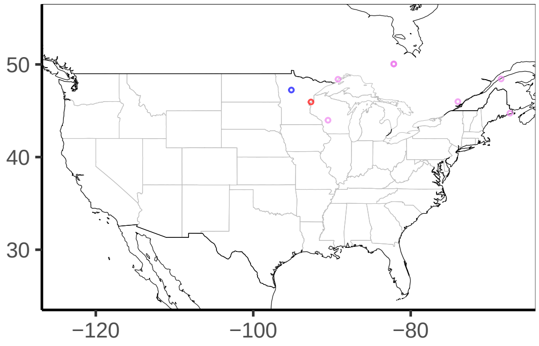
(b) *Agrypnia macdunnoughi*



(c) *Molanna ulmerina*



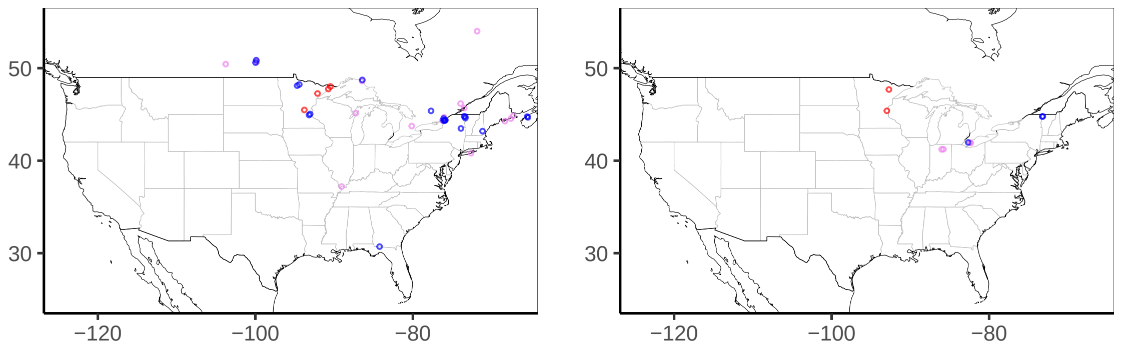
(d) *Agrypnia improba*



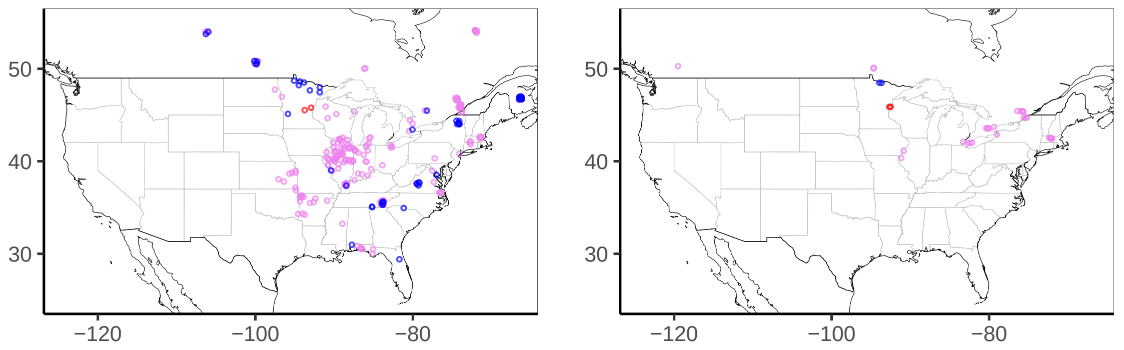
(e) *Hagenella canadensis*

○ BISON ○ BOLD ○ This Study

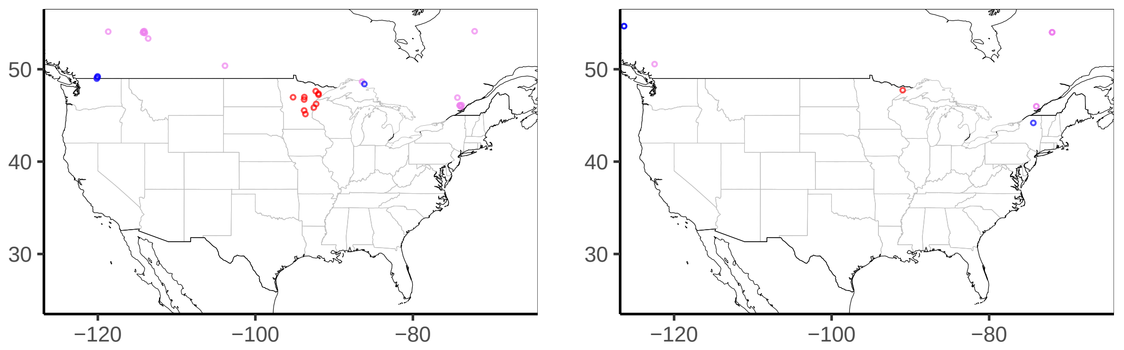
Figure A7: **Phryganeidae, Molannidae, and Hydropsychidae caddisfly observations.** *Agrypnia macdunnoughi (b), A. improba (d)* and *Hagenella canadensis (e)* are giant case-making caddisflies in the family Phryganeidae. All are on the edges of their known ranges in Minnesota with some recent observations in BOLD. *Molanna ulmerina (c)* (Molannidae) is primarily known as an eastern species. The record in this study is the westernmost observation. *Hydropsyche scalaris (a)* (Hydropsychidae) is at the northwestern edge of its range in MN.



(a) *Triaenodes nox* (b) *Triaenodes connatus*



(c) *Ceraclea transversa* (d) *Ceraclea mentiea*



(e) *Asynarchus mutatus* (f) *Lenarchus crassus*

Figure A8: **Leptoceridae and Limnephilidae caddisfly observations.** *Triaenodes nox (a)*, *T. connatus (b)*, *Ceraclea transversa (c)*, and *C. mentiea (d)* are Leptoceridae caddisflies. *Triaenodes connatus* has no observations in either the BOLD or BISON database in MN. Unlike *T. nox* and *C. mentiea*, *C. transversa* is has geographic records in the surrounding states of MN. *Lenarchus crassus* and *Asynarchus mutatus* are Limnephilidae caddisflies with limited distributions. *Lenarchus crassus* has no records in BOLD or BISON in the midwest United States, and *A. mutatus* in MN are farther south than all records in both databases.

Tables

Table A1: **All BINs with species level taxonomic IDs.** Frequencies represent the number of detections that qualified as being prey in the bats *(E) Eptesicus Fuscus*, *(U) Myotis lucifugus*, and *(M) M. septentrionalis*. Arthropod class appears in parantheses with each order: *(A)*: Arachnida, *(D)*: Diplopoda, *(I)*: Insecta, *(M)*: Malacostraca. Species with no observed frequencies were detected either in residual levels or were detected in samples with total 1.5 times above the interquartile range of BIN richness for all samples. They were excluded from diet analyses but used in eDNA analyses.

Taxonomy					Frequency		
Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
(A) Araneae	Anyphaenidae	*Hibana*	*gracilis*	BOLD:AAN6394	-	-	-
	Araneidae	*Araneus*	*bicentenarius*	BOLD:AAI4457	0	0	1
			guttulatus	BOLD:AAN6304	-	-	-
			nordmanni	BOLD:AAB7556	0	0	1
			saevus	BOLD:AAD2248	-	-	-
		Argiope	*aurantia*	BOLD:AAB7933	-	-	-
		Eustala	*anastera*	BOLD:AAB7933	3	0	1
			cepina	BOLD:AAB7935	0	1	0
			emertoni	BOLD:AAB7934	1	2	5
			rosae	BOLD:AAL4913	0	0	2
		Larinioides	*patagiatus*	BOLD:AAA3681	1	1	3
			patagiatus	BOLD:ACX0897	-	-	-
		Mangora	*placida*	BOLD:AAP5578	0	0	0
			placida	BOLD:AAI4456	0	0	0
		Neoscona	*arabesca*	BOLD:AAA4123	0	0	2
	Clubionidae	*Clubiona*	*canadensis*	BOLD:AAB2563	0	3	8
			moesta	BOLD:AAP3591	0	0	1
			obesa	BOLD:AAD5417	0	3	5
		Elaver	*excepta*	BOLD:AAI4088	-	-	-
	Dictynidae	*Dictyna*	*brevitarsa*	BOLD:AAN2653	0	0	1
		Emblyna	*sublata*	BOLD:AAA7272	0	0	3
	Linyphiidae	*Grammonota*	*angusta*	BOLD:AAD1499	0	1	1
			angusta	BOLD:ACC7775	-	-	-
			pictilis	BOLD:AAD1498	0	1	3
		Pocadicnemis	*americana*	BOLD:AAC9060	0	0	1

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
	Lycosidae	*Piratula*	*canadensis*	BOLD:AAB6784	0	1	0
	Philodromidae	*Philodromus*	*imbecillus*	BOLD:AAC6350	-	-	-
			keyserlingi	BOLD:AAI2836	0	0	1
			peninsulanus	BOLD:AAA9991	0	0	2
			praelustris	BOLD:AAD2665	0	0	1
			rufus	BOLD:AAB2768	1	1	2
			vulgaris	BOLD:AAI2835	0	0	1
	Salticidae	*Pelegrina*	*flaviceps*	BOLD:AAD6736	-	-	-
	Tetragnathidae	*Leucauge*	*venusta*	BOLD:AAB8714	0	0	1
		Tetragnatha	*caudata*	BOLD:AAE3958	0	1	0
			elongata	BOLD:AAA4942	-	-	-
			shoshone	BOLD:AAB7995	1	2	3
			shoshone	BOLD:AAN6690	0	0	1
			straminea	BOLD:ACU2889	-	-	-
			viridis	BOLD:AAG5659	1	1	5
			viridis	BOLD:AAN6335	0	0	5
			viridis	BOLD:ACN4170	-	-	-
	Theridiidae	*Dipoena*	*nigra*	BOLD:AAF4974	0	0	1
		Theridion	*albidum*	BOLD:AAV3042	-	-	-
			differens	BOLD:AAC3269	0	1	0
			murarium	BOLD:AAC6350	1	6	7
	Thomisidae	*Xysticus*	*punctatus*	BOLD:AAD2346	0	0	0
(D) Julida	Julidae	*Cylindroiulus*	*caeruleocinctus*	BOLD:AAH7472	0	0	0
(I) Blattodea	Ectobiidae	*Blattella*	*germanica*	BOLD:AAF5944	1	0	0
		Parcoblatta	*uhleriana*	BOLD:AAG9964	0	0	3
(I) Coleoptera	Anthicidae	*Anthicus*	*cervinus*	BOLD:AAH2753	-	-	-
	Anthribidae	*Euparius*	*marmoreus*	BOLD:AAG5219	-	-	-
	Bostrichidae	*Amphicerus*	*bicaudatus*	BOLD:AAH0433	2	0	0
		Lichenophanes	*bicornis*	BOLD:ABW9910	1	0	0
	Brentidae	*Arrhenodes*	*minutus*	BOLD:AAF8687	0	0	0
	Carabidae	*Agonum*	*decorum*	BOLD:AAH2758	1	0	0
			lutulentum	BOLD:AAH2803	-	-	-
			placidum	BOLD:AAC7304	17	1	1
			tenue	BOLD:AAH0099	1	0	0
		Amara	*lacustris*	BOLD:AAQ0060	-	-	-
			latior	BOLD:AAH0443	4	0	0
		Amphasia	*sericea*	BOLD:AAH2757	2	0	0

