UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Sandfly (Diptera:Psychodidae:Phlebotominae) fauna, its ecology, and *Leishmania spp*. infections in the Ecuadorian Chocó forest throughout a degradation gradient.

Tesis de Maestría

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Trabajo de titulación de posgrado presentado como requisito
para la obtención del título de Magíster en Ecología Tropical y Conservación

Quito, May 13th 2025

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COLEGIO DE POSGRADOS

HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

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Lugar y fecha:	Quito, 13 de mayo de 2025.

ACLARACIÓN PARA PUBLICACIÓN

UNPUBLISHED DOCUMENT

AGRADECIMIENTOS

I would like to express my sincere gratitude to the Fundación para la Conservación de los Andes Tropicales (FCAT) for their warm reception and invaluable assistance during the fieldwork stage. Special thanks go to Carlos Aulestia, Luis Carrasco, Luis Zambrano, Alex Gualan, David Cantos, Karlita Zambrano, Darwin Zambrano, Jairo Cabo, Elizabeth Vásquez, Ailin Blasco and Jordan Cruz, who actively participated in the collection of samples and generously shared their local knowledge of the surrounding ecosystem.

I am also deeply grateful to my committee members, Dr. Patricio Rojas Silva, Dr. Renato Leon, Dr. Jordan Karubian and Dr. Veronica Barragán, for their support and guidance throughout the various stages of this process.

RESUMEN

Utilizando trampas miniatura de luz CDC, se capturaron un total de 1,023 especímenes de flebótomos hembras durante un período de 6 meses (marzo de 2024 a agosto de 2024) dentro de la Reserva Ecológica Mache-Chindul en Esmeraldas, Ecuador. Las recolecciones se realizaron en tres sitios (bosque primario, bosque secundario y periurbano). Todos los flebótomos capturados fueron identificados morfológicamente; se encontraron 15 especies: *Lutzomyia lichyi, Lu. hartmanni, Lu. gomezi, Ny. trapidoi, Psathyromyia aclydifera, Ps. shannoni, Sciopemyia sordellii, Pressatia dysponeta, Pr. triacantha, Evandromyia saulensis, Psychodopygus carrerai, Ps. panamensis, Pintomyia serrana y Dampfomyia vespertilionis*. En este estudio, comparamos la abundancia, riqueza y composición de especies en los sitios, y analizamos la infección por el parásito *Leishmania* en flebótomos grávidos y alimentados. De 76 muestras de flebótomos grávidos y con sangre recolectadas, 10 dieron positivo para *Leishmania.* Estos resultados podrían ser útiles para el futuro manejo de la leishmaniasis en la Selva Tropical del Chocó, una zona endémica de la enfermedad.

Palabras clave: Entomología Vectorial, Salud Pública, Ecología, Leishmaniasis, Selva del Chocó, REMACH.

ABSTRACT

Using miniature CDC-light traps, a total of 1,023 female phlebotomine sandflies were captured throughout a 6-month period (March 2024 – August 2024) within the Mache-Chindul Ecological Reserve in Esmeraldas, Ecuador. Collections were done in three sites (primary forest, secondary forest, and peri-urban). All captured sandflies were identified morphologically; 15 species were found: *Lutzomyia lichyi, Lu. hartmanni, Lu. gomezi, Ny. trapidoi, Psathyromyia aclydifera, Ps. shannoni, Sciopemyia sordellii, Pressatia dysponeta, Pr. triacantha, Evandromyia saulensis, Psychodopygus carrerai, Ps. panamensis, Pintomyia serrana, and Dampfomyia vespertilionis.* In this study, we compare species abundance, richness and composition throughout the sites, as well as, tested gravid and engorged sandflies for *Leishmania* parasite infection. Out of 76 gravid and bloodfed samples collected, a total of 10 sandflies tested positive for *Leishmani*a. These results could be helpful in future management of leishmaniasis inside the Chocó tropical rainforest, an endemic area for the disease.

Key words: Vector Entomology, Public Health, Ecology, Leishmaniasis, Chocó Rainforest, REMACH.

TABLA DE CONTENIDO

INTRODUCTION

Phlebotomine sandflies (Diptera: Psychodidae) belong to the family of blood-feeding nematoceran flies involved in the transmission of human and animal pathogens such as *Leishmania,* a protozoan parasite that causes leishmaniasis, *Bartonella bacilliformis,* the etiological agent of Carrion´s diseases and several arboviruses (Carvalho et al., 1941; Shimabukuro et al. 2017) From these diseases, leishmaniasis is the most important causing up to 1.2 million cases every year around the world (Alvar et al., 2012) and significant mortality due to the visceral form (VL) present especially in Brazil, India, Sudan and South Sudan (Belo et al., 2013). In Ecuador, although VL up to date is absent (one parasitological unconfirmed case notified by Leon in 1949 (Leon, 1957; Calvopiña et al., 2004), there have been cases of cutaneous (CL) and mucocutaneous leishmaniasis (MCL), reported from all 24 provinces including Galapagos islands (only imported cases) (Hashiguchi et al._ 2017; Salas et al., 2019). Although the disease is considered a priority of the World Health Organization (WHO), it is significantly neglected and misreported in Ecuador (Morales et al., 2019; Kato et al., 2016). Cutaneous leishmaniasis is the most common form of the disease, causing ulcers and leaving scars on the skin for life (Ramírez et al. 2016). To date, eight *Leishmania* species have been reported in Ecuador from which at least five have been detected in the coast region, namely, *Leishmania (Viannia) panamensis, L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) naiffi, and L. (V.) lainsoni* (Kato et al. 2016; Hashiguchi et al., 2017 ; Hashiguchi et al. 2020). To date, only 80 sand fly species have been reported from Ecuador in comparison to 122 and 156 from Bolivia and Peru respectively (Shimabukuro et al., 2017).

