CLOUDBRIDGE NATURE RESERVE

# Comparative Analysis of Moth Diversity in Different Forest Succession Stages in Costa Rica with Focus on Sphingidae

by

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**Abstract**

The high diversity of moths and complex taxonomic classification present challenges in ecological studies. As bioindicators, moths, particularly the Sphingidae family, are beneficial for monitoring ecosystem health. Cloudbridge Nature Reserve in Costa Rica, a montane cloud forest, served as the study site for analyzing moth abundance, species diversity, and habitat preferences across four distinct forest types. Over ten weeks, 1,230 moth specimens were recorded, with the Geometridae family being the most prevalent. The naturally regenerated forest under 30 years exhibited the highest moth activity, while diversity indices showed consistent moth diversity across different forest succession stages. Environmental factors, including moon phase and cloud cover, were also examined for their impact on moth attraction. These findings highlight the necessity of preserving diverse habitats to maintain moth biodiversity and emphasize the importance of continuous ecological monitoring. Future research should incorporate a broader spectrum of environmental variables and extend sampling periods to gain a more comprehensive understanding of moth communities.

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## Introduction

The order Lepidoptera constitutes a significantly diverse and abundant insect group, intervening vital ecological functions as pollinators, decomposers, and prey (Ouaba et al., 2022). Moths are notably diverse, accounting for over 91% of Lepidoptera (Heppner, 2008) which translates to approximately 165,000 known species worldwide (Perveen, 2018). This large variability results in limited knowledge surrounding behaviors, ecological roles, and composition of specific moth species (Hahn, 2016), although some families are more well-known than others. While difficult, population and diversity studies of moths provide insights for ecosystem health as they're particularly vulnerable to environmental changes such as habitat fragmentation and climate change (Cosma, 2016), and populations rapidly respond to these stressors (Macgregor, 2015). Studies have found that moth numbers have globally declined over the last 25 years, elucidating their sensitivity to ecological disturbances (Blumgart et al., 2022).

Due to the high diversity of moths and difficulty in taxonomic classification processes, this study focuses on the Sphingidae family as an informative model group for moth composition, diversity and habitat association (Sublett et al., 2019). Commonly referred to as hawk moths, sphinx moths, or hummingbird moths, Sphingidae are among the largest and most easily recognized families, identified by their thick abdomens and narrow, rapid-flying wings (Capinera, 2020). These moths exhibit specialized morphological adaptations such as streamlined bodies, powerful flight muscles, and long proboscides, which enable them to hover like hummingbirds while feeding on nectar from deep flowers (Reinwald et al., 2022). While they're well-known taxonomically, Sphingidae additionally possess a broad geographical distribution, making them important bioindicators of ecosystem health (Santos et al., 2014).

Costa Rica in particular harbors a large amount of moth diversity, containing over 75 families and 10,000 documented species, 1,600 of those being Sphingidae (Henerson, 2010). As a montane cloud forest, Cloudbridge Nature Reserve represents a biodiversity hotspot within Costa Rica, yet investigations of moth diversity, abundance, and composition remain highly understudied. Only one previous study was completed by Wells et al. (2019) which examined trap effectiveness and produced a limited species list, necessitating room for future research. To decrease these knowledge gaps, this study examines the relative abundance, species diversity, and habitat preferences of moths across four distinct forest types within the reserve, with a specific emphasis on Sphingidae.

## Methodology

### A. Study Area

Research was conducted at Cloudbridge Nature Reserve (Cloudbridge), a privately owned reserve in the Pérez Zeledón Canton of Costa Rica on the Pacific slope of the Talamanca Mountains. Founded in 2002, Cloudbridge encompasses 283 hectares within a cloudforest ecosystem and represents significant areas of successful regrowth and reforestation, as approximately 255 hectares consist of re-established forest cover from degraded pastureland. Due to its history of reforestation efforts, varying stages of forest succession are present throughout the reserve. These can be classified as primary forest, naturally regenerated forest ranging from 20 to 80 years, and planted forest, while small sections encompass human-dominated areas (Figure 1). Furthermore, Cloudbridge's location in the Talamanca Mountain Range produces a distinct dry season (January-May) and rainy season (June-December), while it lies at an altitude ranging from 1550m to 2600m.