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Order	Family	Genus	Species	BIN	E	U	M
		Bembidion	*rapidum*	BOLD:AAJ8218	4	0	0
		Brachinus	*quadripennis*	BOLD:AAZ0129	1	0	0
		Calosoma	*frigidum*	BOLD:AAK1720	4	1	0
		Carabus	*taedatus*	BOLD:AAT8826	-	-	-
			emarginatus	BOLD:AAJ0149	1	0	0
			sericeus	BOLD:AAH2764	1	0	0
		Colliuris	*pensylvanica*	BOLD:AAH0384	1	0	0
		Cymindis	*platicollis*	BOLD:AAZ1504	1	0	0
		Diplocheila	*striatopunctata*	BOLD:AAX9540	1	0	0
		Harpalus	*caliginosus*	BOLD:AAT8826	4	0	0
			compar	BOLD:AAV6870	5	0	0
			faunus	BOLD:ACO1077	1	0	0
			pensylvanicus	BOLD:AAC3054	12	1	1
		Notiobia	*terminata*	BOLD:AAH2752	11	0	0
		Patrobus	*cinctus*	BOLD:AAY9497	1	0	0
		Platypatrobus	*lacustris*	BOLD:AAH2779	1	0	0
		Selenophorus	*opalinus*	BOLD:AAH2761	1	0	0
		Sericoda	*obsoleta*	BOLD:AAH2827	-	-	-
			ochropezus	BOLD:AAH0147	1	0	0
	Cerambycidae	*Aegomorphus*	*modestus*	BOLD:AAE6659	4	1	0
		Anelaphus	*parallelus*	BOLD:AAG5555	3	0	0
			villosus	BOLD:AAZ1211	3	0	0
		Astylopsis	*collaris*	BOLD:AAE4764	-	-	-
			sexguttata	BOLD:AAJ9523	7	0	0
		Centrodera	*decolorata*	BOLD:AAW6032	1	0	0
		Ecyrus	*dasycerus*	BOLD:AAF6389	3	0	0
		Enaphalodes	*rufulus*	BOLD:AAD5015	1	0	0
		Graphisurus	*fasciatus*	BOLD:AAD6547	10	1	5
		Lepturges	*symmetricus*	BOLD:AAE6802	1	0	0
			carolinensis	BOLD:ABX5367	3	1	1
		Obrium	*rufulum*	BOLD:AAI3406	1	0	1
		Saperda	*calcarata*	BOLD:AAD7174	4	0	0
			tridentata	BOLD:AAD2696	3	1	0
			vestita	BOLD:AAD6865	12	0	2
		Sternidius	*alpha*	BOLD:ACE4064	-	-	-
		Tylonotus	*bimaculatus*	BOLD:AAF6341	1	0	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			sagittatus	BOLD:AAF7394	1	0	1
			undulatus	BOLD:AAB8142	-	-	-
	Chrysomelidae	*Capraita*	*subvittata*	BOLD:ADG8727	-	-	-
		Metachroma	*angustulum*	BOLD:AAU7144	-	-	-
	Cleridae	*Cymatodera*	*bicolor*	BOLD:ACA7844	-	-	-
	Coccinellidae	*Anatis*	*labiculata*	BOLD:AAF3857	4	2	1
		Coccinella	*septempunctata*	BOLD:AAA8933	0	0	1
		Harmonia	*axyridis*	BOLD:AAB5640	0	1	0
		Curculio	*strictus*	BOLD:ADI8773	-	-	-
		Hylobius	*pales*	BOLD:AAG5243	-	-	-
		Hypothenemus	*seriatus*	BOLD:ADD2679	1	0	0
		Pachylobius	*picivorus*	BOLD:AAG5216	0	0	0
		Phyllobius	*oblongus*	BOLD:AAF9187	0	1	0
			formosus	BOLD:ACO8630	0	0	1
	Dytiscidae	*Colymbetes*	*dolabratus*	BOLD:AAA6661	13	0	0
			sculptilis	BOLD:AAP6243	1	0	0
		Coptotomus	*longulus*	BOLD:AAH3847	1	0	0
		Dytiscus	*fasciventris*	BOLD:AAH0231	4	0	0
		Graphoderus	*liberus*	BOLD:AAL3232	-	-	-
			biguttulus	BOLD:AAH0146	5	1	0
			erichsoni	BOLD:AAD6399	4	0	0
			fraterculus	BOLD:AAH2912	8	0	2
			subaeneus	BOLD:AAB8834	0	0	0
		Thermonectus	*nigrofasciatus*	BOLD:AAH3838	1	0	0
	Elateridae	*Ampedus*	*melanotoides*	BOLD:ABW2812	1	0	1
		Athous	*rufifrons*	BOLD:ACA3928	-	-	-
		Denticollis	*denticornis*	BOLD:ACM1366	0	4	1
			denticornis	BOLD:ACM1124	0	2	0
		Elater	*abruptus*	BOLD:AAX7307	1	3	0
		Hemicrepidius	*brevicollis*	BOLD:AAP6367	23	3	5
			memnonius	BOLD:AAH2373	2	1	0
		Melanotus	*castanipes*	BOLD:AAH2392	7	0	4
			decumanus	BOLD:AAZ2240	0	0	0
			similis	BOLD:AAM7567	5	0	1
			similis	BOLD:AAH0376	8	0	0
			similis	BOLD:ABW1291	-	-	-
	Eucnemidae	*Onichodon*	*orchesides*	BOLD:ACN2397	-	-	-

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
	Gyrinidae	*Dineutus*	*nigrior*	BOLD:AAG0708	7	0	0
		Gyrinus	*confinis*	BOLD:AAG0707	1	0	0
			latilimbus	BOLD:ACL2881	1	0	0
	Hydrophilidae	*Cymbiodyta*	*minima*	BOLD:AAN6201	0	1	0
			vindicata	BOLD:ACO5348	0	0	1
		Enochrus	*consors*	BOLD:AAU7145	1	0	0
			hamiltoni	BOLD:AAH2917	3	0	0
			ochraceus	BOLD:AAH2910	1	0	0
		Helocombus	*bifidus*	BOLD:AAM7687	4	0	0
			bifidus	BOLD:AAH2909	2	0	0
		Hydrobius	*fuscipes*	BOLD:AAC5900	12	2	1
			fuscipes	BOLD:AAH0085	1	0	0
		Hydrochara	*obtusata*	BOLD:AAH2908	25	1	1
	Latridiidae	*Melanophthalma*	*inermis*	BOLD:AAN6154	0	1	0
	Leiodidae	*Leiodes*	*subtilicornis*	BOLD:ACK3154	-	-	-
	Lucanidae	*Ceruchus*	*piceus*	BOLD:AAI3970	5	1	1
	Melandryidae	*Dircaea*	*liturata*	BOLD:AAH0363	1	1	0
		Enchodes	*sericea*	BOLD:ACA7524	0	1	2
		Orchesia	*castanea*	BOLD:AAM7670	0	0	1
		Orchesia	*cultriformis*	BOLD:AAP7011	0	1	1
		Serropalpus	*substriatus*	BOLD:AAH9565	2	0	2
	Mycetophagidae	*Mycetophagus*	*punctatus*	BOLD:AAX3298	0	0	1
	Nitidulidae	*Colopterus*	*truncatus*	BOLD:ACA6288	-	-	-
	Ochodaeidae	*Xenochodaeus*	*musculus*	BOLD:ACY6873	1	0	1
	Ptilodactylidae	*Ptilodactyla*	*serricollis*	BOLD:ACR3763	3	1	2
	Pyrochroidae	*Dendroides*	*canadensis*	BOLD:AAG0758	7	1	7
		Dendroides	*concolor*	BOLD:ACI6375	2	0	4
		Neopyrochroa	*femoralis*	BOLD:ACB1646	1	1	1
	Scarabaeidae	*Dichelonyx*	*albicollis*	BOLD:AAH0140	3	0	0
			subvittata	BOLD:AAG4446	0	0	2
		Diplotaxis	*tristis*	BOLD:AAH6793	3	0	0
		Osmoderma	*eremicola*	BOLD:ACX5730	13	0	0
		Pelidnota	*punctata*	BOLD:AAH6809	0	0	0
		Phyllophaga	*anxia*	BOLD:AAH6791	14	0	4
			anxia	BOLD:AAH0136	2	0	0
			crenulata	BOLD:AAY9592	2	0	0
			crenulata	BOLD:AAC4287	3	0	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			drakii	BOLD:AAE4876	23	1	3
			implicita	BOLD:AAC5183	-	-	-
			nitida	BOLD:AAH6804	6	0	2
			rugosa	BOLD:AAJ2312	4	0	0
		Serica	*atracapilla*	BOLD:AAH6805	0	0	4
			intermixta	BOLD:AAH6792	1	0	0
			intermixta	BOLD:AAH6860	-	-	-
			sericea	BOLD:AAH6798	4	1	2
		Tomarus	*gibbosus*	BOLD:AAH6800	-	-	-
	Scirtidae	*Contacyphon*	*fuscescens*	BOLD:ACX3673	0	0	0
			obscurus	BOLD:AAG7259	0	2	0
			ochreatus	BOLD:AAG7261	0	3	0
			variabilis	BOLD:ACX4619	4	5	1
		Scirtes	*tibialis*	BOLD:AAH0215	1	0	0
	Scraptiidae	*Canifa*	*pallipes*	BOLD:ABA9081	0	1	1
			pallipes	BOLD:AAP7039	0	1	0
	Silphidae	*Necrodes*	*surinamensis*	BOLD:AAC2599	5	0	0
		Nicrophorus	*orbicollis*	BOLD:AAE1939	10	0	0
			pustulatus	BOLD:AAC2598	3	0	0
			sayi	BOLD:AAD8009	-	-	-
		Philonthus	*vulgatus*	BOLD:AAG4283	1	0	0
	Stenotrachelidae	*Cephaloon*	*lepturoides*	BOLD:AAI3796	0	2	1
	Synchroidae	*Synchroa*	*punctata*	BOLD:AAH0375	6	0	4
	Tenebrionidae	*Alobates*	*pensylvanicus*	BOLD:AAG3221	6	0	0
		Bolitotherus	*cornutus*	BOLD:AAF0405	1	0	0
		Corticeus	*bicolor*	BOLD:ABW9177	-	-	-
		Hymenorus	*dubius*	BOLD:AAZ4032	1	0	0
			picipennis	BOLD:AAH0473	6	0	0
			pilosus	BOLD:AAZ1878	1	0	0
		Mycetochara	*fraterna*	BOLD:AAU7138	0	0	2
		Xylopinus	*saperdoides*	BOLD:AAH2782	1	0	1
	Tetratomidae	*Penthe*	*pimelia*	BOLD:AAH0351	-	-	-
	Trogidae	*Trox*	*unistriatus*	BOLD:AAD2211	1	0	0
	Trogossitidae	*Tenebroides*	*corticalis*	BOLD:AAH9754	-	-	-
(I) Diptera	Anthomyiidae	*Delia*	*platura*	BOLD:AAA3453	9	2	5
	Asilidae	*Leptogaster*	*flavipes*	BOLD:ADI9443	-	-	-

