

## **Diversity and Status of Butterflies (Lepidoptera: Rhopalocera) Found in PC Hills, Leyte (Philippines)**

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### **Abstract**

Variations in habitat quality is a known factor in the distribution patterns and abundance of butterflies. Research on butterfly population also provide evidence on how vegetation type influence butterfly diversity. This study assessed the Rhopalocera diversity of PC Hills to elucidate the diversity trends in varying vegetation types. PC Hills (Philippines) is a relatively undisturbed area of forests and rivers, transect sampling technique was applied in monitoring and documenting the butterflies' species richness and diversity. Three transects were established based on vegetation type: agroecosystem, dipterocarp forest, and riparian ecosystem. eight sampling sessions resulted to seven hundred forty-six individuals observed, of which twenty-two Rhopalocera genera were identified morphologically. Thirty-five voucher specimen were classified to the species taxonomic level using DNA Barcoding. The highest diversity level was observed in the third transect ( $H' = 3.0449$ ), followed by the second transect ( $H' = 2.7876$ ) and the lowest being in the first transect ( $H' = 2.3593$ ). Of the 36 species of butterflies, only *Notocrypta paralyos volux* was categorized by IUCN conservation status as Vulnerable. This study indicates that butterflies are likely to inhabit dipterocarp and riparian vegetation types which then influences their species composition and diversity.

**Keywords:** Bioiversity; DNA barcoding; Morphology; Rhopalocera; Transect sampling.

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### **1. Introduction**

PC Hills (N11°12'31.7'' E124°57'42.9'') is a series of hills located in Palo, Leyte Philippines. Little to no information can be found relating to the area, as no previous research have been conducted. Barayong, is the lone community located within the forests of PC Hills PC Hills is government owned and military reserved.

Invertebrates are rapidly affected by environmental fluctuations brought by climate change and landscape context compared to other taxa. Thus, they are commonly utilized to assess the impact of changes in the environment. Of these invertebrates, insects are considered as the most diverse group. They are accountable for a number of processes happening in the ecosystem. As the world's most diverse group of organisms, they represent over 50% of the global

terrestrial biodiversity yet they are poorly understood, including their conservation status, and ecologies [1].

Among invertebrates, butterflies are conspicuous components of open habitats and indicators of habitat quality. The results of the study of Nuneza *et al.* [2] on butterflies showed that high to moderate species diversity and a relatively even distribution may be accredited to the micro-habitats within the study area. The Distribution patterns and abundance of butterflies respond in short-term to variations in weather, whereas long-term effects are due to variations in habitat quality [3]. Butterflies are an ideal group for studying the effects of climate change because as poikilothermic organisms, their life cycle, activity, distribution and abundance are influenced by temperature [4]. These factors make butterflies one of the best species group on monitoring changes in biodiversity [1, 5]

Our study aims to determine how varying vegetation types in PC Hills, Palo, Leyte affect the diversity and status of Rhopalocera species which can be narrowed down into (1) the species of Rhopalocera found in PC Hills, (2) the species richness and diversity of Rhopalocera found in each vegetation types and (3) the observed status of the Rhopalocera species found between each of the selected vegetation types.

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Monitoring and determining the Rhopalocera diversity and status in PC Hills, Palo, Leyte will not only contribute to Local Butterfly Index decreasing the gaps in our biodiversity knowledge, but also let us observe how varying vegetation types influence its diversity [6, 7]. Two major factors impact the variability of patterns of species diversity, endemism and distribution [8]. These are temporal – consisting the date and time of sampling, and spatial – consisting the country, region, ecosystem, and habitat. Their study observed that butterfly species richness ought to be richer in forest habitats than in highly disturbed areas. The information gathered from this study will primarily benefit ecologists and taxonomists in the local region by increasing their understanding of Rhopalocera diversity, conservation status, ecology, and insect diversity as a whole.

This study was conducted over the course of two years starting from February 2018. Sample collections were done weekly for the months of February, until July of the same year. Morphological analysis was performed for the first phase of identification Whereas the second phase of classification into species taxonomic level was completed through DNA barcoding. Analysis of data ensued.

This study was limited only to identifying Rhopalocera to the lowest taxonomic level possible along with its diversity and status at varying vegetation types. Sample collections were only done during suitable weather conditions.

