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What is the Diversity of Sub-Canopy Web Building Spiders in Three Separate Regions of Puerto Rico?

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ABSTRACT
Understanding the many aspects of the biodiversity of our planet yields many positive advancements in the conservation and maintenance of its current and future states. This experiment seeks to explore the more intricate facets of this by sampling and comparing the diversity of web building spider species in three separate areas of the island of Puerto Rico. The island is known for its diversity, despite its relatively small size, about 100 by 35 miles. Ecosystems vary from temperate moist forests, to rainforest, to desert and dry forest, just within that space. Several methods of sampling were attempted and evaluated for their efficiency in each environment, but due to difficulties with some methods and a need for standardization for comparisons, only the visual sampling technique was deemed as successful in this case. Specimens were identified down to the classifications of family and genus through identification of morphological structures, as well as other defining traits. After identification, relationships and comparisons were made within and between the areas, and further analysis using Simpson's Index and Community Similarity Index was utilized. It was determined that based off of this collection, the Guanica site housed the greatest diversity in specimens, although there were several similar specimens found in all three of the areas. This may imply the distribution of some types of spiders may be affected by ecological factors that differ in each of the areas, such as resource and habitat availability. The Northeaster Ecological Corridor and El Verde sites were observed to be the most similar samples as expected, but further sampling is necessary for additional comparisons. The samples collected could aid in additional conservation efforts and further research in these areas in the future as they provide information about the presence and distribution of spiders in these areas that may be previously undocumented.

Keywords: *Araneae, Biodiversity, Diversity, Ecology, Ecoregions, El Verde, Guanica, Luquillo Research Station, Northeastern Ecological Corridor, Puerto Rico.*

INTRODUCTION

There are many increasingly important reasons for understanding the biodiversity of our planet today. As part of nature ourselves, it is inherently useful to understand the plethora of ecosystems on the planet and the processes that take place between organisms and environments, as it all in the end affects the world around us in some way. Generally defined, biodiversity can be understood as the variety and variability among living organisms and the ecological complexes in which they occur (US Congress, 1987). Generally speaking, scientists have been interested in protecting this diversity of life for many years (Marsh, 1864), although adequate conservation efforts have been becoming more popular only recently. It is well known in the science community that there is a mass extinction occurring in the world today, which has been occurring at an increasingly more alarming rate even within the last century (Wilson, 1988). This is a great concern for those working to interpret and admire the biodiversity in the world today, and can only be remedied by a thorough understanding of the processes that are taking place. As the first steps of many conservation efforts are working to understand the presence, distribution, abundance, and diversity of species in an area, this process can take significant amounts of time and labor, so organisms that are easier sampled tend to be the most assessed and protected (Tews, et.al, 2004). Although this trend is problematic, we are only in the beginning stages of understanding the incredible variation in the evolution of life we can observe in the world today, and any attempts to pry further into this understanding help to illustrate this diversity more and more.

The assessment of species diversity within given areas can be a very useful tool in understanding the structure and composition of facets of that ecosystem that may have been previously misunderstood. According to the habitat heterogeneity hypothesis, habitats that are more structurally complex tend to contain more living spaces and resources for organisms to take advantage of, therefore, in turn, can express more species diversity(Simpson,1949, MacArthur and Wilson, 1967) . When considering this theory to describe an ecosystem, it is also important to consider the specific organisms' range of habitability and use of resources, as this can provide insight different levels and types of structural complexity within the ecosystem. This is a useful understanding because it can be seen that several groups of organisms that are the easiest to sample, tend to get the most conservation effort, even though they may have a completely different ecological requirements from their habitat than other types of organism that get less attention due to the difficulty of sampling them. Sampling these groups that are difficult and underrepresented is still important in completely illustrating the complexity of an ecosystem, and may yield additional insight into other methods of conservation that could be used to more effectively sustain and enhance biodiversity. A literature review article discussing several papers associated with the relationship between species diversity and habitat heterogeneity (Tews et al., 2004) made a very good point about conservation bias in observing that, out of the 85 papers they reviewed, 61% of them discussed vertebrates, which only encompass about 3% of the observed animal species in the world at this time. Even though this article was largely discussing Habitat heterogeneity studies, this trend is commonly seen in many conservation assessments as well as all sorts of research studies, as protocols and methods of sampling, today are generally specific to easier sampled species. In the same article, it was also recognized that only 31% of the articles reviewed considered arthropods in their assessments, and of these, 19% included arachnids. This again follows the trend for most sampling efforts outside of this review, but in turn, it leaves plenty of opportunities for those interested in putting their time into these underrepresented taxonomic groups to do this type of sampling. Working to achieve a better understanding of those groups and their variability across the globe will hopefully provide a better, and more complete understanding of new and different ecosystems and how to protect them. This is largely the reasoning behind the emphasis of this study, as web building spiders were the primary sampling effort in each of the areas that were considered.