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
	Calliphoridae	*Cynomya*	*cadaverina*	BOLD:AAB0868	0	1	0
		Camptomyia	*heterobia*	BOLD:ABW6095	-	-	-
		Culicoides	*biguttatus*	BOLD:AAG6468	0	0	2
			obsoletus	BOLD:AAG6513	1	1	4
			travisi	BOLD:AAG6436	-	-	-
	Chaoboridae	*Chaoborus*	*flavicans*	BOLD:ADT7894	-	-	-
			punctipennis	BOLD:AAG2647	2	19	1
	Chironomidae	*Ablabesmyia*	*americana*	BOLD:AAC8567	0	7	2
			annulata	BOLD:AAM6231	0	7	0
			aspera	BOLD:AAF3628	0	11	4
		Chironomus	*acidophilus*	BOLD:AAC0903	1	7	2
			bifurcatus	BOLD:AAG5453	0	2	2
			dilutus	BOLD:AAB4658	2	0	0
			maturus	BOLD:AAB4657	0	0	0
			sp. TE12	BOLD:ABA4433	0	1	0
		Cladopelma	*viridulum*	BOLD:ACL4244	0	1	0
		Conchapelopia	*telema*	BOLD:AAN5351	0	0	1
		Cricotopus	*sp. 23ES*	BOLD:AAI6022	1	0	0
			sylvestris	BOLD:AAV1709	-	-	-
		Dicrotendipes	*modestus*	BOLD:ACE8913	0	0	1
			modestus	BOLD:AAL7329	0	3	1
			tritomus	BOLD:AAC0706	0	2	0
		Einfeldia	*synchrona*	BOLD:AAP6213	0	1	1
		Hyporhygma	*quadripunctatus*	BOLD:AAL7335	0	2	0
		Microtendipes	*pedellus*	BOLD:AAE0707	0	1	0
		Paratanytarsus	*laccophilus*	BOLD:ADY5612	-	-	-
			sp. ES01	BOLD:AAH7760	0	1	0
			sp. TE02	BOLD:AAY3407	0	1	0
			denticulatus	BOLD:ADR9580	2	15	1
			signatus	BOLD:AAF6798	-	-	-
			sp. 1ES	BOLD:AAG3920	0	2	0
		Psectrocladius	*barbimanus*	BOLD:AAD0484	0	1	0
			sordidellus	BOLD:AAL7382	0	0	0
		Psectrotanypus	*sp. ES01*	BOLD:AAG0314	0	1	2
		Rheocricotopus	*robacki*	BOLD:AAB6760	0	0	1
		Smittia	*sp. 14ES*	BOLD:ACW5117	0	0	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			mendax	BOLD:ACX2014	2	10	1
			mendax	BOLD:AAA3776	0	1	0
		Xenochironomus	*xenolabis*	BOLD:AAG8483	0	2	1
	Culicidae	*Aedes*	*abserratus*	BOLD:AAA3748	4	20	17
			canadensis	BOLD:AAB5696	0	8	4
			cinereus	BOLD:AAC1222	0	10	8
			cinereus	BOLD:AAP8896	0	1	0
			diantaeus	BOLD:AAF2904	0	0	1
			dorsalis	BOLD:ACE6286	1	0	0
			fitchii	BOLD:AAD8027	1	0	1
			sticticus	BOLD:AAJ5881	3	1	4
			vexans	BOLD:AAA7067	0	1	1
		Anopheles	*walkeri*	BOLD:AAD2594	0	1	2
		Coquillettidia	*perturbans*	BOLD:AAB2539	0	1	0
		Culex	*territans*	BOLD:AAB6943	0	5	2
			territans	BOLD:ABY7666	-	-	-
		Culiseta	*inornata*	BOLD:AAC9132	1	0	0
			melanura	BOLD:AAM8971	1	8	5
			minnesotae	BOLD:AAJ7123	1	2	1
			morsitans	BOLD:AAE3210	3	13	8
			diantaeus	BOLD:ACC5413	1	1	0
	Drosophilidae	*Chymomyza*	*amoena*	BOLD:AAE2703	-	-	-
		Drosophila	*falleni*	BOLD:AAB7507	-	-	-
		Drosophila	*melanogaster*	BOLD:AAA1831	0	0	1
		Scaptomyza	*pallida*	BOLD:AAG8493	1	1	0
	Empididae	*Empis*	*pallida*	BOLD:AAL8965	1	2	1
			sp. 8	BOLD:ABA4861	0	0	0
	Ephydridae	*Hydrellia*	*notata*	BOLD:AAG9656	-	-	-
		Notiphila	*olivacea*	BOLD:AAV4056	1	0	0
			pulchra	BOLD:ABV0852	-	-	-
		Homoneura	*bispina*	BOLD:ABW3687	0	0	1
		Austrolimnophila	*toxoneura*	BOLD:AAI1332	0	3	4
		Cheilotrichia	*stigmatica*	BOLD:ACA9904	0	1	1
		Dicranomyia	*longipennis*	BOLD:AAP8635	2	3	0
		Discobola	*annulata*	BOLD:AAF4967	0	0	0
		Elephantomyia	*westwoodi*	BOLD:AAF9064	0	2	2
		Epiphragma	*fasciapenne*	BOLD:ACL8650	6	13	27

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Order	Family	Genus	Species	BIN	E	U	M
			fasciapenne	BOLD:AAF4617	-	-	-
		Erioptera	*caliptera*	BOLD:AAN5882	0	3	0
			chlorophylla	BOLD:ACM9786	-	-	-
		Euphylidorea	*luteola*	BOLD:ABW5810	-	-	-
			platyphallus	BOLD:AAD6847	11	23	12
		Gnophomyia	*tristissima*	BOLD:ABA9935	1	0	1
		Helius	*flavipes*	BOLD:AAF9008	12	22	8
			immatura	BOLD:ACA9696	2	0	6
			novaeangliae	BOLD:AAF8979	2	0	0
			solitaria	BOLD:ABZ1300	3	3	8
		Molophilus	*forcipulus*	BOLD:ACA9751	0	1	1
			pubipennis	BOLD:AAZ5967	0	1	0
		Ormosia	*affinis*	BOLD:AAU6544	0	3	1
		Pseudolimnophila	*inornata*	BOLD:AAI1351	13	23	9
			maculata	BOLD:AAF9059	0	1	2
			maculata	BOLD:ABY8689	0	1	0
		Shannonomyia	*lenta*	BOLD:ABU6040	0	1	0
	Milichiidae	*Phyllomyza*	*sp. TAW1*	BOLD:AAU5275	0	1	0
		Helina	*evecta*	BOLD:AAC2498	1	0	0
			troene	BOLD:AAG1717	0	0	1
		Limnophora	*narona*	BOLD:AAG1705	-	-	-
		Lispe	*cotidiana*	BOLD:ACE5619	2	0	0
		Mydaea	*nr urbana*	BOLD:ACB9959	0	1	0
		Phaonia	*apicata*	BOLD:AAG1772	0	1	0
			bysia	BOLD:AAG1699	0	0	1
		Exechia	*separata*	BOLD:AAG4880	-	-	-
		Leia	*winthemii*	BOLD:AAG4898	1	0	0
		Leptomorphus	*hyalinus*	BOLD:AAG4957	-	-	-
		Mycomya	*tenuis*	BOLD:AAG4884	0	1	2
			winnertzi	BOLD:AAU4916	0	0	0
		Tarnania	*tarnanii*	BOLD:ABW8792	0	0	1
		Ula	*bolitophila*	BOLD:ABU5946	0	1	1
			sylvatica	BOLD:AAV1814	0	0	1
	Polleniidae	*Pollenia*	*pediculata*	BOLD:AAG6745	-	-	-
	Pyrgotidae	*Sphecomyiella*	*valida*	BOLD:AAC7654	1	0	1
	Rhagionidae	*Chrysopilus*	*quadratus*	BOLD:AAI4082	1	0	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Rhagio	*mystaceus*	BOLD:AAB3308	0	0	1
	Sarcophagidae	*Ravinia*	*derelicta*	BOLD:AAH7161	1	1	0
	Scathophagidae	*Scathophaga*	*furcata*	BOLD:ADY1455	0	1	0
			stercoraria	BOLD:AAD0853	3	0	1
		Atrichomelina	*pubera*	BOLD:AAI7911	1	1	1
		Sepedon	*fuscipennis*	BOLD:AAJ7618	-	-	-
		Tetanocera	*valida*	BOLD:AAN6419	1	0	0
			croxtoni	BOLD:AAB9222	0	0	0
	Sphaeroceridae	*Leptocera*	*erythrocera*	BOLD:AAG7276	-	-	-
	Stratiomyidae	*Odontomyia*	*cincta*	BOLD:AAI3614	0	0	1
		Melanostoma	*mellinum*	BOLD:AAB2866	-	-	-
		Toxomerus	*marginatus*	BOLD:AAA4277	1	1	0
		Xylota	*flavifrons*	BOLD:AAC2125	0	0	1
	Tabanidae	*Tabanus*	*catenatus*	BOLD:ACV2101	-	-	-
		Eutrixa	*exilis*	BOLD:AAG2127	0	1	1
		Hyphantrophaga	*blandita*	BOLD:AAN6460	-	-	-
		Phytomyptera	*melissopodis*	BOLD:AAZ0412	-	-	-
	Tephritidae	*Dioxyna*	*picciola*	BOLD:AAC3022	-	-	-
		Neotephritis	*finalis*	BOLD:AAG9754	-	-	-
	Tipulidae	*Angarotipula*	*illustris*	BOLD:AAD6051	5	0	0
		Dolichopeza	*obscura*	BOLD:AAZ5969	1	1	5
			tridenticulata	BOLD:AAI6345	0	2	0
			walleyi	BOLD:ABA7621	0	0	1
			alterna	BOLD:AAF9035	7	2	6
			eucera	BOLD:ABX5351	2	0	1
			ferruginea	BOLD:ABX6186	7	1	1
			ferruginea	BOLD:ABX6298	3	0	0
			macrocera	BOLD:AAO3948	-	-	-
			occipitalis	BOLD:AAF8998	-	-	-
			sulphurea	BOLD:AAD2626	2	0	0
			coloradensis	BOLD:AAF9052	-	-	-
			entomophthorae	BOLD:AAB0062	0	0	4
			entomophthorae	BOLD:ABY8737	0	0	1
			longiventris	BOLD:AAF9015	1	0	0
			macrolabis	BOLD:AAG4520	0	1	1
			parshleyi	BOLD:AAF8980	1	1	7
			penobscot	BOLD:AAF8983	0	1	2

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			platymera	BOLD:AAC4660	1	1	3
			tephrocephala	BOLD:AAG4532	-	-	-
(I) Ephemeroptera	Baetidae	*Callibaetis*	*ferrugineus*	BOLD:AAC7438	0	0	0
		Procloeon	*sp. JMW4*	BOLD:AAG5056	0	0	0
			diminuta	BOLD:AAA9627	0	5	0
			latipennis	BOLD:AAB4627	22	32	23
			latipennis	BOLD:ADZ9089	-	-	-
	Ephemerellidae	*Eurylophella*	*temporalis*	BOLD:AAB2478	1	3	1
	Ephemeridae	*Hexagenia*	*limbata*	BOLD:AAA5477	5	1	0
			limbata	BOLD:AAI1799	1	1	0
			limbata	BOLD:AAG2645	0	1	0
	Heptageniidae	*Leucrocuta*	*hebe*	BOLD:AAA2791	0	1	0
		Maccaffertium	*exiguum*	BOLD:AAB4365	27	7	10
			mediopunctatum	BOLD:AAC4559	-	-	-
			mediopunctatum	BOLD:ABZ6752	-	-	-
			mexicanum	BOLD:AAE8479	5	1	2
			modestum	BOLD:AAA3409	15	5	5
			pulchellum	BOLD:AAA3407	24	5	10
			vicarium	BOLD:ABY4262	2	2	0
			vicarium	BOLD:ADF6563	-	-	-
		Stenacron	*interpunctatum*	BOLD:AAA8218	10	5	0
			interpunctatum	BOLD:AAA8213	2	3	0
		Stenonema	*femoratum*	BOLD:AAA7920	3	4	0
	Isonychiidae	*Isonychia*	*bicolor*	BOLD:AAA9229	6	1	2
			rufa	BOLD:AAF3494	2	0	1
			sp.DHF1	BOLD:AAI2115	0	1	0
	Potamanthidae	*Anthopotamus*	*verticis*	BOLD:AAZ1943	3	0	2
	Siphlonuridae	*Siphlonurus*	*alternatus*	BOLD:AAA4673	1	4	0
(I) Hemiptera	Acanthosomatidae	*Elasmucha*	*lateralis*	BOLD:AAG8975	0	0	0
	Adelgidae	*Adelges*	*lariciatus*	BOLD:AAC2577	0	1	0
		Pineus	*coloradensis*	BOLD:AAD0872	-	-	-
	Aphididae	*Calaphis*	*betulaecolens*	BOLD:AAC3672	0	0	1
		Chaitophorus	*populifolii*	BOLD:AAB1605	-	-	-
		Eriosoma	*americanum*	BOLD:AAD7955	1	1	1
		Sitobion	*avenae*	BOLD:AAB4894	0	1	0
	Aphrophoridae	*Aphrophora*	*cribrata*	BOLD:AAG3622	-	-	-

