UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Assessment of the diversity of subgenus *Hoffmania* Fox, *Culicoides* (Diptera: Ceratopogonidae) at three different height levels during 2023 at the Tiputini Biodiversity Station.

.

Daniel Die Morejón

Biología

Trabajo de fin de carrera presentado como requisito
para la obtención del título de
Biología

Quito, 9 de mayo de 2025

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

HOJA DE CALIFICACIÓN DE TRABAJO DE FIN DE CARRERA

Daniel Die Morejón

Nombre del profesor, Título académico Sonia Zapata Mena, Ph.D & Juan Daniel Mosquera

Quito, 9 de mayo de 2025

© DERECHOS DE AUTOR

Por medio del presente documento certifico que he leído todas las Políticas y Manuales de la Universidad San Francisco de Quito USFQ, incluyendo la Política de Propiedad Intelectual USFQ, y estoy de acuerdo con su contenido, por lo que los derechos de propiedad intelectual del presente trabajo quedan sujetos a lo dispuesto en esas Políticas.

Asimismo, autorizo a la USFQ para que realice la digitalización y publicación de este trabajo en el repositorio virtual, de conformidad a lo dispuesto en la Ley Orgánica de Educación Superior del Ecuador.

Nombres y apellidos: Daniel Die Morejón

Código: 00321793

Cédula de identidad: 1720891561

Lugar y fecha: Quito, 9 de mayo de 2025

ACLARACIÓN PARA PUBLICACIÓN

UNPUBLISHED DOCUMENT

RESUMEN

El género *Culicoides* (Diptera: Ceratopogonidae) está compuesto por pequeños jejenes hematófagos que son importantes para la salud humana y animal, ya que pueden transmitir virus, parásitos y causar dermatitis. Dentro de este género, el subgénero *Hoffmania* sigue siendo poco conocido, especialmente en los bosques amazónicos. En este estudio, analizamos la abundancia de *Culicoides Hoffmania* y no *Hoffmania* a tres alturas diferentes del bosque (M1: sotobosque, M2: subdosel, y M3: dosel) durante el año 2023 en la Estación de Biodiversidad Tiputini, en Ecuador. También investigamos, mediante métodos moleculares, de qué hospedadores se estaban alimentando las hembras ingurgitadas.

En total, se analizaron 1247 hembras de *Culicoides*. La mayoría de los individuos se encontraron en el dosel (M3), lo que sugiere que las poblaciones de *Culicoides* en general prefieren las capas superiores del bosque. Sin embargo, al observar únicamente a las hembras del subgénero *Hoffmania*, se encontró que eran proporcionalmente más abundantes cerca del suelo (M1), lo que sugiere una posible preferencia de microhábitat para este grupo. La precipitación tuvo un efecto positivo fuerte sobre la abundancia tanto de *Hoffmania* como de no *Hoffmania*, especialmente en el sotobosque y el dosel, lo que resalta la importancia de la lluvia para su reproducción y supervivencia. En cambio, la temperatura tuvo un efecto más débil y variable, aunque se observó una correlación negativa clara entre la temperatura y la abundancia de no *Hoffmania* a nivel del suelo.

A través del análisis de contenido estomacal, encontramos que los *Culicoides* se alimentaban principalmente de mamíferos arbóreos como perezosos (*Choloepus* sp.) y monos (*Lagothrix* sp., *Alouatta* sp.), muchos de los cuales son reconocidos como reservorios de enfermedades zoonóticas. Estos hallazgos sugieren que los *Culicoides* podrían tener un papel más importante en la transmisión de enfermedades en la Amazonía de lo que se pensaba anteriormente. En conjunto, el estudio destaca la complejidad vertical de la distribución de *Culicoides* en los bosques tropicales y revela diferencias ecológicas interesantes entre los subgéneros. Los resultados del análisis trófico también sugieren que *Hoffmania* y sus parientes cercanos podrían estar participando en ciclos de enfermedades zoonóticas.

Palabras clave: *Culicoides*, *Hoffmania*, Estratificación vertical, Bosque amazónico, Análisis de contenido estomacal, Transmisión de enfermedades zoonóticas.

ABSTRACT

The genus *Culicoides* (Diptera: Ceratopogonidae) is made up of tiny blood-feeding midges that are important for human and animal health because they can transmit viruses, parasites, and cause dermatitis. Inside this genus, the subgenus *Hoffmania* is still poorly understood, especially in Amazonian forests. In this study, we looked at the abundance of *Hoffmania* and non-*Hoffmania Culicoides* across three different heights of the forest (M1: understory, M2: subcanopy, and M3: canopy) during 2023 at the Tiputini Biodiversity Station in Ecuador. We also checked what hosts engorged females were feeding on using molecular methods. In total, 1247 female *Culicoides* were analyzed. Most individuals were found at canopy level (M3), which suggests that overall *Culicoides* populations prefer the upper layers of the forest. However, when we looked just at *Hoffmania* females, they were proportionally more abundant closer to the ground (M1), hinting at a possible microhabitat preference for this group. Rainfall had a strong positive effect on both *Hoffmania* and non-*Hoffmania* abundance, especially at the understory and canopy, showing how important rain might be for their reproduction and survival. On the other hand, temperature had a weaker and more variable effect, although a clear negative correlation between temperature and non-*Hoffmania* abundance was found at ground level. Through bloodmeal analysis, we found that *Culicoides* were feeding mainly on arboreal mammals like sloths (*Choloepus* sp.) and monkeys (*Lagothrix* sp., *Alouatta* sp.), many of which are known to be reservoirs for zoonotic diseases. These findings suggest that *Culicoides* could play a bigger role in disease transmission in the Amazon than previously thought. Overall, the study highlights how vertically complex *Culicoides* distribution is in tropical rainforests and shows some interesting ecological differences between subgenera. Bloodmeal results also suggest that *Hoffmania* and its close relatives could be participating in zoonotic disease cycles.

Key words: *Culicoides*, *Hoffmania*, Vertical stratification, Amazon rainforest, Bloodmeal analysis, Zoonotic disease transmission

CONTENT TABLE

TABLE INDEX

FIGURE INDEX

1. INTRODUCTION

Culicoides is a genus of small flies (around 1 -3 mm) commonly known as biting midges within the family Ceratopogonidae, described to possess a worldwide distribution between the latitudes 40° north and 30° south (Fetene et al., 2022). This genus is one of the most diverse, having roughly ~1,300 described species around the globe (Augot et al., 2017a). The genus *Culicoides*, comprises 32 subgenera and 38 species groups, far outnumbering other Ceratopogonids (Augot et al., 2017b). This rich diversity, combined with a wide geographic range, shines a light on the importance of *Culicoides* as a subject of medical, veterinary, and ecological research.

The prevalence of *Culicoides* in the medical and agroindustry fields is noticeable as the genus has been associated with ~40 diseases for both humans and animals, acting as vectors of pathogens and directly causing allergic reactions due to their bite (Crilly et al., 2016; Sick et al., 2019a). Viruses are among the several pathogens transmitted by *Culicoides*. Many species are known vectors of arboviruses that affect humans, wildlife and especially livestock (Sick et al., 2019a). One well known example is *C. insignis, a* vector of Bluetongue virus (BTV) that causes disease in ruminants, resulting in great yearly economic loss (especially in the wool industry) across the globe (Mulholland, 2016). In the Amazon, *C. paraensis* is the main vector of Oropouche virus (OROV), an arbovirus that is responsible for the Oropouche fever in humans (Feitoza et al., 2025). Although rare, human deaths by Oropuche fever have been recorded in recent years (Bandeira et al., 2024). These examples and others, such as African horse sickness, epizootic hemorrhagic disease, and Schmallenberg virus are some of the viruses transmitted by *Culicoides* (Sick et al., 2019b).