## 2. Material and Methods

### 2.1. Sampling sites

A map of PC Hills, Palo, Leyte obtained from the local Mines and Geosciences Bureau (MGB) was used to locate the travel routes, transects, and identify elevation of land features prior to sample collection. Transect belt sampling technique was applied to collect data requisite to this study. Transects were placed in locations that can be mapped, described and relocated easily [7]. A Garmin eTrex 10 GPS receiver with a worldwide basemap, a 25-hour battery life with AA batteries, Global Positioning System (GPS) and Global Navigation Satellite System (GLONASS) satellites, and a paperless geocaching that stores and displays key information was used to preselect the transect locations. Three transects measuring 300m each with a 5m diameter were established on each vegetation type present [9].

### 2.2. Sample collection

A Gratuitous Permit (GP) was secured from the regional office of the Department of Environment and Natural Resources (DENR). A GP is issued to any individual or entity that is engaged in noncommercial scientific, or educational undertaking to collect wildlife in the Philippines [10].

Following the modified pollard transects for tropical butterfly assessment of Caldas and Robbins

[6], sampling was done weekly for six months. Sample collection commenced from 9:00 am until 3:00 pm and was done only in warm and bright weather, with moderate winds and when it was not raining. During sampling sessions, the observer had one collecting net, with a handle length of 1 m and minimum diameter of 30 cm, to capture the butterfly voucher species. Transect walks were done at a slow and consistent pace and observed butterflies were recorded within 2.5 m for both sides of the transect and 5 m ahead of the observer [5]. Each time a sample was collected, the coordinates of where it was found, the frequency of its occurrence, the time of day, and the behaviour of the butterfly were all recorded for documentation. Butterflies observed outside of the transect were classified as “off-transect” but was included to the pool of data on site species list and distribution [11]. Butterflies seen within the transect were identified through morphological and molecular analysis. One sample of each species served as voucher specimen and was subsequently used for DNA barcoding.

### 2.3. Specimen preparation

Each voucher specimen was killed prior to pinning and mounting to ensure reduction of damage to its major parts., The thorax was pinched for 20-30 seconds before placing it into the kill jar for asphyxiation in order to prevent it from flopping and consequently damaging its wings. Kill jars were used to sedate the captured butterflies quickly with minimum damage. Each kill jar had a thin layer of Plaster-of-Paris saturated with acetone (C<sub>3</sub>H<sub>6</sub>O) as killing agent. A Paper towel or cardboard was placed on top of the Plaster-of-Paris to prevent the captured butterflies from touching the killing agent directly (Goforth, 2010). After sedation, the specimen was placed inside a relaxing chamber to regain flexibility. Relaxing jars were prepared to prevent the specimen from breaking apart. Subsequent observations were made after 2-3 days to assess condition of specimen. Relaxing jars consisted of a few pieces of paper towel soaked with water and Lysol placed inside a glass jar. To test pinning viability of specimen the thorax was squeezed gently, and movement of the wings indicated that the butterfly was ready for pinning. Insect pins, spreading boards and pinning blocks were made available for the pinning and mounting of butterflies [12].

### 2.4. Species identification

Pictorial classification through differentiating characters was used for identification of butterfly species from certain geographical areas. Photo-keys acquired from the National Museum of the Philippines and the Illustrated Lists of Philippine Butterflies of Badon *et al.* [13] were used.

Voucher specimen were identified through the aforementioned morphological classification and was cross validated using molecular analysis through DNA Barcoding.

The DNA Barcoding Protocol was based on the Cold Spring Harbor Laboratory DNA Learning Centers' Using DNA Barcodes [14]. The process included DNA isolation, amplification by PCR, and analysis of PCR products by Gel Electrophoresis. The PCR samples were sent to Macrogen Korea for DNA sequencing. DNA Baser and MEGA software applications were utilized for the construction of reliable phylogenetic trees.

### 2.5. Diversity assessment

To determine the species diversity patterns for each of identified habitat, two diversity patterns were calculated to complement species richness - the Shannon index ( $H'$ ) and Simpson index ( $D$ ). Each of these indices calculate an index of species diversity based on their own different prioritization. An open-source software application called EstimateS which offers statistical tools designed to analyse and compare the diversity and composition of groups of species using data gathered from sampling was perused [15]. Biodiversity statistical analyses needed for this study was computed using this application including the non-parametric estimates of species richness, and diversity indices.

