

Inventory of Tropical Bees in the Madre de Dios Region

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Abstract

Insects, particularly bees, hold vital ecological roles as pollinators, decomposers, and components of food webs. However, their populations are declining due to anthropogenic factors and climate change, which threatens biodiversity, food security, and economic stability. This inventory focuses on bee species found in the Madre de Dios region of Peru, a biodiverse hotspot where entomophilous crops and non-timber forest products such as Brazil nuts rely heavily on bee pollination. The research explores the relationship between climate-associated phenological advances and bee pollinators, aiming to understand how these critical species respond to environmental shifts. The methodology involves trail monitoring, bee collection, pinning, and species identification using dichotomous keys. The study's findings shed light on the complex interactions between climate change, bee pollinators, and agricultural ecosystems, emphasizing the urgency of conservation efforts to safeguard both ecological and economic systems. This research underscores the significance of entomological studies in addressing global challenges, ensuring sustainable development, and promoting effective insect management strategies.

Keywords: Insects, bees, pollinators, inventory, climate change, phenological advances, biodiversity, conservation, agriculture, ecosystem services, entomology, Madre de Dios,

Introduction

Insects play a major role in the evolution and preservation of biotic communities. They are key pollinators of plants and crops, consumers and recyclers of decaying organic material, and vital components of food webs. As the world faces new challenges and extreme events of climate change increase, the populations of insects suffer drastically, affecting entire communities in a bottom-up system (Cornelissen, 2011). In order to preserve and improve environmental quality, reduce pesticide usage, increase crop production, control food costs, and increase trade in the global community, societies across the world need to put a larger focus on the study of their insects (Kumar et al. 2022).

Among insects, bees assume a paramount role as primary pollinators for the majority of plant species, therefore playing a vital role in natural ecosystems as well as cultivated environments, ensuring global food security for human populations (Borges et al., 2020). According to the World Economic Forum, the annual economic value attributed to pollination services is estimated at \$235-577 billion, yet the preservation of biodiversity, largely attributable to pollination, is valuable beyond quantification, making the substantial decline of global pollinator populations a cause for massive concern. Anthropogenic factors, including climate change and deforestation, bear responsibility for this decline (Goulson,

2019). The factors contributing to the diminishing populations of bee species encompass habitat loss resulting from alterations in landscape, resource competition with invasive species, emergence of novel species (including pathogens), and the adverse effects of pesticides (Borges et al., 2020).

Pollination in the Tropics:

While some plants and trees can be pollinated by wind, this is more common in ecosystems where neighboring plants are compatible (Snyder, 2009). In tropical regions, plant and tree species are hyperdispersed so the majority of plants and crops in this region are entomophilous, meaning pollinated by insects. This raises bees a prime asset to conservation in the western amazon, the most biodiverse place in the world. Contrary to previous belief that bees were ineffective at long distance pollination, studies including over 70 species of bees have found that some tropical bees will “trap-line” for distances of up to 95 km. (Pokorny et al., 2015 & Regal, 1982). This belief was first questioned and tested in the tropics due to the spacing of species individuals, requiring pollinators that can travel those long distances (Regal, 1982). For these reasons, tropical regions are a high-interest area for bee monitoring.

Inventories:

One way to monitor the insects in an area is through collections and inventories. Inventories provide documentation of the native and non-native insects found within a region and can help scientists to preserve, identify, and classify insects while also exploring the diversity and evolution of the ecosystems they inhabit. (Hendery, 2021). According to Dr. Muniappan, director of the IPM Innovation Lab, despite the immense value of insect collection,

disproportionate obstacles stand in the way of maintaining them in developing countries, further contributing to major agricultural and economic losses (Hendery, 2021).