In addition to viruses, *Culicoides* can also transmit protozoan and filarial parasites. Some species have been recognized to be a part of the life cycles of protozoa from the genera *Haemoproteus* and *Leucocytozoon* (bird parasites) (Yu et al., 2000; Žiegytė et al., 2021). In other instances, *Culicoides* engorged females have been detected carrying parasites such as *Leishmania* (although true vector status hasn't been confirmed) (Kaewmee et al., 2023). Filarial worms responsible for diseases like mansonellosis and dermatozoonoses in humans are also carried by *Culicoides* (Sherlock, 1965; Ta-Tang et al., 2018). *Culicoides* are important actors in different disease-host relationships in the wild, thus indirectly aiding in population regulation of some host species they feed on (Sick et al., 2019a).

Beyond their role in disease transmission, *Culicoides* midges are an integral part of ecological networks such as tropical forests. Biting midges occupy different niches depending on the lifecycle stage they are in (Zimmer et al., 2014). Larvae develop in moist substrates like mud, leaf litter, tree holes or bromeliad axils and are part of the microbiome that helps decompose organic matter . On the other hand, adult male feed on nectar acting as pollinators while the better-known females feed on animal blood, acting as bridges between pathogens and their hosts (Spinelli et al., 2007; Zimmer et al., 2014). More importantly, *Culicoides* act also as a food source for insectivore animals, thus being an important species within tropical food webs (Kar et al., 2022). *Culicoides* aren´t only "pests" but they key participants in ecosystem dynamics and balance.

The taxonomy of *Culicoides* is complex with numerous subgenera and informal species groups (Mosquera et al., 2022). These subgenera, species groups and species of *Culicoides* are identified by morphometric and morphological characteristics based on wings spot patterns, mouthparts, antennae, spermathecae and others (Mosquera et al., 2022; Spinelli et al., 2005). One subgenus of particular interest is *Hoffmania* Fox, 1948, comprised of ~40 species (Trindade & Felippe-Bauer, 2011). This subgenus is strictly Neotropical and includes the known BTV vector *Culicoides insignis* Lutz (Trindade & Felippe-Bauer, 2011; Vigil et al., 2018) . Members of *Hoffmania* generally share a distinctive white spot on the second radial cell of their wings (R2) (Spinelli et al., 1993). In Ecuador the subgenus *Hoffmania* is particularly diverse with 18 species reported in the country, making up 25.7% of the total 70 species of *Culicoides* reported in Ecuador (Moscoso & Sofía, 2014). Other studies carried in Ecuador have also reported high abundances of *Hoffmania Culicoides* when doing surveys, with one study carried in Santo Domingo province even reporting that 75% of females captured belonged to the subgenus (Moscoso & Sofía, 2014). The high diversity and abundance of *Hoffmania* in Ecuador could be underestimated when considering that vertical strata of tropical forest has remained greatly understudied.

Forest habitats and, especially, tropical forests possess canopies that often host different species compositions from the understory, and *Culicoides* are no exception. Studies in the USA and Europe have observed that *Culicoides* species exhibit preferences between forest canopy and ground level habitats (Bernotienė et al., 2024; Erram & Burkett-Cadena, 2021). These studies mention that it could be in response to host availability and microclimatic conditions. In the case of the Amazon, factors such as temperature, humidity, and light availability greatly differ between the upper canopy and understory and in other Diptera members these differences do cause important species partitioning alongside a vertical plain, with many groups showing higher abundance at different strata (de Souza Amorim et al., 2022). Understanding vertical stratification is crucial for ecological studies in the Amazon, as vertical complexity might be even higher than horizontal complexity, since a higher percent of the biomass is in the upper strata (de Souza Amorim et al., 2022). The inaccessibility of the mid and upper canopy has limited sampling causing a huge loss of information, obscuring the true ecological dynamics that might be happening. Located within Yasuní National Park in the Ecuadorian Amazon, the Tiputini Biodiversity Station supports exceptionally high biodiversity across multiple taxa, including Diptera (Linton et al., 2013). Its remote location far from human settlements and its mosaic of microhabitats, notably the Varzea floodplain and Terra Firme Forest, provide a unique setting for examining the intricate ecological interactions that structure lowland tropical rainforest communities (Linton et al., 2013). At Tiputini, which features a multistrata rainforest environment, investigating vertical stratification of *Culicoides* will help determine if the patterns noted on cold regions hold true scale to a tropical context.

This preliminary study seeks to contribute to the understanding of the vertical distribution and species composition of *Culicoides* from the subgenus *Hoffmania* and non *Hoffmania* species across three forest strata at the Tiputini Biodiversity Station during 2023. It also explores the potential influence of climatic variables, such as temperature and precipitation, on the monthly abundance patterns of both *Hoffmania* and non-*Hoffmania* species. In addition, the study examines the trophic preferences of blood-fed females through molecular analysis to identify the vertebrate hosts they utilize and assess whether host associations vary across forest strata.

2. ORIGIN OF THE SAMPLES

The samples were collected at three vertical sampling levels (M1, M2, and M3) in the observation tower of the Tiputini Biodiversity Station with coordinates 0.63778° S, 76.14584° W. The samples were collected monthly from January to December of 2023 and stored in 70% ethanol at -20 °C at the Laboratory of Parasites and Vectors, Universidad San Francisco de Quito. Female *Culicoides* specimens were received and separated from the raw samples taken at Tiputini, except for female *C. paraensis,* which were separated for a different study.

3. METHODOLOGY

3.1. Abundance of *Hoffmania* and non *Hoffmania Culicoides*

3.1.1 Specimen identification of the subgenus *Hoffmania*

In this study, only female *Culicoides* were used for taxonomic identification. *Hoffmania* specimens were identified using the taxonomic keys described by Spinelli et al., (1993). Specimens were observed under a stereoscope (Olympus SZ2-ILST), and the phenotypical trait used for the identification of the subgenus was the presence of a white spot that completely or partially covered the R2 radial cell of the wing. *Hoffmania* and non *Hoffmania* specimens were classified by strata (M1, M2 or M3) and by month of collection in 1.5ml Eppendorf tubes with 70% ethanol.

3.1.2 Abundance analysis

Python (version 3.11.6) was used to calculate monthly total abundances for each group (*Hoffmania* and non *Hoffmania*), as well as their abundances across the three vertical strata. For each stratum and group, monthly trends were visualized using smoothed line plots to identify seasonal patterns. Normality of the abundance data per month was tested using the Shapiro–Wilk test. If the data did not follow a normal distribution, the non-parametric Kruskal–Wallis test was used to compare abundances across strata and months. Analyses and visualizations were performed using the libraries pandas, scipy.stats, matplotlib, seaborn, and statsmodels.

3.1.3 Abundance vs climatic variables

Temperature and precipitation data were obtained from the meteorological station at the Tiputini Biodiversity Station (TBS), which recorded values twice daily at a height of 2 meters, totaling 1,656 measurements. The dataset was filtered to obtain only measurements related to the TBS observation tower. Daily temperature values (°C) were averaged to calculate monthly mean temperatures, while monthly precipitation (mm) is represented as the sum of daily rainfall totals per month.

Monthly abundances of *Hoffmania* and non-*Hoffmania Culicoides* at each vertical stratum (M1, M2, M3) were then compared against the corresponding monthly climatic variables. Due to non-normal data distribution (confirmed via Shapiro–Wilk test), Spearman's rank correlation was used to evaluate the relationship between abundance and each climatic variable. To visualize seasonal trends, line plots were generated showing monthly abundances for each group and stratum alongside temperature (as a red line) and precipitation (as a green line). Abundance and climate data were plotted on dual y-axes to facilitate interpretation of concurrent trends. All analyses and visualizations were performed using Python (version 3.11.6) and the libraries pandas, numpy, scipy, matplotlib, and seaborn.

3.2. Bloodmeals analysis

3.2.1 Species level identification of blood-fed female *Culicoides*

Engorged *Hoffmania* and non-*Hoffmania Culicoides* females were separated and dissected using fine syringe needles. The head, wings, legs, and the terminal three abdominal segments were removed and cleared in a drop of Marc André solution (lactic acid, acetic acid, phenol, and distilled water in a 20:2:1:1 ratio) for 5 minutes to facilitate morphological examination. The remaining abdominal portion, containing the bloodmeal, was preserved in DNA Shield at –20 °C for subsequent molecular analysis.