Of all the sandfly species, only a few species are anthropophilic and have been incriminated as vectors of the disease. For instance, *Nyssomyia trapidoi, Migonemyia migonei, Psathyromyia shannoni, Psathyromyia punctigeniculate, Pintomyia maranonensis*, *Pintomyia robusta*, *Lutzomyia gomezi* and *Lutzomyia spathotrichia* (Hashiguchi et al., 2020). Other species are suspected vectors such as *Lutzomyia hartmanni*, *Psychodopygus panamensis, and Trichophoromyia reburra*. Several past studies on *Leishmania* transmission and respective vectors have focused on *Ny. trapidoi* because of its abundance, competence as a vector, and highly anthropophilic behavior (Duque et al., 2020; Quiroga et al., 2017; Zapata et al., 2012). Due to urbanization and deforestation on the Pacific coast, vector species previously present only in pristine forests have adapted to secondary forests, crop plantations, and peri-urban areas (Barrera et al., 1994; Le Pont et al., 1994; Mouchet et al., 1994). Previous collection and molecular work done at Valle Hermoso, province of Santo Domingo de los Tsáchilas, found several phlebotomine sandfly species near peri-urban areas. The most abundant species was *Ny. trapidoi,* (Anaguano et al., 2015) which was also found infected with a new flavivirus, namely Paraiso Escondido virus (Alkan et al., 2015). Anthropogenic disturbances can affect sandfly distribution, with anthropophilic species like *Ny. trapidoi*, and *Lu. gomezi* that prefers intra-domicile and peri-domicile habitats corroborating previous studies, while zoophilic species like *Tr. reburra* prefer pristine forested habitats but may play a role as alternative vectors as well (Duque et al., 2020). Understanding sandfly ecology can elucidate pathogen transmission dynamics and contribute to a better understanding of pathogen natural cycles and eco epidemiology of the diseases.

Leishmaniasis is a neglected sandfly-borne disease endemic in 23/24 provinces of Ecuador and is significantly underreported (Morales et al., 2019). The transmission dynamics of the disease are poorly understood and complex, as at least eight *Leishmania* parasites and 80 sand fly species have been reported in the country. It is a zoonotic disease associated with forested areas where the parasite inhabits pristine areas and jumps between sylvatic reservoirs through the bite of infected sandflies. It has been reported that seasonality can affect the rate of infection in sandflies, showing that natural infection values ranged from 2.9% to 50% in the dry season, and from 6.3% to 12.5% in the wet season and that peri-urban habitats have higher sandfly diversity and a higher number of *Leishmania*-infected sandflies (Quiroga et al., 2017; Duque et al., 2020; Arrivillaga-Henríquez et al., 2017 and Wassenberg et al., 2003), suggesting that anthropogenic disturbance might enhance abundance and disease transmission by sandflies. We expect a similar trend in our molecular analyses of samples collected at the Fundación para la Conservación de los Andes Tropicales (FCAT).

While site and seasonality are important factors influencing sandfly abundance, elevation and precipitation data could provide deeper insights. Precipitation data can aid in understanding seasonal humidity changes, especially as climate change has made seasonality less predictable. Research on the effects of altitude and precipitation on sandfly composition and abundance is limited, but studies have shown that *Nyssomyia trapidoi* and *Trichophoromyia reburra* prefer areas with lower precipitation (Duque et al., 2020), while higher altitudes are linked to higher rates of *Leishmania* infections (Gomez et al., 2014). Including these factors could reveal important ecological drivers of sandfly distribution and *Leishmania* transmission.

Research into sandfly ecology and infection percentages relating to a habitat degradation gradient have been performed in Ecuador, however, information on phlebotomine sandfly ecology and composition at the Ecuadorian Chocó tropical rainforest in the Esmeraldas province is not clearly defined. Research into sandfly ecology and infection percentages relating to a habitat degradation gradient have been performed in Ecuador, however, information on phlebotomine sandfly ecology and composition at the Ecuadorian Chocó tropical rainforest in the Esmeraldas province is not clearly defined

In this work, we propose to assess the distribution of phlebotomine sandflies present in at the FCAT reserve, located in the Chocó forest, south of the Esmeraldas province, throughout a degradation gradient (primary, secondary forests, and peri-urban areas), as well as determine their status as a leishmaniasis vector. Our study aims to collect sandflies to elucidate their ecology, and *Leishmania* natural infection in sandflies to determine its implications on public health in the area. We also want to take into consideration factors such as temperature, precipitation, and humidity to examine their influence in species composition and abundance. Sand flies are the only vector for leishmaniasis, a neglected tropical disease, and it affects rural communities without access to medical care. The Chocó forest in Esmeraldas, Ecuador is a hotspot for this disease.

The initial Young and Duncan classification of sand flies based exclusively on morphology has been modified using cladistical analyses of morphological characteristics and current molecular data led Galati to propose new genera and raise many *Lutzomyia* subgenera to genera status (Galati, 1995). For the sake of consistency, we will refer to sandfly species using Galati´s classification.

METHODS

Location and Site Description

Data collection will occur at the Fundación para la Conservación de los Andes Tropicales (FCAT) reserve, located within the Mache-Chindul Ecological Reserve (REMACH), Esmeraldas's province. REMACH is a 120,000 ha protected area that contains a transect of untouched Chocó rainforest habitat, a threatened habitat that holds one of Ecuador's most biodiverse communities of species. Elevation throughout REMACH ranges from sea level to 700 m a.s.l. and temperatures vary between 23 °C to 25.5 °C (Clark et al., 2006). FCAT covers an area of 1,500 acres and provides a living research station where researchers can conduct studies and contribute efforts towards its conservation. Four communities currently live near the reserve (Sancudo, Aniceto, Laguna, and Descanso), totaling an estimated 1800 people. Maps of REMACH and FCAT locations are in the Appendix, Figure 1a.

Primary (undisturbed) forest at FCAT is characterized by old growth tree species like Cedar (*Cedrela angusticarpa*), Andiroba (*Carapa guinensis*), and Guayacán (*Handroanthus guayacan*). There are also a few species of palm trees in primary forest sites, that serve as an important food source for several mammal and avian species. These sites have not been affected by anthropogenic activities and have a higher concentration of epiphytic plants like bromeliads and orquids, as well as an abundance of woody vine species.