Connecting Bees to Agricultural Success in the Madre de Dios Region:

Peru, a representative developing nation, substantially relies upon its natural resources as a pivotal driver of its economic growth (OECD, 2019). In the Madre de Dios region, the interdependent relationship between economically important plant species and bees as pollinators are significant for the betterment of environmental and societal communities in this region. Excluding corn, which is wind pollinated, papaya, watermelon, yucca, plantains, bananas, and pineapples are the main entomophilous crops that bring in revenue to small-scale farm holders. Most notably, castanas, or brazil nuts, are responsible for 67% of the annual income of the 30,000 people involved in the castana industry in the Madre de Dios region. (Flores 2002 and Campos 2006, cited in Nunes et al., 2012) While some of these crops have multiple insect pollinators, castanas are solely pollinated by bees due to the size of the flowers. Only large-bodied bees such as those in the genera *Xylocopa*, *Euglossine*, and *Bombus* are strong enough to pry open the flower hood to reach the nectar. Without these species, the Brazil nut trees would not survive (Cavalcante et al., 2012).

Monitoring Phenological Advances:

Rising global temperatures over the last five decades have led to shifts in the timing of biological events, known as phenological advances, across a diverse range of organisms including plants, birds, and insects (Sakai and Kitajima, 2019). It is essential to comprehend how various

species, especially vital pollinators, react to these climate changes. Moreover, numerous ecological functions arise from intricate species interactions (Abarca and Spahn, 2021). Since not all species react uniformly to climate warming, potential mismatches in phenology could occur, such as plants and trees flowering too soon before their pollinators emerge, resulting in the impairment of these functions. Conversely, if interacting species have developed similar responses to environmental shifts, they might be less vulnerable to the impacts of climate variation. (Bartomeus, 2011).

Projected Species:

This study was anticipated to center its focus on the taxonomic family Apidae, encompassing various bee species such as cuckoo bees, carpenter bees, digger bees, bumblebees, and honey bees. There is also a possibility of including representatives from the Megachilidae family, consisting of solitary bees like mason bees and leafcutter bees, as well as the Halictidae family, commonly known as sweat bees (Roubik, 1992). An examination of the species list compiled by the Alliance for a Sustainable Amazon for the Finca Las Piedras region, which was last updated in August 2021, reveals that Apidae exhibited the highest species richness within the area. The species recorded include *Aculeata* sp., *Euglossa* sp., *Eulaema* sp., *Trigona williana*, and *Xylocopa frontalis*. Additionally, the presence of one species of Halictidae, specifically *Augochlora* sp., was documented. Notably, previous studies conducted in the Madre de Dios region also recorded the occurrence of *Euglossa* sp., further emphasizing its significance (Roubik, 2004 & Dressler, 1985). These taxonomic families, comprising both social bees (example *Apis* spp. Honey bees) and stingless bees (meliponines), have specific foraging characteristics, necessitating a

continuous availability of resources throughout their extended foraging periods. However, they are also capable of adapting to patchy resource distributions (Ogilvie and Forrest, 2017). As a result, aseasonal tropical regions like Madre de Dios provide a favorable ecosystem for these bee populations to thrive.

Study Site

Primary and secondary forest ecosystems of the Madre de Dios region in Peru, specifically at Finca Las Piedras. Monitoring and collection took place during the dry season in the months of July and August. The weather on a typical day consisted of high humidity, clear sky, and temperatures ranging between 16.5 and 31 degrees Celsius. It's important to note that the onset of a dry season in many tropical forests promote the emergence of adult bees (Roubik, 2020), suggesting that the dry season is a viable time window for a collection diverse in species.

Methods

Trail monitoring and recording:

Regular monitoring of the trails in both primary and secondary forests was performed to identify locations of high bee density and bee families. This ongoing phase began prior to collection and continued on collection and non-collection days to optimize collection efficiency during the designated time window. High-activity areas were revisited and closely monitored on collection days to increase the diversity of species. Efforts were made to avoid collecting multiple specimens of the same species whenever possible to preserve populations.