For slide preparation, the cleared body parts were removed from the Marc André solution, and a drop of chloral gum was placed on a clean glass slide. The dissected structures were arranged within the drop, after which a coverslip was carefully placed over the specimen. The slides were left to dry at room temperature. Morphological identification to the species level was performed using a Leica DME light microscope and the taxonomic key of Wirth et al., (1988).

3.2.2 DNA extraction

DNA was extracted individually from the remaining abdomen of each engorged *Culicoides* female using the PureLink™ Genomic DNA mini kit (Mammalian Tissue and Mouse/Rat Tail Lysate Protocol), following the manufacturer's instructions. DNA was stored at –20°C for further analysis.

3.2.3 PNOC marker amplification and sequencing

The Prepronociceptin (PNOC) gene, a mitochondrial marker specific to mammals, was used to identify the vertebrate hosts of engorged *Culicoides* females. PCR amplification was performed using the following reaction mix, prepared with the Promega® PCR kit: 1× colorless buffer, 1.2 mM $MgCl_2$, 0.4 mM dNTPs, 0.4 µM of each primer—PNOC forward

(5′-GCATCCTTGAGTGTGAAGAGAA-3′) and reverse (5′-TGCCTCATAAACTCACTGAACC-3′)—and 1 U/µL of Taq DNA polymerase.

PCR conditions were as follows: an initial denaturation at 98 °C for 8 minutes; followed by 50 cycles of denaturation at 96 °C, annealing at 56 °C, and extension at 72 °C (each step lasting 30 seconds); and a final extension at 72 °C for 5 minutes.

Amplicons were analyzed via electrophoresis on a 1.5% agarose gel with a 100 bp DNA ladder (Promega®). Electrophoresis was run at 100 V for 40 minutes. Gels were visualized using a photo-documentation system (BioRad). The presence of a band at approximately 330 bp indicated the presence of vertebrate (mammalian) DNA.

Samples showing a positive PNOC band were lyophilized and sent for Sanger sequencing at Macrogen Inc. (Seoul, South Korea).

4. RESULTS

4.1. Overall abundance patterns of *Hoffmania* vs no *Hoffmania*

A total of 1,247 *Culicoides* females were identified from the Tiputini tower collections in 2023 (Figure 1). Of these, 19.49% were collected at stratum M1, 33.52% at M2, and 46.99% at M3, indicating that nearly half of all females were captured in the uppermost stratum (Figure 2).

To assess vertical stratification in abundance, a Kruskal–Wallis test was conducted for both *Hoffmania* and non-*Hoffmania* specimens across the three strata (M1 $n = 12$; M2 $n = 12$; M3 $n = 12$). No significant differences were detected in abundance among strata for either group (*Hoffmania*: $H = 3.52$, $p = 0.172$; non-*Hoffmania*: $H = 4.26$, $p = 0.119$) (Figure 2).

Seasonal variation in abundance was also evaluated across monthly samples ($n = 3$), revealing no statistically significant differences in either group (*Hoffmania*: $H = 15.33$, $p =$

0.168; non-*Hoffmania*: H = 16.53, p = 0.122), despite visible fluctuations in monthly patterns (Figure 3).

4.2. Climate *Hoffmania* vs no *Hoffmania* Abundance

Climatic variables from 2023, specifically temperature and total monthly precipitation recorded at the Tiputini tower, were compared with the abundance data of *Culicoides* females from the same year.

The Spearman's rank correlation analysis revealed a significant positive association between monthly precipitation and the abundance of *Hoffmania* (ρ = 0.36, n = 36, p = 0.032), as well as for non-*Hoffmania* specimens (ρ = 0.35, n = 36, p = 0.034) (Figure 3). When analyzed by stratum, both groups exhibited a strong positive correlation with precipitation at M1 (*Hoffmania*: ρ = 0.62, n=12, p = 0.032; non-*Hoffmania*: ρ = 0.65, n=12, p = 0.021). At M2, correlations were weak and not statistically significant for either group (*Hoffmania*: ρ = -0.01, n=12, p = 0.975; non-*Hoffmania*: ρ = 0.23, n=12, p = 0.457). At M3, non-*Hoffmania* abundance was significantly correlated with precipitation (ρ = 0.60, n=12, p = 0.040), while *Hoffmania* abundance showed a positive but non-significant trend (ρ = 0.52, n=12, p = 0.083).

In contrast, temperature showed different trends when analyzed by stratum (Figure 4). At M1, a significant negative correlation was observed between temperature and non-*Hoffmania* abundance (ρ = –0.60, n = 12, p = 0.039), while *Hoffmania* showed a non-significant negative trend (ρ = –0.39, n = 12, p = 0.208). At M2, *Hoffmania* exhibited a weak, non-significant correlation with temperature (ρ = –0.22, n = 12, p = 0.474), and non-*Hoffmania* showed a slight positive but non-significant association (ρ = 0.21, n = 12, p = 0.488). No significant correlation with temperature was detected at M3 for either group (*Hoffmani*a: ρ = –0.27, n = 12, p = 0.395; non-*Hoffmania*: ρ = –0.12, n = 12, p = 0.725).

4.3. Blood-fed female analysis

Of the 64 blood-fed *Culicoides* females analyzed, 22 species were identified across nine subgenera or species groups, including Daedalus, Diphaomyia, Eublepharus, Fluvialis, Haematomydium, *Hoffmania*, Leoni, Mohave, and Reticulatus (Table 1). Twenty of these had been previously recorded in Ecuador (Mosquera et al., 2022). *Culicoides mirsae* and one unidentified *Culicoides* from the Daedalus group are newly reported for the country. *PNOC* amplification yielded four positive specimens. Sequencing identified *C. leopoldi* feeding on *Choloepus sp.*, *C. frestasi* and *C. mirsae* on *Lagothrix sp.*, and *C. propinquus* on *Alouatta sp.* The respective collection strata were M1, M2, and M3 (Table 1).

5. DISCUSSION

Although differences in the total abundance of *Hoffmania* and non-*Hoffmania* females across the three vertical strata were not statistically significant, a clear trend in spatial distribution was observed, with a greater proportion of specimens captured in the canopy (M3). Specifically, 46.99% of all female *Culicoides* were collected in M3, followed by 33.52% in the mid-canopy (M2) and only 19.49% in the understory (M1) . These findings are consistent with previous studies in temperate forests, where most *Culicoides* were also captured in higher strata. For instance, McGregor et al. (2018) reported 79–89.2% of all *Culicoides* collected in the canopy in a Florida reserve, and Bernotienė et al. (2024) observed 80.6% of *Culicoides* captured at mid- and high-canopy levels in Lithuania.

Despite this similarity, the mentioned studies were conducted in temperate systems, which differ ecologically from the tropical rainforests of the Amazon. As such, while they provide proper context, they may not fully explain the mechanisms influencing vertical distribution in this region. One possible explanation for the canopy preference in Tiputini could be the significantly greater biomass in upper forest strata. Nascimento and Laurance (2002) reported that up to a quarter of all aboveground biomass in central Amazonian forests is stored in the canopy, supporting higher host and resource availability for *Culicoides*.

Although *Culicoides* females were more abundant in the canopy overall, the vertical distribution of the subgenus *Hoffmania* contrasted with this pattern. *Hoffmania* was more abundant in the understory, comprising 60.1% of females at M1 but only 23.2% at M3. This distribution suggests a potential adaptation or ecological preference for the lower strata. Supporting this, Erram and Burkett-Cadena (2021) demonstrated that *C. insignis*, a member of the *Hoffmania* subgenus, preferentially oviposits in muddy substrates. Although this behavior may not be universal across all *Hoffmania* species, such substrate preferences may help explain their increased abundance in understory habitats.