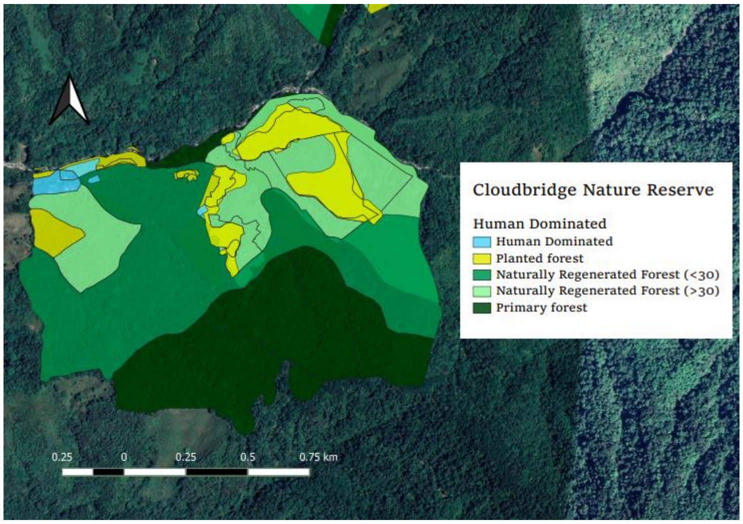


**Figure 1:** Map of Cloudbridge Nature Reserve and its different forest types

A stratified sampling design was employed to capture the variability in forest types across the reserve. As depicted in Figure 2, four sampling sites were established, each situated within a distinct forest type: human-dominated, planted, naturally regenerated under 30 years, and naturally regenerated over 30 years. This approach ensures that the data collected encompasses the range of the restored forest conditions present within Cloudbridge, allowing for a comparative analysis of habitat types. To maximize individuals attracted to the light traps, sampling sites were selected in open areas with minimal canopy cover, alongside habitat classification.

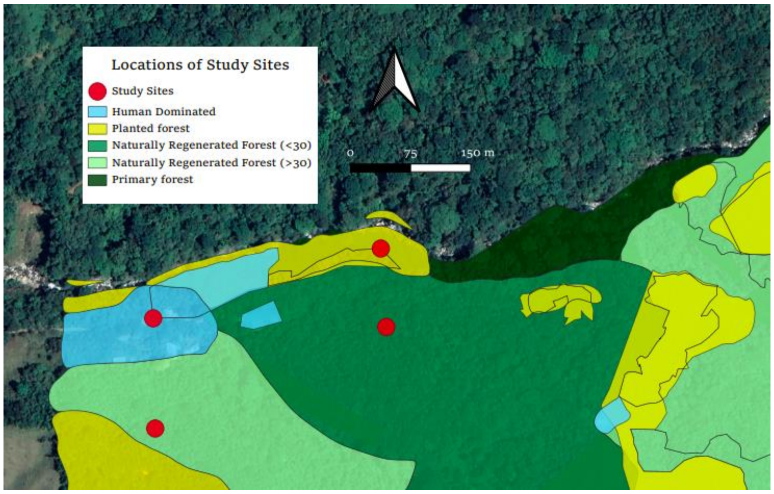


**Figure 2:** Map of study site locations within Cloudbridge Nature Reserve

B. Trapping Method

A standardized sheet light trap was deployed at each sampling site, consisting of a large white bedsheet suspended horizontally between two trees using ropes, approximately 1.5m above the ground (Figure 3). To illuminate the bedsheet, four battery-powered UV LED light bars, each containing 10W of power, were positioned along the top edge. The power source consisted of two portable cell phone power banks possessing a 15,000mAh capacity. This configuration maximized light sheet illumination while maintaining a consistent height for specimen attraction across all sampling locations. Furthermore, utilizing sheet light traps offered a cost-effective and straightforward approach to evaluate moth diversity, yielding reliable results as compared to other insect sampling methods (White et al., 2016).

