Diversity and Distribution of the Asilidae in Ohio

Undergraduate Research Thesis

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by
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Abstract

Asilidae are important predatory insects that eat other insects. To understand their diversity, abundance, and habitat associations in Ohio, passive water bowl traps were set across 149 sites in Ohio from May to October 2020 by volunteer community scientists. All specimens were sent to the Goodell laboratory at The Ohio State Newark to be pinned and identified. A total of 1,705 asilid specimens were collected representing 25 species. The most common species was *Atomosia puella*, which accounted for 80% of all specimens. To understand habitat influences on abundance and diversity, the presence of forest, grassland/shrub, cropland, and developed land in a 500 m buffer around each site was calculated. These landscape variables were analyzed to determine which habitat was most important for overall Asilidae species richness and abundance. Both cropland and developed land were associated with a decrease in Asilidae abundance and species richness. Forested habitat was positively associated with species richness. Grasslands and open landscapes were positively associated with abundance, largely due to the increase in *Atomosia puella* abundance with the percent of open habitat. Asilid diversity was not significantly associated with grassland or open habitat. The anthropogenic factors of cropland and developed land negatively influence Asilidae potentially because of pesticides and habitat damage, suggesting the need for conservation management. Forested areas contain greater habitat heterogeneity than grasslands, potentially contributing to the increase in species richness. These findings provide insight into the distribution of Asilidae within Ohio and contribute to future conservation management.

Chapter 1: Introduction to the World of Asilidae

Asilidae, also known as the robber fly, are predatory Diptera. There are 7,003 described species globally (Geller-Grimm 2008); North America has 1,000 asilid species (Finn 2018). Asilids reside on all other continents except Antarctica. Asilidae occur most commonly in warm, dry climates, especially in rocky, sandy, and grassland habitats. Some can be found in woodland landscapes, but they tend to aggerate towards the edge of forests (McCravy & Baxa 2011).

Asilidae Life Cycle

The Asilidae lifecycle begins with copulation. Copulation involves the connection of both genitalia from tail-to-tail, often inhibiting flight. Males have aggressive mating behaviors that include landing on a female as it would prey. Once mating is successful, the female robber fly oviposits small white eggs in grasses (Figure 1A), rock openings, wood, soil, and even bark. Once the egg hatches, the predatory larva feed on other insect eggs or soft-bodied insects to survive (Finn 2018). As larval asilids, they develop in the soil, allowing for them to survive in colder climates. After four to six instars, the larvae aggregate towards the top of the soil and begin to molt into a prepupal stage. The pupae stage involves the production of a cocoon-like structure, allowing for metamorphosis, over a four-to-five-week period. Adult asilid flies eventually eclose, leaving behind their casing, and begin the reproductive phase of their life cycle. The duration of the Asilidae life cycle ranges from one to three years; it can be accelerated in warmer climates. The most common life span of an asilid is one year (Theodor 1980).

Predatory Behavior

Adult Asilidae vary in length from 3 mm to 50 mm. All adults have a set of compound eyes, like most insects, and strong bristled legs that function in capturing prey. Asilidae also vary in integument color and hairiness, with many black or brown, but some species are yellow, orange, or even red. Many species closely resemble bees, wasps, and even mosquitoes; they are both Batesian and aggressive mimics of these stinging and biting insects (Bhuiyan, et al. 2022). Batesian mimics are protected from predators by resembling stinging and venomous insects (Figure 1B). The predators of asilids associate the color (often yellow and black) with the painful sting of bees and wasps and avoid pursuing them. Aggressive mimicry is beneficial as well in that asilids can reside closer to their prey and capture their meal without being detected. Aggressive mimicry occurs when a predator, this being Asilidae, emulates the identification of another species to take advantage of this species. This allows Asilidae to catch prey easily; therefore, they can often be found eating the insects that they mimic.

Asilids capture their prey with precise measurement and strategy (Wardill, et al. 2017). They perch at variable heights, scoping for suitable prey insects in flight. Asilidae attack their prey mid-flight, using their strong legs to stabilize food. When adult asilids seize their meal, they stab the prey with dagger-like mouthparts, called a hypotharynx. The hypotharynx releases neurotoxic and proteolytic enzymes, paralyzing and secreting a deadly fluid. These digestive enzymes liquify the prey’s body, allowing them to suck up their food using their straw-like mouthparts.

Asilidae research is important to understand not only their populations, but also insect community dynamics and ecosystem services. Both adult and larval Asilidae are predators that can help control pest insect populations by consuming crop herbivores and mosquitoes. In South

Dakota, they were used as biological control agents against mountain pine beetles limiting damage to forests (Schmid 1969). Prey availability likely affects Asilidae abundance; therefore, asilid populations may respond to environmental factors that influence prey densities, such as land use (Uhler et al. 2021) and plant diversity (Haddad et al. 2011).

Asilidae Distribution within the State of Ohio

Ohio includes diverse ecosystems such as forests, prairies, grasslands, oak savannas, freshwater marshes, wetlands, and shrubby areas (Slack, et al. 2003). Ohio lacks desert landscapes, recognized as a preferred landscape for some asilid species (Forbes 1995); however, the grasslands and shrubs provide an important habitat for asilids to thrive. Various Asilidae species are habitat specialists with a specific niche (Shelly 1985); therefore, it is very important to conserve these areas to avoid potential harm to their populations. Studies that provide baseline species abundance and distribution data for Ohio's Asilidae are needed to fully understand their diversity population status, and habitat associations. This information will aid future conservation efforts.

Chapter 2: Landscape Distributions Affect Asilidae Across Ohio

Introduction

Recent reports that insects are in decline are alarming and suggest that we risk losing critical ecosystem components (Van der Sluijs, 2020). A quantitative synthesis of many studies found that > 40% of insect species are threatened with extinction (Sánchez-Bayo and Wyckhuys, 2019). To detect declines and manage for persistence, species checklists provide critical baseline data about insect distributions, community diversity and the population status. This research is the first published statewide study in Ohio on Asilidae in 73 years (Bromley 1950). There is a major gap in knowledge about Asilidae, inspiring the need for updated research. This research will deliver key data on species distributions, habitat preferences, and responses to land use at landscape levels. The analyses performed will provide foundation for conservation management of asilids and help identify which habitats support Ohio's Asilidae taxa.

Understanding the habitat use of Asilidae will facilitate conservation decisions. Ohio is geographically and topographically diverse and spans various climates, habitats, and vegetation zones (EPA 2010). Anthropogenic factors, such as agriculture and urban development, threaten asilid habitats. Asilid species that occupy narrow niches or show patterns of habitat specialization may be particularly vulnerable to anthropogenic threats, such as urbanization and agriculture (McCravy and Baxa, 2011).

In this study, I examine the distribution of Asilidae across the state of Ohio. In addition to providing species distributions and adult phenological information, I explore the effects of various land use types on the abundance and diversity of asilids. In particular, I asked how open fields and grasslands, as well as forest, urban areas, and agriculture, are associated with asilid abundance and diversity across the state. Natural history information about Asilidae suggests that they reach their highest diversity in dry habitats with abundant sunlight (McCravy and Baxa

2011). I hypothesized that Asilidae abundance and diversity would be highest in sites near open areas and grasslands, but lower in forested sites. Additionally, I predicted that urban and agricultural lands, because of their lower insect diversity in general, would harbor fewer asilids.

Methods

Starting in May 2020 – October 2020, volunteers throughout Ohio set water bowl traps at 149 sites weekly on non-rainy days (Figure 2). Each sampling kit included eight 3.25 oz painted Solo™ souffle bowls in three different colors (white, fluorescent blue, or fluorescent yellow) for a total of 24 bowls (Droege 2015). These bowls were filled with approximately 1.5 oz of dilute soapy water solution (blue Dawn™ or generic equivalent) on the ground. The bowls were deployed for 24 h, and then the insect specimens within were strained and frozen until they could be turned in for processing. Samples were turned in to the Native Bee Biology Laboratory (Dr. K. Goodell, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University Newark) in the fall of 2020 and sorted by taxonomic group. I pinned, labeled, and identified all asilid specimens using available keys (Baker and Fischer 1975; Barnes 2008; Fisher 2001; Lindsay 2019; Martin 1957; McKnight 2019; Wilcox 2021; Wood 2022). Once identified, the distribution of each species was mapped in ArcGIS Pro (ESRI 2022) using the latitude and longitude coordinates of the site as indicated by each volunteer collector (Appendix A) and verified by Google Earth Pro. Specimens are archived at The Ohio State University Triplehorn Insect Collection at the Museum of Biological Diversity.

Data Analysis

To evaluate the completeness of the sampling, I calculated a collector's curve using vegan package in R with "specaccum" (version 2022.12.0; Kindt and Oksanen). Varying collecting effort at sites across Ohio was standardized by dividing the number of specimens collected by the number of days sampled. The abundance of *Atomosia puella* was analyzed separately for impacts of landscape because I had a larger number of specimens to work with.

Rarefaction was a method used to assess species richness in an unbiased way. This technique was used due to the large variation across sites in the number of specimens collected. I used specimen-based rarefaction of species richness based on a random sample of six individuals per site, the maximum number of species found at one site. For sites with fewer than six specimens collected, the non-rarefied species richness value was used. Rarefaction of species richness was performed in R using vegan package and rarefy function (version 4.2.2, Appendix B).

To investigate the influence of surrounding land use, I used data from the National Land Use and Land Cover Database (NLCD) (Dewitz 2021) to calculate the proportion of the land surrounding each site categorized as forest (a combination of deciduous, mixed, and evergreen NLCD categories), open fields and meadows (a combination of shrub, grasslands, herbaceous, pasture, and hay NLCD categories), agriculture (cultivated crops), and urban land (a combination of medium and high density NLCD categories) within a buffer zone of 500m radius of each site. I dropped six sites from the landscape analyses because they were within 1 km of each other, leaving 143 sites. The 500m radius was chosen because Asilidae have relatively short foraging areas (Lavigne 1964).

A generalized regression analysis was used to test hypotheses regarding the impact of two uncorrelated sets of landscape land use variables on the species richness and abundance of asilid flies per site: habitat-related landscape variables (percent forest and percent cropland within 500m) or anthropogenic land uses (percent cropland and percent developed land within 500m). The dependent variables were species richness, rarefied species richness, and abundance per sample. We tested the dependent variables against various distributions using a Goodness-of-Fit test and found that a Poisson distribution best fit all variables. Therefore, I assumed a Poisson distribution in all regression analyses. For each regression analysis, I initially tested a saturated model with main effects and an interaction term. Backward selection was used to sequentially eliminate the least explanatory non-significant variable. I compared AIC values among models, accepting the model that had the lowest AIC value. I centered and standardized the independent variables to estimate parameters in the model. These analyses were run using JMP Pro v. 17.0.0 (JMP 2022). I used an α-value equal to or less than 0.05 to assess whether a finding was significant.

Results

The survey produced 1,705 asilid specimens, all of which were identified to genus, and all but four damaged specimens were identified to species. I determined sex on some, but not all specimens; the larger specimens were easier to determine sex. The dataset resulted in 25 species, representing 12 different genera (Table 1). The most abundant species, representing 80% of all specimens in the project (1,368) was *Atomosia puella.* Asilidae were more prevalent within the months of June through September, with some species spanning May through October (Figure 3).

To evaluate completeness of sampling, we produced a species accumulation curve, which determines the number of species expected per number of sites sampled. The curve shows a steep increase in numbers of species up to about 40 sites, then continues to increase slowly through 140 sites when it reached 25 species (Figure 4). Although it did not reach a horizontal asymptote, the pattern suggests diminishing returns on collections beyond 140 sites using the trapping method employed in this study.

Species Distributions

National Land Use and Land Cover data base in ArcGIS was used to formulate a map that compares landscape variables to sites with Asilidae present or absent (Figure 2). Asilids tended to be present within the southeastern part of the state that is more heavily forested but not in the northwestern part of the state that is highly agricultural. I also constructed maps to display the distribution of each species (Figure 5). Species with fewer than 30 specimens I considered too rare to interpret for ranges and those maps are provided in the appendix (Appendix C). *Atomosia puella*, the most abundant species, occurred at 95 of the sites across Ohio. This species had a wide range of distribution all throughout Ohio but was limited in areas with cropland (Figure 5A). *Atomosia glabrata*, present at 15 sites, and *Atomosia rufipes*, present at 14 sites, had distributions that were more restricted to the southern and southeastern parts of the state that are still predominantly forested (Figure 5B-C). *Laphria sicula* was documented at 35 sites across Ohio and appeared to be absent in heavily agricultural sites (Figure 5D). Similarly, *Efferia aestuans*, occurring at 22 sites, appeared mostly in forested regions (Figure 5E).