Monthly fluctuations in the abundance of both *Hoffmania* and non-*Hoffmania* groups were observed, but these differences were not statistically significant. However, previous research show the strong seasonal behavior of many *Culicoides* species. For example, *C. paraensis* in Rondônia, Brazil, exhibited clear seasonal peaks corresponding to transitions between dry and rainy seasons (Feitoza et al., 2023). Gusmão et al. (2019) also found that 88% of captures of 26 *Culicoides* species occurred during the rainy season, which supports this study's significant seasonal patterns, as more rainfall increased the abundance of both groups. Longer-term monitoring in various sites is needed to robustly assess the effect of seasonality in Tiputini *Culicoides* community.

A significant positive correlation was found between total monthly precipitation and the abundance of *Hoffmania* and non-*Hoffmania*. This result is consistent with numerous studies (Blackwell et al., 1999; Gusmão et al., 2019; Veggiani Aybar et al., 2010) and aligns with the aquatic or semi-aquatic larval development of *Culicoides*, which depends on moist substrates. When stratified by height, precipitation remained a significant positive predictor of abundance at M1 and M3 but not at M2. This absence of correlation in the mid-canopy may reflect its transitional ecological role. Flores et al. (2024) suggest that the understory (M2) possesses a reduced structural complexity and simplified interaction networks compared to the canopy and understory. *Culicoides* may use this stratum primarily as a transit zone, which could explain the weaker response to precipitation.

In contrast to precipitation, temperature exhibited weaker and more variable effects on *Culicoides* abundance. At M1, a significant negative correlation was observed for non-*Hoffmania*, whereas *Hoffmania* showed a non-significant negative trend. These patterns may be linked to larval dependence on moist substrates. Blackwell et al. (1999) reported increased mortality of *C. impunctatus* larvae in drying soils under high temperatures. Similarly, Feitoza et al. (2023) noted that *C. paraensis* populations increased with rising precipitation and lower temperatures.

At M2, both groups showed weak, non-significant associations with temperature, possibly due to the buffering effects of the mid-canopy microclimate (Flores et al., 2024). At M3, no significant temperature correlation was detected. All temperature data were collected at ground level, which may have limited the detection of vertical temperature gradients. Future studies should include microclimatic monitoring across all vertical strata.

Of the 64 engorged *Culicoides* females analyzed, 22 species were identified. Two taxa, *C. mirsae* and an unidentified species within the Daedalus group, appear to be new records for Ecuador. *C. mirsae* Ortiz., previously reported from Venezuela, Panama, Trinidad and Tobago, and Colombia (Wirth et al., 1988). The blood meal source of one *C. mirsae* specimen was also successfully sequenced identified, marking this species first recorded feeding preference. The unidentified Daedalus *sp.* shared several morphological features with *C. daedaloides* Wirth and Blaton., but exhibited a PR approximately twice as large, suggesting it may represent a new, undescribed species (Wirth et al., 1988). *C. daedaloides* has been reported in Colombia and Panama, meaning that the Daedalus group could harbor unreported or new species in Ecuador (Wirth et al., 1988). Further morphological and molecular analyses are required to confirm this.

Nine of the 22 species had known trophic preferences. Species such as *C. castillae*, and *C. paraensis* have been recorded feeding on mammals, including humans, primates, birds, and livestock (Felippe-Bauer & Silva, 2006; Perruolo et al., 2016; Pinheiro et al., 1981). As expected, better-studied vector species had more dietary records, while non-vector taxa remained poorly characterized. Expanding blood-fed female analyses is thus essential to understanding the vectorial potential and host range of *Culicoides* species in Amazonian ecosystems.

The four successfully sequenced engorged female samples revealed ecologically significant host associations. *C. leopoldi*, collected at M1, fed on a two-toed sloth (*Choloepus sp.*), a canopy-dwelling species known to descend to the ground periodically to defecate, a behavior hypothesized to reduce olfactory detection by predators (Monge Nájera, 2021) . *Choloepus* has also been proposed as a sylvatic reservoir of *Leishmania spp.* (Herrer & Christensen, 1980). Although *Culicoides* are not confirmed vectors of *Leishmania*, reports of DNA from these parasites in species like *C. foxi* suggest potential for interaction (Rebêlo et al., 2016). At M2 and M3, *C. frestasi* and *C. mirsae* were found to have fed on *Lagothrix sp.* (woolly monkeys), while *C. propinquus* (M2) fed on *Alouatta sp.* (howler monkeys). These findings align with the known vertical movement patterns and habitat use of these primates in Tiputini (Marsh, 1997). Reports mention *Lagothrix* and *Alouatta* as recognized hosts of filarial parasites such as *Mansonella* and *Dipetalonema* (Conga et al., 2018; Laidoudi et al., 2020).

These findings suggest that some *Culicoides* species in the Neotropics may exhibit host preferences for arboreal mammals, particularly primates. Previous studies have documented such associations in *C. atelis, C. hollensis,* and *C. paraensis* (Farias et al., 2024), reinforcing the notion of vertical stratification in host use. In the present study, three identified hosts were primates, suggesting a potential trophic bias among canopy-dwelling midges. Further molecular analyses using broader markers like *Cytochrome B* (*CytB*) are needed to capture non-mammalian hosts and increase species-level resolution. This preliminary study will help build a more complete picture of the feeding ecology and disease transmission potential of *Culicoides* species in tropical forest environments.

6. CONCLUSIONS

This preliminary study provides a detailed account of the spatial and temporal distribution of *Culicoides* species in an Amazonian rainforest, integrating environmental variables and trophic patterns into a stratified ecological framework. The results confirm that the canopy supports the highest abundance of non-*Hoffmania* species, reinforcing the view that upper forest strata act as key reservoirs of diversity for this group. Vertical stratification was evident, with each forest level harboring a distinct community likely shaped by its specific microhabitat conditions.

From a climatic perspective, the sub-canopy appeared to function as a transitional zone, where *Culicoides* abundance was relatively unaffected by either temperature or precipitation. In contrast, rainfall was positively correlated with total abundance, particularly during April and May, suggesting that precipitation may serve as a seasonal cue for adult emergence or activity.

Molecular analysis of blood-fed females confirmed that certain *Culicoides* species feed on arboreal vertebrates, including known reservoirs of zoonotic pathogens. These findings highlight the importance of incorporating vertical forest structure into biodiversity surveys and disease ecology frameworks. The evidence suggests that the canopy plays a pivotal role in shaping ecological dynamics and influencing potential transmission cycles of zoonotic agents.

Collectively, these results highlight the value of a vertically stratified approach in vector ecology. They reveal that abundance, activity patterns, and feeding behavior vary significantly across forest levels. Such differences are crucial for advancing ecological understanding and informing public health interventions in tropical forest systems.

7. BIBLIOGRAPHY

Augot, D., Mathieu, B., Hadj-Henni, L., Barriel, V., Mena, S. Z., Smolis, S., Slama, D., Randrianambinintsoa, F. J., Trueba, G., Kaltenbach, M., Rahola, N., & Depaquit, J. (2017a). Molecular phylogeny of 42 species of Culicoides (Diptera, Ceratopogonidae) from three continents. *Parasite*, *24*, 23. https://doi.org/10.1051/parasite/2017020

Augot, D., Mathieu, B., Hadj-Henni, L., Barriel, V., Mena, S. Z., Smolis, S., Slama, D., Randrianambinintsoa, F. J., Trueba, G., Kaltenbach, M., Rahola, N., & Depaquit, J. (2017b). Molecular phylogeny of 42 species of Culicoides (Diptera, Ceratopogonidae) from three continents. *Parasite*, *24*, 23. https://doi.org/10.1051/parasite/2017020

Bandeira, A. C., Pereira, F. M., Leal, A., Santos, S. P. O., Barbosa, A. C., Souza, M. S. P. L., Souza, D. R. de, Guimaraes, N., Fonseca, V., Giovanetti, M., Alcantara, L. C. J., Lessa, A. A. A., Saavedra, R. C., Tomé, L. M. R., Iani, F. C. M., Barros, R. M., Purificação, S. M. O., Jesus, J. P. de, Fonseca, R. R., & Araújo, M. L. V. (2024). *Fatal Oropouche Virus Infections in Nonendemic Region, Brazil, 2024—Volume 30, Number 11—November 2024—Emerging Infectious Diseases journal—CDC*. https://doi.org/10.3201/eid3011.241132