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			saratogensis	BOLD:ACF3839	-	-	-
	Cicadellidae	*Athysanus*	*argentarius*	BOLD:AAG2892	-	-	-
			brevita	BOLD:ACE9691	1	0	0
		Ponana	*rubida*	BOLD:AAG2889	7	1	4
	Clastopteridae	*Clastoptera*	*obtusa*	BOLD:AAG8823	1	1	0
	Corixidae	*Callicorixa*	*audeni*	BOLD:AAB0657	5	1	1
			audeni	BOLD:AAB0656	-	-	-
		Hesperocorixa	*atopodonta*	BOLD:AAE3786	5	0	0
		Palmacorixa	*buenoi*	BOLD:AAG8833	0	0	0
		Sigara	*decorata*	BOLD:ADZ9867	2	0	0
			decoratella	BOLD:AAC6473	-	-	-
	Derbidae	*Apache*	*degeerii*	BOLD:ACA9002	-	-	-
	Flatidae	*Metcalfa*	*pruinosa*	BOLD:ABW2936	-	-	-
	Lygaeidae	*Kleidocerys*	*resedae*	BOLD:AAD4820	0	0	0
			resedae	BOLD:ABY8347	-	-	-
	Miridae	*Deraeocoris*	*aphidiphagus*	BOLD:AAV0156	1	0	0
			pinicola	BOLD:ACN6093	-	-	-
		Hyaliodes	*harti*	BOLD:AAG8824	-	-	-
		Megaloceroea	*recticornis*	BOLD:ACD9085	-	-	-
			quercalbae	BOLD:AAB2216	-	-	-
			tiliae	BOLD:ABX5095	0	2	0
		Neurocolpus	*nubilus*	BOLD:AAN8390	-	-	-
		Orthotylus	*necopinus*	BOLD:AAZ2093	1	0	0
			driesbachi	BOLD:AAD5662	1	0	0
			lasiomerus	BOLD:AAD9724	0	1	0
			neglectus	BOLD:AAE4645	0	1	0
			purvus	BOLD:ACM6388	1	0	1
		Stenotus	*binotatus*	BOLD:AAC0635	1	0	0
		Tropidosteptes	*pettiti*	BOLD:AAW8008	1	0	0
	Pentatomidae	*Banasa*	*calva*	BOLD:AAG1817	8	2	1
(I) Hymenoptera	Braconidae	*Aleiodes*	*terminalis*	BOLD:AAG5007	2	0	0
	Figitidae	*Melanips*	*opacus*	BOLD:AAU8950	0	1	0
	Ichneumonidae	*Agrypon*	*flexorium*	BOLD:AAL8261	0	0	1
		Dirophanes	*hariolus*	BOLD:AAA9337	-	-	-
		Enizemum	*cf. ornatum*	BOLD:ADD1920	-	-	-
		Lissonota	*sexcincta*	BOLD:AAD8806	0	0	1
		Netelia	*sayi*	BOLD:AAG8112	1	0	0

Continued on next page

Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			bilineatus	BOLD:AAG8323	1	0	0
		Zatypota	*cingulata*	BOLD:AAW5215	0	0	1
(I) Lepidoptera	Argyresthiidae	*Argyresthia*	*aureoargentella*	BOLD:AAJ8381	0	1	0
			canadensis	BOLD:AAF5128	0	1	0
			goedartella	BOLD:AAA8886	0	1	0
			oreasella	BOLD:AAA7249	0	1	0
	Autostichidae	*Glyphidocera*	*septentrionella*	BOLD:AAH4943	0	1	5
		Oegoconia	*deauratella*	BOLD:AAB8271	0	1	0
		Taygete	*attributella*	BOLD:AAC7542	0	0	1
	Batrachedridae	*Batrachedra*	*praeangusta*	BOLD:AAC6252	0	0	1
	Blastobasidae	*Asaphocrita*	*aphidiella*	BOLD:AAA8940	3	9	9
			aphidiella	BOLD:AAC4277	0	6	6
			aphidiella	BOLD:AAC4238	2	1	8
			busckiella	BOLD:AAA8938	0	0	1
		Blastobasis	*floridella*	BOLD:AAB1097	0	1	0
			glandulella	BOLD:AAB1096	5	4	4
		Calosima	*dianella*	BOLD:AAD5993	1	1	1
		Holcocera	*chalcofrontella*	BOLD:AAA8504	0	2	4
			immaculella	BOLD:AAB2463	0	2	1
		Hypatopa	*binotella*	BOLD:AAB7100	0	0	1
			simplicella	BOLD:AAB9632	0	1	3
			spoliatella	BOLD:AAG8597	0	1	2
			vestaliella	BOLD:AAG8581	1	0	1
		Pigritia	*fidella*	BOLD:AAG8600	0	1	2
			fidella	BOLD:AAD5152	-	-	-
			laticapitella	BOLD:AAC6102	1	2	0
	Carposinidae	*Bondia*	*comonana*	BOLD:AAC1052	0	0	1
			alnifoliae	BOLD:AAB7467	-	-	-
			pruniella	BOLD:AAA7822	0	2	1
			salicivorella	BOLD:AAB9344	-	-	-
			trifolii	BOLD:AAA7670	1	0	0
			versurella	BOLD:ABZ0013	1	0	0
	Cosmopterigidae	*Limnaecia*	*phragmitella*	BOLD:AAA7368	5	1	3
	Cossidae	*Acossus*	*centerensis*	BOLD:AAB5740	-	-	-
		Prionoxystus	*macmurtrei*	BOLD:AAD9795	1	0	0
	Crambidae	*Anageshna*	*primordialis*	BOLD:AAA8310	0	1	1

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Chrysoteuchia	*topiarius*	BOLD:AAA2518	1	0	0
		Crambus	*albellus*	BOLD:AAB1584	3	0	1
			lyonsellus	BOLD:AAH4937	0	1	0
			saltuellus	BOLD:ACE4701	1	0	0
		Eudonia	*alpina*	BOLD:AAB0095	0	0	1
		Evergestis	*pallidata*	BOLD:AAB4832	-	-	-
		Palpita	*magniferalis*	BOLD:AAA2490	0	0	0
		Petrophila	*canadensis*	BOLD:AAB8838	0	1	0
		Scoparia	*biplagialis*	BOLD:AAA1518	1	2	0
	Depressariidae	*Agonopterix*	*curvilineella*	BOLD:AAB2503	0	1	0
		Antaeotricha	*leucillana*	BOLD:AAA8103	1	1	0
		Bibarrambla	*allenella*	BOLD:AAA4417	0	3	7
		Ethmia	*zelleriella*	BOLD:AAC6363	1	0	1
		Nites	*grotella*	BOLD:AAW5248	1	0	0
	Drepanidae	*Drepana*	*arcuata*	BOLD:AAA3083	0	0	1
		Eudeilinia	*herminiata*	BOLD:AAB0338	-	-	-
		Pseudothyatira	*cymatophoroides*	BOLD:AAA2148	0	0	1
	Epermeniidae	*Ochromolopis*	*ramapoella*	BOLD:AAD7535	0	1	0
	Erebidae	*Dyspyralis*	*illocata*	BOLD:AAB6432	0	1	0
		Halysidota	*tessellaris*	BOLD:AAA3425	0	1	0
		Hypena	*baltimoralis*	BOLD:AAA2330	-	-	-
			palparia	BOLD:ABY9635	-	-	-
		Hypenodes	*n. sp. 4*	BOLD:AAA3002	-	-	-
			palustris	BOLD:AAA3001	0	0	1
			sombrus	BOLD:AAA3005	0	0	1
			rotundalis	BOLD:AAA3326	0	0	1
		Metalectra	*quadrisignata*	BOLD:AAB4859	-	-	-
		Pangrapta	*decoralis*	BOLD:ACF1497	0	0	1
		Parallelia	*bistriaris*	BOLD:AAA8563	-	-	-
		Phalaenophana	*pyramusalis*	BOLD:AAA5643	-	-	-
		Virbia	*laeta*	BOLD:AAA7234	-	-	-
		Zale	*duplicata*	BOLD:ABY8158	0	0	1
	Gelechiidae	*Agnippe*	*prunifoliella*	BOLD:AAC3010	0	0	1
		Aristotelia	*rubidella*	BOLD:AAA9175	-	-	-
		Aroga	*argutiola*	BOLD:AAE6617	2	0	0
			trialbamaculella	BOLD:AAB1382	0	1	0
		Carpatolechia	*belangerella*	BOLD:AAA6351	0	3	1

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Chionodes	*fondella*	BOLD:ABA4737	-	-	-
			fuscomaculella	BOLD:AAD2564	0	0	1
			mediofuscella	BOLD:AAA5871	0	7	15
			mediofuscella	BOLD:AAA5872	0	0	1
			molitor	BOLD:AAI4284	-	-	-
			praeclarella	BOLD:AAB1901	1	0	0
			terminimaculella	BOLD:AAE2222	0	1	1
			thoraceochrella	BOLD:AAA4438	0	0	1
			occidentis	BOLD:ACI3511	0	0	1
			quercivorella	BOLD:AAA4970	0	1	0
		Dichomeris	*punctipennella*	BOLD:AAB7772	0	1	0
		Exoteleia	*pinifoliella JFL1*	BOLD:AAA8974	0	0	1
		Gelechia	*lynceella*	BOLD:AAB2057	0	5	5
		Glauce	*pectenalaeella*	BOLD:AAE7137	0	3	3
		Neotelphusa	*querciella*	BOLD:AAH4952	0	0	1
			quercinigracella	BOLD:AAB0603	0	0	1
		Scrobipalpula	*manierreorum*	BOLD:AAG9100	0	2	0
		Xenolechia	*ontariensis*	BOLD:AAC6357	3	0	1
	Geometridae	*Campaea*	*perlata*	BOLD:AAA2078	0	1	0
		Caripeta	*divisata*	BOLD:AAA2638	0	0	1
		Costaconvexa	*centrostrigaria*	BOLD:AAA4271	-	-	-
			truncata	BOLD:AAA2864	0	0	2
		Ectropis	*crepuscularia*	BOLD:AAA2073	-	-	-
			crepuscularia	BOLD:AAA2074	0	1	1
		Eufidonia	*convergaria*	BOLD:AAA6717	0	0	1
			columbiata	BOLD:AAA6295	0	0	0
		Eusarca	*confusaria*	BOLD:AAA9811	0	0	0
		Hydriomena	*divisaria*	BOLD:AAA2032	2	2	0
			perfracta	BOLD:AAB2461	0	0	0
			renunciata	BOLD:ACE5766	-	-	-
		Hypagyrtis	*piniata*	BOLD:AAA4057	-	-	-
		Iridopsis	*ephyraria*	BOLD:AAC2550	-	-	-
			vellivolata	BOLD:AAB0284	0	1	0
		Lobophora	*nivigerata*	BOLD:AAA3392	0	1	0
		Lomographa	*vestaliata*	BOLD:AAA4964	1	0	0
			bisignata	BOLD:AAA3983	-	-	-