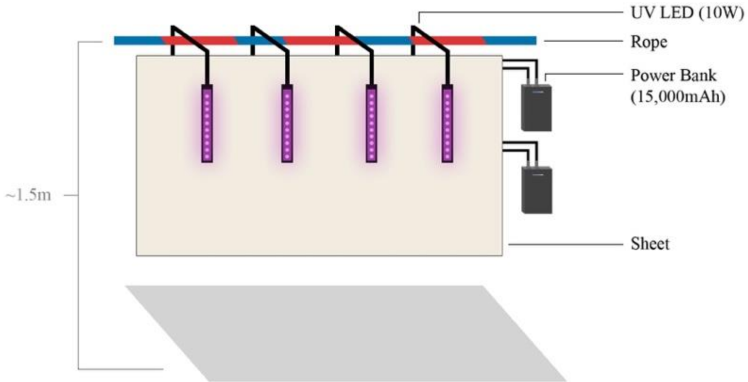


**Figure 3:** Sheet trap configuration

C. Data Collection and Methodology

Surveys were completed bimonthly, resulting in a sampling frequency of one survey every two weeks per site. Each site underwent five independent sampling nights, with surveys commencing approximately one hour after sunset from 1900 to 2030 hours. Prior to each sampling event, potential influences on moth attraction were documented, including moon phase, percentage of moon illumination, and cloud cover, visually estimated using the standardized Okta scale. The light trap configuration was prepared for activation at the designated start time (1900 hours) by assembling its components shortly beforehand. During the sampling period, all moth specimens exceeding 3 centimeters in wingspan attracted to the light trap were photographed and assigned a unique identification number (Wells et al., 2019). Sphingidae specimens, as the focal point of this research, were captured by hand using appropriate handling techniques. This ensured minimal harm and allowed for careful hindwing examination, facilitating species-level identification. Following data collection, moths were identified to the lowest possible taxonomic level using a variety of online resources including iNaturalist, Tropicleps.ch, and LEPS.

## Results

Throughout ten weeks, surveys were completed across the four sampling locations, utilizing the light trap methodology to produce a comprehensive dataset. The analysis includes overall moth composition by family, habitat distribution patterns, and diversity indices. Emphasis is placed on the Sphingidae family, which were separately analyzed due to their role as ecological indicators. Finally, the influence of environmental factors such as lunar cycles and cloud coverage on moth activity is examined.

**Result A:** Overall Moth Composition

During the research period, a total of 1,230 individual moth specimens, each measuring three centimeters or larger, were attracted to the light trap and recorded. The overall composition demonstrates a notable dominance of the Geometridae family, which accounted for 56% of all recorded specimens, totaling 683 individuals (Figure 4). The second most abundant family was Erebidae, comprising 16% of the specimens, followed by Crambidae at 9%. The remaining ten moth families observed, along with unidentified specimens, constituted a smaller proportion of the total, each ranging from 0% to 5%.

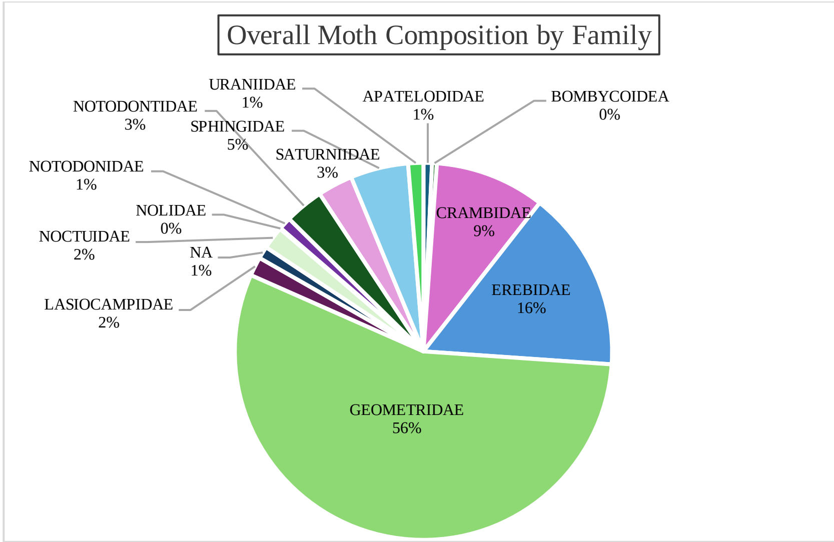


**Figure 4:** Pie chart demonstrating overall moth composition at the family level

**Result B:** Habitat Distribution

Among the four distinct study sites, each representing varying stages of forest succession, the naturally regenerated forest less than 30 years (NR<30) had the highest number of recorded specimens, comprising 39.19% of the dataset. The other three sampling locations exhibited relatively similar specimen counts. The human-dominated habitat accounted for 24.55% of the total, while the naturally regenerated over 30 years (NR>30) constituted 18.70%, and the planted forest had the fewest specimens, making up 17.56% (Figure 5).