Bernotienė, R., Treinys, R., & Bukauskaitė, D. (2024). Vertical Distribution of Culicoides Biting Midges in Temperate Forests. *Diversity*, *16*(9), 585. https://doi.org/10.3390/d16090585

Blackwell, A., Lock, K. A., Marshall, B., Boag, B., & Gordon, S. C. (1999). The spatial distribution of larvae of Culicoides impunctatus biting midges. *Medical and Veterinary Entomology*, *13*(4), 362-371. https://doi.org/10.1046/j.1365-2915.1999.00180.x

Carvalho, L. P., Pereira Júnior, A., Paulo, P., Silva, G., Costa, G., Freitas, M., Pessoa, F., & Medeiros, J. (2021). DNA-based blood meal analysis of Culicoides (Diptera: Ceratopogonidae) species from Jamari National Forest, Southwestern Amazon, Brazil. *Acta Tropica*, *221*. https://doi.org/10.1016/j.actatropica.2021.106025

Conga, D. F., Mayor, P., Furtado, A. P., Giese, E. G., & Santos, J. N. dos. (2018). Occurrence of *Dipetalonema gracile* in a wild population of woolly monkey *Lagothrix poeppiigii* in the northeastern Peruvian Amazon. *Revista Brasileira de Parasitologia Veterinária*, *27*, 154-160. https://doi.org/10.1590/S1984-296120180014

Costa, J., Lorosa, E., Moraes, J., & Rebêlo, J. M. (2013). Espécies de Culicoides (Diptera; Ceratopogonidae) e hospedeiros potenciais em área de ecoturismo do Parque Nacional dos Lençóis Maranhenses, Brasil. *Revista Pan-Amazônica de Saúde*, *4*, 11-18. https://doi.org/10.5123/S2176-62232013000300002

Crilly, J. P., Nuttall, T., del Pozo, J., Hopker, A., Tomlinson, M., & Sargison, N. (2016). Hypersensitivity to *Culicoides* midges causing seasonal dermatitis in sheep. *Veterinary Parasitology: Regional Studies and Reports*, *3-4*, 53-56. https://doi.org/10.1016/j.vprsr.2016.06.001

de Souza Amorim, D., Brown, B. V., Boscolo, D., Ale-Rocha, R., Alvarez-Garcia, D. M., Balbi, M. I. P. A., de Marco Barbosa, A., Capellari, R. S., de Carvalho, C. J. B., Couri, M. S., de Vilhena Perez Dios, R., Fachin, D. A., Ferro, G. B., Flores, H. F., Frare, L. M., Gudin, F. M., Hauser, M., Lamas, C. J. E., Lindsay, K. G., … Rafael, J. A. (2022). Vertical stratification of insect abundance and species richness in an Amazonian tropical forest. *Scientific Reports*, *12*(1), 1734. https://doi.org/10.1038/s41598-022-05677-y

Erram, D., & Burkett-Cadena, N. (2021). Oviposition of Culicoides insignis (Diptera: Ceratopogonidae) under laboratory conditions with notes on the developmental life history traits of its immature stages. *Parasites & Vectors*, *14*(1), 522. https://doi.org/10.1186/s13071-021-05025-5

Farias, E. de S., Souza, C. R. L. de, Costa, R. C. M., Carvalho-Fernandes, S. P., Santana, E. W. de P., Oliveira, L. da C. de, Ríos-Velásquez, C. M., & Pessoa, F. A. C. (2024). Diversity of Biting Midges *Culicoides* (Diptera: Ceratopogonidae) in Pacoti Municipality, Baturité Highland, Ceará State, Northeast of Brazil. *Biota Neotropica*, *24*, e20241668. https://doi.org/10.1590/1676-0611-BN-2024-1668

Feitoza, L. H. M., de Carvalho, L. P. C., da Silva, L. R., Meireles, A. C. A., Rios, F. G. F., Silva, G. S., de Paulo, P. F. M., Pessoa, F. A. C., de Medeiros, J. F., & Julião, G. R. (2023). Influence of meteorological and seasonal parameters on the activity of *Culicoides paraensis* (Diptera: Ceratopogonidae), an annoying anthropophilic biting midge and putative vector of Oropouche Virus in Rondônia, Brazilian Amazon. *Acta Tropica*, *243*, 106928. https://doi.org/10.1016/j.actatropica.2023.106928

Feitoza, L. H. M., Gasparelo, N. W. F., Meireles, A. C. A., Rios, F. G. F., Teixeira, K. S., da Silva, M. S., Paz, M. de A., Roca, T. P., Moreira, H. M., de França, K. P., Dall'Acqua, D. S. V., Julião, G. R., & de Medeiros, J. F. (2025). Integrated surveillance for Oropouche Virus: Molecular evidence of potential urban vectors during an outbreak in the Brazilian Amazon. *Acta Tropica*, *261*, 107487. https://doi.org/10.1016/j.actatropica.2024.107487

Felippe-Bauer, M. L., & Silva, C. (2006). *Alterações morfológicas em Ceratopogonidae (Diptera) Neotropicais*. *23*.

Fetene, E., Teka, G., Dejene, H., Mandefro, D., Teshome, T., Temesgen, D., Negussie, H., Mulatu, T., Jaleta, M. B., & Leta, S. (2022). Modeling the spatial distribution of Culicoides species (Diptera: Ceratopogonidae) as vectors of animal diseases in

Ethiopia. *Scientific Reports*, *12*(1), 12904. https://doi.org/10.1038/s41598-022-16911-y

Flores, B. M., Montoya, E., Sakschewski, B., Nascimento, N., Staal, A., Betts, R. A., Levis, C., Lapola, D. M., Esquível-Muelbert, A., Jakovac, C., Nobre, C. A., Oliveira, R. S., Borma, L. S., Nian, D., Boers, N., Hecht, S. B., Ter Steege, H., Arieira, J., Lucas, I. L., … Hirota, M. (2024). Critical transitions in the Amazon forest system. *Nature*, *626*(7999), 555-564. https://doi.org/10.1038/s41586-023-06970-0

Gusmão, G. M. C., Brito, G. A., Moraes, L. S., Bandeira, M. D. C. A., & Rebêlo, J. M. M. (2019). Temporal Variation in Species Abundance and Richness of Culicoides (Diptera: Ceratopogonidae) in a Tropical Equatorial Area. *Journal of Medical Entomology*, *56*(4), 1013-1018. https://doi.org/10.1093/jme/tjz015

Herrer, A., & Christensen, H. A. (1980). *Leishmania Braziliensis in the Panamanian Two-Toed Sloth, Choloepus Hoffmanni*. https://doi.org/10.4269/ajtmh.1980.29.1196

Kaewmee, S., Mano, C., Phanitchakun, T., Ampol, R., Yasanga, T., Pattanawong, U., Junkum, A., Siriyasatien, P., Bates, P. A., & Jariyapan, N. (2023). Natural infection with Leishmania (Mundinia) martiniquensis supports Culicoides peregrinus (Diptera: Ceratopogonidae) as a potential vector of leishmaniasis and characterization of a Crithidia sp. isolated from the midges. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1235254

Kar, S., Mondal, B., Pal, A., Mondal, A., & Mazumdar, A. (2022). Report of spiders preying on adult Culicoides spp. Using molecular based marker with notes on its feeding activity. *International Journal of Tropical Insect Science*. https://doi.org/10.1007/s42690-021-00715-0

Laidoudi, Y., Medkour, H., Levasseur, A., Davoust, B., & Mediannikov, O. (2020). New Molecular Data on Filaria and its Wolbachia from Red Howler Monkeys (Alouatta macconnelli) in French Guiana—A Preliminary Study. *Pathogens*, *9*(8), 626. https://doi.org/10.3390/pathogens9080626