Collection:

Bees were carefully collected using a bug net and kill jar. During observation, the bug net was always held upright in a ready

position to quickly capture any bees as they are quick to move locations. A sweeping motion over the bee moved them down into the net which was then closed by gathering the open end of the net with the other hand. Another technique was commonly used to collect specimens that were stationary on a plant or flower. The open end of the net was carefully placed over the bee and plant and closed with the opposite hand. Bees typically fly upward, so when the bee leaves the flower, they are trapped in the net. Once the bee was in the net, the bottom of the net is held upwards while the other hand gathers more net until the bee is in a small area of closed net. From here, the bee was moved into the kill jar. (Droege, 2010)

Three kill jars were brought into the field for each collection segment to allow for higher specimen counts. The kill jar consists of a glass jar with a layer of hardened plaster of paris on the bottom. The porous plaster was faintly covered with 10 drops of ethyl acetate immediately before placing the bee in the kill jar, this chemical quickly relaxes and kills the bee. This chemical is typically used in higher amounts to fully “charge” the kill jar prior to collection but was used sparingly in this collection due to the availability of resources. Acetone was eventually used as a substitute due to the low availability of ethyl acetate. With this new chemical, the kill jar was fully charged every collection segment with a full pipet dropper prior to going out into the field. This method avoids damaging any identifying parts of the bee. To move the bee into the kill jar, remove the lid and slide the jar up into the net so there are no gaps for the bee to escape. Once the bee flies down into the jar, quickly place the lid over the jar and close it. It takes about 20 minutes for the specimen to fully perish and can then be moved into the collection of bees ready to be pinned. (Droege, 2010). Observation and collection

were repeated until the collection window was concluded.

Pinning:

Collected bees were pinned according to Purdue’s entomology guidelines for proper preservation and identification (www1). A pinning station was prepared with a polystyrene block, insect pins, tweezers/forceps, magnifying glass, a headlamp, and a table lamp (Due to available resources, a stereo microscope was not used but is preferred to maneuver the small specimens into a more precise position). It is important to avoid using common pins as they tend to rust and can damage valuable specimens. While insect pins come in various sizes, size No. 2 was used for this collection.

It was made sure the bee was large enough to be supported on a pin without breaking or distortion. In cases where the bee was too small to be pinned, a small triangle card-point and nail hardener was used to glue the specimen to the tip of the paper. The specimen was placed with the triangle tip under the right side of the body on the mesothorax, allowing for diagnostic characteristics to remain visible. A pin was then placed through the large end of the paper triangle to display the specimen. When pinning all other specimens, the pin was inserted through the body, starting from the top and going through to the bottom. To assist in pinning, a 1-inch-thick polystyrene sheet was used. After placing the pin in the correct location, the pin was inserted into the foam until the bee rests on the surface. Approximately one-quarter to one-third of the pin protruded above the insect to facilitate handling and allow for space for identification tags below the specimen. Forceps or pin tips were used to adjust the position of the legs, antennae, and wings, securing them with additional pins as necessary. Each bee was adjusted to portray

its position in flight to show its features, and when possible, its mouth parts were pinned in a visible location. The specimen was allowed to dry in the desired position for 7 to 8 days before moving it. Once the specimen was dry, it retained its proper position, and the temporary supports were removed.

Species Identification and Display:

Identification of the collected bees was performed using relevant dichotomous keys, allowing classification down to highest taxonomical level possible. During this process, very limited keys were

available to assist in identification and without the use of a stereo microscope, many identifying characteristics were not clearly visible. Most of the specimens were identified down to family and tribe. Identification tags were pinned alongside each specimen. The first tag consists of collection location, elevation, date, gps coordinates, and the name of the collector. The second tag consists of the scientific name of the specimen. To display the collected specimens, they were categorized by family and tribe. All unidentified species were grouped together.