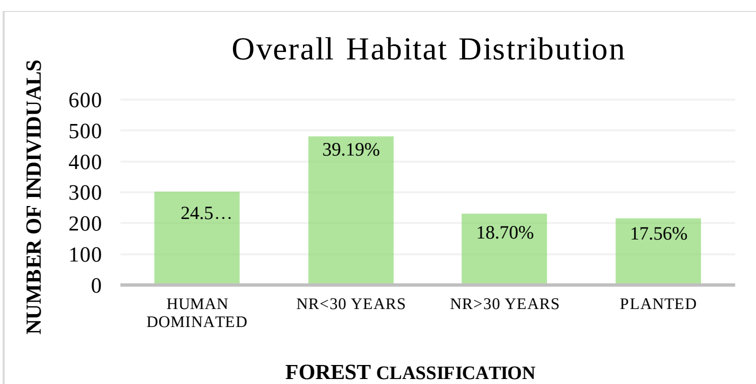


**Figure 5:** Bar chart representing general habitat preference of moth specimens

**Result C.1:** Overall Diversity

The Shannon-Weiner Diversity Index (H') was calculated for the entire dataset to assess overall species diversity, which accounts for both the number of species present (richness) and the evenness of their distribution (Nolan & Callahan, 2006). Higher values of H' indicate greater diversity, where a larger number of species are evenly distributed. The diversity index was found at three taxonomic levels: family, genus, and species. At the family level, the diversity index was calculated at 1.524 suggesting that while several families were present, certain families, such as Geometridae, accounts for much of the population. The diversity index increased to 2.983 at the genus level, and 4.064 at the species level.

**Result C.2:** Diversity by Forest Type

Diversity indices were additionally calculated for each of the four sampling sites. The human-dominated location showed a diversity index of 3.573, while NR<30 exhibited the highest diversity with an index of 3.721. NR>30 had a diversity index of 3.431, and the planted forest type showed an index of 3.256.

**Result D:** Environmental Factors

The analysis of the lunar cycle's influence on moth attraction suggests a relationship between the number of moth individuals recorded and moon phase. A scatter plot (Figure 6) was created with moth presence plotted against the percentage of moon illumination. The plot demonstrates a negative trend line, indicating that the number of moths recorded tends to decrease as moonlight increases. The coefficient of determination, $R^2=0.2359$, suggests a slight influence of moonlight on moth activity, with lower illumination levels being more conducive to higher moth attraction.

In addition to documenting the moon phase for each sampling event, cloud coverage was recorded, as it often impacts the moon's visibility or intensity of illumination. Cloud coverage was measured using the okta scale, a unit of measurement that quantifies the amount of sky covered by clouds, ranging from 0 to 8 oktas. A reading of 0 oktas indicates completely clear skies, while 8 oktas signify total cloud cover. The scatter plot in Figure 7 shows a positive relationship between cloud coverage and moth activity, with the number of moth presence increasing as cloud cover increases. This relationship is represented by a linear regression line with a coefficient of determination $R^2=0.7539$, demonstrating a strong correlation.

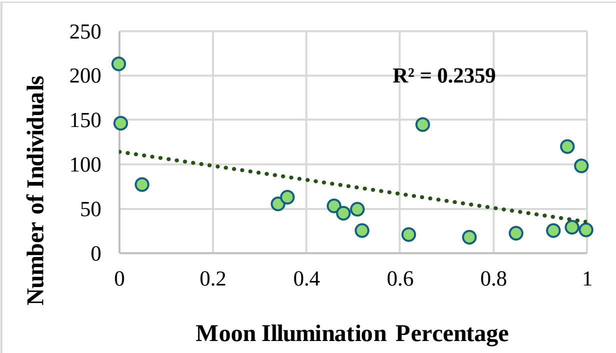


**Figure 6**: Relationship of lunar cycle with number of specimens recorded

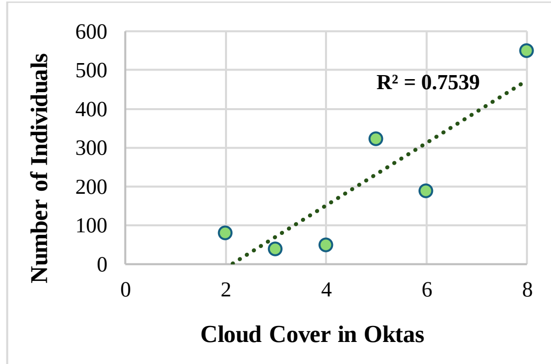


**Figure 7**: Relationship of cloud coverage with number of specimens recorded

**Result E.1:** Sphingidae Composition

A total of 61 individual Sphingidae specimens were identified, representing seven distinct genera and fifteen species. Among the most frequently recorded genera were *Xylophanes* with 24 individuals, and *Adhemarius* with 22, while the remaining five had significantly less sightings (Figure 8).