Linton, Y.-M., Pecor, J. E., Porter, C. H., Mitchell, L. B., Garzón-Moreno, A., Foley, D. H., Pecor, D. B., & Wilkerson, R. C. (2013). Mosquitoes of eastern Amazonian Ecuador: Biodiversity, bionomics and barcodes. *Memórias Do Instituto Oswaldo Cruz*, *108*, 100-109. https://doi.org/10.1590/0074-0276130440

Marsh, L. (1997). Primate species at the Tiputini Biodiversity Station, Ecuador. *Neotropical Primates Biol. Conserv. Oryx Am. J. Primatol. Ecology and Behavior of Neotropical Primates World Wildlife Fund*, *12*, 70-99192. https://doi.org/10.62015/np.2004.v12.575

McGregor, B. L., Runkel, A. E., Wisely, S. M., & Burkett-Cadena, N. D. (2018). Vertical stratification of Culicoides biting midges at a Florida big game preserve. *Parasites & Vectors*, *11*(1), 505. https://doi.org/10.1186/s13071-018-3080-5

Milián-García, Y., Janke, L., Young, R., Ambagala, A., & Hanner, R. (2021). Validation of an Effective Protocol for Culicoides Latreille (Diptera: Ceratopogonidae) Detection Using eDNA Metabarcoding. *Insects*, *12*, 401. https://doi.org/10.3390/insects12050401

Monge Nájera, J. (2021). Why sloths defecate on the ground: Rejection of the mutualistic model. *UNED Research Journal*, *13*, 4. https://doi.org/10.22458/urj.v13i1.3438

Moscoso, T., & Sofía, A. (2014). *Identificación molecular de las preferencias tróficas de género Culicoides en cinco provincias del Ecuador* [bachelorThesis, Quito, 2014]. http://repositorio.usfq.edu.ec/handle/23000/3443

Mosquera, J. D., Zapata, S., Spinelli, G., Gualapuro, M., León, R., & Augot, D. (2022). An updated list of the Culicoides (Diptera, Ceratopogonidae) fauna from Ecuador. *Parasite*, *29*, 63. https://doi.org/10.1051/parasite/2022061

Mulholland, C. (2016). *New and improved methods for the detection of bluetongue virus (BTV) infection.*

Nascimento, H. E. M., & Laurance, W. F. (2002). Total aboveground biomass in central Amazonian rainforests: A landscape-scale study. *Forest Ecology and Management*, *168*(1-3), 311-321. https://doi.org/10.1016/S0378-1127(01)00749-6

Perruolo, G., Chacón-Ortiz, A., & Rodríguez, W. T. (2016). The bloodsucking biting midges Culicoides (Diptera: Ceratopogonidae) of Yaracuy state, Venezuela. *Boletin de Malariologia y Salud Ambiental*, *56*, 131-144.

Pinheiro, F. P., Hoch, A. L., Gomes, M. L., & Roberts, D. R. (1981). Oropouche virus. IV. Laboratory transmission by Culicoides paraensis. *The American Journal of Tropical Medicine and Hygiene*, *30*(1), 172-176.

Rebêlo, J. M. M., Rodrigues, B. L., Bandeira, M. da C. A., Moraes, J. L. P., Fonteles, R. S., & Pereira, S. R. F. (2016). Detection of Leishmania amazonensis and Leishmania braziliensis in Culicoides (Diptera, Ceratopogonidae) in an endemic area of cutaneous leishmaniasis in the Brazilian Amazonia. *Journal of Vector Ecology: Journal of the Society for Vector Ecology*, *41*(2), 303-308. https://doi.org/10.1111/jvec.12227

Romero-Álvarez, D., & Escobar, L. E. (2018). Emergent viruses in America: The case of Oropouche virus. *International Journal of Infectious Diseases*, *73*, 98. https://doi.org/10.1016/j.ijid.2018.04.3644

Sherlock, I. (1965). Dermatozoonosis by Culicoides' bite (Diptera, Ceratopogonidae) in Salvador, State of Bahia, Brazil: IV - A clinical study. *Memorias Do Instituto Oswaldo Cruz - MEM INST OSWALDO CRUZ*, *63*. https://doi.org/10.1590/S0074-02761965000100003

Sick, F., Beer, M., Kampen, H., & Wernike, K. (2019a). Culicoides Biting Midges—Underestimated Vectors for Arboviruses of Public Health and Veterinary Importance. *Viruses*, *11*(4), 376. https://doi.org/10.3390/v11040376

Sick, F., Beer, M., Kampen, H., & Wernike, K. (2019b). Culicoides Biting Midges—Underestimated Vectors for Arboviruses of Public Health and Veterinary Importance. *Viruses*, *11*(4), 376. https://doi.org/10.3390/v11040376

Spinelli, G. R., Greiner, E. C., & Wirth, W. W. (1993). *The Neotropical bloodsucking midges of Culicoides guttatus group of the subgenus Hoffmania (Diptera: Ceratopogonidae)*. Contributions of the American Entomological Institute.

Spinelli, G. R., Ronderos, M. M., Díaz, F., & Marino, P. I. (2005). The bloodsucking biting midges of Argentina (Diptera: Ceratopogonidae). *Memórias Do Instituto Oswaldo Cruz*, *100*(2), 137-150. https://doi.org/10.1590/S0074-02762005000200006

Spinelli, G. R., Ronderos, M. M., Marino, P. I., Carrasco, D. S., & Ferreira, R. L. M. (2007). Description of Culicoides (Mataemyia) felippebauerae sp. n., Forcipomyia musae immatures, and occurrence of F. genualis, breeding in banana stems in Brazilian Amazonia (Diptera: Ceratopogonidae). *Memórias Do Instituto Oswaldo Cruz*, *102*, 659-669. https://doi.org/10.1590/S0074-02762007005000083

Ta-Tang, T.-H., Crainey ,James L, Post ,Rory J, Luz ,Sergio LB, & and Rubio, J. M. (2018). Mansonellosis: Current perspectives. *Research and Reports in Tropical Medicine*, *9*, 9-24. https://doi.org/10.2147/RRTM.S125750

Trindade, R. L. D., & Felippe-Bauer, M. L. (2011). <p>Culicoides parauapebensis, a new species of the subgenus Hoffmania Fox from northern Brazil (Diptera: Ceratopogonidae)</p>. *Zootaxa*, *2999*(1), Article 1. https://doi.org/10.11646/zootaxa.2999.1.4

Veggiani Aybar, C. A., Dantur Juri, M. J., Lizarralde De Grosso, M. S., & Spinelli, G. R. (2010). Species diversity and seasonal abundance of *Culicoides* biting midges in northwestern Argentina. *Medical and Veterinary Entomology*, *24*(1), 95-98. https://doi.org/10.1111/j.1365-2915.2009.00846.x

Vigil, S. L., Ruder, M. G., Shaw, D., Wlodkowski, J., Garrett, K., Walter, M., & Corn, J. L. (2018). Apparent Range Expansion of Culicoides (Hoffmania) insignis (Diptera: Ceratopogonidae) in the Southeastern United States. *Journal of medical entomology*, *55*(4), 1043-1046. https://doi.org/10.1093/jme/tjy036

Wirth, W. W., & Blanton, F. S. (1959). *Biting Midges of the Genus <I>Culicoides</I> from Panama (Diptera: Heleidae)*. http://repository.si.edu/xmlui/handle/10088/16674

Wirth, W. W., Dyce, A. L., & Spinelli, G. R. (1988). *AN ATLAS OF WING PHOTOGRAPHS, WITH A SUMMARY OF THE NUMERICAL CHARACTERS OF THE NEOTROPICAL SPECIES OF CULICOIDES (DIPTERA: CERATOPOGONIDAE)* (Vol. 25). Contributions of the American Entomological Institute.