The Simpson index focuses on the relative abundance of the most abundant species [16]. Equation (1) is the Simpson's index of Dominance.

$$D = \sum(n/N)^2 \quad (1)$$

where  $n$  is the total number of individuals of a particular species and  $N$  is the total number of individuals of all species. The Simpson's Diversity Index in Equation (2), on the other hand, is the reciprocal of the Simpson's Index of Dominance.

$$\frac{1}{D} = \frac{1}{\sum(n/N)^2} \quad (2)$$

The Shannon-Wiener index prioritizes on species richness and dominant species.

$$H' = -\sum(pi \ln pi) \quad (3)$$

where  $pi$  is equal to the total number of individuals of a particular species over the total number of individuals of all species.

The complementarity, or the similarity and dissimilarity between two different transects was determined with the use of Jaccard's coefficient ( $J$ ). This method measures species overlaps between two species.

$$J = A/(A + B + C) \quad (4)$$

where  $A$  is the sum of joint species occurrences,  $B$  is the unique occurrence in transect a, and  $C$  is the unique occurrence in transect b. A value of 1 means that the two sampling sites contain identical species. The converse

therefore is a measure of complementarity ( $1 - J$ ) which correlates to greater differences between two sampling sites. The species overlap between each of the habitats was compared to determine which habitats provide greater contribution to overall species diversity.

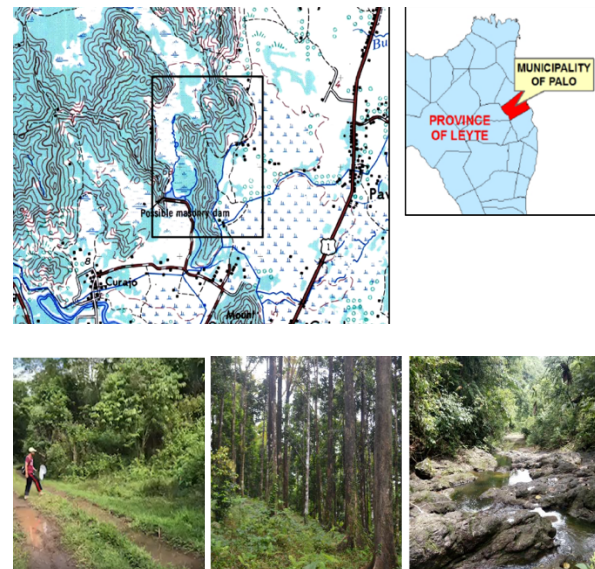
### 2.6. Assessment of status

The scale of occurrences by Mohagan and Treadaway [7] was used to evaluate the local status of butterflies found in PC Hills. Taxonomic status of butterflies was assessed using Treadaway and Schröder Revised Checklist [17]. Ecological and conservation status of butterflies was assessed through the Philippine Lepidoptera website of Badon *et al.* [13].

## 3. Results and Discussion

### 3.1. Site identification

The transects were placed based on the observed dominant types of plants found in the area (Figure 1). Transect I was stationed within an agroecosystem where agricultural crops were observed to be dominant. Transect II was surrounded with trees under the family Dipterocarpaceae, a plant family which dominates lowland tropical forests. Transect III was situated on an area with an interface between land and a river, a riparian ecosystem.



**Figure 1.** Top: Geographic map of PC Hills, Palo, Leyte, Philippines. Map obtained from the Mines and Geo Sciences Bureau Regional Office 8. The selected study sites at PC Hills, Palo, Leyte. Bottom left: transect I; Bottom center: transect II; Bottom right: transect III.

### 3.2. Species composition

The data collected after eight successful sampling sessions (Table 1) recorded a total of 737 individuals. Thirty-five *Rhopalocera* species were collected in PC Hill, Palo, Leyte, Philippines. These species belong to 25 genera and six families of butterflies.