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Metanema	*inatomaria*	BOLD:ACF5552	-	-	-
		Nematocampa	*resistaria*	BOLD:AAA3400	0	0	1
		Orthonama	*obstipata*	BOLD:AAA3430	0	1	0
		Perizoma	*basaliata*	BOLD:AAB1700	-	-	-
		Protoboarmia	*porcelaria*	BOLD:AAA2077	-	-	-
		Scopula	*limboundata*	BOLD:AAA4208	-	-	-
		Sicya	*macularia*	BOLD:AAA5207	-	-	-
		Speranza	*pustularia*	BOLD:AAA4456	-	-	-
		Tacparia	*detersata*	BOLD:AAB9199	0	1	0
		Xanthorhoe	*ferrugata*	BOLD:AAA3817	-	-	-
			iduata	BOLD:AAB7253	-	-	-
			lacustrata	BOLD:AAA8660	0	1	0
	Gracillariidae	*Caloptilia*	*alnivorella*	BOLD:AAB7940	0	1	0
			bimaculatella	BOLD:AAB9096	0	0	0
			burgessiella	BOLD:AAE7389	0	1	0
			coroniella	BOLD:AAC1801	0	1	0
			invariabilis	BOLD:AAH4508	0	1	0
			stigmatella	BOLD:AAA9982	0	0	0
		Parornix	*betulae*	BOLD:ADE7141	0	1	0
			betulae	BOLD:AAE3418	0	1	0
			apparella	BOLD:AAD4914	0	0	2
			latus	BOLD:AAN8980	0	0	1
			nipigon	BOLD:AAI2946	0	1	0
			salicifoliella	BOLD:AAD4915	0	0	1
	Hepialidae	*Korscheltellus*	*gracilis*	BOLD:AAB5838	0	0	1
	Lasiocampidae	*Malacosoma*	*disstria*	BOLD:AAA4130	9	8	3
	Limacodidae	*Lithacodes*	*fasciola*	BOLD:ABY7387	-	-	-
		Packardia	*geminata*	BOLD:ABY5125	0	0	1
		Tortricidia	*flexuosa*	BOLD:AAA9275	5	0	1
			testacea	BOLD:AAA5967	1	0	1
	Momphidae	*Mompha*	*JFL02*	BOLD:AAF3087	-	-	-
		Zimmermannia	*grandisella*	BOLD:AAH4721	-	-	-
	Noctuidae	*Acronicta*	*dactylina*	BOLD:AAA2802	1	0	2
			fragilis	BOLD:AAA9541	0	0	1
			grisea	BOLD:AAA7688	0	1	1
			innotata	BOLD:AAA3813	0	0	1
			laetifica	BOLD:AAB3866	-	-	-

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
			radcliffei	BOLD:AAC4642	-	-	-
		Apamea	*cristata*	BOLD:AAF1717	-	-	-
			unanimis	BOLD:AAA8789	0	0	0
			verbascoides	BOLD:AAD6273	-	-	-
		Callopistria	*cordata*	BOLD:AAA7113	-	-	-
		Chytonix	*palliatricula*	BOLD:AAA6619	-	-	-
		Elaphria	*versicolor*	BOLD:AAA4393	-	-	-
		Eueretagrotis	*perattentus*	BOLD:AAA8151	-	-	-
		Euplexia	*benesimilis*	BOLD:AAA4097	0	0	1
		Feralia	*comstocki*	BOLD:AAB2071	1	0	0
		Lacinipolia	*lorea*	BOLD:AAA5449	0	0	1
			renigera	BOLD:ABZ4680	-	-	-
			renigera	BOLD:ACE7010	-	-	-
		Lycophotia	*phyllophora*	BOLD:AAA7117	-	-	-
		Neoligia	*crytora*	BOLD:AAF0862	-	-	-
		Orthodes	*detracta*	BOLD:AAA6122	-	-	-
		Orthosia	*hibisci*	BOLD:AAA4128	-	-	-
		Protodeltote	*albidula*	BOLD:AAA2331	-	-	-
	Nolidae	*Baileya*	*ophthalmica*	BOLD:AAA6592	0	0	0
	Notodontidae	*Gluphisia*	*septentrionis*	BOLD:AAA2247	1	0	0
		Heterocampa	*guttivitta*	BOLD:AAA3773	-	-	-
		Nadata	*gibbosa*	BOLD:AAA2279	-	-	-
		Nerice	*bidentata*	BOLD:AAB3168	-	-	-
		Oligocentria	*semirufescens*	BOLD:AAA8308	-	-	-
		Peridea	*basitriens*	BOLD:ABZ5648	-	-	-
		Schizura	*leptinoides*	BOLD:AAB0904	-	-	-
	Oecophoridae	*Epicallima*	*argenticinctella*	BOLD:AAA9667	0	2	1
			argenticinctella	BOLD:ABY9153	1	3	2
			argenticinctella	BOLD:AAA9668	0	0	0
	Pterophoridae	*Amblyptilia*	*pica*	BOLD:AAB3477	0	1	1
		Geina	*sheppardi*	BOLD:AAC2637	0	0	1
	Pyralidae	*Acrobasis*	*carpinivorella*	BOLD:ACE8773	-	-	-
		Dioryctria	*reniculelloides*	BOLD:AAA5470	0	0	1
		Ephestiodes	*erythrella*	BOLD:AAH4272	0	0	1
		Meroptera	*abditiva*	BOLD:AAA6817	0	1	0
			pravella	BOLD:AAA6818	1	0	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Pococera	*aplastella*	BOLD:AAA3814	0	0	1
		Pyralis	*farinalis*	BOLD:AAB3316	1	0	0
		Vitula	*broweri*	BOLD:AAB2094	-	-	-
	Saturniidae	*Antheraea*	*polyphemus*	BOLD:AAA8413	1	0	0
	Schreckensteiniidae	*Schreckensteinia*	*festaliella*	BOLD:ACE3537	-	-	-
	Sphingidae	*Ceratomia*	*undulosa*	BOLD:AAA6709	1	0	0
		Lapara	*bombycoides*	BOLD:AAB1249	1	0	1
		Pachysphinx	*modesta*	BOLD:AAA9265	2	0	1
		Smerinthus	*cerisyi*	BOLD:AAA1635	0	1	1
			jamaicensis	BOLD:AAA6395	0	1	0
		Sphinx	*poecila*	BOLD:AAB0355	1	0	1
	Tineidae	*Acrolophus*	*arcanella*	BOLD:AAF6158	6	2	0
		Homosetia	*marginimaculella*	BOLD:AAQ3212	0	1	2
			tricingulatella	BOLD:ABY0631	0	1	1
		Nemapogon	*acapnopennella*	BOLD:AAE4335	0	1	0
			DRD045	BOLD:AAE4233	0	1	0
			molybdanella	BOLD:AAG0121	-	-	-
			ophrionella	BOLD:AAE4217	0	0	1
			tylodes	BOLD:AAF1562	1	0	2
		Philonome	*clemensella*	BOLD:AAF6833	0	0	1
		Xylesthia	*pruniramiella*	BOLD:AAC5286	0	0	1
	Tischeriidae	*Coptotriche*	*citrinipennella*	BOLD:AAC7129	-	-	-
	Tortricidae	*Acleris*	*cervinana*	BOLD:AAB2296	0	0	2
			chalybeana	BOLD:AAA7667	1	1	1
			cornana	BOLD:ABZ7431	-	-	-
			implexana	BOLD:AAB6464	0	1	1
			logiana	BOLD:AAB0754	0	1	4
			maccana	BOLD:AAA8391	0	1	0
			nigrolinea	BOLD:AAD4689	-	-	-
			schalleriana	BOLD:AAB2825	1	0	0
			semiannula	BOLD:AAB4304	0	1	0
			semipurpurana	BOLD:AAB9410	4	0	0
			sp. 1	BOLD:ACF5694	1	0	0
			subnivana	BOLD:ABZ7432	0	1	1
		Adoxophyes	*negundana*	BOLD:AAB5285	2	0	0
		Aethes	*interruptofasciata*	BOLD:AAJ1300	0	0	2

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Amorbia	*humerosana*	BOLD:AAA8591	1	0	2
		Ancylis	*diminuatana*	BOLD:AAA7190	0	1	0
			divisana	BOLD:AAA9071	0	2	4
			subaequana	BOLD:ABZ7855	0	1	1
		Anopina	*ednana*	BOLD:AAE5367	1	0	0
		Apotomis	*capreana*	BOLD:ACE4055	1	1	3
			funerea	BOLD:AAC1890	0	2	1
			infida	BOLD:AAA4846	0	2	1
		Archips	*argyrospila*	BOLD:AAA7035	5	0	2
			grisea	BOLD:AAB9405	3	0	3
			packardiana	BOLD:AAA7690	1	1	0
			semiferanus	BOLD:AAC0733	3	1	5
			strianus	BOLD:ABZ2280	0	1	1
		Argyrotaenia	*alisellana*	BOLD:AAB4731	3	0	1
			mariana	BOLD:AAA4119	0	1	2
			quercifoliana	BOLD:AAB1667	1	0	1
		Aterpia	*approximana*	BOLD:AAD0026	-	-	-
		Bactra	*furfurana*	BOLD:AAA7927	1	0	0
		Catastega	*aceriella*	BOLD:AAA7894	1	0	3
			reticulatana	BOLD:AAA9619	-	-	-
			conflictana	BOLD:AAA3301	1	3	4
			fractivittana	BOLD:AAA2986	0	5	6
			rosaceana	BOLD:AAA1517	11	15	29
		Clepsis	*melaleucanus*	BOLD:AAA5620	2	1	4
			peritana	BOLD:ABY9168	1	6	10
			virescana	BOLD:AAA3055	0	1	6
			virescana	BOLD:AAA3057	0	2	4
			virescana	BOLD:AAA3056	0	4	4
		Coelostathma	*discopunctana*	BOLD:AAC0126	0	2	0
		Corticivora	*parva*	BOLD:AAC3888	0	0	1
		Cydia	*populana*	BOLD:AAA6911	0	1	0
			toreuta	BOLD:AAC6105	0	1	2
		Epiblema	*otiosana*	BOLD:AAB2495	-	-	-
			normanana	BOLD:AAE1162	0	2	2
			solicitana	BOLD:AAA2953	0	1	0
		Eucopina	*tocullionana*	BOLD:AAA3067	1	0	1
		Eulia	*ministrana*	BOLD:AAA7315	0	0	1

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Gretchena	*delicatana*	BOLD:AAA6260	1	1	3
		Gymnandrosoma	*punctidiscanum*	BOLD:AAB4070	4	1	0
			adjuncta	BOLD:AAB0712	1	5	7
			substitutionis	BOLD:AAB0709	0	0	1
		Larisa	*subsolana*	BOLD:AAB6961	0	0	1
			appendiceum	BOLD:AAB1224	2	0	2
			astrologana	BOLD:AAC2508	-	-	-
			concinnana	BOLD:AAB7993	-	-	-
			fasciatana	BOLD:AAC2533	1	6	7
			glaciana	BOLD:AAA6005	3	4	4
		Pammene	*bowmanana*	BOLD:AAE3071	0	1	1
		Pandemis	*lamprosana*	BOLD:AAA8840	0	1	2
			limitata	BOLD:AAA3659	6	3	4
		Pelochrista	*derelicta*	BOLD:AAA9420	0	1	0
			fiskeana	BOLD:AAE1001	1	0	0
		Phalonidia	*lepidana*	BOLD:AAB5962	1	0	1
		Platynota	*idaeusalis*	BOLD:ABY7901	5	5	14
			idaeusalis	BOLD:AAA5192	0	0	2
		Proteoteras	*aesculana*	BOLD:AAA6740	0	0	1
		Pseudosciaphila	*duplex*	BOLD:AAA2940	6	3	8
		Retinia	*albicapitana*	BOLD:AAA6914	0	0	1
			gemistrigulana	BOLD:AAB9815	0	1	2
		Rhopobota	*dietziana*	BOLD:AAB8879	1	0	1
			finitimana	BOLD:AAB9899	1	0	0
			naevana	BOLD:AAA9812	0	0	1
		Syndemis	*afflictana*	BOLD:AAA3369	0	0	1
		Thyraylia	*nana*	BOLD:AAB3573	-	-	-
			canadensis	BOLD:AAB0484	0	1	1
		Zomaria	*interruptolineana*	BOLD:AAC0407	-	-	-
	Urodidae	*Wockia*	*asperipunctella*	BOLD:AAB1378	0	0	2
	Yponomeutidae	*Swammerdamia*	*caesiella*	BOLD:AAB0224	0	0	1
		Zelleria	*celastrusella*	BOLD:AAJ9159	-	-	-
			haimbachi	BOLD:AAB0436	1	0	0
(I) Megaloptera	Corydalidae	*Chauliodes*	*pectinicornis*	BOLD:AAH3593	19	3	4
			rastricornis	BOLD:AAH3594	7	1	2
(I) Neuroptera	Chrysopidae	*Chrysopa*	*chi*	BOLD:AAG2018	-	-	-
		Meleoma	*signoretii*	BOLD:AAG2022	0	1	0