Secondary (previously disturbed) forest is characterized by having previously been primary forest but was cut down and replaced with a grass species (*Brachicera sp*), and cacao species (*Theobrema cacao*) for agriculture. This area was bought by FCAT, and natural reforestation has been in place for two decades. The first succession plants are mostly from the Piperaceae family, followed by Secropias and some species from the Lauraceae family. Trees on this site have not been long enough to see large presence of epiphytic plants and woody vines.

Peri-urban (currently disturbed) sites were characterized by proximity to homes, inside a 50-meter radius, and domesticated animals like dogs, pigs, chickens and cattle. Vegetation is minimal, or dominated by commercial agricultural species like cacao, plantain and introduced invasive grass species. Map and sample points locations are in the Appendix, Figure 1b.

Sandfly Collection

Sandfly surveys were carried out during a 6-month period using CDC miniature light traps according to the methods described in Anaguano et al. 2015. Traps were placed at dusk (6:00 pm), left overnight, and collected at dawn (6:00 am) when sandflies are known to be most active. Two separate traps were placed on each site, four nights a month resulting in 24 collections per month, 144 collections in total for 6 months. The collected insects were stored in a freezer (30 min at -20°C), placed in 75% ethanol or Shield solution, transported to the Medical Entomology Laboratory (LEMMT) at USFQ, and stored at -20°C.

FCAT aided with sandfly collections by sampling once a month for 6 months, starting March 2024, and ending August 2024.

Morphological Identification

Marc André solution was used for tissue clarification of the head and the last abdominal segments. Female sandfly spermatheca, cibarium, horizontal and vertical teeth, and male genitalia including the style, coxite, paramere, lateral lobe, genital filaments, and genital pump were key morphological structures used for identification through keys by Young & Duncan, 1984 and Galati, 1995. For this study, we only morphologically identified females because of their bloodfeeding behavior. Gravid and engorged females were separated individually in Shield solution for DNA extraction and *Leishmania* detection.

Statistical Analyses

We utilized R studio to assess and visualize differences in abundance, diversity, and composition of sandflies across the three sites: Primary forest (PF), secondary forest (SF), and Peri-urban (PU). Bar graphs and plots were made to help visualize the abundance and distribution of female sandflies, while linear models quantify each independent variable's influence and interactions with total abundance. ANOVA tests how explanatory variables (Precipitation, Temperature and Humidity) interact and impact sandfly abundance and composition. To evaluate the completeness of our sampling effort, we applied rarefaction and extrapolation curves using the iNEXT R-studio package.

FCAT has a meteorological station within its reserve, which provided us with daily precipitation, temperature and humidity data for the 6 months of sandfly collections. The average of the values for all the independent variables was calculated by month, and those values were used for all statistical analyses.

iNEXT coverage-based rarefaction/extrapolation & Composition Similarity

In ecological studies, abundance data are often used to compare species across sites, but it's important to account for differences in sampling effort, especially when comparing sites like Primary forest and Peri-urban areas. iNEXT coverage rarefaction/extrapolation helps estimate species richness while correcting for unequal sampling efforts. The values 0, 1, and 2 in the iNEXT graphs represent different diversity indices: Species Richness (0) measures the total number of species, the Shannon Index (1) accounts for both richness and evenness, and the Simpson Index (2) focuses on species dominance.

Species composition similarity was analyzed using the SpadeR package. This package allows for the comparison of species composition on each site (PF, SF and PU) by grouping them in pairs and using diversity-based similarity indices (C12 (Horn), C22 (Morisita), and U22 (Regionally adjusted)). C12 index is solely based on species relative abundance, C22 emphasizes dominant species in their analysis, and U22 is a regionally adjusted extension of C22.

Molecular Work on Engorged and Gravid Sandflies

DNA extraction of engorged and gravid females was done with Qiagen Extraction Kit. Engorged and gravid female sandflies were dissected, and their abdomens stored separately in Shield solution (Zymo) to detect *Leishmania* infection. Detection of the *Leishmania* parasitic infection was performed with Polymerase Chain Reaction (PCR) using the JW-11F and JW-12R primers, to amplify a small fragment of *Leishmania* kinetoplastoic minicircles. These primers are

120 base pairs long: JW11, 5'-CCT ATT TTA CAC CAA CCC CCA GT-3' and JW12, 5'-GGG TAG GGG CGT TCT GCG AAA-3' (Zapata et al. 2012). Positive controls used were segments of DNA from *Leishmania guyanensis* and *Leishmania mexicana.* PCR protocol followed these conditions: Initial Denaturation at 94 for 3 minutes. Then, 35 cycles of denaturation at 94 for 30 seconds, annealing at 58 for 30 seconds and extension at 72 for 30 seconds. Final extension at 72 for 5 minutes.

RESULTS

Abundance & Distribution

Samples collected from March through August 2024 resulted in 1, 669 sandflies, of which 1,023 were female. Samples analyzed revealed 17 different sandfly species, 15 of which could be identified morphologically: *Lutzomyia lichyi, Lu. hartmanni, Lu. gomezi, Ny. trapidoi, Psathyromyia aclydifera, Ps. shannoni, Sciopemyia sordellii, Pressatia dysponeta, Pr. triacantha, Evandromyia saulensis, Psychodopygus carrerai, Ps. panamensis, Pintomyia serrana, and Dampfomyia vespertilionis.* The most abundant species is *Tr. reburra* with 303 individuals, followed by *Ny.trapidoi* with 257 and *Lu. hartmanni* with 115 collected specimens.

Trichophoromyia reburra was most abundant in primary forest, with only 4 individuals found in peri-urban areas. *Nyssomyia trapidoi* was abundant in both primary and secondary forests with 9 individuals found in peri-urban areas. *Lutzomyia hartmanni*, *Ps. aclydifera, Lu. gomezi, Ny. trapidoi, Pi. serrana* are the six species currently shared between the three sites, primary and secondary forests and peri urban. Distribution of sandfly species between sites looking at individuals collected in March through August shows an expected trend. *Trichophoromyia reburra* is a species typically associated with primary forests and is not considered highly anthropophilic.