Although they are important to almost any ecosystem as insect population control, as well as a food source, it is easy to notice that spiders are largely ignored when considering conservation efforts and big-picture ecological studies. This is likely due to the fact that there is not a lot of research out there that completely describes the prevalence and distribution of this group of invertebrates, and the prioritization process of conservation efforts demands a thorough understanding of these variables (Cardoso, 2004). The abundance of spiders has been observed to be unequal in places such as urban areas that are more commonly disturbed from day to day life, rather than rural areas, which require less moving around and reestablishment (Sattler, et al., 2010). So, regardless of the flexibility in spiders hunting techniques and their ability to locate and relocate living spaces virtually anywhere rather quickly, common disturbances tend to push these groups of organisms out of those areas. This may imply that habitat structure and consistency may be predicted by assessing spider populations and abundances in an area, but it should not be used as the only factor considered when predicting how common disturbances occur. Also, evaluating the diversity of spider groups can create a better idea of what sorts resources are being used there (prey type, plants utilized for webs, etc.), as well as what sorts of competition are possibly present between species, as it is very common for a spider's worst predator to be another spider. As spiders are present in almost every ecosystem besides the open ocean and Antarctic regions (Foelix, 1996), a better understanding on a larger scale of their habitat preferences and biogeography could yield many more insights as to their role in different ecosystems, as well as provide a better illustration of how these organisms may have evolved and adapted to be where and how they are today.

As far as sampling areas go, Puerto Rico is a particularly interesting location to study, in that within its area of only about 100 miles by 35 miles, the geography of the island allows it to encompass a large array of very diverse ecoregions. The island is also home to many endemic species of plants, animals, and invertebrates, several of which are found only in specific regions. As it is located in the middle latitudes, around 18.4500 degrees N, the climate in this area supports year-round growth of vegetation, due to the temperatures' constant range from an average low of 65 to an average high of 85 across the island (Colon, 2009). Although there is a wet and dry season, which can affect the population of invertebrates, there is a substantial amount of rainfall year round across the island, averaging around 70-80 inches annually, (Colon, 2009). The rainfall is more focused in the northeastern and central parts of the island, whereas some southern areas get average rainfall closer to 35-40 inches (Colon, 2009). These conditions on different ends on the island are also very supportive of the rich diversity of ecoregions and forest types found on the island, from deserts and dry forests, to rain forests, lowland forests and lower montane forests. The overall climate of the island contributes greatly to the upkeep of several different types of these systems across the island. This in turn results in the presence of about 20 nature reserves on the island, all conserved to aid in the preservation of the biodiversity of these systems.

In sampling for the diversity of spiders, we were interested in utilizing this variety of ecoregions to better explore the possibility that each region may produce entirely different results depending on the processes and resources occurring there. For this collection, it was decided that there would be a sample taken from three different areas that would represent three different types of ecoregions, all within the confines of the island. The ecoregions sampled in this project include the dry forests of Guanica, the subtropical moist forests of the Northeastern ecological corridor, and the subtropical rain forest at the El Verde research site in Luquillo. Utilizing these different regions works to assess for possible differences, as well as any similarities that can be observed between the areas. Seeing as the biogeography of island species patterns is another related subject that has not been widely studied, especially so for spiders in Puerto Rico, the island is a great place to attempt observing these relationships, because of the large amount variation between habitats within such a small area.

MATERIALS AND METHODS

Sampling Area Descriptions:
The three sites being sampled in this study represent three very different ecosystems found on the island. The differences between the sites give rise to the expectation that each location will yield very different ecological systems and produce interesting contrasts in abundances and species of spiders identified (Rosenzweig, 1995). They are described in the following paragraphs.

The Luquillo site, also described as El Verde, is largely a subtropical rainforest setting and of the three sites was expected to have the highest density of spider population due to its wetter nature year round and noted diversity of other organisms (Environmental Setting, 2012). Also, because there is a cited scarcity of insects and spiders in the understory and canopy of the forest due to the presence of many Anoline lizards and coqui frogs, spiders tend to thrive in the sub-canopy areas. This is because the forest floor in this zone does not contain many potential perches for these larger sized predators (Reagan, 1996). This makes Arachnids the major invertebrate predators in the forest in this area as the only main competition for sub-canopy prey is other arachnids. The area around the Luquillo site has been previously sampled, and the study showed mean spider densities of around 25,00 per Ha-1 in the dry period, and about 52,500 per Ha-1 in the wetter seasons, showing the vast difference between the population densities through the seasons (Reagan and Wade, 1996). Despite all this, overall, the site is promising for species diversity and should be easily sampled with positive results.