Habitat Associations

The best fit models included both forest and grassland, but not their interactions. The amount of forested land and the amount of grassland within a 500m radius both positively influenced the species richness, rarefied species richness, and abundance per sample of asilid flies (Table 2, Figure 6).

The effect of forest was greater than that of open land on species richness variables. Raw species richness was not significantly influenced by the percent of grassland within 500m (Table 2). The abundance per sample responded to the interaction between forest and open habitat (Table 2), indicating that the response of abundance to percent forest depends on the percent grassland. Examination of the data showed a stronger positive effect of grassland habitat in unforested landscapes, probably because grasslands offered natural habitat in these areas that were otherwise developed or cropland, but of which negatively impacted abundance (below).

The most common species of Asilidae, *Atomosia puella*, responded positively to forested land and grasslands within a 500m radius; however, this species was more influenced by open land than forested sites (Table 2, Figure 7).

Anthropogenic Land Use

The best fit models assessing the effects of anthropogenic land use on asilid species richness and abundance retained the percent of developed land and the percent of cropland within 500m, but not the interaction terms (Table 2). The quantity of cropland and developed land within a 500m radius negatively impacted the species richness, rarefied species richness, and abundance per sample (Table 2, Figure 8). The negative effect of cropland on rarefied species richness and abundance per sample effort was greater than that of developed land, while the opposite was true for the raw richness data (Table 2). Both land uses strongly affected the abundance per sample with most asilids collected from sites with < 10 percent of developed land within 500m and < 25 percent of cropland within 500m (Figure 8 C, F). As the developed land increased beyond about 50%, we found few asilid specimens, and species diversity declines to zero (Figure 8 C).

The best fit models assessing the effects of anthropogenic land use on *Atomosia puella* abundance retained the percent of developed land and the percent of cropland within 500m, but not the interaction terms (Table 2). *Atomosia puella* abundance per sample was significantly negatively associated with both anthropogenic land use classes, with a greater effect of developed than cropland (Table 2, Figure 7).

Discussion

Ohio was historically forested (Deines, et al. 2016), but it was heavily deforested following European colonization and much of Ohio's landscape remains open today. I hypothesized that open grasslands of Ohio may provide suitable habitat for species of Asilidae that thrive in open land. In sites surrounded by a large proportion of open landscape, we expected to find an abundance of these species. On the other hand, only a small percentage of Ohio land is unmanaged grasslands. Anthropogenic activities can degrade open land in agricultural and urban landscapes, which can reduce abundance and diversity of Asilidae in open habitats. I found a general trend for a positive effect of open grasslands on Asilidae richness, but a greater positive impact of forest. Other open habitats include those heavily impacted by human land uses: agriculture and development. The lower richness and abundance of Asilidae in landscapes with developed and cropland highlight the importance of natural habitats for supporting these predators (Pyle, et al. 1981).

Forested land surrounding sites had a stronger impact on Asilidae species richness than sites surrounded by open habitat. This pattern could reflect a higher habitat heterogeneity in forests than open lands. The temperate hardwood forests of Ohio are characterized by high diversity of plants that provide structural heterogeneity with many niches for insects (Sobek, et al. 2009). Each layer of forest vegetation has unique characteristics that provide different resources to different species. For example, insect abundance and diversity were positively associated to multi-layered forests, allowing for mixed species strands to positively impact abundance (Knuff, et al. 2020). Forests also have canopy gaps where trees have fallen or died, providing open habitats for species that thrive in high light environments. Therefore, forests offer a variety of habitats and resources that can support a diverse assemblage of asilid species (Hilmers, et al. 2018).

The lower abundance and richness of Asilidae in developed and agricultural landscapes suggests that anthropogenic factors associated with urbanization and crop production reduce habitat suitability for Asilidae. Development of land can degrade habitat through loss of vegetation, use of pesticides, and pollution (Isenring 2010). As cities and populated areas develop, the species richness and abundance of varying insect taxa decrease (Fenoglio, et al. 2021). A study performed on Lepidoptera, Orthoptera, and other insect taxa found that as the local urbanization (building infrastructure) in the landscape increased, terrestrial arthropods decreased in abundance especially for orthopterans and lepidopterans that experienced a 67% and 86% decline respectively (Piano, et al. 2019). Decline in total insect species richness was also observed. Asilidae species, as well as other insects, are in decline due to expanding development, revealing the potential harm of these areas (Corcos, et al. 2019).

A longitudinal study in Rome analyzing literature and museum collections indicated significant declines, even extinction, in species richness of butterflies and other insects (Fattorini 2011). The overall trend was a negative impact of urban development on insect richness, raising concern for conserving this important component of ecosystems.

Agriculture also poses risks to asilids and was negatively associated with their species richness and abundance in Ohio. Agriculture degrades natural habitat, simplifies vegetation structure, and introduces harmful chemicals into the environment in the form of pesticides. The use of pesticides is common in modern commercial scale agriculture. Pesticides pose a threat at interrupting survival, insect maturation, and decreased production of offspring. Pesticide use was investigated via a long-term, region-wide analysis spanning a duration of 21 years on the change of butterfly abundance and population trends (Wepprich, et al. 2019). It was found that the abundance decreased at an annual rate of 2%, resulting in a total 33% decline. This declining trend for butterflies provides an estimate for other insect population levels. Both Asilidae and butterflies are impacted by these agricultural areas due to the increased use of insecticides. Neonicotinoids, a common class of systemic insecticide, has increased in the state of Ohio for croplands with corn and soybeans (Goulson 2013). Neonicotinoids can accumulate in soils, waterways, nectar, and pollen. Pesticide usage associated with contamination yields damaging exposure to insects.

The negative effects of agriculture and urbanization highlights the possible threats that these anthropogenic factors have on Asilidae. This study revealed strong declines in species richness and abundance per sampling effort with higher proportions of agriculture and urbanization. Further studies that are specifically designed to test some of the factors influenced by these land uses are needed to understand why asilids are poorly represented in these landscapes. Future conservation research is needed to understand how to improve on the interaction of humans with the environments that insects rely on.

The dominance of *Atomosia puella* in my dataset likely reflects the sampling method. Asilids were trapped using water bowl traps designed to attract bees; the asilids were bycatch from this effort. The traps were placed directly on the ground, which could have biased the collection toward lower-flying, smaller asilids. Asilids that perch to catch prey and generally reside on branches and rocky areas were likely biased against with this sampling method. The trap size also may have allowed larger insects to escape, resulting in a greater abundance in smaller Asilidae captured.

This method of sampling also influences the estimate of the number of species expected in Ohio. A species accumulation curve was analyzed to represent how well the study sampled the Asilidae genera as a function of number of sampled sites. The species accumulation curve conducted shows diminishing returns on species richness of sampling over 140 sites tapering off at a moderately low species richness. The curve likely underestimates true richness that might be improved with the use of additional sampling methods, such as netting or malaise traps. This curve suggests that additional sampling with water bowl traps would be unlikely to add many more species, especially if they are set on the ground.

To improve upon the trapping method of this study, malaise traps and hand netting could be used in addition to bowl traps. Malaise traps are a tent like structure that is useful for collecting flying insects. An analysis testing the effectiveness of these traps for Asilidae found that observed species richness from these traps was more than 85% greater than the richness that was estimated before the study, proposing that Malaise traps are effective in providing a representative asilid sample compared to netting (McCravy 2017). Malaise traps for insect sampling provides a method that is effective for a variety of Asilidae species and can be used throughout Ohio to provide a future expansive survey to demonstrate the distribution of Asilidae.

The most recent asilid species analysis in Ohio was conducted in 1966 and analyzed the genus *Diogmites* (Artigas 1966), ignoring all other genera of Asilidae. My data will help fill this knowledge gap of the Asilidae in Ohio by contributing baseline asilid abundance and species richness data. It will allow conservation management to obtain insight on the status of the Asilidae populations.

Asilidae provide a critical ecosystem service of balancing the food chain. They allow for pest control and depletion of invading species due to their wide range of diet. Knowledge of their habitat preference within Ohio allows humans to gain insight on how they are impacting the environment and what areas are essential to protect. My research provides Ohio with updated distribution, habitat preference, and anthropogenic factors for the vital insect Asilidae.

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Table 1. Phenology of Each Species. Change in shading indicates abundance for each month, with darker shading indicating more Asilidae collected.

Species Name	May	June	July	August	September	October
Atomosia glabrata		2	4	33	30	
Atomosia puella	2	186	1029	93	57	1
Atomosia rufipes			20	26	15	
Atomosia sayii			4	2		
Cerotainia albipilosa					1	
Cerotainia macrocera		1	3	3		
Diogmites misellus			1			
Efferia aestuans			6	23	2	
Eudioctria brevis		2				
Heteropogon macerinus				1	1	
Holcocephala fusca				4	4	
Laphria aktis species complex		2				
Laphria canis canis		5	3			
Laphria flavicollis			1			
Laphria sicula		6	60	13	3	
Laphria thoracica		1				
Laphria undescribed species 2		1				
Laphria winnemana		2	8	2		
Machimus antimachus			2	4	1	
Machimus maneei			3			
Machimus sadyates					5	
Machimus snowii/paropus			8	7	5	
Promachus hinei					1	
Promachus rufipes					1	
Psilonyx annulatus			1			
Asilidae	2	208	1153	211	126	1

Table 2. Results of generalized regression to test for the effects of the proportion of cropland and developed land within a 500m radius of each site on the Asilidae species richness, rarefied species richness, and abundance per sample effort. The models assumed a Poisson distribution of the dependent variables. Models that had the lowest AIC values are presented. For each factor, the degrees of freedom = 1. Shown are standardized parameter estimates (SE), Wald Chi-Squared statistics, * = p<0.05, **=p<0.01, ***p=<0.001.

Source	Species Richness	Rarefied Richness	Abundance per Sample	*Atomosia puella* abundance
Habitat Type				
Forest	6.07 (0.78), 60.50 ***	5.11 (0.85), 35.80 ***	6.48 (0.13), 22.92 ***	1.88(0.45), 17.68 ***
Open	1.70 (0.82), 4.37*	1.36 (0.90), 2.31	8.25 (1.11), 55.49 ***	5.47(0.62), 77.87***
Forest x Open	removed	removed	-3.11 (0.85), 13.35 *	removed
Anthropogenic Factors				
Cropland	-1.66 (0.35), 23.01 ***	-6.28 (1.55), 13.06 ***	-6.86 (1.60), 18.51 ***	-2.18(0.57), 14.60***
Developed	-5.45 (1.11), 24.31***	-3.99 (1.10), 16.38 **	-1.94 (2.81), 18.07***	-8.81(2.41), 13.35***
Crop x Developed	removed	removed	removed	removed

Figures

Figure 1. (A) Female Asilidae depositing her eggs in grass. (B) *Laphria thoracica* photographed by MaLisa Spring in 2019.