Lutzomyia gomezi and *Lu. hartmanni* are considered highly anthropophilic and vectors of *Leishmania*, and they are both found in peri-urban sites. Individuals who could not be identified morphologically will be identified using molecular analyses in the future. The monthly distribution of female sandflies is illustrated in Figure 2.

Statistical Analyses

Abundance data for all species across the three sites were totaled by month to create a total abundance dataset. This dataset was used to visualize the monthly total abundance of female sandflies, categorizing the months by name. Once the total abundance of sandflies per month was calculated, it was followed by a Shapiro-Wilk normality test on the abundance values. The test yielded a p-value of 0.0001636, indicating the data were not normally distributed. Given this, A Kruskal-Wallis rank sum test was applied to compare abundance across months, which revealed a p-value of .5152 suggesting no significant difference in total sandfly abundance between months.

A Kruskal-Wallis rank sum test was applied to compare abundance across sites, resulting in a p-value of 0.004784, suggesting a significant difference in abundance between sites. To identify which specific site pairs contributed to this difference, a Dunn's test was conducted. The PF-SF pair showed no significant difference, but the PF-PU and SF-PU pairs had significant p-values of .0012 and .005, respectively. These results indicate that PU sites are driving significant differences in abundance between sites.

Linear Model

A generalized linear model was performed in r, using Total_Sum (total # of Individuals) by month as the response variable with site, precipitation, temperature and humidity as the independent variables. This model allows us to see how the interaction of the independent variables affects the total abundance of sandflies, and which has the most impact.

A non-binomial model was used, to fit the overdispersion of the abundance data:

```
nb_model <- glm.nb(Total_Abundance ~ Precipitation + Temperature + RelHumidity + Site,
data = SSP)
```

This model considers all independent variables and reveals that the site has the strongest relationship with total abundance. A negative coefficient and p-value of 1.26×10^{-9} for the peri-urban site suggests a significant negative correlation between PU sites and sandfly abundance. Precipitation also showed a marginal negative correlation with abundance with a negative coefficient and p-value of .0786. Although the value is not significant, it's close to .05. Temperature and humidity did not show a significant relationship with abundance.

An ANOVA was performed on the generalized linear model above to test the interactions between the independent variables and total sandfly abundance per month. While the ANOVA suggested that relative humidity likely contributes to the variation in total abundance ($p = 0.125$), further analysis with likelihood ratio test comparing models with and without humidity showed that models with humidity did not improve the model fit significantly ($p = 0.207$).

Species distribution by site

A total of 15 morphologically identified species were found across the primary forest, secondary forest, and peri-urban sites in FCAT. The distribution of eight species differed significantly across certain site pairs. *Lutzomyia hartmanni* differed in the SF-PU pair. *Sc. sordellii, Ps. aclydifera, Py. panamensis, Ny. trapidoi* in both PF-PU and SF_PU pairs. *Psychodopygus carrerai* in PF-SF and SF-PU pairs. *Lutzomyia lichyi* was the only species to differ in all site pairs, this might be due to only being present in the PF site. Species distribution and site pairs can be visualized in Figure 3.

iNEXT coverage-based rarefaction/extrapolation & Composition Similarity

Sample coverage values of the primary forest, secondary forest and peri-urban area are .99, .99 and 1, respectively. Coverage-based analysis revealed that PF has a qD value of 8.41, a q=1 value of 5.87 and a q=2 value of 4.53. This suggests the primary forest site has an average of 8 species, in which 5 are constant and 4 are dominant. The SF site has a qD value of 9.48, a q=1 value of 5.87 which suggests a less even species distribution than PF sites, and a q=2 value of 4.54. Compared to the PF sites, SF sites might have an average of 9 species but have a slightly higher species richness and evenness. PU has a qD value of 5.92, a q=1 value of 5.06 and a q=2 value of 4.53. Values can be visualized in Figure 5.

Composition similarity between sites was determined using the SpadeR package in R. The results show that the mean estimate of similarity across pairwise comparisons is .815, indicating moderate similarity between sites, but not identical. The site pair with the least composition similarity is PF-PU with a C12 value of .674, C22 value of .696 and U22 value of .821. SF sites, however, showed an interesting similarity to PU sites with a C12 value of .810, C22 value of .959 and U22 value of .979. Composition results can be found in Figure 4.

Molecular Analyses

DNA was extracted from a total of 76 gravid and blood-fed sandflies collected from all three sites. PCR on 75 samples revealed a total of 10 positive *Leishmania* infected sandflies. Positive samples can be seen in Table 3. Six positive samples came from the PF location, and four from the SF location. Of the positive samples, four belonged to *Lutzomyia hartmanni*, three to

Nyssomyia trapidoi, One from *Psychodopygus carrerai*, *Sciopemyia sordellii,* and *Pressatia triacantha.*

DISCUSSION

Sandfly Fauna in the Pacific Coast

The results of this research revealed a high number of sandfly species spread through primary forest, secondary forest and peri-urban areas at and around FCAT. Five anthropophilic species: *Psychodopygus panamensis*, *Nyssomyia trapidoi, Lutzomyia gomezi, Lutzomyia hartmanni, and Lutzomyia shannoni.* Ten non-anthropophilic species were also recorded: *Trichophoromyia reburra, Pressatia dysponeta, Pressatia triancantha, Psychodopygus carrerai, sciopemyia sordellii, Psathyromyia aclydifera, Dampfomyia vespertilionis, Pintomyia serrana, Lutzomyia lichyi, Evandromyia saulensis.* Sandfly collections in provinces near Esmeraldas, and along the Pacific coast, report that the most abundant species in this region are *Ny. trapidoi, Tr. reburra, Ps. panamensis, Lu. hartmanni, Lu. aclydifera* (Hashiguchi et al., 2014; Hashiguchi et al., 2020; Enriquez et al., 2024; Anaguano et al., 2015; Duque et al., 2020; Le Pont et al., 1994; Zapata et al., 2015). Collections and analysis in (Hashiguchi et al., 2020) in Manabí during November 2016 and 2017 reported 11 species, of which 8 were anthropophilic. Collections by (Anaguano et al., 2015) were also done during 2 months in Santo Domingo de los Tsáchilas, July 2013 and March 2014. Six anthropophilic sandfly species were described in this locality, although the paper only mentions the species in which female sandflies were found engorged. *Nyssomyia trapidoi* was one of the most abundant species in both publications. These studies help compare species composition at FCAT with those found in other areas throughout the Northern Pacific coast region.