Yu, C. Y., Wang, J. S., & Yeh, C. C. (2000). Culicoides arakawae (Diptera: Ceratopogonidae) population succession in relation to leucocytozoonosis prevalence on a chicken farm in Taiwan. *Veterinary Parasitology*, *93*(2), 113-120. https://doi.org/10.1016/s0304-4017(00)00362-9

Žiegytė, R., Platonova, E., Kinderis, E., Mukhin, A., Palinauskas, V., & Bernotienė, R. (2021). Culicoides biting midges involved in transmission of haemoproteids. *Parasites & Vectors*, *14*(1), 27. https://doi.org/10.1186/s13071-020-04516-1

Zimmer, J.-Y., Brostaux, Y., Haubruge, E., & Francis, F. (2014). Larval development sites of the main *Culicoides* species (Diptera: Ceratopogonidae) in northern Europe and distribution of coprophilic species larvae in Belgian pastures. *Veterinary Parasitology*, *205*(3), 676-686. https://doi.org/10.1016/j.vetpar.2014.08.029

8 TABLES AND FIGURES

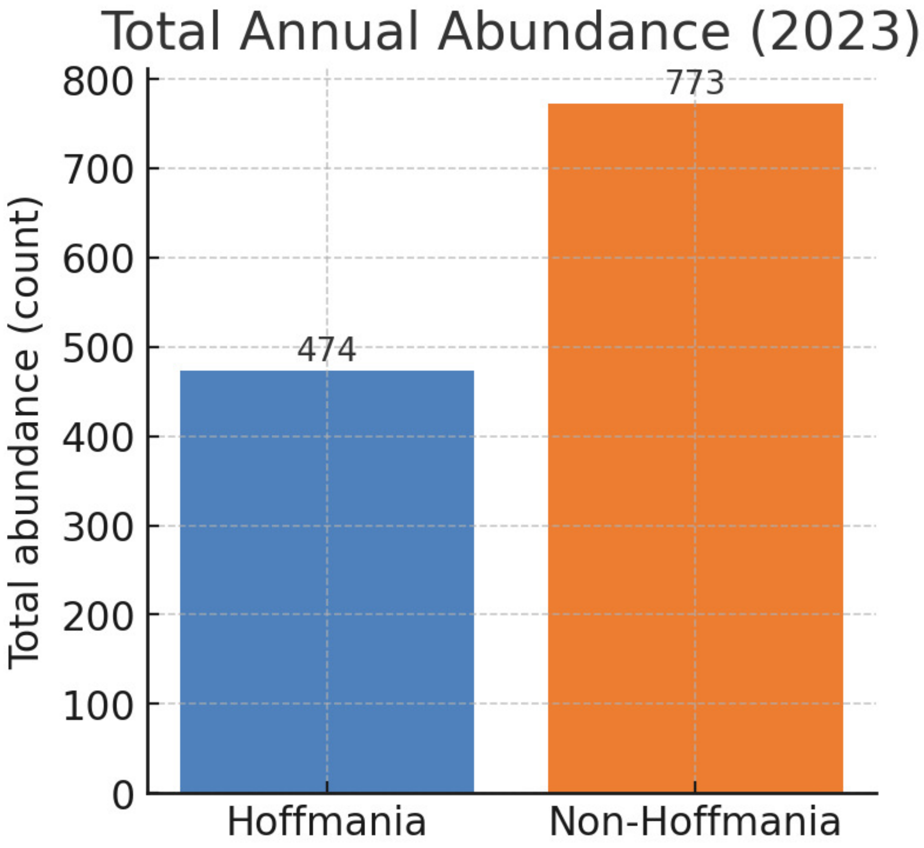

Figure 1. Total *Hoffmania* vs non *Hoffmania* abundance comparison

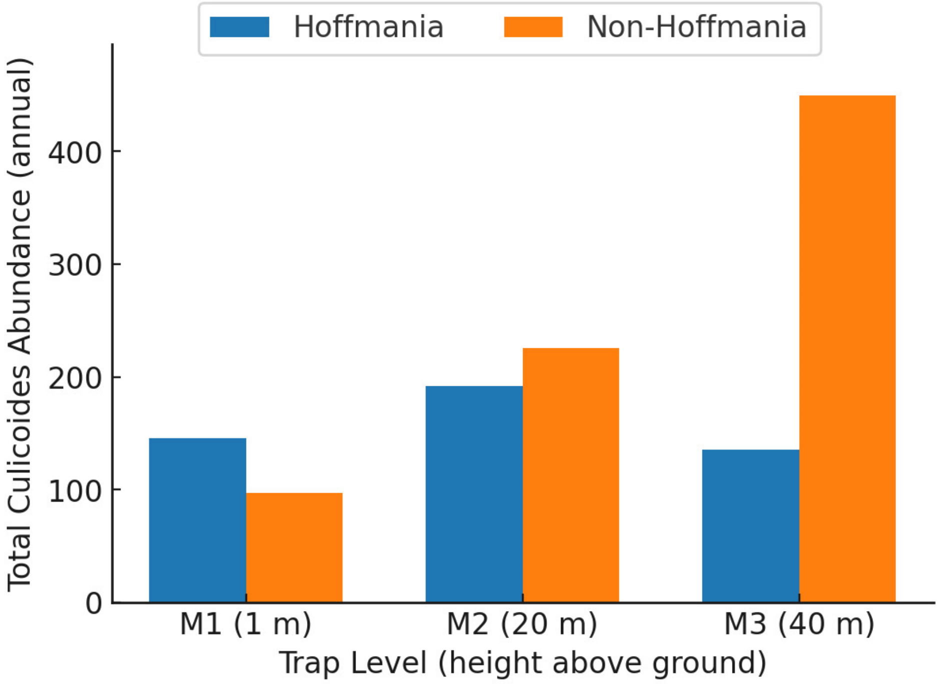

Figure 2. Total *Hoffmania* vs non *Hoffmania* abundance across strata

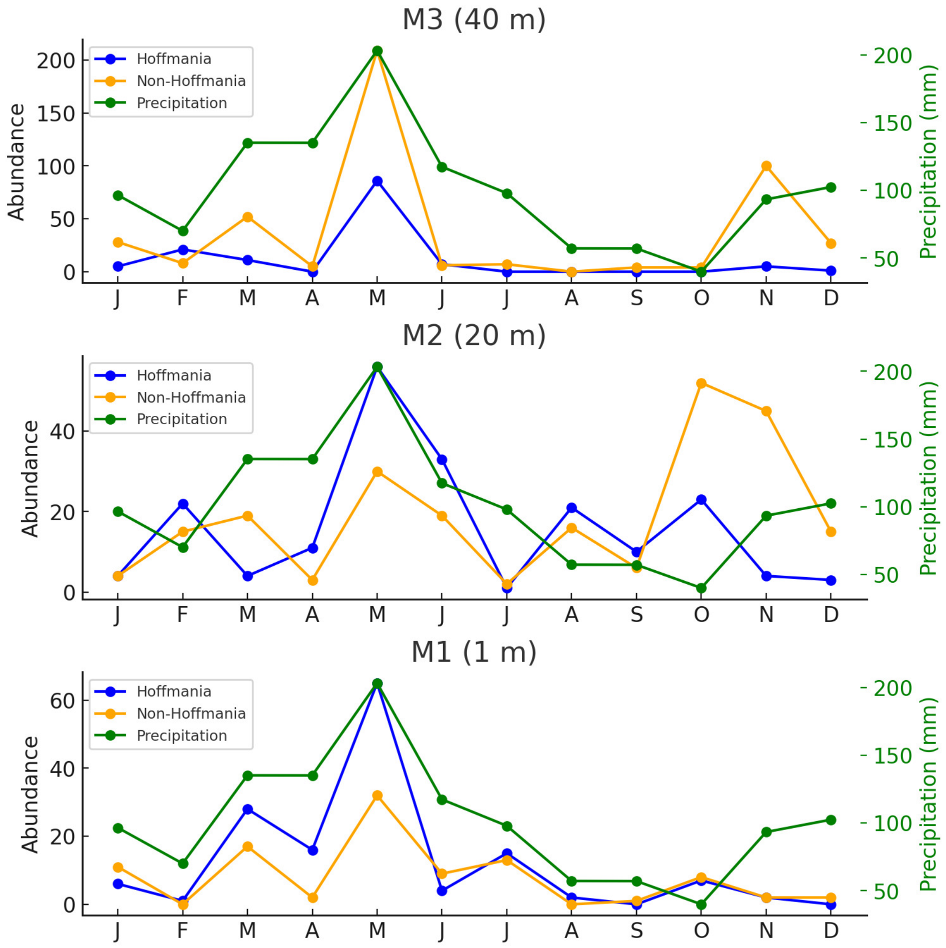

Figure 3. Seasonal abundance of *Hoffmania* and non *Hoffmania* vs total monthly precipitation (mm), per strata