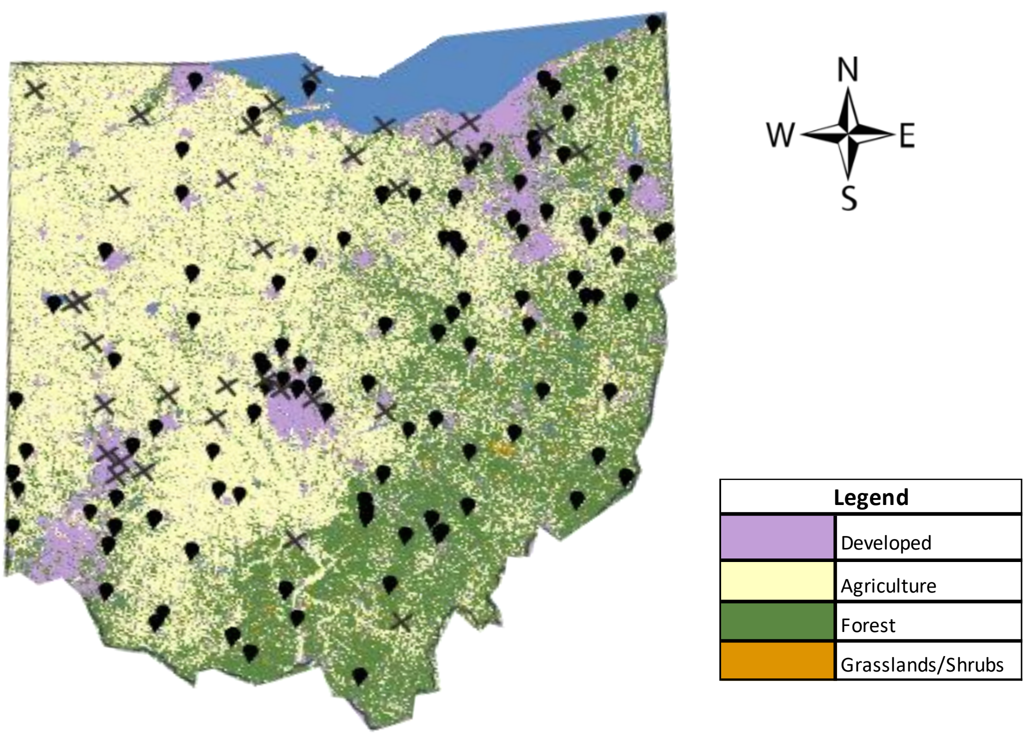


Figure 2. Distribution of sites sampled for Asilidae. Base layer is the USA National Land Use and Land Cover data. X denotes with zero Asilidae, and pinpoints denotes presence of Asilidae. Location of sites were verified via Google Earth

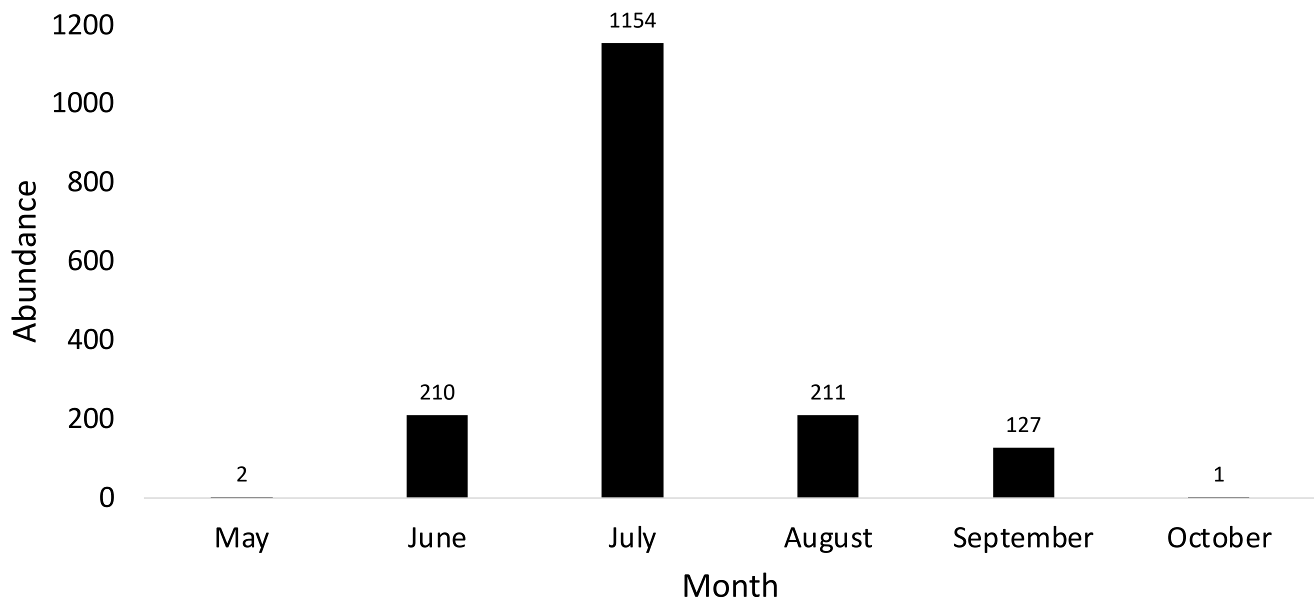


Figure 3. Phenological distribution of the abundance of Asilidae.

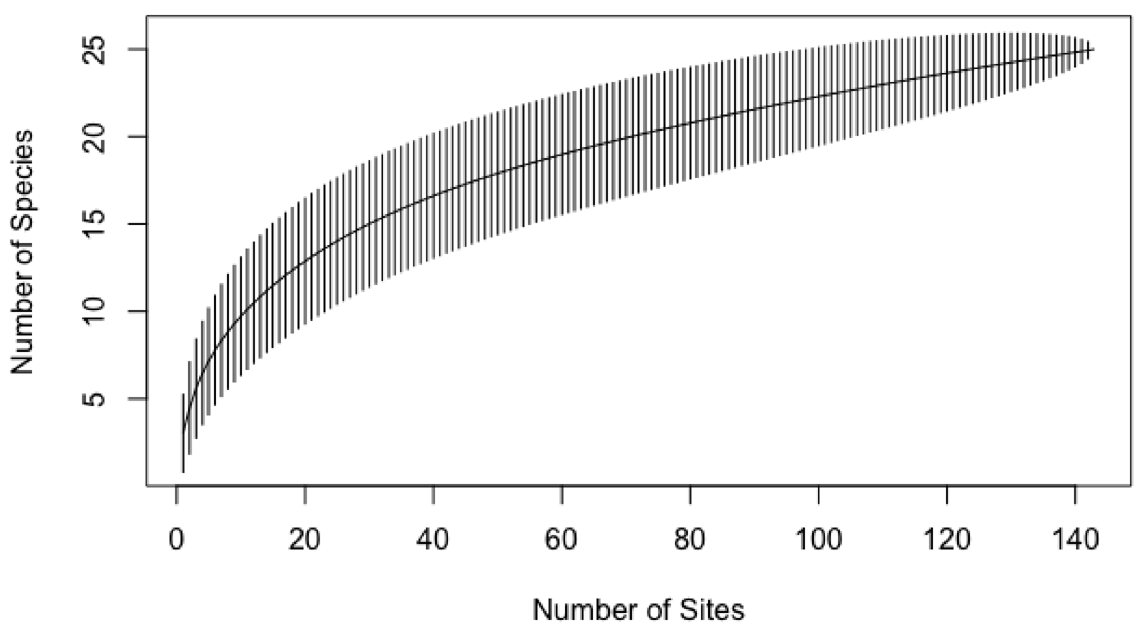
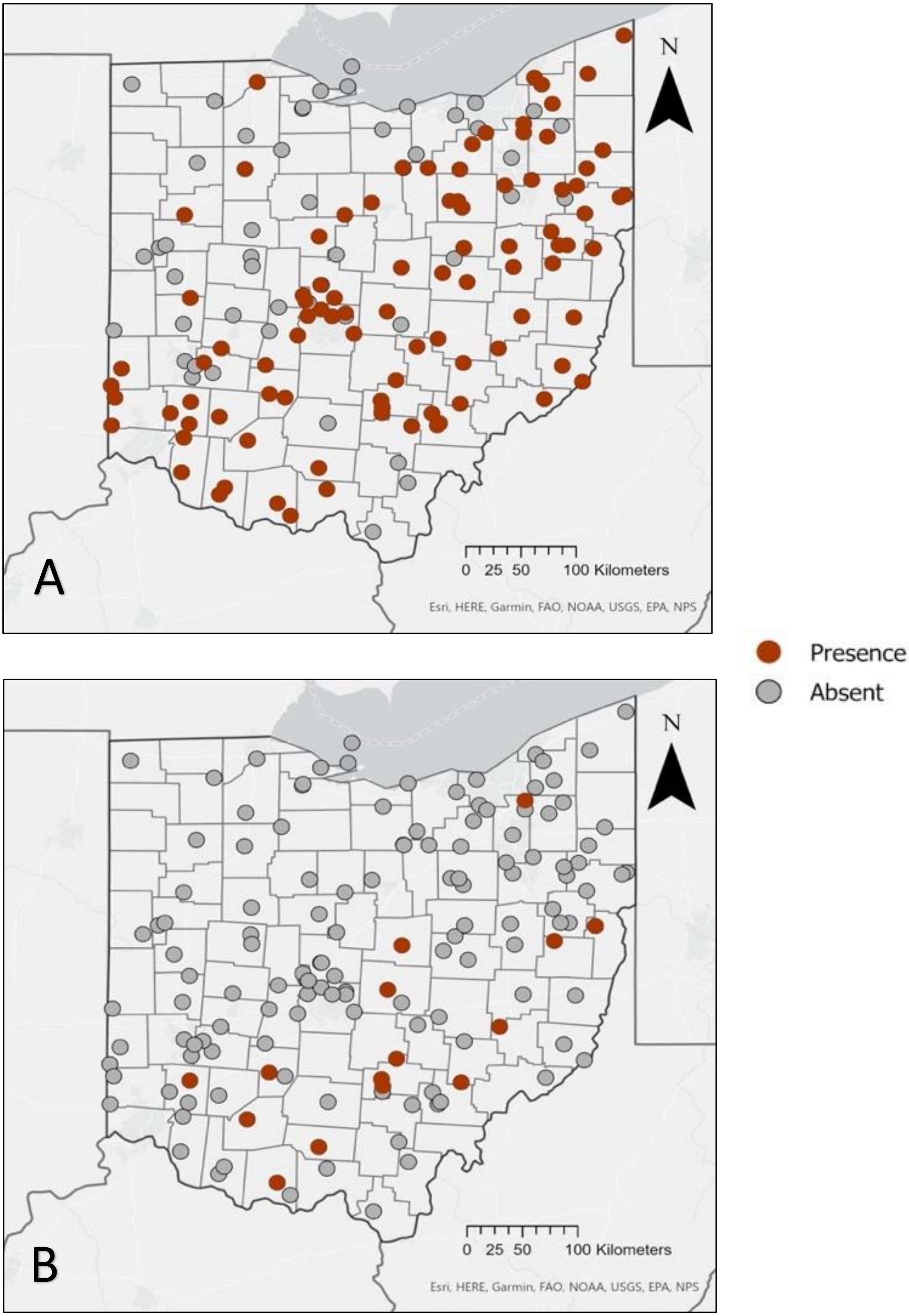
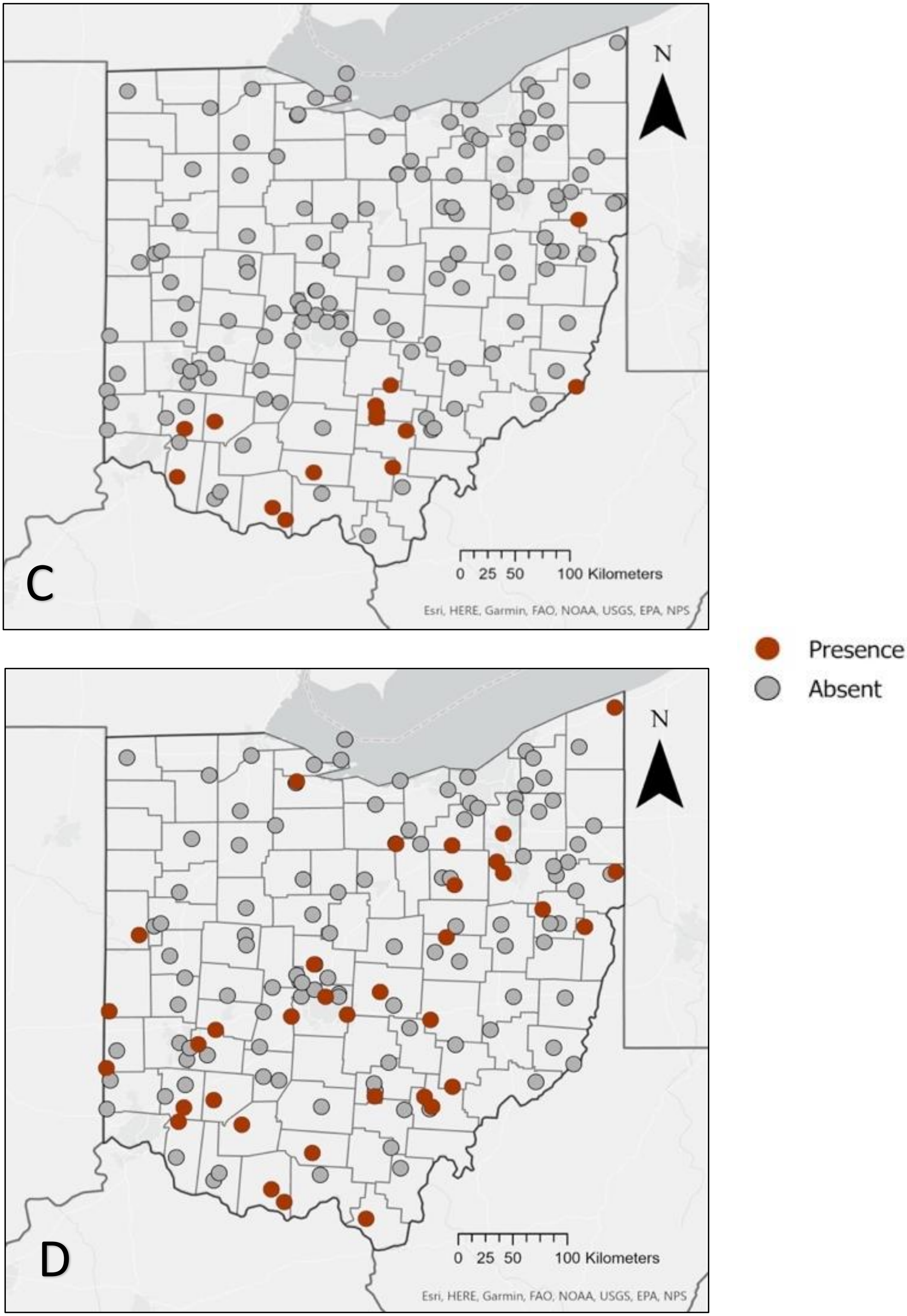


Figure 4. Species accumulation curve showing mean change in species richness as the number of sites increase. For complete samples, the curve asymptotes at the expected number of species. Error bars represent the standard deviation around the site mean predicted richness.