Sandfly species composition in FCAT closely resembles that of other collection sites done in the Northern Pacific coast, with the most abundant anthropophilic and vector species being *Ny. trapidoi and Lu. hartmanni*.

Some of the same studies that report sandfly fauna along the Pacific coast also test for *Leishmania* parasite infections. Two of these studies found *Ny. trapidoi* and *Lu. hartmanni* infected (Le Pont et al., 1994), (Hashiguchi et al., 1985a) in the North-western Andes. Out of the 10 infected individuals that tested positive for the parasite, 3 were *Ny. trapidoi* and 4 were *Lu. hartmanni.* Results obtained from testing engorged and gravid sandflies in this research corroborate results from the papers above and emphasize *Ny. trapidoi* as a possible vector and further incriminates *Lu. hartmanni* as a *Leishmania* vector in this site.

Sites species composition and assemblages

In this study, *Ny. trapidoi* was the second most abundant species found throughout secondary forest and peri-urban sites, and second most abundant after *Tr. reburra* in primary forest. In terms of species composition, SF and PU sites showed a more similar species composition than PF and PU sites. Secondary forest has a similar abundance composition (Horn) when compared to primary forest, but presence/absence composition indices (Morisita and Regionally adjusted) are closer to those of PU sites. These results highlight the change of species composition and how they can still be observed after decades of natural reforestation. Also, when you consider the dominant species present in SF and PU sites, *Ny. trapidoi* and *Lu. hartmanni* are two of the main dominant species in terms of abundance on both sites, while in PF *Tr. reburra* is the most dominant species, overpassing *Ny. trapidoi* by almost 100 individuals.

Ecuador has a system of nationally protected areas (SNAP), which area areas characterized by high biodiversity and is managed by the Ministerio del Ambiente, Agua y Transición Ecológica

(MAAE). REMACH, which contains a small fraction of the Chocó rainforest stretch that runs from southwest Panama to Northwest Ecuador is part of SNAP territory. These protected areas are vulnerable to anthropogenic activities such as deforestation, forest degradation, and fragmentation. Between the years 1990 and 2018, approximately 4% of accumulated deforestation occurred within SNAP boundaries (Kleeman et al., 2022). Research on the effects of deforestation of vector infectivity has been conducted on endemic areas in South America. A study done in Argentina investigating the edge effect (deforestation) on populations of sandflies, found increased abundance of vectors of tegumentary leishmaniasis (TL) in the buffer zone between primary vegetation and crop vegetation (Quintana et al., 2012). Another study in Northeastern Brazil emphasized that the expansion of anthropological activities that relate to changes in land use produces occupiable niches, affects fauna composition and vector reservoir that could lead to change in local leishmaniases epidemiology (Pinheiro et al., 2021). Secondary forest sites in our study were previously agricultural land, which changed the composition of sandfly species and provided a surge of occupiable niches as natural regeneration took hold.

Future Work

Gravid and engorged female sandflies were prioritized for molecular work and *Leishmania* infections in this study. Next steps for these samples are to test for host feeding behavior by elucidating what host DNA is found in the blood-fed specimens. Further analysis involving DNA sequencing will be done with the sandflies that tested positive for *Leishmania* to determine the parasite species. Knowing what *Leishmania* species are found at these sites can help build a more contextual image of transmission dynamics at REMACH.

In order to gather more ecological context on sandfly ecology and *Leishmania* parasite transmission dynamics, all sandflies collected need to be identified. Male sandflies collected throughout the sites will be morphologically identified to verify the composition of species at each site and further analyze species composition and abundance. Knowing sandfly abundance, both female and male) can provide ecological context that can lead to a plan for vector management and monitoring.