Tag ID Number	Family	Tribe	Scientific Name	Forest Type
7	<i>Apidae</i>	Apini		secondary
14	<i>Apidae</i>	Apini		secondary
17	<i>Apidae</i>	Apini		secondary
18	<i>Apidae</i>	Apini		primary
19	<i>Apidae</i>	Apini		secondary
26	<i>Apidae</i>	Apini		primary
11	<i>Apidae</i>	Centridini	<i>Centris lanipes</i>	secondary
4	<i>Apidae</i>	Euglossini	<i>Euglossa sp.</i>	secondary
30	<i>Apidae</i>	Euglossini		secondary
1	<i>Apidae</i>	Meliponini		secondary
2	<i>Apidae</i>	Meliponini		secondary
3	<i>Apidae</i>	Meliponini		secondary
9	<i>Apidae</i>	Meliponini		secondary
10	<i>Apidae</i>	Meliponini		secondary
16	<i>Apidae</i>	Meliponini		secondary
6	<i>Apidae</i>	Xylocopini	<i>Xylocopa latipes</i>	secondary
12	<i>Apidae</i>	Xylocopini	<i>Xylocopa similis</i>	secondary
25	<i>Apidae</i>	Xylocopini	<i>Xylocopa frontalis</i>	secondary
24	<i>Halictidae</i>	Augochlorini	<i>Megalopta genalis</i>	secondary
8	<i>Halictidae</i>			secondary
15	<i>Halictidae</i>			secondary
22	<i>Halictidae</i>			secondary
23	<i>Halictidae</i>			secondary
29	<i>Halictidae</i>			primary
5	Unknown			secondary
13	Unknown			secondary
20	Unknown			secondary
21	Unknown			secondary
27	Unknown			primary
28	Unknown			primary

Figure 1: Collected bees categorized by tag ID number, family, tribe, scientific name, and forest type.

Results

30 different species of bees were collected between the dates of July 6th and August 8th, 2023.

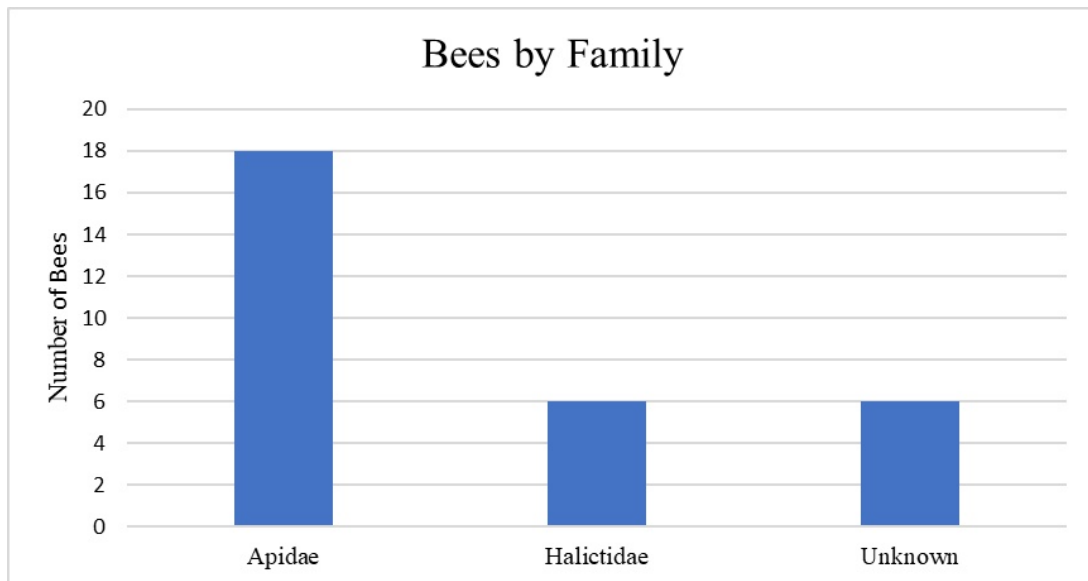


Figure 2: Collected bees categorized by Family.