N
0 25 50 100 Kilometers
Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS
A
Presence
Absent
N
0 25 50 100 Kilometers
Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS
B


C
N
0 25 50 100 Kilometers
Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS
Presence
Absent
D
N
0 25 50 100 Kilometers
Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS

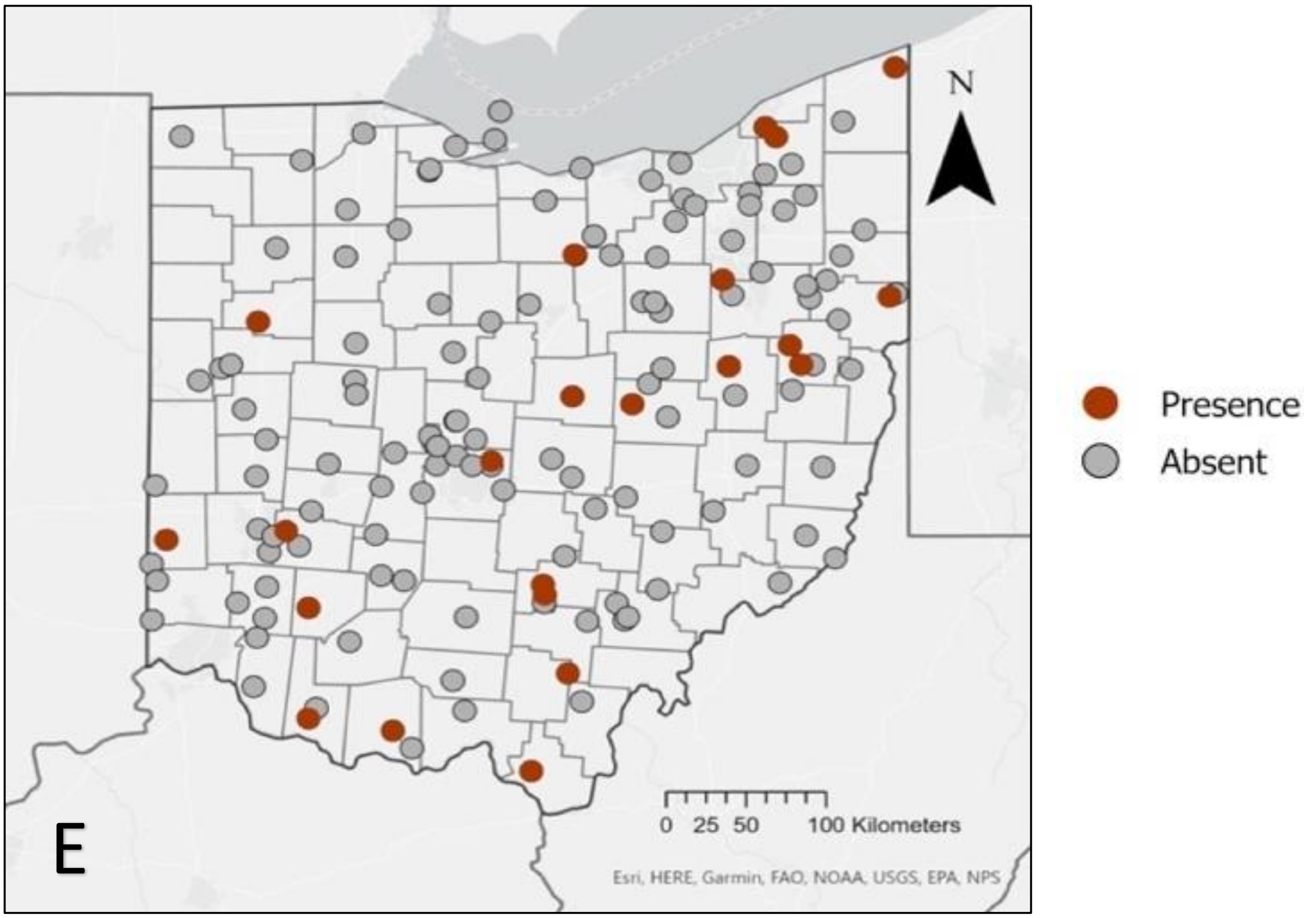


Figure 5. Distribution map of species with an abundance greater than 30 specimens. (A) *Atomosia puella* (n = 1,368), (B) *Atomosia glabrata* (n = 69), (C) *Atomosia rufipes* (n = 61), (D) *Laphria sicula* (n=82), (E) *Efferia aestuans* (n = 31).

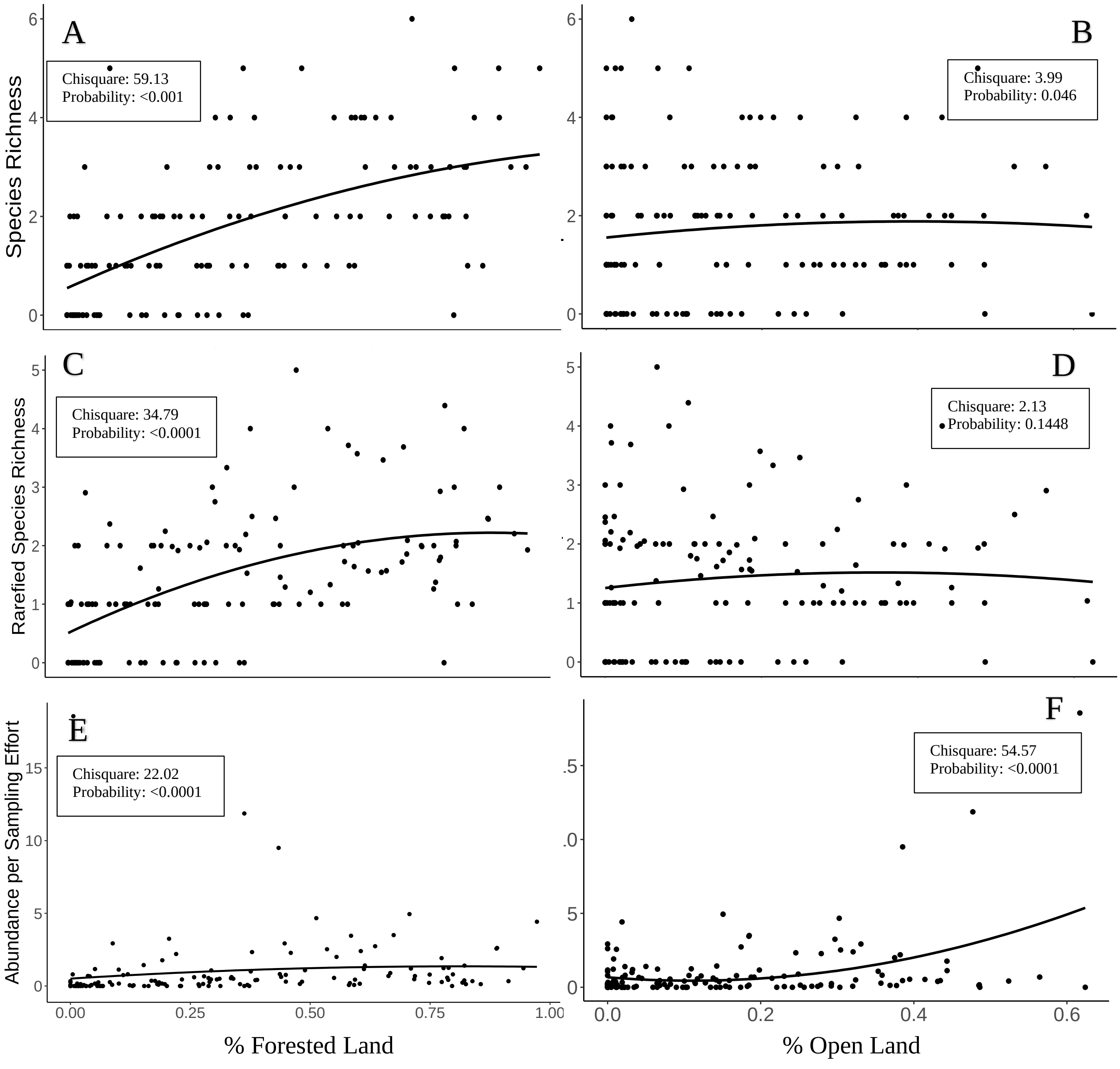


Figure 6. Graphs comparing independent variables of landscape type versus dependent variables. (A) Forested land versus species richness. (B) Open land versus species richness. (C) Forested land versus rarefied species richness. (D) Open land versus rarefied species richness. (E) Forested land versus abundance per sampling effort. (F) Open land versus abundance per sampling effort.

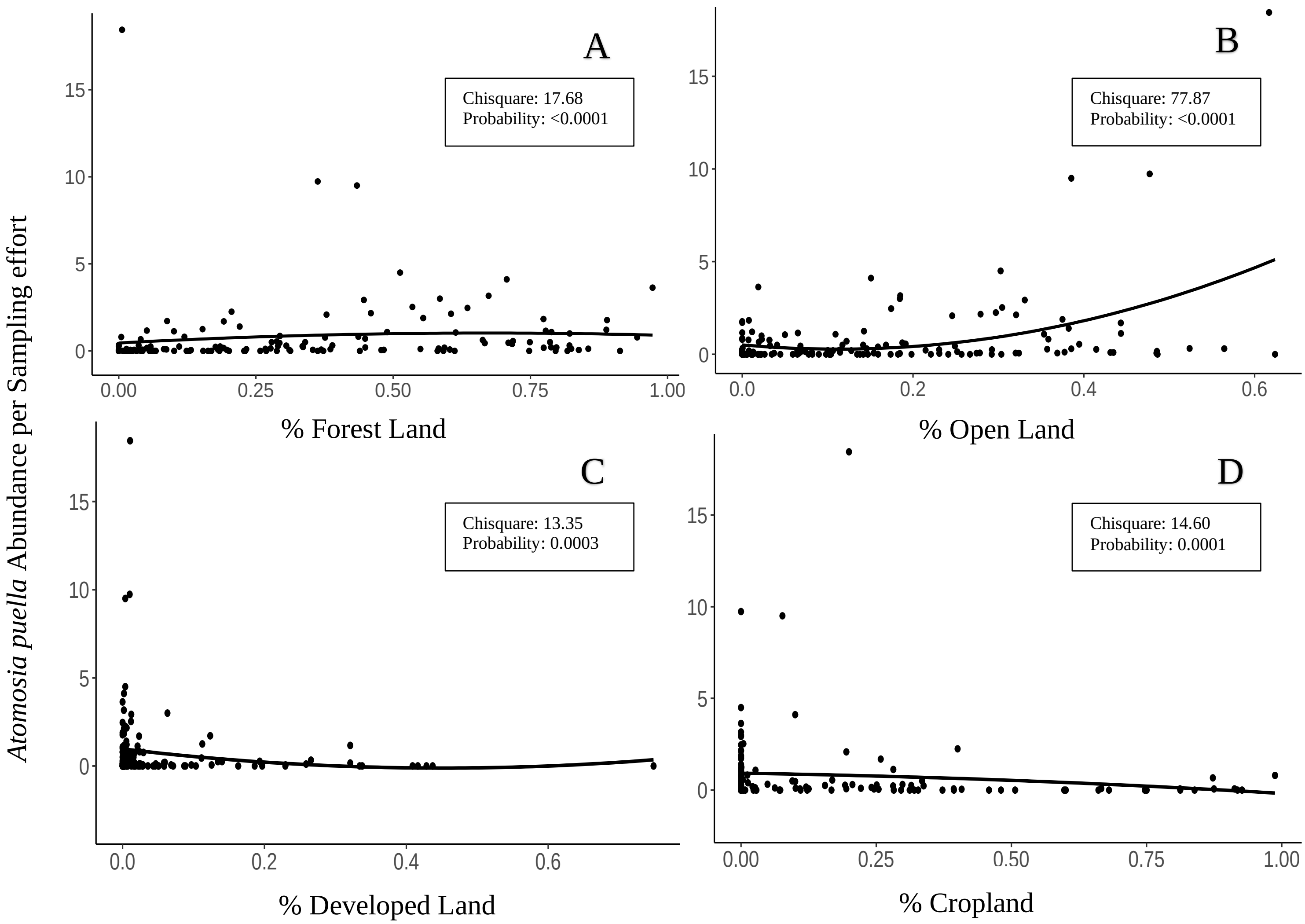


Figure 7. *Atomosia puella* data displayed against varying anthropogenic and habitat types. Both habitat types are displayed on the left of the figure; (A) forest and (C) open land. The two anthropogenic factors are shown on the right; (C) developed land and (D) cropland.