**Table 1.** List of Rhopalocera species observed in each transect of PC Hills (Philippines).

| Species   | Transect Frequency |            |            | Total      |
|---|--------------------|------------|------------|------------|
|   | I                  | II         | III        |            |
| <b>Hesperiidae</b>                                  |                    |            |            |            |
| <i>Borbo cinnara</i> Wallace 1866                   | 0                  | 2          | 5          | 7          |
| <i>Notocrypta paralysos volux</i> Mabille 1883      | 0                  | 0          | 2          | 2          |
| <i>Tagiades japetus titus</i> Plotz 1884            | 0                  | 0          | 1          | 1          |
| <b>Lycaenidae</b>                                   |                    |            |            |            |
| <i>Caleta roxus angustior</i> Staudinger 1889       | 2                  | 4          | 10         | 16         |
| <i>Jamides alecto manilana</i> Toxopeus 1930        | 1                  | 29         | 15         | 45         |
| <i>Jamides zebra</i>                                | 5                  | 20         | 6          | 31         |
| <b>Papilionidae</b>                                 |                    |            |            |            |
| <i>Graphium 28gamemnon</i> Linnaeus 1758            | 2                  | 4          | 6          | 12         |
| <i>Papilio polytes</i> (♂)                          | 1                  | 2          | 3          | 6          |
| <i>Papilio polytes</i> (♀)                          | 9                  | 11         | 17         | 37         |
| <i>Papilio palinurus</i>                            | 0                  | 3          | 14         | 17         |
| <i>Papilio paris</i> Linnaeus 1758                  | 0                  | 0          | 2          | 2          |
| <i>Troides vandepolli</i>                           | 0                  | 0          | 1          | 1          |
| <b>Pieridae</b>                                     |                    |            |            |            |
| <i>Appias libythea</i>                              | 8                  | 4          | 1          | 12         |
| <i>Eurema hecabe</i> Linnaeus 1758                  | 62                 | 53         | 33         | 148        |
| <i>Pareronia valeria calliparga</i> Fuhstorfer 1910 | 2                  | 0          | 1          | 3          |
| <i>Pieris naganum</i>                               | 0                  | 0          | 2          | 2          |
| <i>Pieris rapae</i>                                 | 66                 | 24         | 2          | 92         |
| <b>Riodinae</b>                                     |                    |            |            |            |
| <i>Melanis smithiae</i>                             | 2                  | 2          | 0          | 4          |
| <i>Panara phereclus</i>                             | 0                  | 4          | 0          | 4          |
| <b>Nymphalidae</b>                                  |                    |            |            |            |
| <i>Amathusia phidippus cebuensis</i> Okano 1986     | 1                  | 1          | 1          | 3          |
| <i>Acrophtalmia leuce</i>                           | 2                  | 34         | 17         | 53         |
| <i>Cyrestis nivea</i>                               | 0                  | 0          | 1          | 1          |
| <i>Danaus melanippus edmondii</i> Lesson 1837       | 9                  | 5          | 3          | 17         |
| <i>Euploea mulciber mindanensis</i> Staudinger 1885 | 1                  | 5          | 1          | 7          |
| <i>Euploea tulliolus pollita</i> Erichson 1834      | 16                 | 13         | 2          | 31         |
| <i>Hypolimnas anomala</i> Wallace 1869              | 1                  | 3          | 2          | 6          |
| <i>Hypolimnas bolina</i> Butler 1874                | 4                  | 4          | 4          | 12         |
| <i>Junonia atlites</i> Linnaeus 1758                | 1                  | 3          | 3          | 7          |
| <i>Junonia hedonia</i> Cramer 1775                  | 18                 | 10         | 11         | 39         |
| <i>Neptis jumbah</i>                                | 5                  | 4          | 4          | 13         |
| <i>Neptis taiwana</i>                               | 2                  | 3          | 3          | 8          |
| <i>Orsotriaena medus</i>                            | 2                  | 1          | 2          | 5          |
| <i>Parantica vitrina</i> Felder & Felder 1861       | 2                  | 3          | 2          | 7          |
| <i>Parthenos sylvia</i>                             | 0                  | 0          | 1          | 1          |
| <i>Ypthima baldus selinuntius</i> Fruhstorfer 1911  | 10                 | 16         | 2          | 28         |
| <i>Ypthima pandocus</i>                             | 8                  | 45         | 3          | 56         |
| <b>TOTAL</b>  | <b>242</b>         | <b>312</b> | <b>183</b> | <b>737</b> |

Transect I: agroecosystem, Transect II: diptocarp forest ecosystem, Transect III: riparian ecosystem