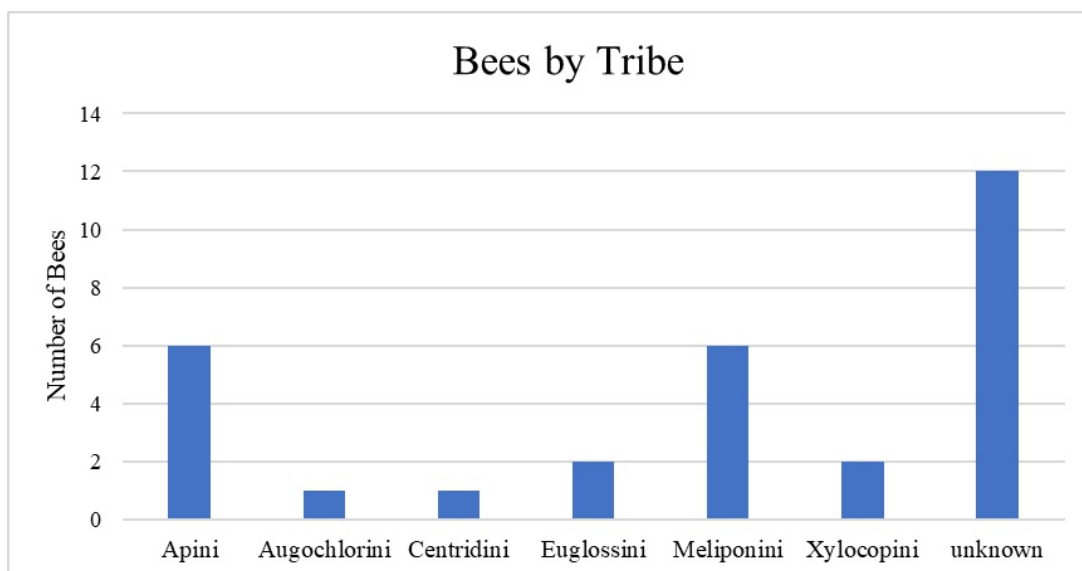


Figure 3: Collected bees categorized by Tribe.