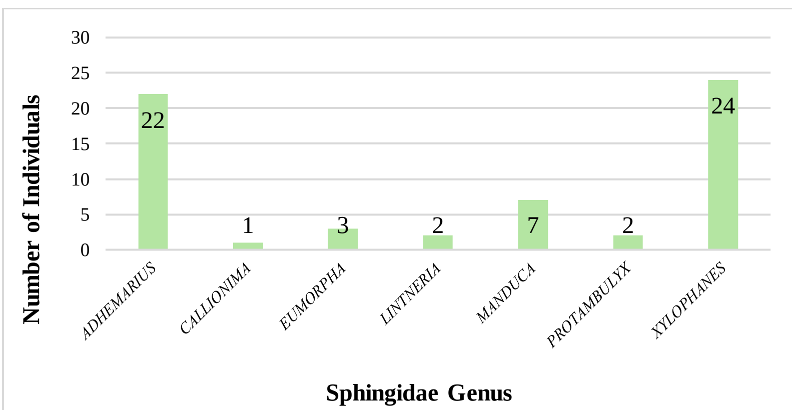


**Figure 8:** Bar chart of Sphingidae composition by genus

**Result E.2:** Sphingidae Habitat Distribution

Demonstrating similar results to the overall habitat distribution, Sphingidae specimens were most frequently recorded in the NR<30 forest type, followed by human dominated. Both the NR>30 and planted habitat classifications harbored significantly fewer Sphingidae specimens, with NR>30 showing the lowest amount (Figure 9).

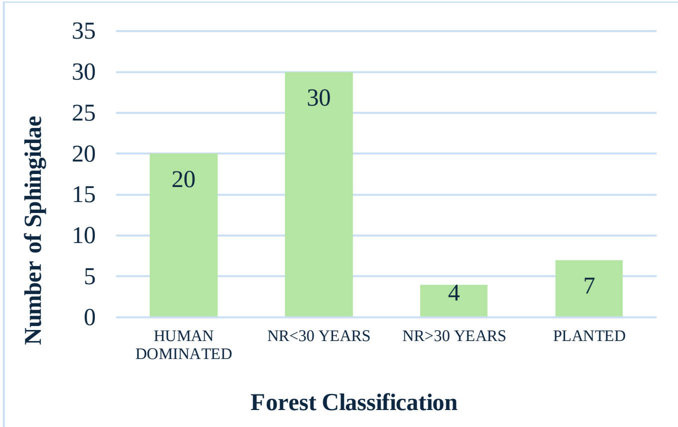


**Figure 9:** Bar chart visualizing the habitat distribution of Sphingidae

**Result E.3:** Sphingidae Diversity

The Shannon-Weiner Diversity Index for Sphingidae was calculated at 2.322.. When assessing Sphingidae diversity by habitat type, the human-dominated site had the highest index at 2.069, followed by planted forest with a diversity index of 1.875. The NR<30 habitat type showed a diversity index of 1.734, while the NR>30 habitat type had the lowest diversity index at 1.099.

**Result E.4:** Influence of Environmental Factors on Sphingidae

Figure 10 demonstrates the relationship between the number of Sphingidae specimens and the moon illumination percentage. The scatter plot shows a weak negative correlation, as indicated by the $R^2$ value of 0.0547. This suggests that as the moon illumination increases, the number of Sphingidae specimens slightly decreases; however, the low $R^2$ value indicates that moon illumination is not a strong predictor of Sphingidae specimen numbers.

Conversely, a positive correlation can be depicted from the relationship between the number of Sphingidae specimens and cloud coverage measured in oktas (Figure 11). The scatter plot shows an $R^2$ value of 0.6239, suggesting that higher cloud coverage is associated with an increase in the number of Sphingidae recorded. This indicates that cloud coverage has a more substantial impact on Sphingidae presence compared to moon phase.