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FIGURES

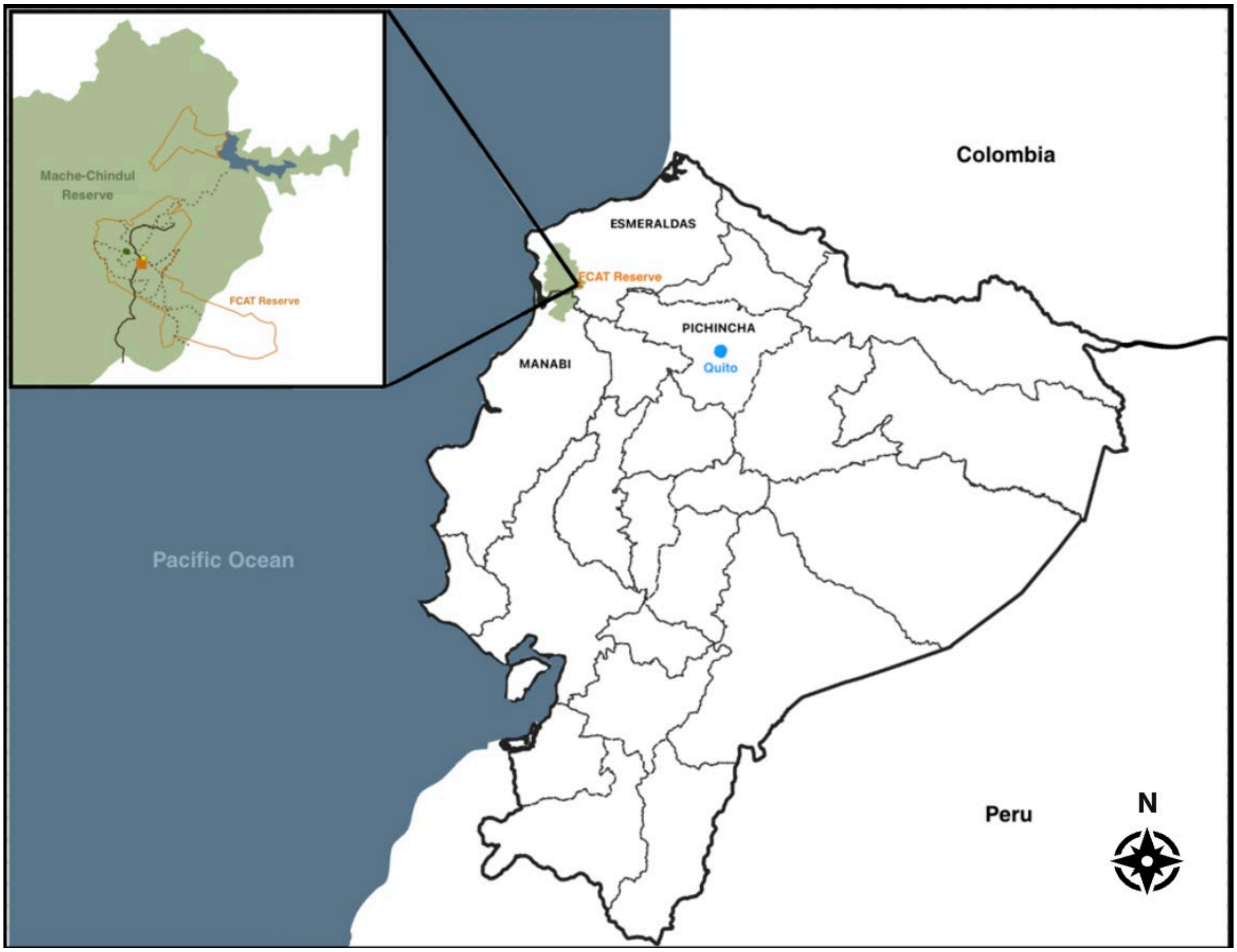

Figure 1a. National Map depicting the location of the Mache-Chindul (REMACH) Reserveand Fundacion para la Conservación de los Andes (FCAT) reserve. Located near the Pacific Coast, West of the Northern Andes.