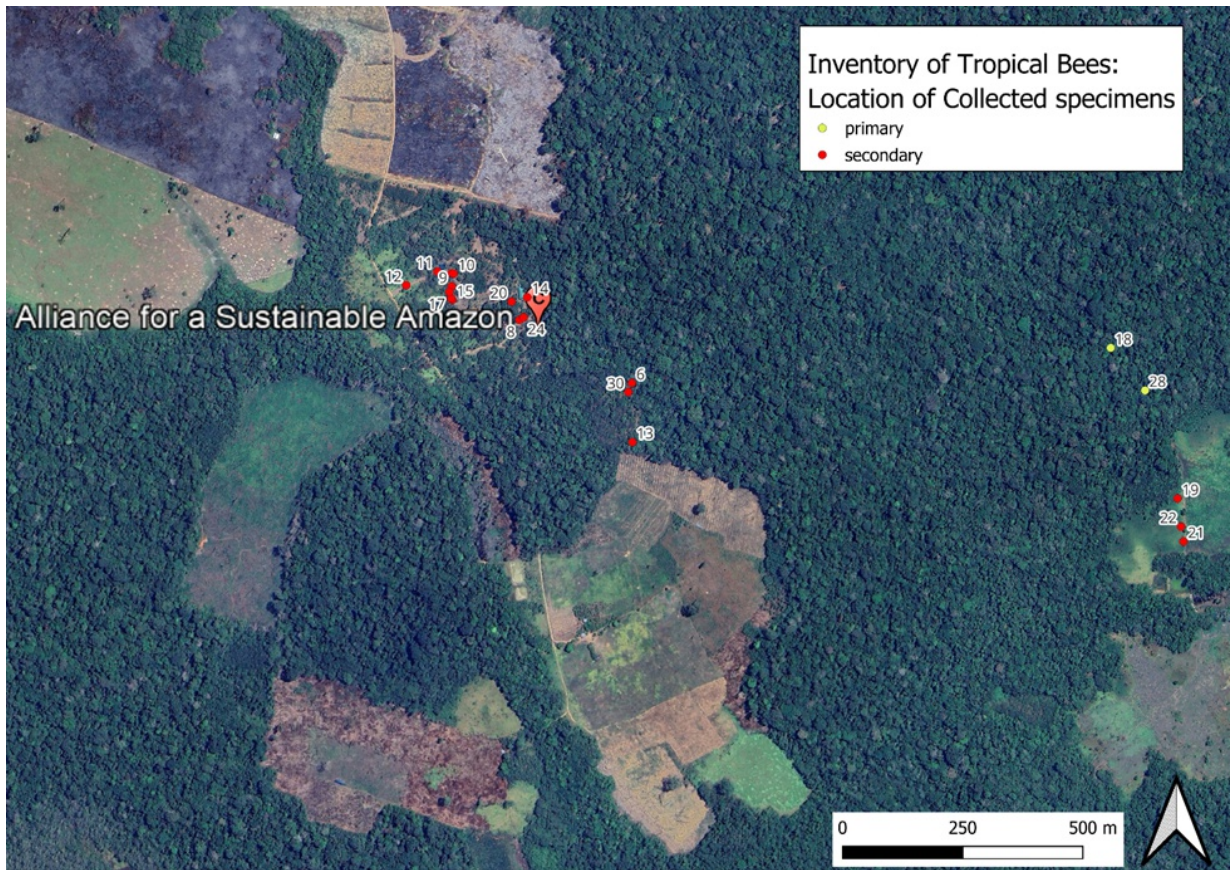


Figure 4: Map showing the locations of where each bee was collected, categorized by tag ID number and forest type.

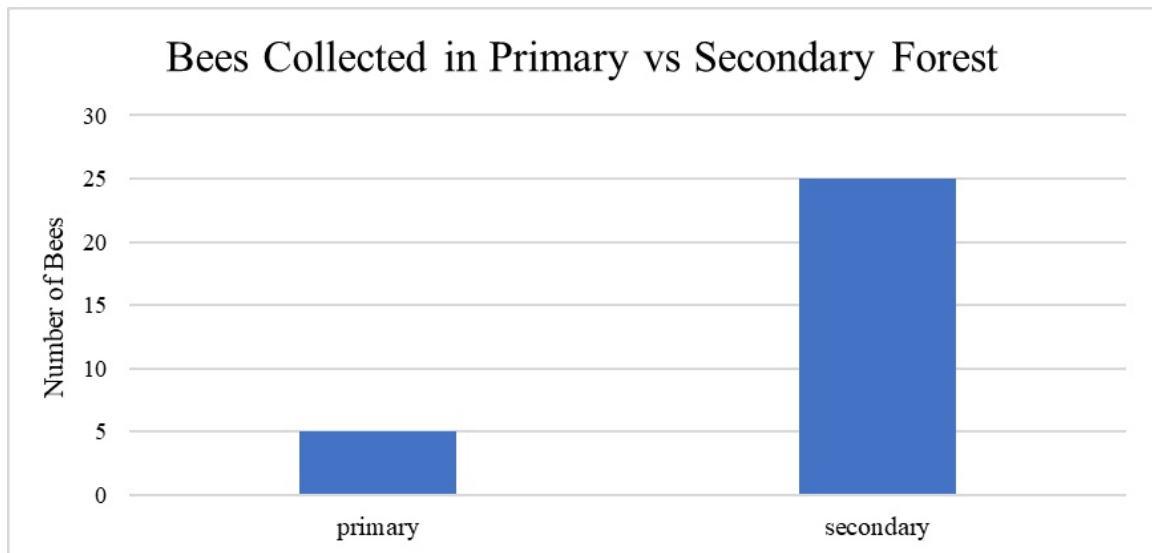


Figure 5: Bees categorized by forest type they were collected in.

Discussion

It's anticipated that this inventory combined with the research serves as a steppingstone to further bee collection and monitoring in the Madre de Dios region. The biodiversity, spatial dispersion of plant species, entomophilous dependent cash crops, and ecological interactions all serve as strong reasonings to understand and preserve the functioning and populations of tropical bees. Monitoring the phenological advances of bees in comparison to the plants and trees they pollinate is a top priority, as these ecological processes are the backbone of environmental and societal communities in this region. The Onset of the dry season promotes the emergence of many adult bees in tropical forests (Roubik, 2020), making this a prime time to begin monitoring and collecting to record their annual emergence dates. In addition, the onset of the wet season begins the emergence of flowers and fruits for many of the plants and trees in the western amazon. A collection window during both seasons would allow for a diverse inventory, and to take it a step further, the interactions of collected species with various entomophilous plant species can be recorded. This would allow for monitoring of these plants and trees, providing data for phenological research and mutualistic relationships.