The Northeastern Ecological Corridor is a 13 mile stretch of subtropical moist forest along the Northeastern corner of Puerto Rico. It is known to contain vast biodiversity largely because of the types of environmental changes it experiences year round, which support a wide array of different types of life. This area also supports at least 54 endemic plants and animal species from Puerto Rico and it could lead to interesting discoveries along these lines when sampled for spider populations as well. Past surveys of the area have yielded only 30 species of spiders in the area, six of those being endemic species (Northeastern Ecological Corridor- Fauna, 2013). Also, much of the vegetation in the area is typical of dry forests and wetlands and because of its temperate nature. All of these factors, including the lack of previous sampling, facilitate a belief that spider populations and diversity may be expected to be moderate to high (Northeastern Ecological Corridor-Flora, 2013).

The Guanica site, a NEON site, may have a completely different representation of species due to the fact that it is a different ecosystem entirely and it is also on the opposite end of the island. Most of the area is seen as a seasonally dry forest, and compares to seasonal temperate forests in climate, although many areas around it are like a desert in nature (Atlantic Neotropical). The research for this project will be done in desert area, and that type of ecosystems tend to contain smaller densities of spider populations, and many of those species tend to be ground dwelling or nighttime hunting spiders, due to the intense heat during the day. Sampling for these species may be more difficult but any data collected from this site would useful in comparison with data from the other areas.

Capturing and Storing Methods:
To be efficient at sampling as much diversity as possible, two methods of capturing specimens were attempted, as conditions permitted. Several methods of sampling were prepared before departure on the trip, such as net sweeping and litter sampling, but after seeing the habitats and locations of the webs being sampled, as well as adhering to time constraints, two methods were ultimately attempted, and only one produced useful results.

Ramp Trapping- Ramp traps were brought to assess for the possibility of spiders moving across the ground and leaf litter in each of the locations. These traps were assembled from Tupperware containers with ramps leading into them that were camouflaged with litter from the area being sampled. A 50-50 propylene glycol and water mixture was used as the solution in the traps, and the specimens were to be later transferred into vials with 70% Isopropyl Alcohol for shipment and identification. Twelve Pitfall traps were set up evenly throughout a 15 by 20 meter plot, with one or two in significantly different habitats within the system if applicable, and locations were marked with flags. Traps were set out for two days in each area, and each trap was checked each day for samples.

After attempts in both the Northeastern Ecological Corridor and the Luquillo site, the lack of significant results from ramp trapping, as well as the collateral sampling of unintended fauna, led to the decision that only the data collected from the visual sampling would be included in the following tables and figures. The only successful captures in the ramp traps were two similar, but unidentifiable, juvenile Mygalomorphs.

Walks/ Visual Sampling- Walks were performed in each location in several different areas during the day. Nighttime sampling was omitted to keep the sampling effort conserved between the sites. This was due to the nighttime moisture in the Luquillo site interfering with attempts to use a headlamp to scope for eye shine and glare from webs, as well as time constraints in each area during the trip. General searching of each area was utilized as well to account for possible diversity in different types of habitats within the sampling area. Specimens were captured by hand using storage vials and transferred into separate vials of Isopropyl alcohol storing solution to be transported and analyzed for taxonomic classification at the college.

Identification of specimens:

For cost reasons, family and genus were identified through visualization of defining morphological structures in mature specimens instead of the more expensive DNA sequence identification techniques. Several pictures were taken of each individual and initial identifications of the family of each were obtained by following keys presented by public spider collection sources, previous data collected, and by other entomological sources. (Ubick, et al., 2009; Sackett, Buddle, and Vincent, 2008; Williams and Howe, 2013). Also, in all of the sites, there were several juveniles that were identifiable down to the Genus level. Juveniles that were not identifiable to genus were processed as far into the taxa as could be identified and used in observations of family diversity, but results from more complex diversity calculations were not produced with these specimens.

Data Analysis Techniques:

The samples were taken individually from the labeled storage vials and identified using proper methods, according to gender and maturity (Ubick, et al., 2009; Sackett, Buddle, and Vincent, 2008; Williams and Howe, 2013). Data was recorded for each location and analyzed for family and genus abundance and sampling efficiency with standardized statistical techniques (Simpsons Index, Community similarity index a.k.a Proportional similarity Index)(Equations 1 and 2).

Equation 1. Equation used for Simpsons Diversity Index. Taking 1-D gives the diversity value. The range of diversity increases as the value approaches 1 on a 0 to 1 scale.