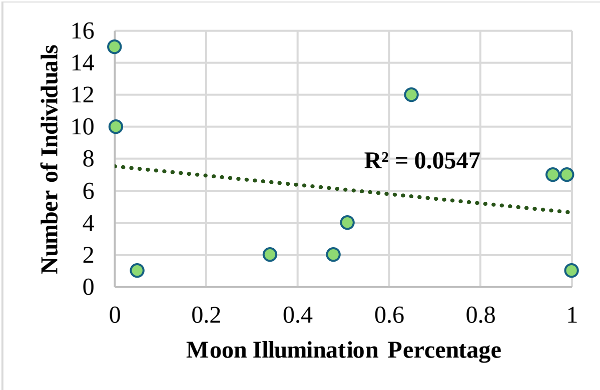


**Figure 10:** Relationship of Sphingidae with the lunar cycle

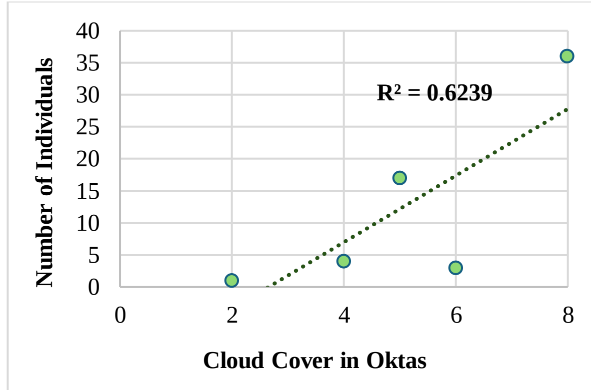


**Figure 11:** Relationship of Sphingidae with cloud coverage

**Discussion**

A. Ecological and Biological Implications

Among the 1,230 moth specimens recorded, the Geometridae family was the most dominant, representing 56% of the dataset. This indicates that Geometridae is the most prevalent moth family (with specimens measuring 3 cm or larger) in the study area, followed by the Erebidae family at 16%. The prominence of these families aligns with the Smithsonian Institution's findings on the global distribution of moths, where Geometridae and Erebidae are among the largest moth families worldwide (Smithsonian Institution, n.d.), reflecting the overall composition observed in this research. Within the Sphingidae family, *Adhemarius* and *Xylophanes* were the most frequently recorded genera. This prevalence suggests that *Adhemarius* and *Xylophanes* may have specific ecological advantages or preferences within Cloudbridge. Further investigation into their habitat requirements and behavioral patterns is needed, as understanding these aspects can help examine their roles within the ecosystem.

The NR<30 sampling location showed the highest count for both overall moth presence and Sphingidae specimens, with the human-dominated area being the second most abundant. This suggests that these sites provided the most favorable conditions for moth activity, potentially due to the availability of microhabitats and pollination sources nearby; however, the distribution across these distinct forest types may also be influenced by specific site characteristics, such as the degree of openness and canopy cover, which were not controlled for in this study but visually estimated prior to site selection. As these factors may have limited moth attraction, no definitive conclusions can be drawn about the preferred forest type for moths.

The high biodiversity, particularly at the species level, illustrates the ecological richness within Cloudbridge, as analyzing diversity within this classification provides the most detailed view of the ecosystem's health and stability. The consistent diversity indices across the different stages of forest succession suggest an overall healthy moth community, but again, the potential influence of site-specific characteristics calls for further investigation under controlled conditions. The diversity index for Sphingidae showed a more moderate figure than overall species diversity. This informs that while overall species diversity was considerably high, there was less diversity within the Sphingidae family, with more frequent occurrences of the same species, particularly those in the *Adhemarius* and *Xylophanes* genera. Coulthard et al. (2019) found that habitat preferences and ecological traits such as voltinism (number of generations per year) significantly influence moth populations, which could potentially explain the dominance of certain genera. Additionally, Sphingidae exhibited the highest species richness in the human-dominated habitat-type, though no significant variations were observed between each location, reinforcing the need for further study to understand habitat preferences fully. Research on the diversity of moths in Sri Lanka emphasized the importance of habitat diversity in maintaining moth populations and indicated that moth species richness and abundance are closely linked to the availability of suitable habitats (Gunathunga et al., 2022) while Krishnan (2016) similarly found that that the presence of host plants was the most critical influence on Sphingid moth diversity and abundance in Kerala, India. These findings suggest that future research should also consider the availability and distribution of relevant plant species and microhabitats to better understand their role on moth diversity and abundance in Cloudbridge. Although this research did not account for all applicable environmental factors, the findings corroborate the observed high species richness and even distribution of moths across different forest succession stages. This illustrates the necessity of preserving diverse habitats to maintain overall diversity.