Fires:

During the collection window for this inventory, interactions were recorded when applicable. While most interactions were herbivory and pollination. Notably on three different occasions, meliponini (stingless) bees were found in fighting swarms with the same species. The dates these observations took place were July 17th, 20th, and 24th and all occurred at different locations. A study on nest defense in stingless bees concluded that these fighting swarms typically comprise two colonies and are a response to the entry of non-nestmate

conspecific workers to a nest. (Gloag et al, 2008). These swarms can last for days, pausing at dusk and resuming at dawn, resulting in a high mortality rate for both the offensive and defensive colonies. Nest usurpation is the most likely cause for the fight, as the benefits for a reproducing colony taking over an existing, pre-provisioned nest can outweigh the costs of the attack. Gloag et al also hypothesizes that periods of low resources can incite these attacks, which could connect directly to the observations seen at Finca las Piedras. On July 19th, 20th, 21st, 26th, and 27th, farmers in the surrounding area were burning their land to make way for new crops. With these dates overlapping, it's reasonable to question if these fires destroyed their resources, resulting in the fighting swarms. While the first observation on the 17th serves as an outlier, this would be an interesting hypothesis to test as farmers continue to burn their land surrounding the Amazon rainforest.

Tips for Future Collections

Many things were learned through the process of creating this inventory of tropical bees at Finca Las Piedras. Being the first study and collection of bees at this site, necessary resources were not fully accessible creating some roadblocks along the way. In order to generate a larger and more diverse collection, there is a necessity for more thorough methods of collection, euthanasia, preservation, and identification. These are the issues that were faced during the process.

Euthanasia:

The research station was equipped with a small amount of ethyl acetate, the chemical most commonly used by entomologists to euthanize specimens in a kill jar. This meant that only small amounts could be used to charge the kill jar each day, resulting in a failure to kill the specimens in a timely manner. This put a strain on

collection and pinning. After testing various methods, acetone was eventually used as a substitution for the ethyl acetate. While this was a fair substitution in a time of desperation, obtaining a suitable amount of ethyl acetate for future collections is ideal as the acetone has the possibility to degrade and damage specimens.

Identification:

Dichotomous keys and proper technology for identifying bee species were limited, so some specimens were not able to be identified. Many species of insects are identified through characteristics such as mouth parts, antennae segments, and size of various body parts. Due to the small size of insects, a stereo microscope is used to get a clear enhanced view of these parts. With the absence of the microscope, a hand lens and head torch were used, but did not always provide a large enough view. In addition, the scarcity of dichotomous keys made it impossible to confidently identify all specimens. Obtaining a stereo microscope and various dichotomous keys for tropical bees would generate more precise data.

Pinning and Display Materials:

Insect pins and materials for display were also limited during this collection. In the absence of multiple insect pin sizes, a display box, and polyethylene foam sheets, a cardboard box, Styrofoam blocks, and insect pin size 2 were used. While these all served as a good substitute for these materials, obtaining a kit comprised of these materials would allow for better pinning and a more professional inventory that can be well preserved over a long period of time.

Collection Methods:

For this inventory, a bug net was used for collection in areas of high activity. This was a great method that allowed for the collection of 30 different species of bees but the inclusion of other collecting methods such as bowl traps, and the use of transects

would have helped to cover more ground, possibly resulting in greater species diversity.

Future Resources:

For future researchers that intend to expand on this inventory, it would be advisable to use these tips in combination with further research on bee monitoring methods and inventory guides/management to increase the quality of the collection and data. The National Protocol Framework for the Inventory and Monitoring of Bees, created by the National Wildlife Refuge System (2016) is a great place to start.

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