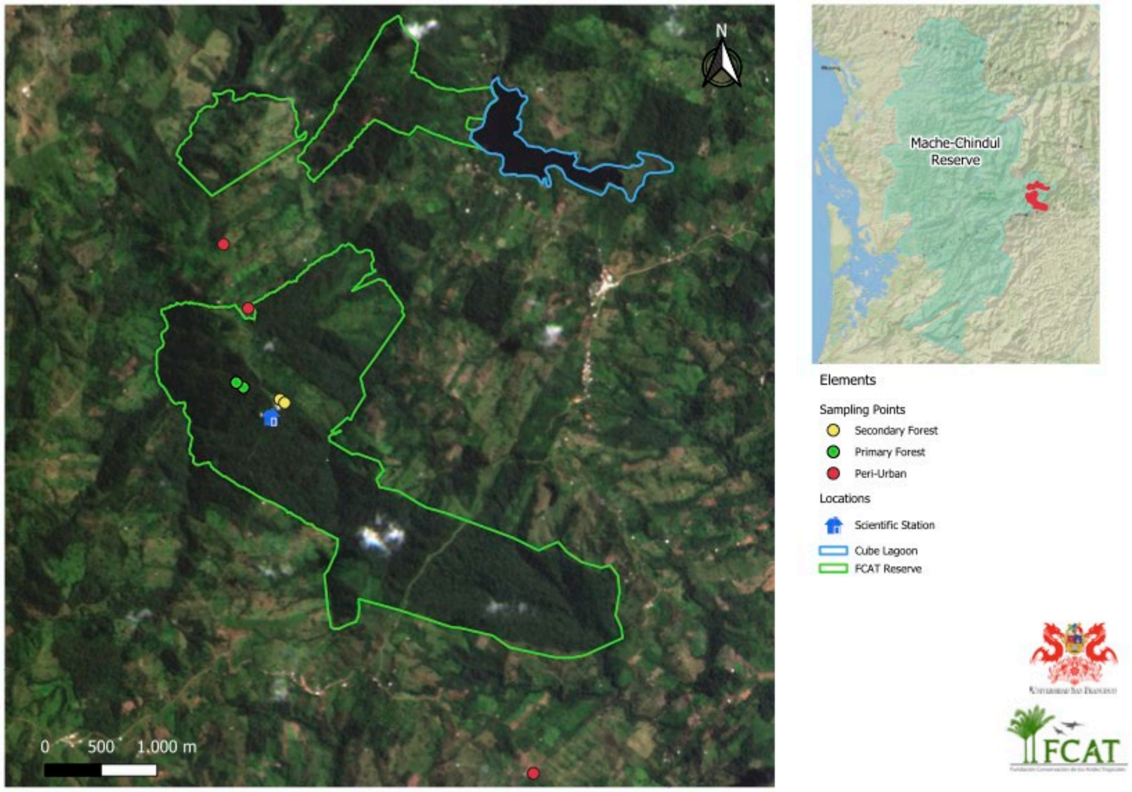

Figure *1b. Collection Site locations within the FCAT reserve.*

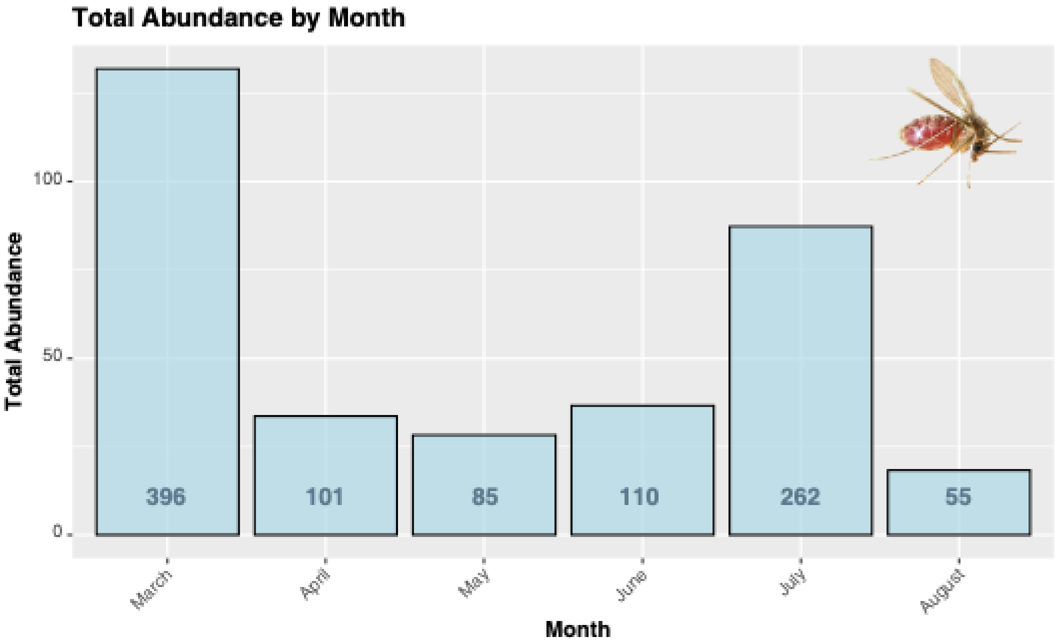

Figure 2. Bar graph depicting sandfly abundance per month collected from the months of March 2024 through August 2024.

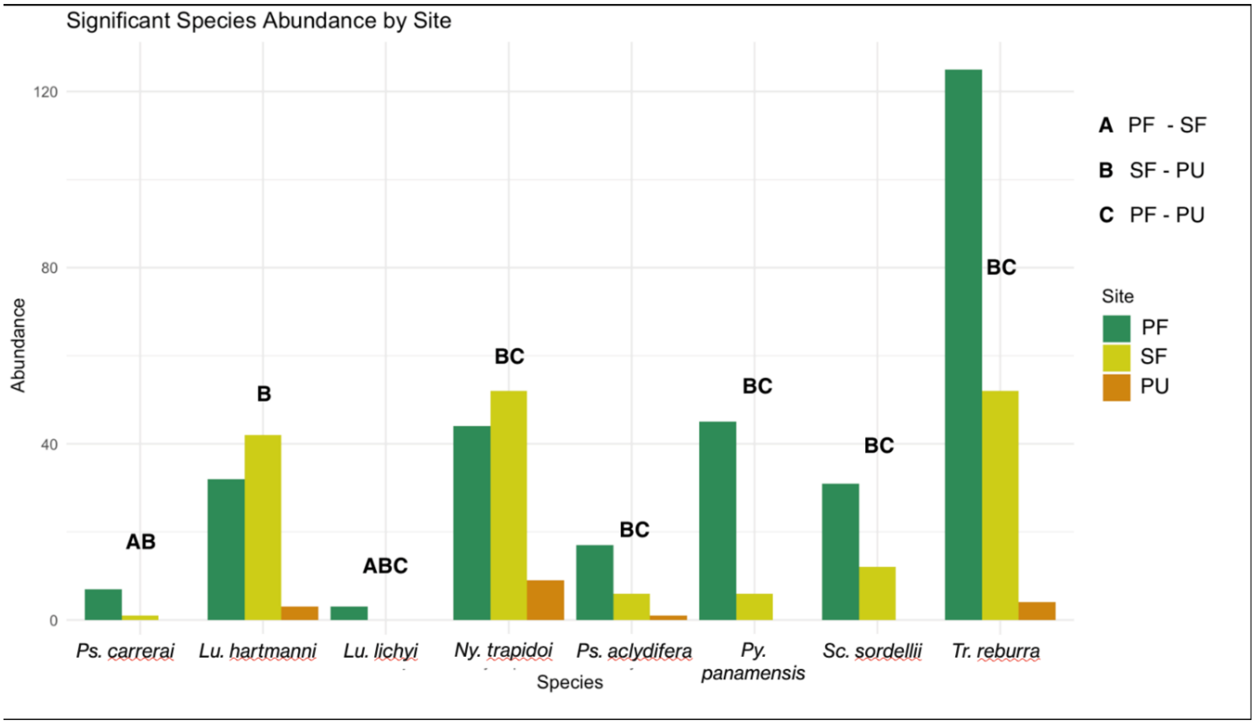

Figure 3. Species that showed a significant abundance difference between site pairs. A total of 8 species out of the 15 found throughout the sites showed an important difference in abundance between sites.

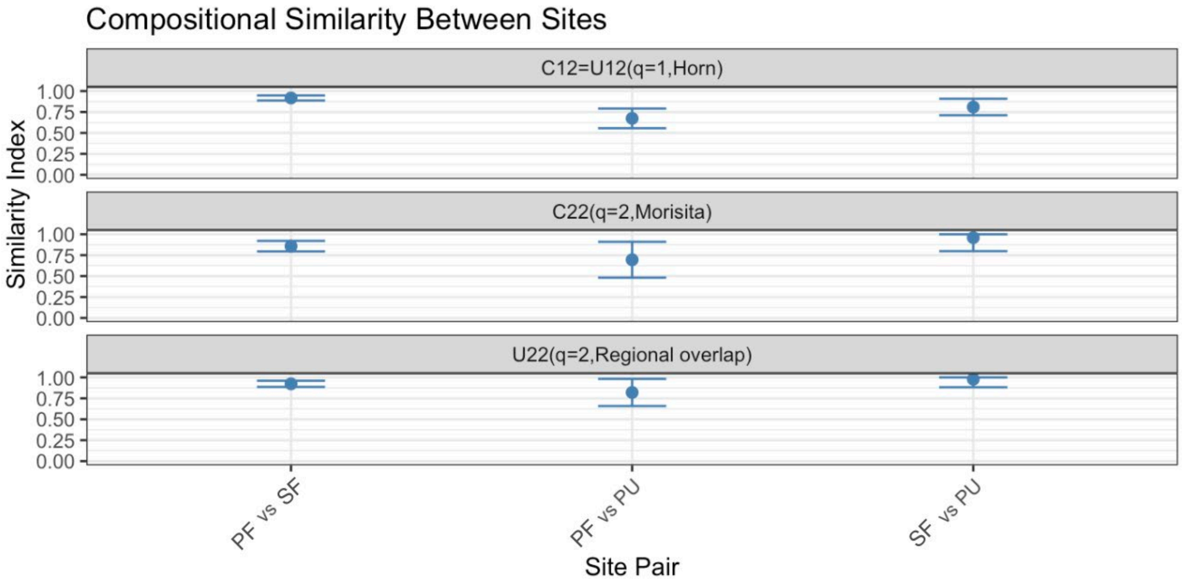

Figure 4. Composition similarity values between site pairs. PF and SF show a high species composition similarity; however, SF also shows a high similarity to PU sites in Morisita and Regional overlap indexes.