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Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
	Hemerobiidae	*Hemerobius*	*marginatus*	BOLD:AAP2910	0	1	1
			pinidumus	BOLD:AAG0904	0	1	2
			simulans	BOLD:ACM1832	-	-	-
		Micromus	*angulatus*	BOLD:ACZ4162	1	0	0
			posticus	BOLD:AAG0906	1	0	1
			subanticus	BOLD:AAG0893	1	1	0
		Wesmaelius	*longifrons*	BOLD:AAP4512	4	4	3
			pretiosus	BOLD:ACI5934	-	-	-
(I) Odonata	Aeshnidae	*Anax*	*junius*	BOLD:AAC9113	1	0	0
	Coenagrionidae	*Enallagma*	*civile*	BOLD:AAB3741	1	0	0
			signatum	BOLD:AAD5238	1	0	0
			vesperum	BOLD:AAE0330	0	2	0
		Nehalennia	*irene*	BOLD:AAA5874	-	-	-
(I) Orthoptera	Tettigoniidae	*Conocephalus*	*fasciatus*	BOLD:AAG2716	-	-	-
(I) Plecoptera	Perlidae	*Agnetina*	*flavescens*	BOLD:AAC7929	-	-	-
		Perlinella	*ephyre*	BOLD:AAD4053	-	-	-
(I) Psocodea	Amphipsocidae	*Polypsocus*	*corruptus*	BOLD:AAM8933	0	0	1
	Caeciliusidae	*Valenzuela*	*flavidus*	BOLD:AAH3228	0	0	1
	Psocidae	*Amphigerontia*	*bifasciata*	BOLD:AAP4629	0	1	0
			bifasciata	BOLD:ACK5479	-	-	-
		Blaste	*sp. 2KJEM*	BOLD:AAH3230	0	0	0
			sp. 2KJEM	BOLD:ACZ1284	-	-	-
		Metylophorus	*novaescotiae*	BOLD:AAH3225	0	0	1
		Trichadenotecnum	*majus*	BOLD:AAP4635	0	1	0
(I) Trichoptera	Dipseudopsidae	*Phylocentropus*	*placidus*	BOLD:AAB6237	1	4	3
	Glossosomatidae	*Glossosoma*	*intermedium*	BOLD:AAA9475	-	-	-
	Helicopsychidae	*Helicopsyche*	*borealis*	BOLD:AAA4316	-	-	-
		Hydropsyche	*alternans*	BOLD:AAA3236	2	0	1
			betteni	BOLD:AAA1669	2	2	0
			morosa	BOLD:AAA3678	18	1	2
			morosa	BOLD:AAA3679	2	1	0
			phalerata	BOLD:AAC3243	11	3	3
			placoda	BOLD:AAB0996	1	0	0
			scalaris	BOLD:AAC1715	-	-	-
			simulans	BOLD:AAD0911	1	0	0
			slossonae	BOLD:AAA2527	-	-	-

Continued on next page

Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Macrostemum	*zebratum*	BOLD:AAB8050	14	1	3
		Potamyia	*flava*	BOLD:AAB3702	5	1	2
			cancellata	BOLD:AAA8032	1	3	1
			cancellata	BOLD:AAA8031	0	2	0
			cancellata	BOLD:ABZ0710	-	-	-
			mentiea	BOLD:AAI7427	1	0	0
			transversa	BOLD:ABZ3976	1	0	0
		Leptocerus	*americanus*	BOLD:AAB9376	-	-	-
		Nectopsyche	*albida*	BOLD:AAB5769	0	1	0
		Oecetis	*cinerascens*	BOLD:AAA5653	0	1	0
			cinerascens	BOLD:AAA5651	0	0	0
			inconspicua	BOLD:AAA1532	1	3	0
			inconspicua	BOLD:AAA1525	-	-	-
			inconspicua	BOLD:AAA1534	0	1	0
			inconspicua	BOLD:AAK2989	-	-	-
			osteni	BOLD:AAC3953	0	1	0
			injustus	BOLD:AAA1540	0	3	0
			nox	BOLD:AAB9652	1	0	1
			tardus	BOLD:AAA5398	1	1	0
	Limnephilidae	*Asynarchus*	*mutatus*	BOLD:AAA4199	0	1	1
		Lenarchus	*crassus*	BOLD:AAJ1878	0	1	0
			moestus	BOLD:AAA4226	0	1	0
			ornatus	BOLD:AAA7732	8	6	4
			submonilifer	BOLD:AAA3080	0	1	1
			submonilifer	BOLD:ACY2979	-	-	-
		Nemotaulius	*hostilis*	BOLD:AAB8595	1	1	3
		Platycentropus	*radiatus*	BOLD:AAA8153	5	4	3
	Molannidae	*Molanna*	*uniophila*	BOLD:AAA7977	0	1	0
	Phryganeidae	*Agrypnia*	*improba*	BOLD:ACK0044	2	5	1
			improba	BOLD:ACF1659	-	-	-
			improba	BOLD:ACF0941	-	-	-
			improba	BOLD:AAA6582	-	-	-
			macdunnoughi	BOLD:AAB6649	0	1	0
			vestita	BOLD:AAC0360	0	0	2
		Banksiola	*crotchi*	BOLD:AAA4801	5	5	1
		Hagenella	*canadensis*	BOLD:AAF7726	-	-	-
		Phryganea	*cinerea*	BOLD:AAA7906	6	5	7

Continued on next page

Order	**Family**	**Genus**	**Species**	**BIN**	E	U	M
		Ptilostomis	*ocellifera*	BOLD:AAA9748	3	1	2
			semifasciata	BOLD:AAB5504	2	1	0
	Polycentropodidae	*Plectrocnemia*	*cinerea*	BOLD:AAA3441	1	2	0
			cinerea	BOLD:ACL7631	0	1	0
	Psychomyiidae	*Psychomyia*	*flavida*	BOLD:ABZ2387	-	-	-
(M) Isopoda	Armadillidiidae	*Armadillidium*	*vulgare*	BOLD:AAH4111	0	1	0

Table A2: **All BINs without species level taxonomic IDs.** NA in the 'Type' column indicates that no species (or genus) level identification was assigned to the BIN in the BOLD reference database. NC indicates that no consensus taxonomy was reached using the Wang method. Arthropod class precedes each order: *(C)*: Collembola, *(I)*: Insecta. Frequencies represent the number of detections that qualified as being prey in the bats *(E) Eptesicus Fuscus*, *(U) Myotis lucifugus*, and *(M) M. septentrionalis*. Species with no observed frequencies were detected either in residual levels or were detected in samples with total 1.5 times above the interquartile range of BIN richness for all samples. They were excluded from diet analyses but used in eDNA analyses.

Taxonomy					**Frequency**		
BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:AAN6522	(C) Poduromorpha	Hypogastruridae	NA	NA	1	0	0
BOLD:AAH0357	(I) Coleoptera	Carabidae	*Chlaenius*	NC	1	0	0
BOLD:AAH2778			*Platynus*	NC	-	-	-
BOLD:AAC0661			*Pterostichus*	NC	1	0	0
BOLD:AAE9009			*Stenolophus*	NA	7	1	1
BOLD:AAA9568		Cerambycidae	*Monochamus*	NC	4	0	0
BOLD:AAB8141			*Xylotrechus*	NC	1	0	0
BOLD:ABW9931		Cleridae	*Neorthopleura*	NA	3	0	0
BOLD:ACY3454		Curculionidae	*Conotrachelus*	NA	0	0	1
BOLD:AAO4332			*Polydrusus*	NC	-	-	-
BOLD:ABW8719		Dytiscidae	*Coptotomus*	NA	3	0	0
BOLD:AAA9163			*Ilybius*	NC	8	0	0
BOLD:ABX7717		Elateridae	*Ampedus*	NC	0	0	1
BOLD:AAF3428			*Melanotus*	NA	37	3	1
BOLD:ABW9945				NA	-	-	-
BOLD:AAH2916		Hydrophilidae	*Hydrochara*	NA	-	-	-
BOLD:AAM7640		Ptinidae	*NA*	NA	0	0	0
BOLD:ACH2408			*Oligomerus*	NC	-	-	-
BOLD:AAG4300		Staphylinidae	*Bledius*	NA	-	-	-
BOLD:ABA6337			*Tetartopeus*	NA	0	1	0
BOLD:AAP7866		Tenebrionidae	*Hymenorus*	NA	1	0	0
BOLD:AAV0892			*Isomira*	NA	1	0	0
BOLD:ABW6144			*Strongylium*	NA	-	-	-
BOLD:AAL9120	(I) Diptera	Anthomyiidae	*Delia*	NA	-	-	-

Continued on next page

BIN	Order	Family	Genus	Type	E	U	M
BOLD:ACC5677		Cecidomyiidae	*Asteromyia*	NA	0	0	1
BOLD:AAV5552				NA	0	0	1
BOLD:ACV2679			NA	NA	0	0	1
BOLD:AAQ0655				NA	0	2	1
BOLD:AAH3725				NA	0	1	0
BOLD:AAN5237				NA	0	1	0
BOLD:ADE1424				NA	0	0	1
BOLD:ACM4099				NA	1	0	0
BOLD:AAH3704				NA	0	0	2
BOLD:ADE8132				NA	-	-	-
BOLD:AAM6098				NA	0	0	0
BOLD:ACI8787				NA	0	1	0
BOLD:ACH3035				NA	0	1	1
BOLD:ACC8702				NA	1	0	0
BOLD:ABA0854				NA	0	1	0
BOLD:ACA5146				NA	0	0	1
BOLD:AAM6097				NA	0	0	1
BOLD:ADB4988				NA	0	0	1
BOLD:ACK8692				NA	1	0	0
BOLD:ACB3116				NA	0	0	1
BOLD:ABV9078				NA	-	-	-
BOLD:ACK1651				NA	-	-	-
BOLD:ADH9777				NA	0	0	0
BOLD:AAG6519		Ceratopogonidae	*Atrichopogon*	NA	0	2	0
BOLD:AAG6543				NA	0	1	1
BOLD:AAG3631				NA	0	1	0
BOLD:ACR1960			*Bezzia*	NA	0	1	0
BOLD:ACB9270				NA	-	-	-
BOLD:ACG7735				NA	-	-	-
BOLD:AAG6442			*Brachypogon*	NA	0	0	1
BOLD:AAG6451			*Clinohelea*	NA	0	1	0
BOLD:ACC3892			*Culicoides*	NA	0	0	1
BOLD:ACI4841				NA	0	0	0
BOLD:ACF3277			*Forcipomyia*	NA	0	1	1
BOLD:AAG5509				NA	0	1	0
BOLD:AAG6526				NA	1	0	0
BOLD:AAV5181				NA	-	-	-
BOLD:AAG6429				NA	0	0	1
BOLD:AAG6433				NA	0	0	1