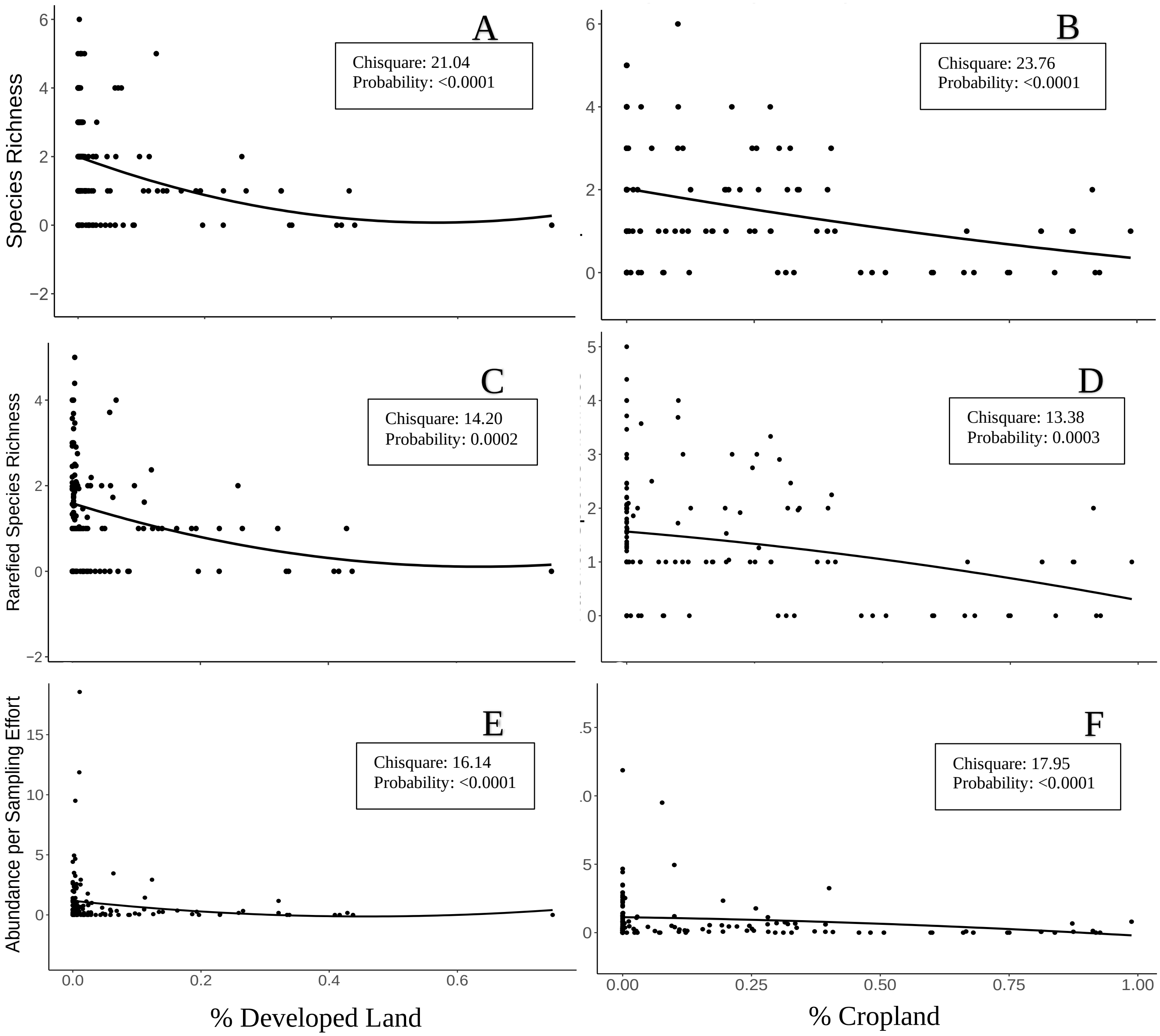


Figure 8. Graphs comparing independent variables of anthropogenic type versus dependent variables. (A) Developed land versus species richness. (B) Cropland versus species richness. (C) Developed land versus rarefied species richness. (D) Cropland versus rarefied species richness. (E) Developed land versus abundance per sampling effort. (F) Cropland versus abundance per sampling effort.

Appendix A.

Table displays the sites that found Asilidae specimens, their abundance, and what type of species. The level of accuracy on locations is within 100 m of transect center.

Site ID	GPS Coordinates	Scientific Name	Number of Specimens
1	**39.501, -81.251**	*Atomosia puella*	6
		Cerotainia macrocera	1
		Laphria sp.	1
3	**41.567, -81.273**	*Atomosia puella*	37
		Efferia aestuans	2
		Laphria aktis species complex	1
		Laphria canis canis	1
		Laphria sp.	1
6	**39.836, -81.626**	*Atomosia glabrata*	1
		Atomosia puella	17
8	**40.727, -80.918**	*Atomosia puella*	1
		Atomosia rufipes	1
9	**40.187, -83.219**	*Atomosia puella*	1
10	**38.896, -83.027**	*Atomosia puella*	1
		Diogmites misellus	1
11	**40.519, -81.062**	*Atomosia puella*	1
12	**40.049, -82.984**	*Atomosia puella*	24
		Cerotainia albipilosa	1
		Laphria sicula	14
		Machimus snowii/paropus	1
		Promachus hinei	1
13	**40.946, -81.354**	*Atomosia puella*	2
		Machimus sadyates	3
14	**41.140, -80.771**	*Atomosia puella*	7
17	**39.403, -82.167**	*Atomosia puella*	33
		Laphria sicula	3
		Laphria winnemana	1
		Psilonyx annulatus	1
18	**39.955, -84.765**	*Laphria canis canis*	2
		Laphria flavicollis	1
		Laphria sicula	7
19	**41.027, -82.404**	*Atomosia puella*	4

		Efferia aestuans	2
		Holcocephala fusca	4
		Laphria aktis species complex	1
		Laphria sicula	1
		Laphria winnemana	2
		Machimus antimachus	4
20	**41.018, -83.693**	*Atomosia puella*	1
21	**40.909, -80.985**	*Atomosia puella*	2
22	**41.235, -83.685**	*Machimus snowii/paropus*	1
23	**41.442, -81.185**	*Atomosia puella*	13
24	**40.718, -84.187**	*Atomosia puella*	4
		Efferia aestuans	2
26	**39.492, -82.583**	*Atomosia glabrata*	7
		Atomosia puella	69
		Atomosia rufipes	6
		Efferia aestuans	1
		Eudioctria brevis	1
27	**39.009, -84.212**	*Atomosia puella*	54
		Atomosia rufipes	2
28	**39.339, -82.104**	*Atomosia puella*	2
		Laphria sicula	1
29	**39.382, -83.903**	*Atomosia puella*	1
		Atomosia rufipes	1
		Efferia aestuans	1
		Laphria sicula	1
		Laphria winnemana	1
30	**40.883, -81.103**	*Atomosia puella*	17
32	**40.372, -82.416**	*Atomosia glabrata*	14
		Atomosia puella	74
		Efferia aestuans	1
33	**38.717, -83.322**	*Atomosia puella*	20
		Atomosia rufipes	1
		Laphria sicula	7
34	**41.555, -82.854**	*Machimus maneei*	1
35	**39.406, -84.304**	*Atomosia puella*	9
36	**41.419, -83.219**	*Laphria sicula*	1
37	**39.442, -82.574**	*Atomosia glabrata*	1

		Atomosia puella	17
		Atomosia rufipes	16
		Efferia aestuans	1
		Eudioctria brevis	1
38	**39.408, -82.576**	*Atomosia puella*	14
		Atomosia rufipes	7
		Laphria sicula	1
39	**41.254, -81.418**	*Atomosia puella*	1
40	**39.848, -82.291**	*Atomosia puella*	57
41	**39.726, -83.527**	*Atomosia puella*	8
42	**39.742, -84.033**	*Atomosia puella*	12
		Efferia aestuans	1
		Laphria sicula	2
43	**39.038, -83.092**	*Atomosia glabrata*	15
		Atomosia puella	146
		Atomosia rufipes	11
		Atomosia sayii	4
		Laphria sicula	2
44	**40.765, -81.922**	*Atomosia puella*	4
		Laphria sicula	4
45	**38.799, -83.428**	*Atomosia glabrata*	5
		Atomosia puella	7
		Atomosia rufipes	2
		Efferia aestuans	1
		Laphria sicula	2
		Machimus antimachus	1
47	**41.024, -82.199**	*Atomosia puella*	5
49	**39.626, -82.462**	*Atomosia glabrata*	4
		Atomosia puella	23
		Atomosia rufipes	6
		Heteropogon macerinus	1
50	**41.583, -83.594**	*Atomosia puella*	1
		Machimus snowii/paropus	1
51	**40.258, -83.076**	*Atomosia puella*	3
		Laphria sicula	1
		Machimus sadyates	1
		Machimus snowii/paropus	2
53	**39.480, -84.139**	*Atomosia glabrata*	3
		Atomosia puella	4
		Atomosia sayii	2
55	**40.430, -81.987**	*Cerotainia macrocera*	1

		Laphria sicula	1
		Machimus sadyates	1
56	**40.519, -81.132**	*Atomosia puella*	4
		Cerotainia macrocera	2
		Efferia aestuans	1
		Holcocephala fusca	1
57	**39.615, -80.936**	*Atomosia puella*	18
		Atomosia rufipes	5
		Laphria winnemana	1
58	**40.337, -82.081**	*Atomosia puella*	13
		Efferia aestuans	1
		Holcocephala fusca	1
59	**41.636, -80.895**	*Atomosia puella*	41
61	**40.512, -81.538**	*Atomosia puella*	22
		Efferia aestuans	1
62	**39.772, -81.103**	*Atomosia puella*	10
		Promachus rufipes	1
63	**40.799, -82.661**	*Atomosia puella*	2
65	**41.181, -81.836**	*Atomosia puella*	2
		Machimus snowii/paropus	1
66	**40.842, -81.517**	*Laphria sicula*	4
67	**40.070, -82.874**	*Atomosia puella*	1
		Efferia aestuans	1
		Machimus maneei	1
		Machimus snowii/paropus	3
71	**39.702, -84.703**	*Atomosia puella*	1
		Efferia aestuans	2
72	**40.833, -80.630**	*Atomosia puella*	1
		Efferia aestuans	1
73	**40.053, -83.182**	*Atomosia puella*	1
74	**39.510, -83.367**	*Atomosia puella*	8
75	**41.139, -80.771**	*Atomosia puella*	1
76	**40.849, -80.596**	*Atomosia puella*	25
		Laphria sicula	3
77	**40.719, -82.879**	*Atomosia puella*	1
80	**41.884, -80.601**	*Atomosia puella*	4
		Efferia aestuans	2
		Laphria sicula	2
81	**40.811, -82.020**	*Atomosia puella*	1
82	**39.468, -81.937**	*Atomosia glabrata*	1

		Atomosia puella	19
		Laphria sicula	1
84	**41.092, -81.518**	*Laphria sicula*	2
85	**39.224, -83.673**	*Atomosia glabrata*	6
		Atomosia puella	18
		Laphria sicula	2
86	**40.496, -80.847**	*Atomosia glabrata*	2
		Atomosia puella	32
		Laphria canis canis	1
		Laphria sicula	1
87	**40.277, -81.882**	*Atomosia puella*	5
90	**39.509, -84.755**	*Atomosia puella*	4
92	**39.838, -83.887**	*Atomosia puella*	6
		Laphria sicula	2
93	**38.609, -82.648**	*Efferia aestuans*	2
		Laphria sicula	1
94	**40.049, -81.434**	*Atomosia puella*	7
		Cerotainia macrocera	1
95	**41.023, -80.903**	*Atomosia puella*	166
		Laphria thoracica	1
96	**39.535, -83.495**	*Atomosia glabrata*	1
		Atomosia puella	1
97	**39.335, -84.150**	*Atomosia puella*	6
		Atomosia rufipes	1
		Laphria canis canis	1
		Laphria sicula	1
99	**40.094, -83.074**	*Atomosia puella*	4
100	**39.242, -84.193**	*Atomosia puella*	9
		Laphria sicula	1
		Machimus snowii/paropus	1
101	**39.936, -82.806**	*Atomosia puella*	1
		Laphria sicula	2
		Machimus antimachus	1
		Machimus snowii/paropus	1
102	**38.859, -83.903**	*Atomosia puella*	20
		Efferia aestuans	3
103	**39.323, 84.785**	*Atomosia puella*	6
104	**39.320, -82.334**	*Atomosia puella*	15
		Atomosia rufipes	1
106	**40.376, -81.503**	*Atomosia puella*	1
107	**40.445, -84.518**	*Laphria sicula*	1