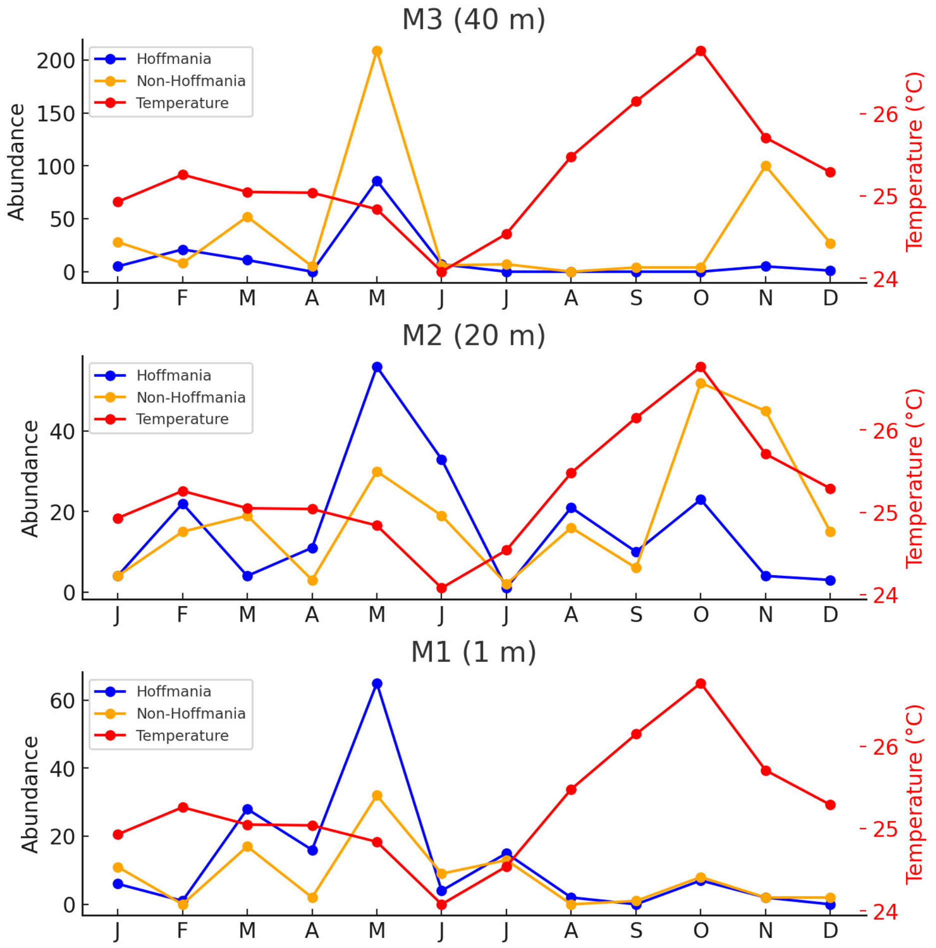

Figure 4. Seasonal abundance of *Hoffmania* and non *Hoffmania* vs average monthly temperature, per strata

Table. 1. Reported species of engorged female *Culicoides* with background on trophic preferences and new trophic preferences

Species	Group/Subgenus	Reported Diet		Our Study
Culicoides aureus	*Reticulatus*	No data reported	-	*N/A*
Culicoides castillae	*Fluvialis*	Human blood (anthropophilic; caught biting humans)	(Wirth & Blanton, 1959)	*N/A*
Culicoides crucifer	*Haematomyidium*	No data reported	-	*N/A*
Culicoides sp.	*Daedalus*	No data reported	-	*N/A*
Culicoides eublepharus	*Eublepharus*	No data reported	-	*N/A*
Culicoides evansi	*Diphaomyia*	No data reported	-	*N/A*
Culicoides fieldi	*Leoni*	No data reported	-	*N/A*
Culicoides filarifer	*Hoffmania*	Generalist – mammals and birds	(Milián-García et al., 2021)	*N/A*
Culicoides fittkaui	*Reticulatus*	Mammals (presumed; no direct data)	-	*N/A*
Culicoides florenciae	*Eublepharus*	No data reported	-	*N/A*
Culicoides frestasi	*Diphaomyia*	No data reported	-	*Lagothrix sp.*
Culicoides glabrior	*Haematomyidium*	No data reported	-	*N/A*
Culicoides guttatus	*Hoffmania*	No data reported	-	*N/A*
Culicoides horticola	*Haematomyidium*	No data reported	-	*N/A*
Culicoides leopoldi	*Fluvialis*	No data reported	-	*Choleopus sp.*
Culicoides mirsae	*Diphaomyia*	No data reported	-	*Lagothrix sp.*
Culicoides paucienfuscatus	*Reticulatus*	Mammals (livestock; dominant near cattle)	(Costa et al., 2013)	*N/A*
Culicoides pinafoi	*Reticulatus*	No data reported	-	*N/A*
Culicoides propinquus	*Mohave*	No data reported	-	*Alouatta sp.*
Culicoides propriipennis	*Eublepharus*	No data reported	-	*N/A*
Culicoides reticulatus	*Reticulatus*	Mammals – including humans (also large mammals)	(Carvalho et al., 2021)	*N/A*
Culicoides paraensis	*Haematomyidium*	Humans, sloths (Bradypus tridactylus), rodents, marsupials, non-human primates, birds (low evidence), livestock (occasionally); confirmed via molecular analyses and virus reservoir studies.	(Pinheiro et al., 1981; Romero-Álvarez & Escobar, 2018)	*N/A*

9. APPENDIX A: TABLE OF HOFFMANIA AND NON HOFFMANIA WITH CLIMATIC VARIABLES

Month	Strata	No_*Hoffmania*	*Hoffmania*	Avg_Temperature_C	Total_Precip_mm
Nov	M1	2	2	25.71	93.32
Nov	M2	45	4	25.71	93.32
Nov	M3	100	5	25.71	93.32
Dec	M1	2	0	25.29	102.45
Dec	M2	15	3	25.29	102.45
Dec	M3	27	1	25.29	102.45
Jan	M1	11	6	24.93	96.49
Jan	M2	4	4	24.93	96.49
Jan	M3	28	5	24.93	96.49
Feb	M1	0	1	25.26	70.03
Feb	M2	15	22	25.26	70.03
Feb	M3	8	21	25.26	70.03
Mar	M1	17	28	25.05	135.21
Mar	M2	19	4	25.05	135.21
Mar	M3	52	11	25.05	135.21
Apr	M1	2	16	25.04	135.16
Apr	M2	3	11	25.04	135.16
Apr	M3	5	0	25.04	135.16
May	M1	32	65	24.84	203.37
May	M2	30	56	24.84	203.37
May	M3	209	86	24.84	203.37
Jun	M1	9	4	24.08	117.39
Jun	M2	19	33	24.08	117.39
Jun	M3	6	7	24.08	117.39
Jul	M1	13	15	24.54	97.91
Jul	M2	2	1	24.54	97.91
Jul	M3	7	0	24.54	97.91
Aug	M1	0	2	25.48	57.12
Aug	M2	16	21	25.48	57.12
Aug	M3	0	0	25.48	57.12
Sept	M1	1	0	26.15	57.03
Sept	M2	6	10	26.15	57.03
Sep	M3	4	0	26.15	57.03
Oct	M1	8	7	26.77	39.89
Oct	M2	52	23	26.77	39.89
Oct	M3	4	0	26.77	39.89