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

Equation 2. Equation for the Community similarity index.

CS= ∑(Lowest proportional value of a genus similar between the two areas)

These indices were taken of the three locations sampled and then were compared for further analysis of relationships between the areas. Each Genus present was interpreted and compared geographically, as well as in conjunction with previous research for further interpretation of the results.

RESULTS

Overall, there were 155 total spiders collected from the three sampling sites representing 7 different families, and at least 12 different genera, not including the unidentified juveniles.

The Guanica sample encompassed 59 of the total spiders, within 3 families and 6 genera (Table 1). The NEC sample was comprised of 32 spiders, within 4 families and 5 genera (Table 2). The El Verde sample had the largest collection of 64 spiders, and spanned 6 families and 7 or more genera if the unknown genera of some of the juveniles were to be included (Table 3).

It was found that of the three sites sampled, the Guanica sample was the most diverse, the NEC sample was the least diverse, and the El Verde sample was not significantly more diverse than the NEC (Figure 1). This supports the observation that the NEC and El Verde samples were the most similar at 75% similarity, while the Guanica sample was more different, only receiving a 46.7% similarity with the NEC and an even lower 30.5% similarity with the El Verde Sample (Figure 2).

Table 1. Genus abundances from the Guanica Dry Forest Site.

Family	Genus	Abundance (# Juveniles)
Araneidae	*Argiope*	4 (0)
	Cyclosa	4 (0)
	Cyrtaphora	3 (0)
	Damaged/ unknown	7(6)
	Neoscona	9(0)
Tetragnathidae	*Leucauge*	18(3)
Uloboridae	*Zosis*	14(1)

Table 2. Genus Abundance of samples from the Northeastern Ecological Corridor.

Family	Genus	Abundance (# Juveniles)
Araneidae	*Argiope*	4(0)
	Neoscona	2(1)
Selenopidae	*Selenops*	1(0)
Tetragnathidae	*Leucauge*	24(9)
Uloboridae	*Zosis*	1(0)

Table 3. Genus abundances from the El Verde site.

Family	Genus	Abundance (# Juveniles)
Araneidae	*Gasteracantha*	1(0)
	Verrucosa	1(0)
	Unknown	1(1)
Pholcidae	*Crossopriza*	5(0)
	Unknown	2(2)
Salticidae	Unknown	1(1)
Tetragnathidae	*Alcimosphenus*	1(0)
	Leucauge	48(6)
Theridiidae	*Nesticodes*	2(0)
Uloboridae	*Miagrammopes*	1(0)
Unknown	damaged juvenile	1(1)

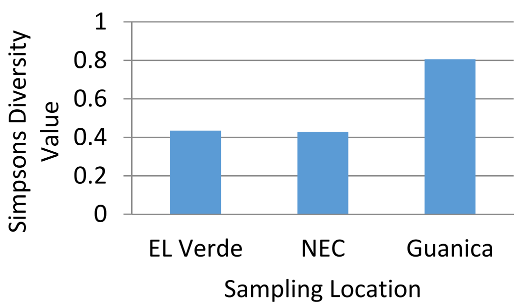

Figure 1. Simpsons Diversity Index results of the three sites sampled. Values range from 0 to 1, with values closer to one being the most diverse sample.

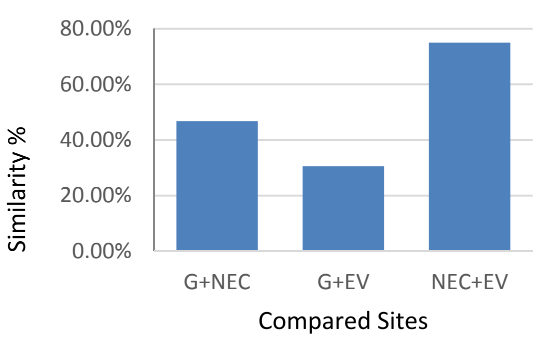

Figure 2. Community Similarity Index (CSI) between the three sites. G=Guanica, NEC=Northeastern Ecological Corridor, EV= El Verde.