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:ACX4395				NA	-	-	-
BOLD:ACF3206			NA	NA	0	1	0
BOLD:ABW1511				NA	0	0	0
BOLD:ACC8862				NA	0	0	1
BOLD:AAG6377				NA	1	0	0
BOLD:ADI0672				NA	0	1	0
BOLD:ACG7949				NA	0	1	0
BOLD:ACO2265				NA	0	0	1
BOLD:AAG6519				NA	0	2	0
BOLD:ACC5373			*Palpomyia*	NA	0	1	0
BOLD:AAG6831		Ceratopogonidae	*Sphaeromias*	NA	1	3	0
BOLD:AAG5462		Chaoboridae	*Chaoborus*	NA	4	14	1
BOLD:AAG5471				NA	0	4	0
BOLD:AAM6294				NA	0	1	0
BOLD:AAI3268			*Mochlonyx*	NA	0	1	2
BOLD:AAM6295			NA	NA	0	2	0
BOLD:AAG5471				NA	0	4	0
BOLD:ACE6563		Chironomidae	*Ablabesmyia*	NC	-	-	-
BOLD:ACG3931				NA	1	6	1
BOLD:ABZ1582				NA	0	2	2
BOLD:ACX4522				NA	0	2	1
BOLD:ACH2330				NA	0	3	1
BOLD:AAN7576				NA	0	0	0
BOLD:ABV1232				NA	0	2	0
BOLD:ACB9385				NA	0	0	1
BOLD:AAN5313				NA	0	0	0
BOLD:ACJ5479				NA	-	-	-
BOLD:AAG7003			*Axarus*	NA	-	-	-
BOLD:AAB7030			*Chironomus*	NA	1	12	1
BOLD:AAC0597				NA	1	6	1
BOLD:AAB7436				NA	10	11	0
BOLD:ACL4512				NA	2	8	0
BOLD:AAG5478				NA	1	8	1
BOLD:AAG5515				NA	0	0	1
BOLD:AAP3004				NA	1	3	2
BOLD:ACA6708				NA	1	0	1
BOLD:ACQ6990				NA	0	3	1
BOLD:AAZ0144				NA	1	4	1
BOLD:ACJ2946				NA	0	0	0

Continued on next page

BIN	Order	Family	Genus	Type	E	U	M
BOLD:ABV1236				NA	1	0	0
BOLD:ACF2043				NA	0	0	0
BOLD:AAM6291				NA	0	1	0
BOLD:ADA8002				NA	0	1	0
BOLD:ADA7562				NA	-	-	-
BOLD:ACL4081				NA	-	-	-
BOLD:AAC0594				NA	-	-	-
BOLD:ACJ6539			*Conchapelopia*	NA	0	0	1
BOLD:AAI6007			*Cryptochironomus*	NA	1	5	0
BOLD:ACC9856			*Dicrotendipes*	NA	0	2	1
BOLD:AAQ0607				NA	1	2	1
BOLD:AAN5383				NA	0	0	0
BOLD:ACN7117				NA	-	-	-
BOLD:ACP7121			*Einfeldia*	NA	1	4	2
BOLD:ADZ1183			*Glyptotendipes*	NA	0	1	1
BOLD:AAM6271			*Krenopelopia*	NA	0	0	1
BOLD:AAQ0621			*Lasiodiamesa*	NA	0	0	1
BOLD:ACC7609			*Metriocnemus*	NA	0	0	2
BOLD:ACK8276			*Micropsectra*	NA	0	0	1
BOLD:ABY9871			*Microtendipes*	NA	0	3	0
BOLD:ACN7933				NA	0	2	0
BOLD:AAG5479				NA	1	1	0
BOLD:ACU7060				NA	-	-	-
BOLD:ACL4841				NA	-	-	-
BOLD:AAG5457			NA	NA	4	17	4
BOLD:ABW4221				NA	0	11	3
BOLD:ADD0962				NA	2	4	0
BOLD:ACA7733				NA	0	1	1
BOLD:ACL4152				NA	0	2	0
BOLD:AAM6293				NA	0	5	1
BOLD:AAN5311				NA	0	6	0
BOLD:ACN1969				NA	0	2	0
BOLD:ACL9301				NA	2	1	0
BOLD:ACL8154				NA	0	2	1
BOLD:ACX6139				NA	0	3	0
BOLD:AAM6234				NA	1	1	0
BOLD:AAG5465				NA	0	5	1
BOLD:ACN4910				NA	1	2	0
BOLD:ACA4559				NA	1	0	0

Continued on next page

BIN	Order	Family	Genus	Type	E	U	M
BOLD:ACE1684				NA	0	2	0
BOLD:AAM6286				NA	1	3	1
BOLD:ACA7555				NA	0	1	0
BOLD:ACC8785				NA	1	4	1
BOLD:AAN5352				NA	0	1	1
BOLD:ACH2244				NA	0	0	2
BOLD:AAM6290				NA	0	2	1
BOLD:ACI4917				NA	0	1	1
BOLD:AAG5441				NA	1	2	1
BOLD:ACA3183				NA	0	0	1
BOLD:AAL7327				NA	0	0	1
BOLD:ACC8171				NA	0	0	0
BOLD:ACA8455				NA	0	2	0
BOLD:AAP5113				NA	0	5	1
BOLD:ACK5507				NA	0	1	1
BOLD:AAL7346				NA	0	3	0
BOLD:ACV5349				NA	0	0	1
BOLD:AAM6279				NA	0	2	3
BOLD:AAP6878				NA	0	2	1
BOLD:ACB9392				NA	0	4	0
BOLD:AAP2998				NA	0	0	1
BOLD:ACL5405				NA	0	2	0
BOLD:AAH0044				NA	0	1	0
BOLD:AAP3003				NA	0	0	0
BOLD:AAG5464				NA	0	1	0
BOLD:AAL7333				NA	0	1	0
BOLD:AAM6296				NA	0	1	0
BOLD:ADC6510				NA	0	2	0
BOLD:AAM6241				NA	0	1	0
BOLD:ACL3837				NA	0	1	0
BOLD:AAN5354				NA	0	1	1
BOLD:ACA2938				NA	0	1	0
BOLD:ACW1301				NA	0	0	0
BOLD:ACU5359				NA	0	0	1
BOLD:AAN5335				NA	0	0	1
BOLD:ACH3390				NA	0	1	0
BOLD:AAH0040				NA	0	1	0
BOLD:ACM8046				NA	0	0	1
BOLD:ACL6399				NA	0	1	0
BOLD:AAG5517				NA	0	0	0

Continued on next page

BIN	Order	Family	Genus	Type	E	U	M
BOLD:AAG5468				NA	0	1	0
BOLD:ACX4822				NA	0	0	0
BOLD:ACI3742				NA	-	-	-
BOLD:ADF7148				NA	0	1	0
BOLD:AAZ0144				NA	1	4	1
BOLD:AAP7556				NA	0	1	0
BOLD:ACA8801				NA	0	0	0
BOLD:ACI3711				NA	0	1	0
BOLD:ACL5054				NA	0	1	0
BOLD:ACK2202				NA	0	0	1
BOLD:AAM6278				NA	0	1	0
BOLD:ACF8490				NA	0	1	0
BOLD:ACN6816				NA	0	1	0
BOLD:ACI9234				NA	0	1	0
BOLD:AAG5506				NA	0	1	0
BOLD:ACY4145				NA	0	1	0
BOLD:AAG5512				NA	-	-	-
BOLD:ACL8737				NA	0	1	0
BOLD:ACL3824				NA	-	-	-
BOLD:ACD0546				NA	-	-	-
BOLD:AAV5938				NA	-	-	-
BOLD:ACL4497				NA	-	-	-
BOLD:ACA3190				NA	-	-	-
BOLD:AAN5373				NA	-	-	-
BOLD:ACL3651				NA	-	-	-
BOLD:ADI4110				NA	-	-	-
BOLD:ADA7186				NA	-	-	-
BOLD:AAG5505				NA	-	-	-
BOLD:AAC0706				NA	-	-	-
BOLD:ADI3463				NA	-	-	-
BOLD:ACX4522				NA	0	2	1
BOLD:ACN5893				NA	-	-	-
BOLD:ACH3707				NA	-	-	-
BOLD:ACP3545				NA	-	-	-
BOLD:AAP8991				NA	-	-	-
BOLD:ABX7479			*Parachironomus*	NA	0	4	1
BOLD:ACB9399				NA	0	1	0
BOLD:ACE5981				NA	-	-	-
BOLD:AAI2688			*Parametriocnemus*	NA	-	-	-
BOLD:ADI1867			*Paratendipes*	NA	-	-	-

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:ACC0467			*Phaenopsectra*	NA	0	1	0
BOLD:AAM6287				NA	-	-	-
BOLD:AAG5516			*Polypedilum*	NA	0	1	1
BOLD:AAD1395				NA	1	1	0
BOLD:ACG8783				NA	0	0	2
BOLD:AAP1268				NA	0	1	0
BOLD:ABW2707				NA	-	-	-
BOLD:AAM6227			*Procladius*	NA	1	9	0
BOLD:AAG3918				NA	0	1	0
BOLD:AAL7370				NA	0	1	0
BOLD:AAG2646				NA	-	-	-
BOLD:ACL7098				NA	-	-	-
BOLD:AAG5477			*Pseudochironomus*	NA	0	0	1
BOLD:ACA4847			*Tanytarsus*	NA	0	0	1
BOLD:AAG5463				NA	0	2	0
BOLD:ACD0612				NA	0	0	1
BOLD:ABV1188				NA	0	1	0
BOLD:AAG5467				NA	-	-	-
BOLD:AAG0920			*Tribelos*	NA	-	-	-
BOLD:AAL7323			*Xylotopus*	NA	0	1	0
BOLD:AAA3751		Culicidae	*Aedes*	NC	0	1	0
BOLD:AAB1098				NC	0	0	1
BOLD:AAC1238				NC	0	13	9
BOLD:AAC9062				NC	0	0	0
BOLD:AAA6148				NA	0	3	4
BOLD:AAC0584			*Anopheles*	NC	-	-	-
BOLD:AAB2539			NA	NA	39	48	41
BOLD:AAC9062				NA	2	3	2
BOLD:AAB5696				NA	0	0	2
BOLD:AAI1618				NA	0	1	0
BOLD:AAP8896				NA	-	-	-
BOLD:AAC1222				NA	-	-	-
BOLD:AAJ5881				NA	-	-	-
BOLD:AAC9132				NA	-	-	-
BOLD:ABY7666				NA	-	-	-
BOLD:AAA7067				NA	-	-	-
BOLD:AAA3748			*Ochlerotatus*	NC	-	-	-
BOLD:AAM9000		Diadocidiidae	NA	NA	-	-	-
BOLD:ACD1787		Dixidae	NA	NA	0	0	1

Continued on next page

BIN	Order	Family	Genus	Type	E	U	M
BOLD:ACG9958				NA	0	0	0
BOLD:AAG9709		Dolichopodidae	NA	NA	1	0	0
BOLD:AAG9733				NA	0	0	1
BOLD:ACL3272		Empididae	NA	NA	0	3	6
BOLD:AAF9785				NA	1	3	0
BOLD:AAF9755				NA	0	1	1
BOLD:AAF9756				NA	0	2	1
BOLD:ACL6209				NA	0	0	0
BOLD:AAF9873				NA	0	1	0
BOLD:ABW1189			*Rhamphomyia*	NA	0	1	0
BOLD:AAF9805				NA	0	1	0
BOLD:AAJ0306		Fanniidae	*Fannia*	NC	0	0	0
BOLD:AAV4274				NA	0	0	1
BOLD:AAG0464		Heleomyzidae	NA	NA	-	-	-
BOLD:AAF9772		Hybotidae	*Platypalpus*	NA	0	3	2
BOLD:AAP6357				NA	0	0	1
BOLD:AAG4933		Keroplatidae	NA	NA	0	1	0
BOLD:AAG4943				NA	-	-	-
BOLD:AAG6753		Lauxaniidae	*Camptoprosopella*	NA	-	-	-
BOLD:AAF8995		Limoniidae	*Antocha*	NA	1	0	0
BOLD:AAI1345			*Dicranomyia*	NA	4	1	0
BOLD:AAI1350				NA	1	4	2
BOLD:ABV1401				NA	6	4	1
BOLD:ACL8547			*Epiphragma*	NA	0	0	1
BOLD:AAF9060				NA	-	-	-
BOLD:AAF9055			*Erioptera*	NA	6	9	5
BOLD:ABV1765				NA	3	9	5
BOLD:ABU6610				NA	0	6	0
BOLD:AAG7012			*Geranomyia*	NA	2	1	0
BOLD:ACC2561			*Gnophomyia*	NA	0	1	0
BOLD:AAI1323			*Limnophila*	NA	3	2	2
BOLD:AAK8831			*Limonia*	NA	0	0	0
BOLD:AAB3507			*Metalimnobia*	NC	1	0	0
BOLD:AAF8971			NA	NA	16	15	4
BOLD:ABV1767				NA	0	1	1
BOLD:ABV9305				NA	14	3	4
BOLD:ACI7550				NA	1	1	0
BOLD:ACC8213				NA	2	1	0