		Laphria sp.	1
108	**40.382, -83.635**	*Holcocephala fusca*	1
109	**40.079, -82.534**	*Atomosia glabrata*	1
		Atomosia puella	39
		Laphria sicula	1
110	**40.502, -81.907**	*Atomosia puella*	48
114	**39.590, -84.787**	*Atomosia puella*	1
		Laphria sicula	1
		Laphria winnemana	1
115	**41.253, -81.727**	*Atomosia puella*	9
117	**41.611, -81.332**	*Atomosia puella*	12
		Efferia aestuans	1
118	**41.230, -81.226**	*Atomosia puella*	2
		Machimus snowii/paropus	7
119	**40.911, -81.571**	*Atomosia puella*	10
		Efferia aestuans	1
		Laphria sicula	2
120	**40.400, -81.180**	*Atomosia glabrata*	4
		Atomosia puella	7
		Promachus sp	1
121	**40.809, -81.956**	*Atomosia puella*	2
		Cerotainia macrocera	1
122	**39.740, -81.913**	*Atomosia puella*	6
123	**41.016, -81.940**	*Atomosia puella*	1
		Laphria sicula	3
		Laphria winnemana	4
124	**40.170, -84.140**	*Atomosia puella*	1
126	**40.153, -83.198**	*Atomosia puella*	6
		Machimus antimachus	1
		Machimus snowii/paropus	1
128	**38.909, -83.859**	*Atomosia puella*	2
129	**41.312, -81.417**	*Atomosia glabrata*	4
		Atomosia puella	7
130	**40.171, -82.963**	*Atomosia puella*	5
131	**40.043, -81.009**	*Atomosia puella*	22
		Laphria winnemana	1
132	**40.826, -81.081**	*Holcocephala fusca*	1
133	**40.609, -81.194**	*Atomosia puella*	2

		Cerotainia macrocera	1
		Efferia aestuans	2
		Laphria sicula	2
		Laphria undescribed species 2	1
134	**39.073, -82.443**	*Atomosia rufipes*	1
		Efferia aestuans	1
		Machimus maneei	1
135	**40.576, -83.088**	*Atomosia puella*	1
137	**39.923, -83.263**	*Atomosia puella*	1
		Laphria canis canis	1
		Laphria sicula	1
		Machimus snowii/paropus	1
138	**39.324, -82.125**	*Atomosia puella*	4
		Heteropogon macerinus	1
140	**40.618, -83.637**	*Laphria canis canis*	2
		Laphria winnemana	1
141	**39.902, -82.117**	*Atomosia puella*	2
		Laphria sicula	1
	Grand Total		**1705**

Appendix B.

R-Code:

Graphing code:

```
ggplot(data = matrix, mapping = aes(x = variable, y = variable)) + geom_point() +
        geom_smooth(method = "lm", formula = y ~ poly(x,2), color = "black") + ggtitle("Title")
        + xlab("x-variable label + ylab("y-variable label") + theme(text= element_text(size = 20))
```

Species Accumulation Curve Code:

```
plot(specaccum(data, method = "exact", permutations = 100, conditioned =TRUE, gamma =
        "jack1",  w = NULL))
```

Regression Analysis Code:

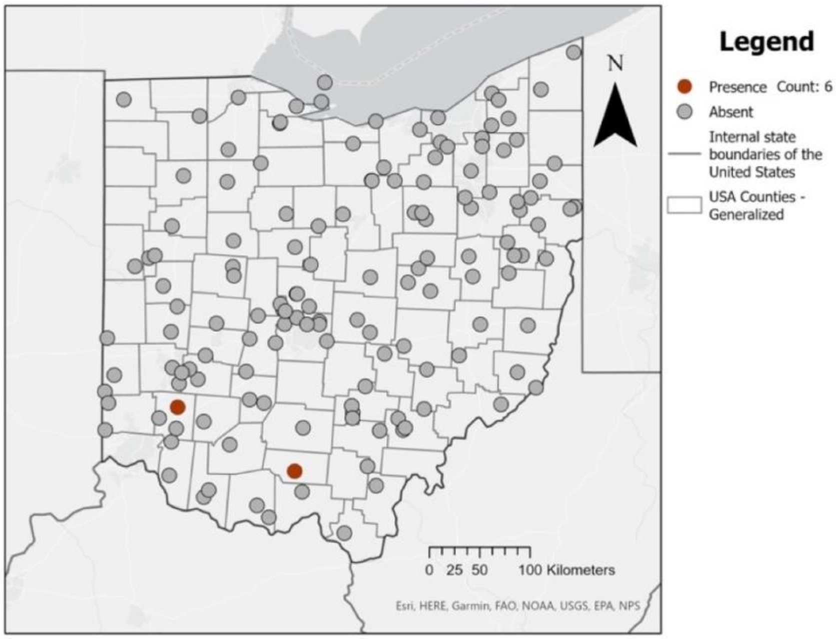
```
regression=lm(rrichness~Developed, data=data)
summary(regression)
plot(rrichness~Developed, data=data)
abline(regression, col="blue")
```

Rarefaction Code using vegan:

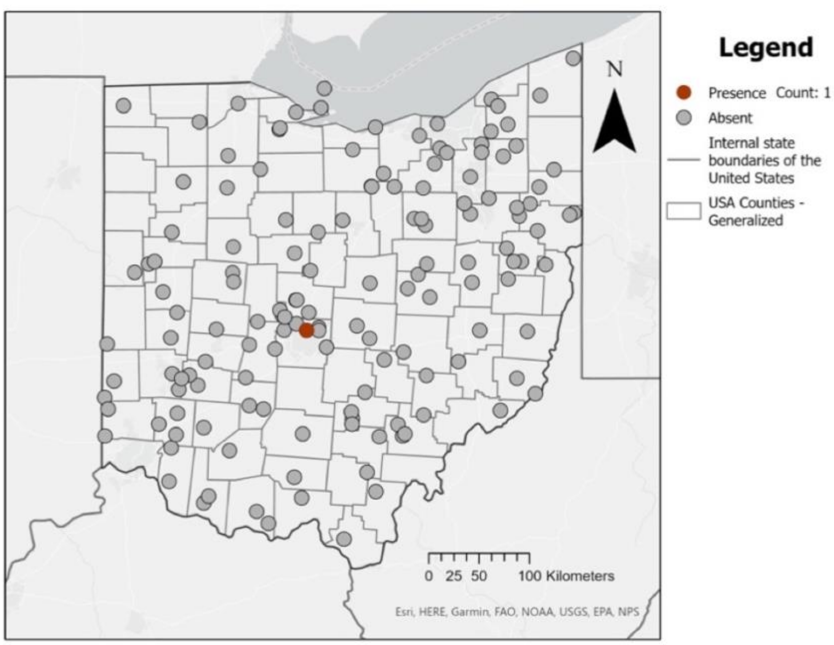
```
install.packages("vegan")
library(vegan)
dat <- read.csv("Book3.csv")

subsample = 6 # Or some number that is relevant for your study/data
# Use MARGIN = 2 if your species are given in the rows.
# Use MARGIN  = 1 if your species are given in the columns
rare_richness <- rarefy(dat, subsample, MARGIN=1)
"To write the results as a csv file"
df <- data.frame(rare_richness)
write.csv(df,"C:\\Users\\vanessachilcoat\\Desktop\\Robber Flies\\rarefied.csv",
row.names=FALSE)
```

Appendix C.
Maps of Species with Fewer than 30 specimens:

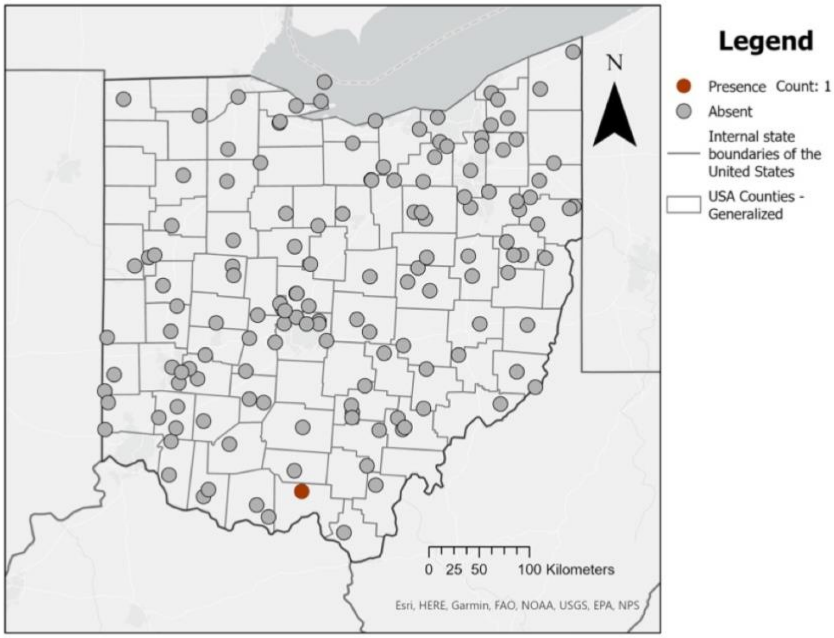
Atomosia sayii



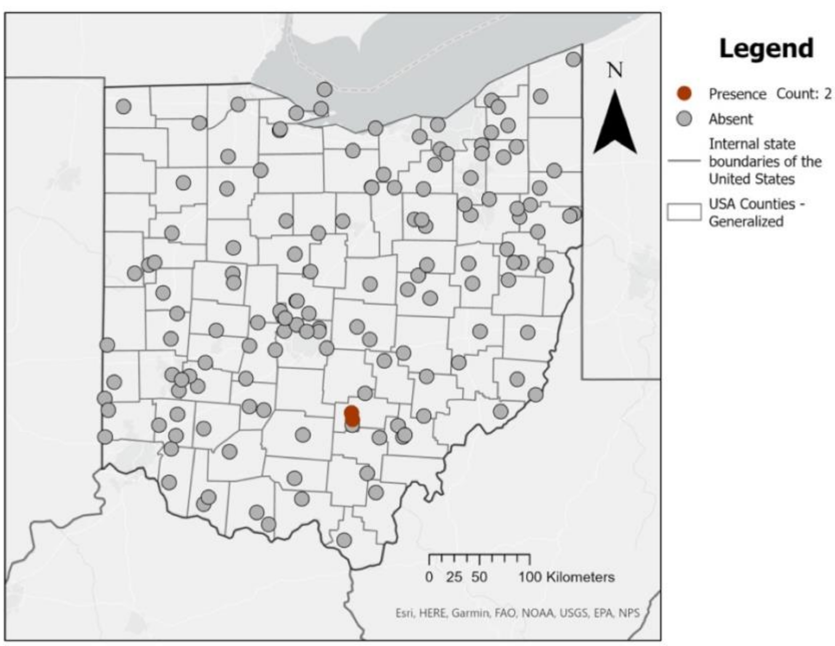
Cerotainia albipilosa



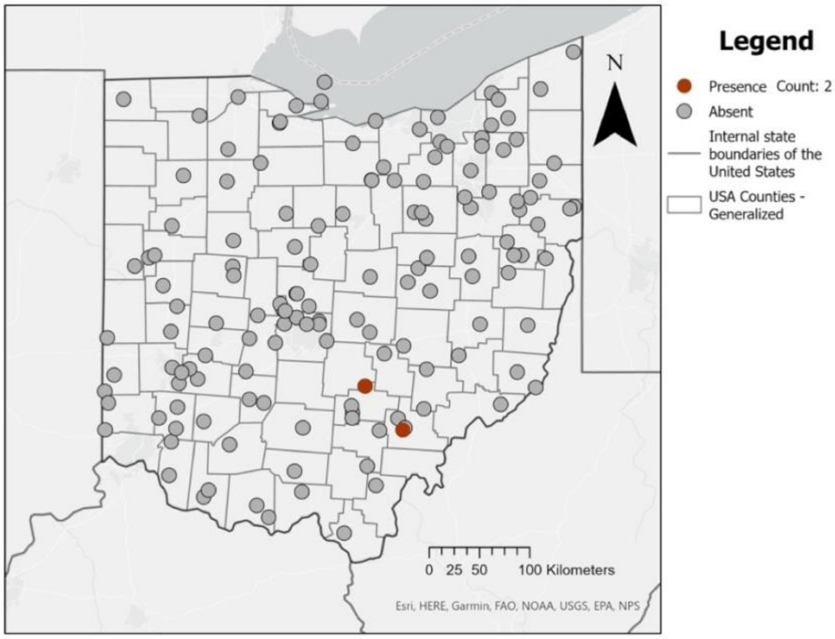
Diogmites misellus



Eudioctria brevis



Heteropogon macerinus



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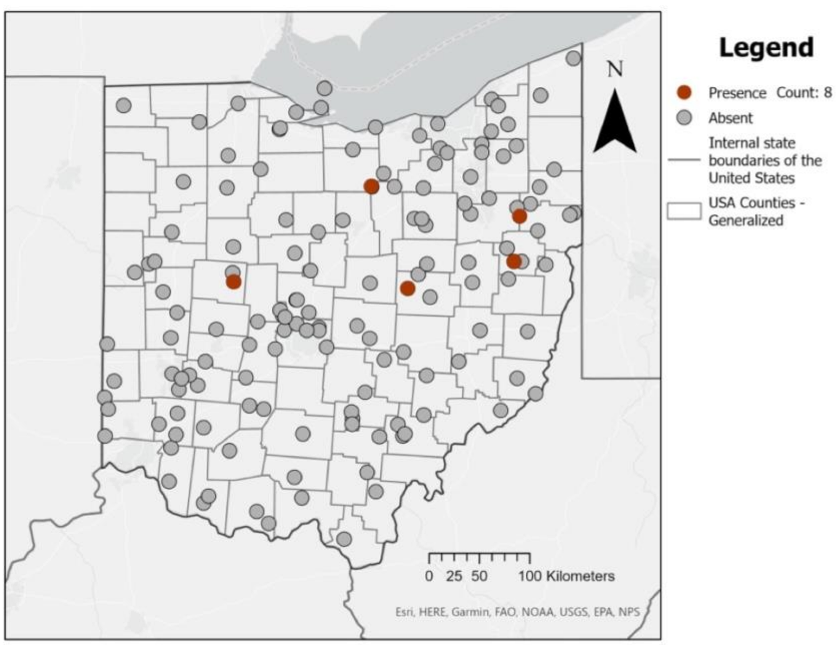
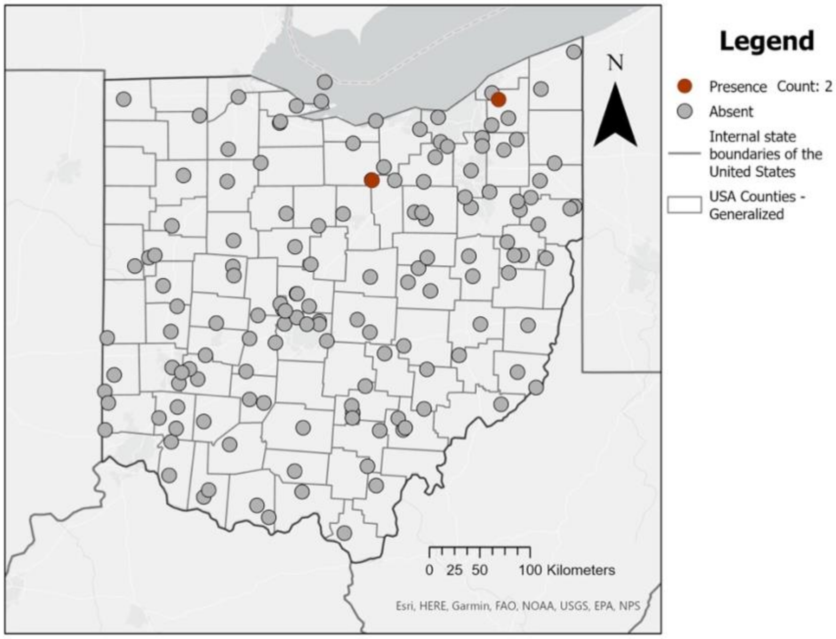
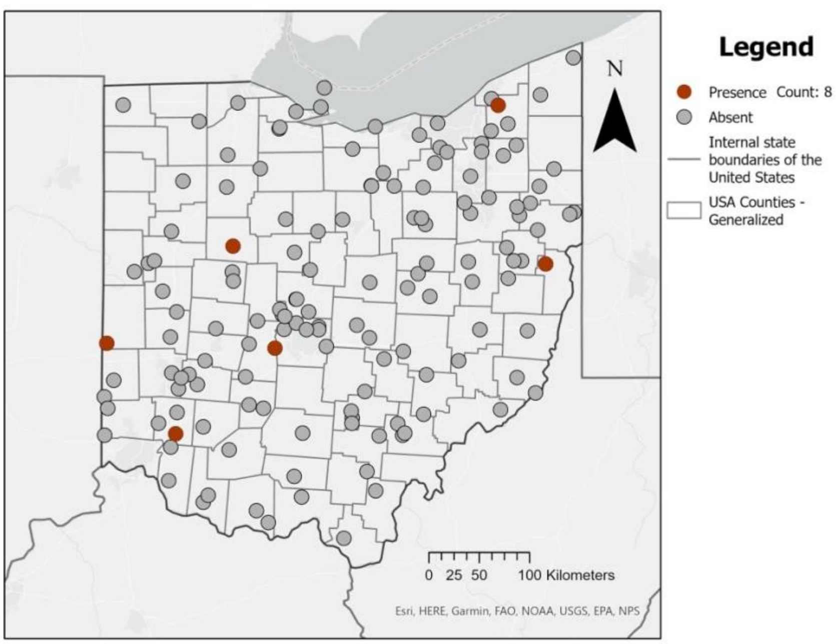


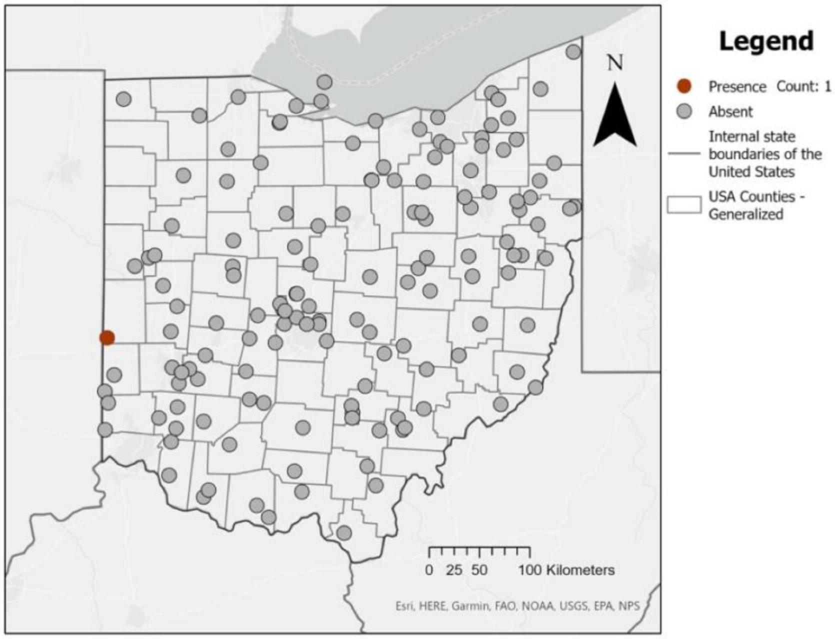
Laphria aktis species complex



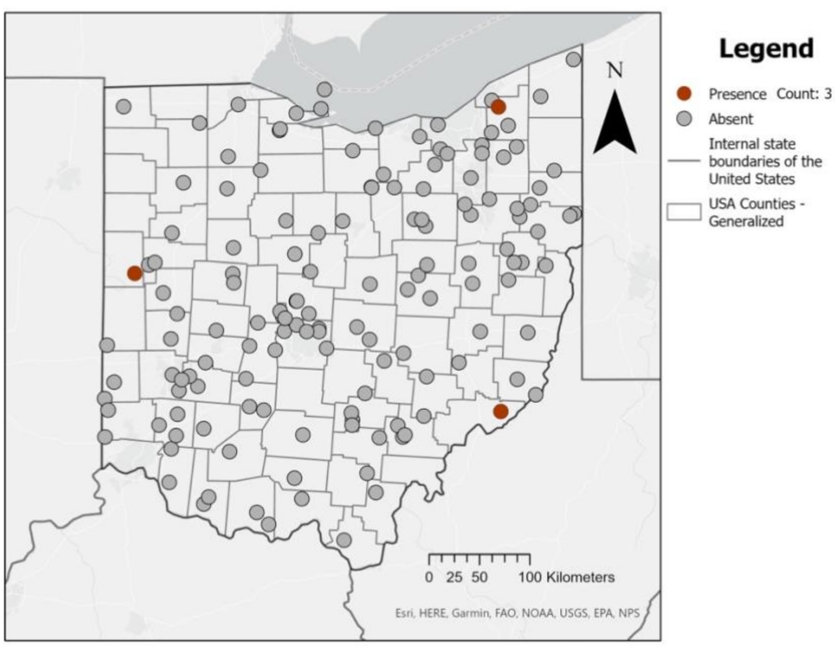
Laphria canis canis



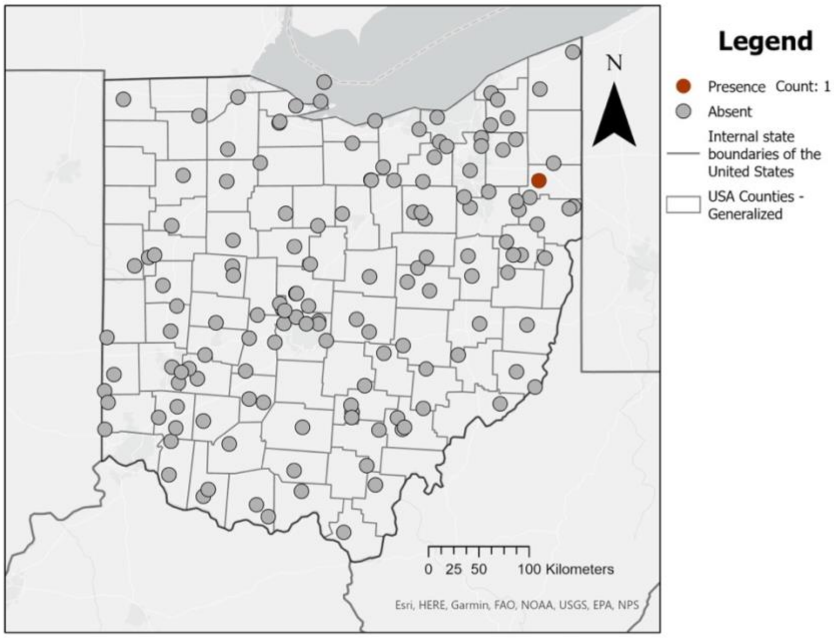
Laphria flavicollis



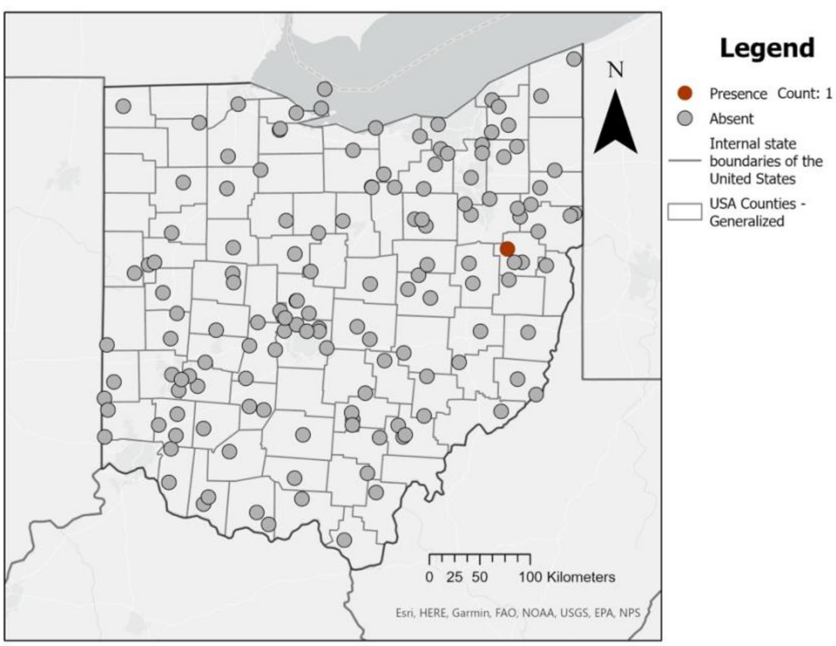
Laphria sp.