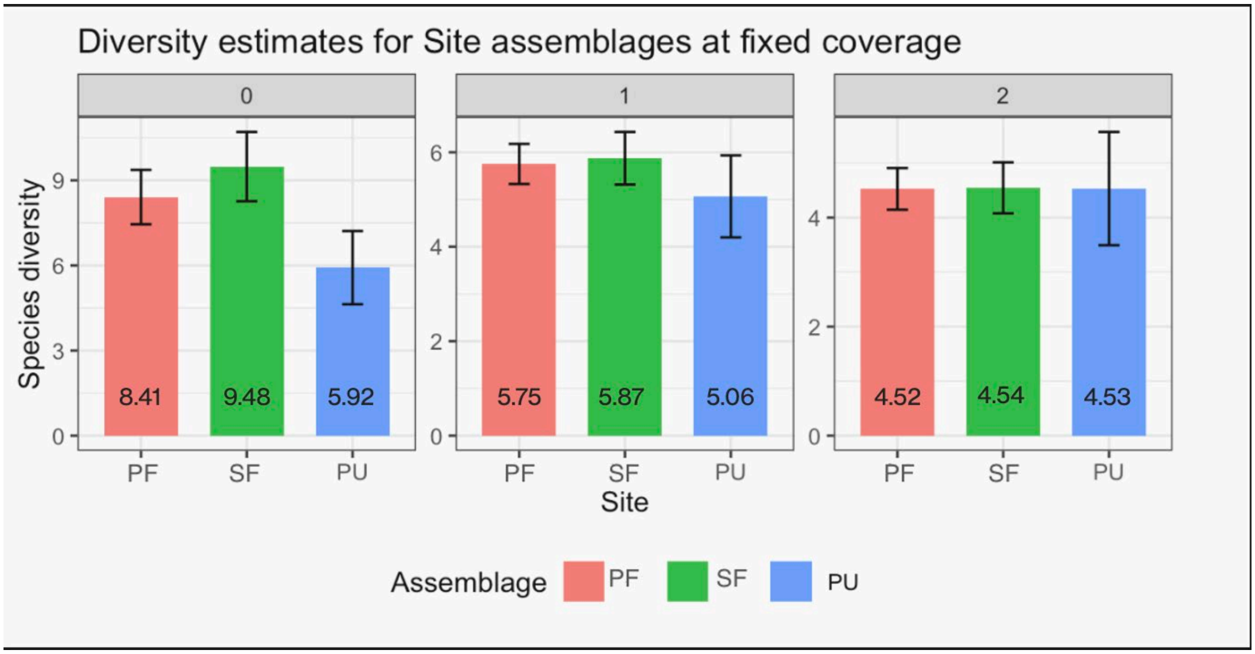

Figure 5. Assemblage diversity estimates for each site, normalized at 0.96 coverage. Secondary forest shows a slightly higher species richness and evenness than primary forest.

TABLES

Species	# of individuals per site			Total Female Sandflies
	PF	**SF**	**PU**	
Trichophoromyia reburra	216	83	4	303
Nyssomyia trapidoi	117	131	9	257
Lutzomyia hartmanni	43	66	6	115
Sciopemyia sordellii	74	26	0	100
Psychodopygus panamensis	74	21	0	95
Psathyromyia aclydifera	38	22	1	61
Psychodopygus carrerai	19	1	0	20
Dampfomyia vespertilionis	3	12	0	15
Pintomyia serrana	3	4	3	10
Pressatia triacantha	5	5	0	10
Lutzomyia gomezi	1	1	4	6
Lutzomyia lichyi	5	0	0	5
Psathyromyia shannoni	2	2	0	4
Pressatia dysponeta	2	2	0	4
Evandromyia saulensis	0	3	0	3
UNK.	10	4	0	14

Table 1: These numbers are derived from samples collected monthly in March – August 2024. Traps were placed weekly on each site, resulting in 24 samples total per month.

Species	Site Pairs		
	PF-SF	PF-PU	SF-PU
Lutzomyia hartmanni	NS	NS	Significant
Trichophoromyia reburra	NS	Significant	Significant
Sciopemyia sordellii	NS	Significant	Significant
Psathyromyia aclydifera	NS	Significant	Significant
Psychodopygus carrerai	Significant	NS	Significant
Psychodopygus panamensis	NS	Significant	Significant
Nyssomyia trapidoi	NS	Significant	Significant
Lutzomyia lichyi	Significant	Significant	Significant

Table 2: Species that show significant differences in abundance between site pairs.

Sample #	Site	Code (Site_especimen#+Date)	Species	*Leishmania* spp. (JW: 120 bp)
4	BP1	BP1_00120240323	*Ny. trapidoi*	+
2	BS1	BS1_00220240324	*Ny. trapidoi*	+
9	BS2	BS2_00220240324	*Lu. hartmanni*	+
18	BP2	BP2_00420240325	*Lu. hartmanni*	+
25	BP1	BP1_00120240426	*Lu. hartmanni*	+
27	BS1	BS1_00220240426	*Ny. trapidoi*	+
45	BP2	BP2_00420240616	*Sc. sordellii*	+
59	BS2	BS2_00220240729	*Pr. triacantha*	+
63	BP1	BP1_00120240730	*Lu. hartmanni*	+
72	BP2	BP2_00520240829	*Ps. carrerai*	+

Table 3: Leishmaniasis infections on gravid sandflies. Ten samples tested positive for the parasite, five in PF and five in SF.