DISCUSSION

The results from this project could possibly lead to several different aspects of understanding the spiders present in Puerto Rico. In addition to providing a baseline for further sampling and research in these areas, the findings could also lead to some potential theories of the movement and development of spider populations on the island. The families that were present in all three of the areas sampled, such as the *Araneidae, Uloboridae, and Tetragnathidae*, including the specific genus *Leucauge*, the most abundant genus in all three sites, may be considered somewhat more generalist groups of spiders in terms of their ecological needs, therefore could be better adapted for any of the ecoregions available to them on the island. This in turn gives way to the additional theory that some of the other groups sampled, that were just present in one if the three areas or in small abundance, for example, *Pholcidae, Selenopidae,* and *Salticidae*, may be more specialized as to what type of structures they need for habitat, or what kinds of resources they utilize. An example of this specialization may be seen in the case of the family *Pholcidae*, which have no adhesive property in their webs, and rely on the tangled nature of the web, as well as their own response speed for prey capture. This implies that areas with a higher density of small insects moving through may be more hospitable for this type of hunting strategy, as they are more likely to miss a feeding opportunity, whereas many other web building spiders utilize adhesive in their webs to secure prey in areas that may be less densely populated with insects.

The sites did show a large difference in diversity from one side of the island to the other, as the Guanica site, present on the southern side of the island, was calculated to be significantly more diverse than either of the other two sites on the Northeastern side, as well as being more distinct from the other two samples based on the CSI. As coppicing, or the regrowth of additional stems after stem falling, is a very common regeneration style of trees in this type of forest, an impenetrable maze of tangled stems and plant matter covers much of the terrain, and creates many opportunities to establish webs that will not be frequently disturbed. Also, dry forest ecoregions like the Guanica forest are said to be capable of high levels of diversity due to the fact that they are seasonally different when it comes to rainfall and climate, and can account for two or more different types of water and/or vegetation regimes, allowing different types of organisms to thrive in either the wet or the dry seasons. Although these seasonal wet and dry periods are present throughout the island, the dry season is especially accentuated in Guanica, and may in turn be an underlying factor for the high level of diversity calculated for the area. This is a possibility, but because there was only one sample collected in the NEC site on the opposite side of the island that was from a different family than those collected in Guanica, this leads to the belief that there may be other factors more likely affecting the increased diversity of the Guanica site, such as the increased habitat availability and less frequently disturbed areas.

The NEC and El Verde sites were observed to be fairly similar in the diversity of their samples with a community similarity of 75%, although both sites had markedly lower calculated diversity. This could have many possible explanations. There is a possibility that the frequent disturbances on the Northeastern side of Puerto Rico due to the prevalence of severe weather such as hurricanes may have an effect on the abundance of spider populations in those areas, specifically the NEC, as it is close to the Coast, and spiders tend to not be as abundant in commonly disturbed areas. The common disturbances could also result in a less complex ecosystem structure, and produce decreased habitat availability and structure that is not as hospitable for certain groups of spiders.

As the representative for the subtropical moist forest in the sampling, the NEC, seemed to have a moderate amount of habitat availability, in the form of small trees and vines, and had much more ground covered by growth than the El Verde site. This may be a factor of the moderately diverse sample collected, but also it is likely that because this was the first area sampled, the effort may not have been as efficient or effective as the other sites due to initial sampling hang-ups in the process of the changing sampling methods.

The El Verde site was initially expected to have a high diversity of spiders due to the renowned diversity of wet, subtropical rainforests. This changed quickly as the ecological impact of frequent and heavy rainfall was considered, as its effect on the establishment and maintenance of webs would likely hinder certain groups from thriving there. Also, with the presence of an extensive canopy cover, comes a lack of well-established shrubbery and plants in the understory and the presence of several large spaced out trees. This gives way to webs being built very high in between trees and in and among epiphytes or very low to the ground between saplings or rocks. In comparison to the other sites, the El Verde site did not seem to have as much habitat availability within the range of heights that were being sampled visually for this experiment, and due to standardization of sampling techniques, this may have led to its lower calculated diversity.

In contrast to these theories of ecological differences, it was observed that in the El Verde Site there were more examples of Families and Genus present there than the other two sites sampled, but its diversity was still low, which seemed incorrect in initial data exploration. This is likely due to the fact that abundances of each group found were very low, and in turn may imply that a more thorough sampling of all of these areas is necessary to be able to make any further, or more decisive, conclusions and comparisons about and between these sites.

Both the Northeastern Ecological Corridor and the Guanica site do not have extensive research done on this subject previously, so the data produced should be an exciting and beneficial baseline to understanding these ecoregions more. Also, because the island supports an impressive variety of organisms, and some of the areas being sampled have not been surveyed thoroughly for the type of organisms collected in this project, they may contain species that were previously not known to be present on the island. Further identification of the samples is necessary to be sure of this, but was not able to be completed within the scope of this project. But, as there is always an interest in supporting endemic and endangered species, the type of data collected in this study could aid in a better understanding of the island and its ecoregions, as well as the conservation of these areas, because as was said above, any information supplied by assessment of present organisms and their distributions supports conservation efforts very effectively.

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