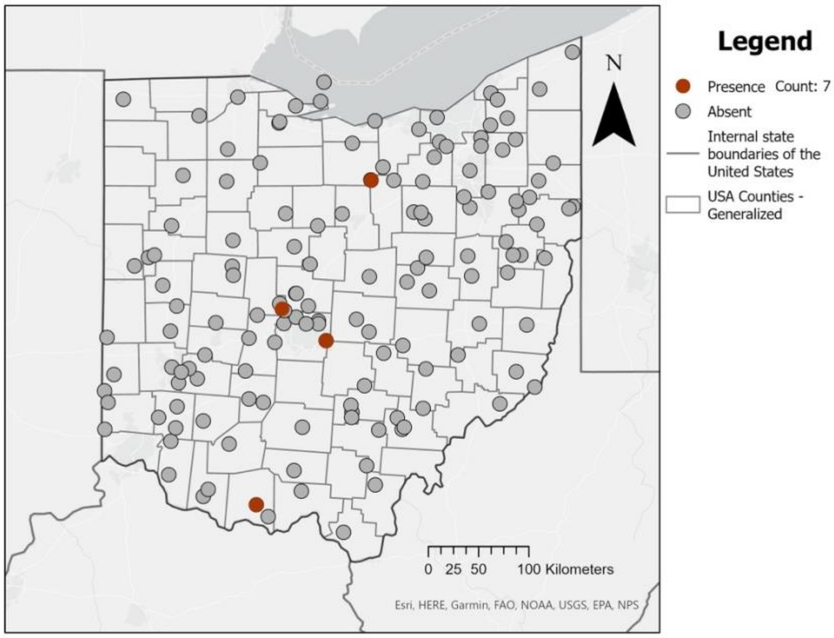
Laphria thoracica



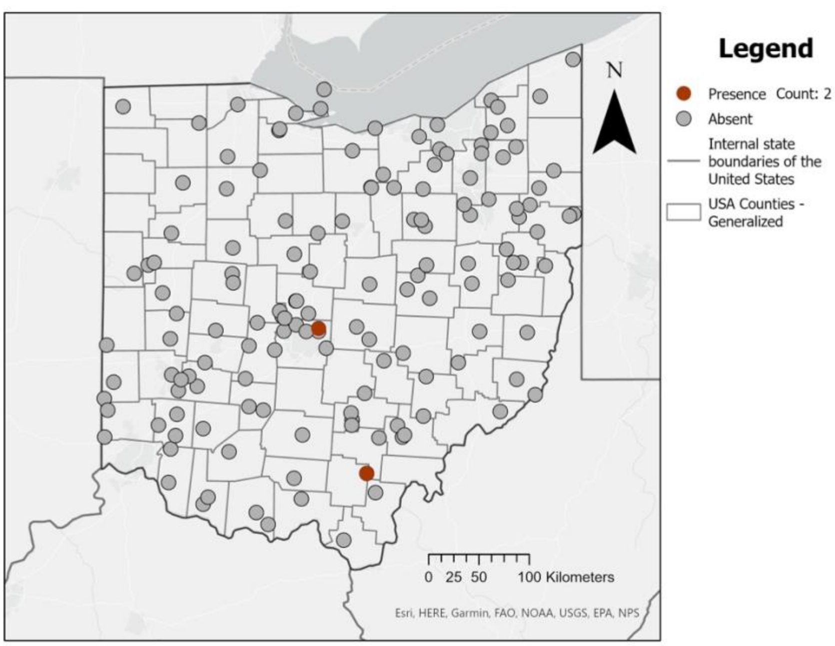
Laphria undescribed species 2



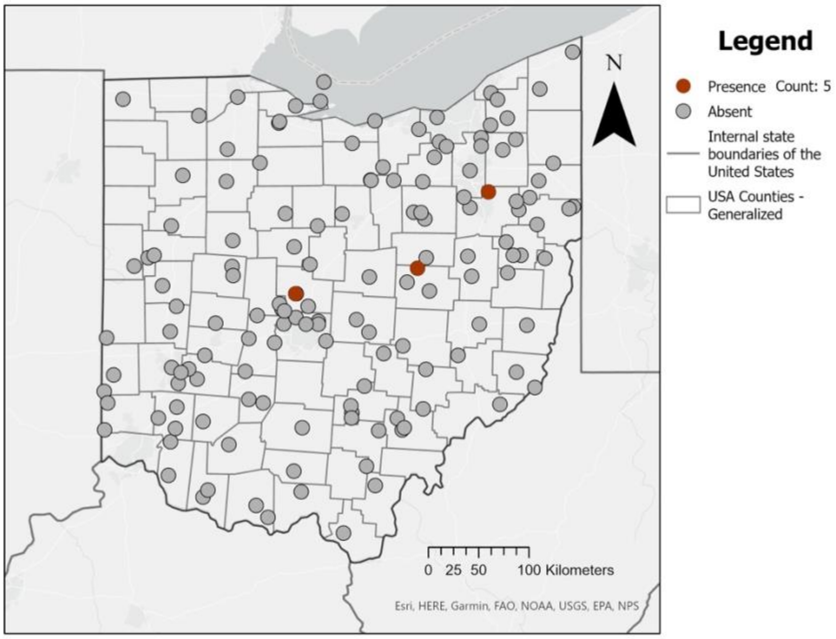
Machimus antimachus



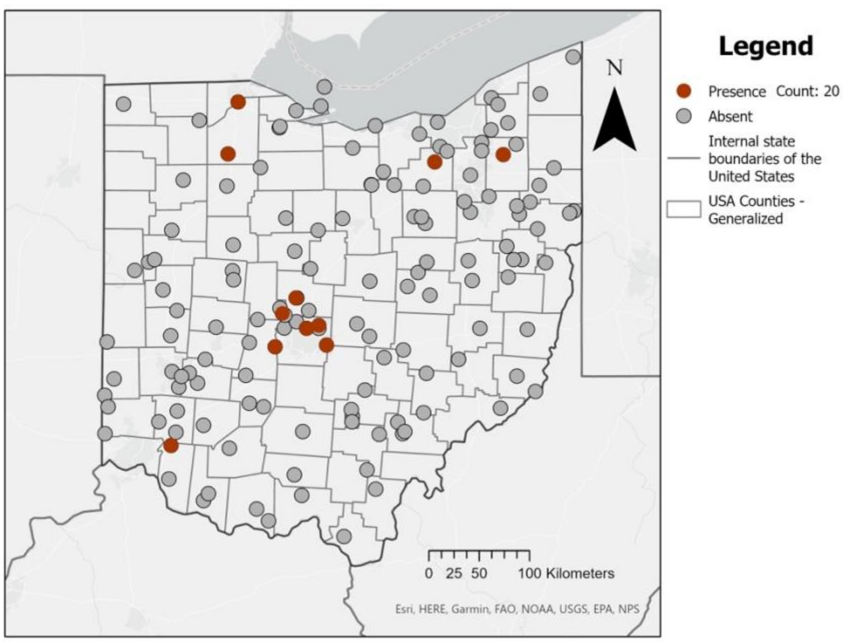
Machimus maneei



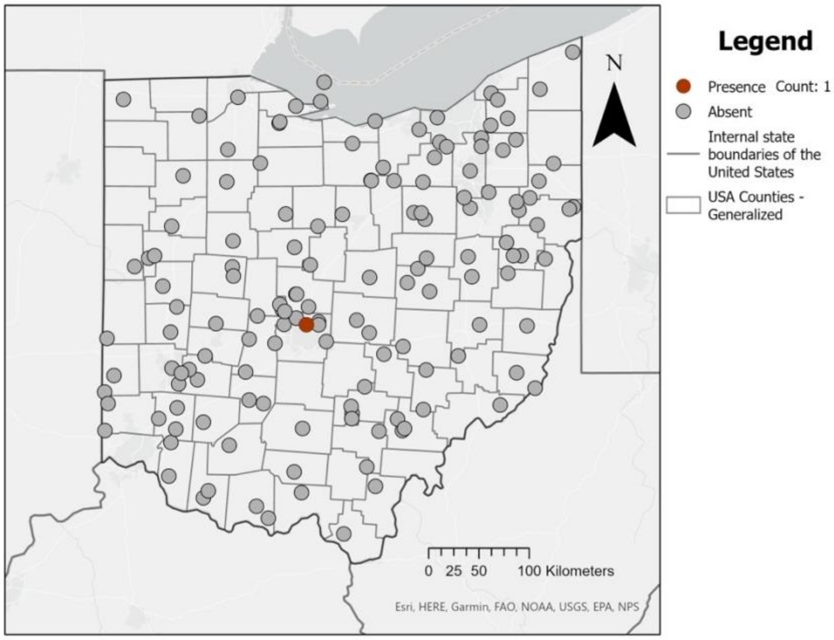
Machimus sadyates



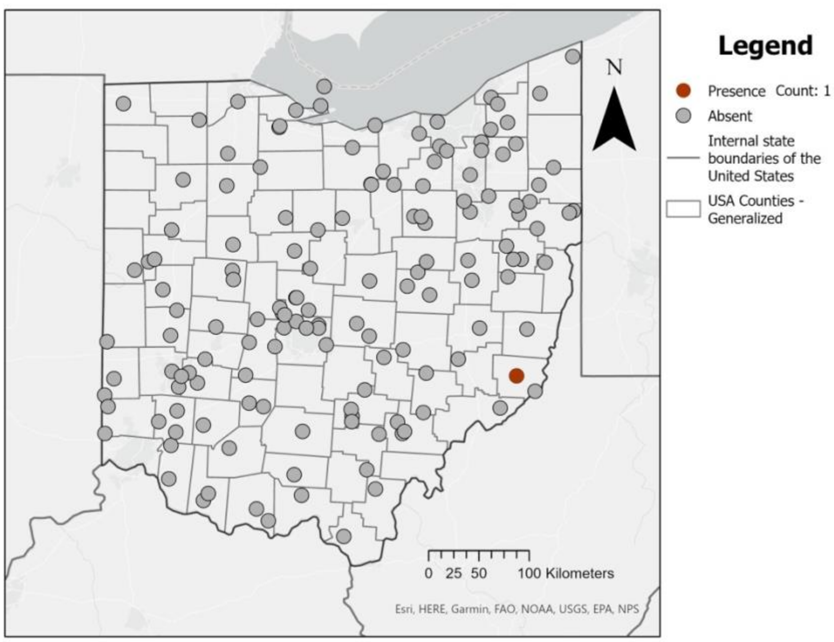
Machimus snowii/paropus



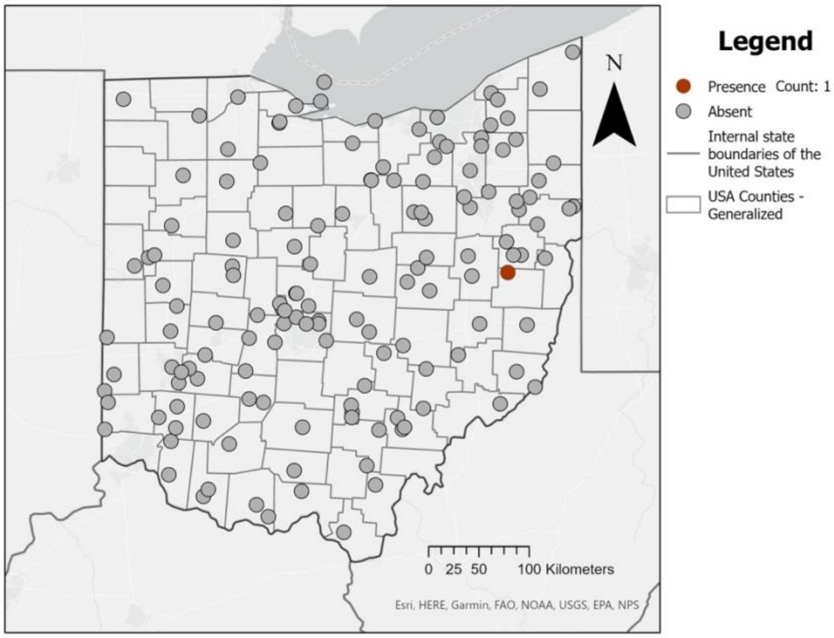
Promachus hinei



Promachus rufipes



Promachus sp



Psilonyx annulatus