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:ACH0642				NA	0	6	1
BOLD:AAZ5968				NA	0	0	1
BOLD:AAN5879				NA	5	2	1
BOLD:AAF9040				NA	0	1	0
BOLD:AAP6567				NA	1	1	0
BOLD:AAF9028				NA	0	1	0
BOLD:ACP3905				NA	0	0	1
BOLD:ACI7617				NA	0	1	0
BOLD:ACR5231				NA	-	-	-
BOLD:ACP0710				NA	-	-	-
BOLD:ABX9225			*Rhipidia*	NA	2	1	2
BOLD:ACG7891				NA	0	1	1
BOLD:AAF9014			*Symplecta*	NC	3	0	1
BOLD:AAC8885		Muscidae	*Coenosia*	NA	-	-	-
BOLD:ABX9224			*Helina*	NA	0	0	0
BOLD:AAU4987		Mycetophilidae	*Boletina*	NA	0	0	1
BOLD:ACL8268				NA	0	0	1
BOLD:ACI3894			*Epicypta*	NA	0	0	1
BOLD:AAM8991			*Exechia*	NA	0	2	3
BOLD:AAM8997				NA	0	1	0
BOLD:AAU6631			*Exechiopsis*	NA	0	1	1
BOLD:AAG4956			*Greenomyia*	NA	0	1	2
BOLD:ACK1664			*Mycetophila*	NA	0	0	1
BOLD:AAM8964				NA	0	1	0
BOLD:AAG4978				NA	-	-	-
BOLD:AAG4890			*Mycomya*	NA	0	0	1
BOLD:ACD1769				NA	0	1	2
BOLD:AAM8970				NA	0	1	0
BOLD:ACD1267				NA	0	0	1
BOLD:AAG4934				NA	0	0	0
BOLD:AAG4866				NA	0	1	0
BOLD:AAK8903				NA	-	-	-
BOLD:ACD2295			*Phronia*	NA	0	0	1
BOLD:AAG4944			*Sciophila*	NA	0	1	0
BOLD:AAV1798		Pediciidae	NA	NA	0	1	1
BOLD:AAF8992				NA	0	0	2
BOLD:AAV1780				NA	0	0	1
BOLD:AAD9688			*Tricyphona*	NA	1	0	0
BOLD:AAD3176		Pipunculidae	*Nephrocerus*	NA	0	0	1

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:ADZ9121		Psychodidae	NA	NA	0	2	0
BOLD:AAV1295		Sciaridae	*Bradysia*	NC	0	2	0
BOLD:AAG6971		Sciomyzidae	*Anticheta*	NA	0	1	0
BOLD:ACZ5284			*Dictya*	NA	-	-	-
BOLD:ACI6940			NA	NA	0	1	0
BOLD:AAG6845			*Pherbellia*	NA	-	-	-
BOLD:AAN6420				NA	-	-	-
BOLD:AAA1697		Simuliidae	*Simulium*	NC	0	0	1
BOLD:AAA4121				NC	1	0	0
BOLD:AAG9572				NA	0	1	0
BOLD:AAB2384		Syrphidae	*Eupeodes*	NC	-	-	-
BOLD:AAK7221			*Melangyna*	NC	-	-	-
BOLD:AAA9506			*Platycheirus*	NC	1	0	0
BOLD:ADZ7596		Tachinidae	*Cryptomeigenia*	NA	2	5	3
BOLD:AAG2128				NA	2	0	0
BOLD:AAF6259			*Lypha*	NC	0	1	0
BOLD:AAG2182			NA	NA	-	-	-
BOLD:AAZ8513		Tipulidae	*Nephrotoma*	NC	1	1	1
BOLD:ACP9125			NA	NA	-	-	-
BOLD:AAA8248			*Tipula*	NC	6	4	1
BOLD:AAC9088				NC	0	0	4
BOLD:ABX5520				NC	4	1	1
BOLD:ACV1559				NC	-	-	-
BOLD:ABX5931				NA	1	5	6
BOLD:AAV1787				NA	0	0	1
BOLD:AAF9037				NA	0	0	1
BOLD:AAC4491				NA	1	0	0
BOLD:AAG4526				NA	1	0	0
BOLD:ACI9876				NA	-	-	-
BOLD:ABA7148		Trichoceridae	*Trichocera*	NA	-	-	-
BOLD:AAA7515	(I) Ephemeroptera	Caenidae	*Caenis*	NC	25	33	17
BOLD:ACX3924				NA	0	1	0
BOLD:AAG5760		Heptageniidae	*Maccaffertium*	NA	8	1	7
BOLD:AAK5110		Leptophlebiidae	*Paraleptophlebia*	NC	0	0	1
BOLD:AAG2883	(I) Hemiptera	Cicadellidae	*Draeculacephala*	NA	2	0	0
BOLD:AAG2878			*Gyponana*	NC	3	0	0
BOLD:ABA8101			NA	NA	0	1	0
BOLD:AAB0657		Corixidae	NA	NA	-	-	-

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:ABX5969		Miridae	*Neolygus*	NC	1	1	0
BOLD:ACF2629				NA	-	-	-
BOLD:AAA5803			NA	NA	-	-	-
BOLD:AAH9372			*Phytocoris*	NC	-	-	-
BOLD:ACF1942				NC	1	0	0
BOLD:AAD6352		Pentatomidae	NA	NA	-	-	-
BOLD:AAF0124			*Podisus*	NA	0	1	1
BOLD:ACI5513	(I) Hymenoptera	Braconidae	*Centistes*	NA	0	1	0
BOLD:AAC3666		Formicidae	*Formica*	NC	-	-	-
BOLD:AAA1468			NA	NA	3	0	0
BOLD:AAG8100		Ichneumonidae	*Enicospilus*	NA	1	0	0
BOLD:AAH1928			*Mesochorus*	NA	-	-	-
BOLD:ACB1513			*Netelia*	NA	1	0	0
BOLD:AAG8106				NA	1	0	0
BOLD:AAI3087				NA	0	0	1
BOLD:ACY3166			NA	NA	0	0	1
BOLD:ACM3032				NA	0	1	0
BOLD:ABA6131				NA	-	-	-
BOLD:AAI3367			*Ophion*	NA	1	0	0
BOLD:AAI3365				NA	2	0	0
BOLD:AAF8717				NA	1	0	0
BOLD:AAH4708	(I) Lepidoptera	Argyresthiidae	*Argyresthia*	NA	0	0	2
BOLD:AAF5253				NA	0	1	0
BOLD:AAB9343		Coleophoridae	*Coleophora*	NC	0	0	1
BOLD:ABY6070				NC	0	1	1
BOLD:AEA3805		Crambidae	*Argyria*	NC	-	-	-
BOLD:AAA2323			*Herpetogramma*	NC	-	-	-
BOLD:AAA2229		Erebidae	*Idia*	NC	-	-	-
BOLD:AAA5206			*Zanclognatha*	NC	0	0	0
BOLD:AAG5259		Gelechiidae	*Athrips*	NA	0	1	0
BOLD:ACX5784			*Chionodes*	NA	0	1	0
BOLD:AAA3569			*Coleotechnites*	NC	0	0	1
BOLD:AAA5953				NC	0	5	4
BOLD:AAI5898				NA	0	0	0
BOLD:AAH4282				NA	-	-	-
BOLD:AAH6284				NA	-	-	-
BOLD:AAH4283				NA	-	-	-
BOLD:AAH4942			*Dichomeris*	NA	0	0	2

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:AAB0607			*Pseudotelphusa*	NC	0	3	1
BOLD:ABZ7191		Geometridae	*Dysstroma*	NC	-	-	-
BOLD:AAA2320			*Eupithecia*	NC	0	1	1
BOLD:AAA9176				NC	0	0	0
BOLD:AAB4027			*Homochlodes*	NC	0	0	1
BOLD:AAA4056			*Hypagyrtis*	NA	-	-	-
BOLD:AAA1255			*Macaria*	NC	0	1	2
BOLD:ACE4468			*Metarranthis*	NC	-	-	-
BOLD:AAA2077			NA	NA	-	-	-
BOLD:AAA6610			*Xanthotype*	NC	1	0	0
BOLD:AAC7941		Gracillariidae	*Caloptilia*	NA	0	1	0
BOLD:AAC7831				NA	0	0	0
BOLD:AAC1756			*Phyllonorycter*	NA	0	1	0
BOLD:AAB2016		Hepialidae	*Sthenopis*	NC	1	1	6
BOLD:AAH4721		Nepticulidae	*Ectoedemia*	NA	0	0	1
BOLD:AAB4103		Notodontidae	*Paraeschra*	NC	-	-	-
BOLD:ABY6654		Pterophoridae	*Amblyptilia*	NA	-	-	-
BOLD:AAB3656			*Hellinsia*	NC	0	1	1
BOLD:ACV0710		Tineidae	*Homosetia*	NA	-	-	-
BOLD:ABW0987			*Nemapogon*	NA	0	1	4
BOLD:AAB6465		Tortricidae	*Acleris*	NC	1	0	0
BOLD:AAA8534			*Ancylis*	NC	1	1	4
BOLD:AAB2076				NC	1	0	0
BOLD:AAB2077				NC	0	1	0
BOLD:ABZ6958			*Apotomis*	NC	0	2	0
BOLD:AAA2955			*Argyrotaenia*	NC	0	1	2
BOLD:ACN6207				NC	0	0	1
BOLD:AAB0124			*Cenopis*	NC	6	4	11
BOLD:AAB7926				NC	0	1	5
BOLD:ABX5883			*Choristoneura*	NC	6	13	19
BOLD:AAB1331			*Epinotia*	NC	1	8	5
BOLD:AAB8744				NC	0	0	2
BOLD:AAA5486			*Eucosma*	NC	0	1	0
BOLD:AAB1285			*Gypsonoma*	NC	0	2	2
BOLD:ACF0609			*Olethreutes*	NC	-	-	-
BOLD:AAB7869			*Phtheochroa*	NC	0	0	0
BOLD:AAA7907			*Zeiraphera*	NC	1	11	7
BOLD:AAB0483				NC	1	6	6

Continued on next page

BIN	**Order**	**Family**	**Genus**	**Type**	**E**	**U**	**M**
BOLD:AAB0373	(I) Neuroptera	Chrysopidae	*Chrysoperla*	NC	-	-	-
BOLD:AAG0901		Hemerobiidae	*Hemerobius*	NA	0	0	3
BOLD:AAG0891				NA	1	0	4
BOLD:AAP2550		Sisyridae	*Sisyra*	NA	-	-	-
BOLD:AAB4412	(I) Odonata	Corduliidae	*Epitheca*	NC	0	0	1
BOLD:AEA6705	(I) Plecoptera	Perlidae	*Perlesta*	NA	0	0	1
BOLD:AAG2693				NA	-	-	-
BOLD:AAA5695	(I) Trichoptera	Hydropsychidae	*Cheumatopsyche*	NC	-	-	-
BOLD:ABY5833			*Cheumatopsyche*	NC	2	1	0
BOLD:AAA2325		Lepidostomatidae	*Lepidostoma*	NC	-	-	-
BOLD:AAA5876		Leptoceridae	*Ceraclea*	NC	4	0	0
BOLD:ABY4173			*Triaenodes*	NC	0	1	0
BOLD:AAA1543		Limnephilidae	*Asynarchus*	NC	0	4	1
BOLD:AAA7041			*Limnephilus*	NC	1	1	1
BOLD:AAD6530		Sericostomatidae	*Agarodes*	NC	-	-	-

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