### 3.3. Diversity assessment

Butterfly diversity patterns were determined using the data gathered from the sampling sites with differing vegetation types. The sampling efforts were summarized by assessing the butterfly abundance, richness, and the diversity of the Rhopalocera species encountered. Consequently, the Simpson's Diversity Index and Shannon -Weiner Index values contribute to the richness in diversity of the transect areas. Transect 3 recorded the highest diversity index value. On the other hand, transect 1 had the lowest species richness among all three transects. It is to be noted that Transect 1 was situated within an agroecosystem while Transect 3 was part of a riparian ecosystem. Mohagan and Treadaway [7] posited that butterfly abundance was highest in agroecosystems and it decreases with increased elevations suggesting that butterfly species in higher elevations have the tendency to become rare or unique. Two major factors influence the variability of patterns of species diversity, endemism and distribution. These are temporal – consisting the date and time of sampling, and spatial – consisting the country, region, ecosystem, and habitat. The study observed that butterfly species richness ought to be richer in forest habitats than in highly disturbed areas. Though sampling sites with a good amount of species richness compared to the others tend to have better chances of having higher endemism, it did not seem to be the case. High endemism of butterfly species may be due to unique location, higher elevation, and unique habitat that influence the existence of the butterfly fauna and flora [9]. Transect 2 recorded the most number of butterfly sightings. Of the six families observed, Family Nymphalidae showed the greatest number of species. In another study, habitats near a side stream have high records of diversity. The stream sides inside the disturbed forests, on the other hand, have less vegetation layers and simpler vegetation than the disturbed forest, thus the stream sides have less species number than the disturbed forest. However, these sites were found to host a great number of butterfly individuals and high proportion of common species with a wide geographical distribution range because of their openings and the availability of water in rocks, sand, and mud along the stream [18]. Meanwhile, the species under the family Nymphalidae, *Ypthima baldus*, as well as the ones under the family Pieridae, *Eurema hecabe* and *Pieris rapae*, were most occurring among all butterflies observed in the three transects throughout the whole sample collection.



**Figure 2.** Three most occurring Rhopalocera species along the three transects.

For each sampling, species composition similarity between paired transects was assessed using the Jaccard Similarity Coefficient.

The similarity rate of Rhopalocera species between paired transects was determined using the computed Jaccard Similarity Coefficient values (Table 2). Transects 2 and 1 showed the highest values which means that they have much more concordant species than being paired with other transects. The butterflies which favor an agroecosystem vegetation (Transect 1) is also likely to fly into a dipterocarp vegetation (Transect 2) and vice versa. Transects 1 and 3 showed the least value meaning butterflies living in each vegetation have a lesser tendency to favor the other transect due to the differences in vegetation.

**Table 2.** Jaccard Similarity Coefficient

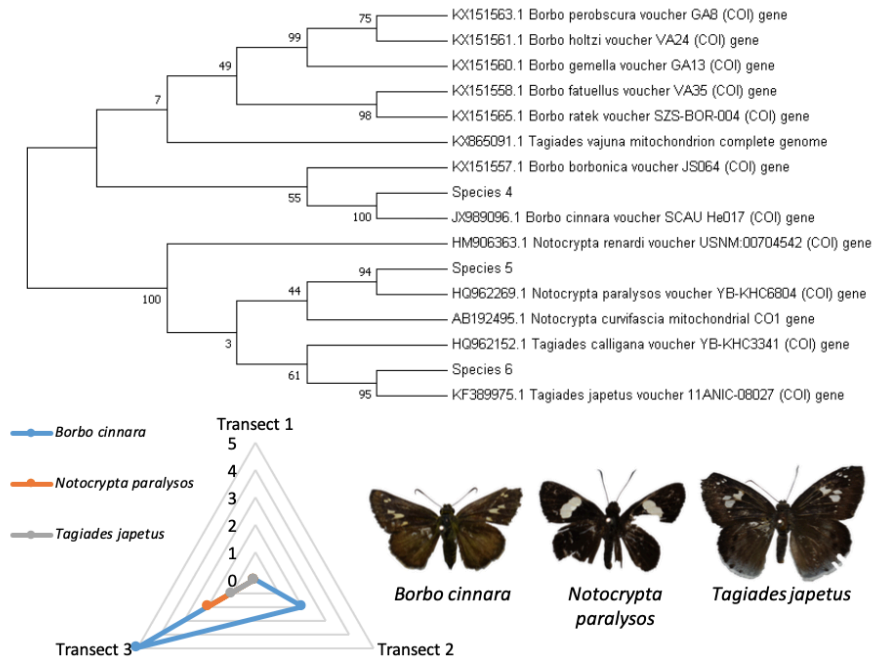
|            | Transect I | Transect II | Transect III |
|------------|------------|-------------|--------------|
| Transect 1 |            | 0.806       | 0.676        |
| Transect 2 | 0.806      |             | 0.737        |
| Transect 3 | 0.676      | 0.737       |              |