The negative impact of moonlight on overall moth activity suggests that sampling during a new moon or when moon illumination is low may slightly increase moth presence and diversity; however, this correlation is considerably weaker for Sphingidae. In contrast, cloud coverage showed a stronger relationship with moth activity for both overall counts and Sphingidae specimens. As cloud cover increased, so did the number of individuals, indicating that cloud coverage is more effective than moon phase for increasing moth presence and diversity during light-trapping. This phenomenon is likely due to increased cloud cover blocking moonlight intensity, which otherwise might inhibit moth attraction (Nowinszky et al., 2023).

### B. Limitations

This study presents several limitations that require consideration for interpretation of the results. Firstly, the exclusion of moth specimens smaller than 3 cm likely overlooked a substantial segment of the moth community. Future studies should incorporate smaller moth species to achieve a more comprehensive understanding of moth diversity and community dynamics. Additionally, this study only accounted for a limited range of environmental variables. To strengthen the validity of future research, it’s recommended to measure a broader spectrum of environmental factors, including temperature, rainfall, wind speed and direction, as well as seasonal variations, as these can potentially influence moth activity and behavior.

The constrained sampling duration and timing, restricted to 1.5 hours post-sunset, may have also biased the results. Extending the duration and varying the sampling periods would likely capture a more diverse array of species, particularly those active later in the night, thereby providing a more accurate representation of the moth community. Furthermore, the selection of sampling sites based on visual estimates of openness and canopy cover might have introduced bias. Systematic control of these site-specific characteristics in future studies is advised to ensure the reliability and validity of the findings. Sites with greater openness and reduced canopy cover might improve light trap visibility and effectiveness, potentially enhancing moth capture rates.

While the UV light-traps produced substantial results, budget limitations restricted the use of more effective but expensive mercury vapor lights. These are known to be more attractive to moths compared to UV LED lights, as the bulbs emit a larger proportion of their light as ultraviolet (Pendleton, n.d.). The use of UV traps in this study might have resulted in lower attraction rates and potentially biased the assessment of moth diversity. Additional research conducted at Cloudbridge should consider the inclusion of mercury vapor light traps to improve attraction efficiency and obtain a more representative sample of the moth community.

**Conclusion**

This study provides a comprehensive analysis of moth diversity and habitat preferences within Cloudbridge Nature Reserve, with a specific focus on the Sphingidae family. The results show a notable dominance of the Geometridae family across the reserve, highlighting its prevalence within this ecosystem. The NR<30 forest type exhibited the highest number of recorded specimens, suggesting favorable conditions for moth activity in this sampling location. The diversity indices calculated for the various forest types show that moth diversity remains relatively consistent across different stages of forest succession, emphasizing the importance of preserving a range of habitat types to maintain overall biodiversity. The high species-level diversity index reflects the ecological richness of Cloudbridge, demonstrating a balanced distribution of species within the reserve. The Sphingidae family showed moderate diversity with the highest species richness observed in the human-dominated habitat, warranting further investigation into their specific habitat requirements. Environmental factors such as moon phase and cloud coverage were found to influence moth activity. The negative correlation between moon illumination and moth presence suggests that sampling during periods of low moonlight may slightly increase capture rates, while increased cloud cover positively correlated with higher moth activity, indicating that cloud coverage enhances moth attraction to light traps. Despite producing considerable results, the study faced limitations, including the exclusion of smaller moth specimens and a constrained sampling duration, which may have influenced the results. Future research should incorporate a broader spectrum of environmental variables and extend sampling periods to capture a more comprehensive representation of the moth community. Overall, this study illustrates the importance of continuous monitoring and conservation efforts within Cloudbridge. The observed diversity and habitat preferences of moths, particularly the Sphingidae family, provide baseline data for future ecological studies and conservation strategies aimed at assessing and preserving biodiversity.

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