Table 3 shows measurements of the evenness and dominance of the Rhopalocera Diversity were computed through the Simpson's Index of Dominance. Values show that the Agroecosystem vegetation (Transect 1) has about twice as much than among all the other transects. A higher value of dominance level in the agroecosystem vegetation type indicates that Rophalocera species richness is low, and the population bulk is due to the high number of individuals of *E. hecabe* and *M. smithiae* which make up about 32% of the population when combined.

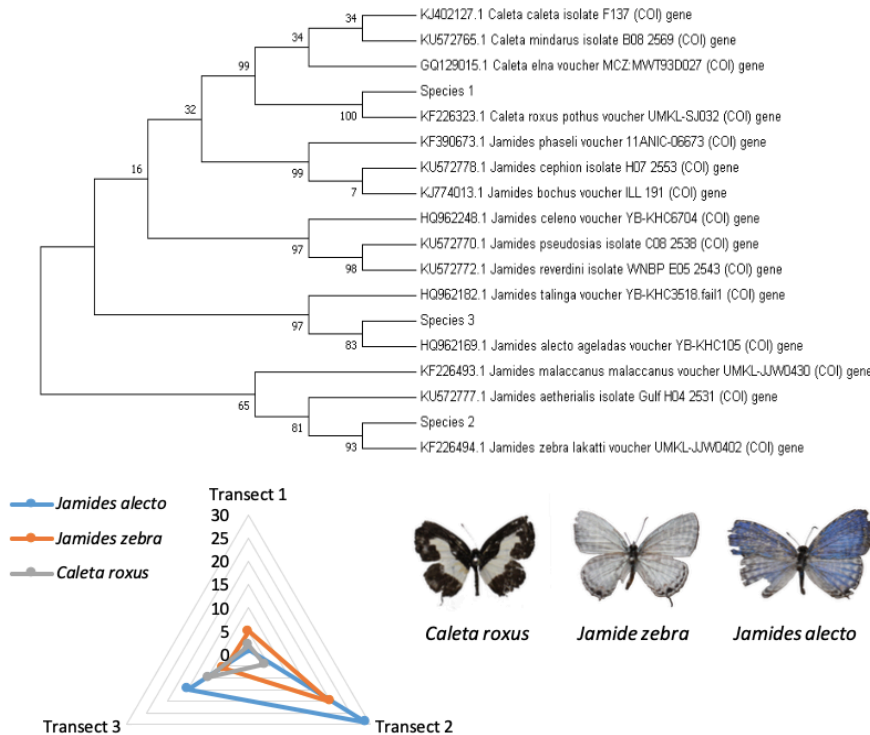
**Table 3.** Diversity of the Rhopalocera species found in PC Hills, Palo, Leyte

|            | Simpson's Index of Dominance (D) | Simpson's Diversity Index (1/D) | Shannon - Weiner Index |
|------------|----------------------------------|---------------------------------|------------------------|
| Transect 1 | 0.1584                           | 6.3149                          | 2.3593                 |
| Transect 2 | 0.0873                           | 11.4539                         | 2.7876                 |
| Transect 3 | 0.0713                           | 14.0247                         | 3.0449                 |

On the other hand, the Simpson's Diversity Index and Shannon - Weiner Index values contribute to the richness in diversity of the transect areas. Transect 3 showed the highest diversity index value thus stating that it is the most diverse among all the other transects. Since Transect 1 is situated nearby a village and the abundant plant types were agricultural its richness in diversity is lower while the other transects were in dipterocarp and riparian vegetation types. Caldas [6] accounted this due to the lack of disturbance in the area.



**Figure 3.** Butterfly distribution for family Hesperiiidae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Hesperiiidae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Hesperiiidae along the selected sampling sites. Bottom right: Rhopalocera species under the family Hesperiiidae.



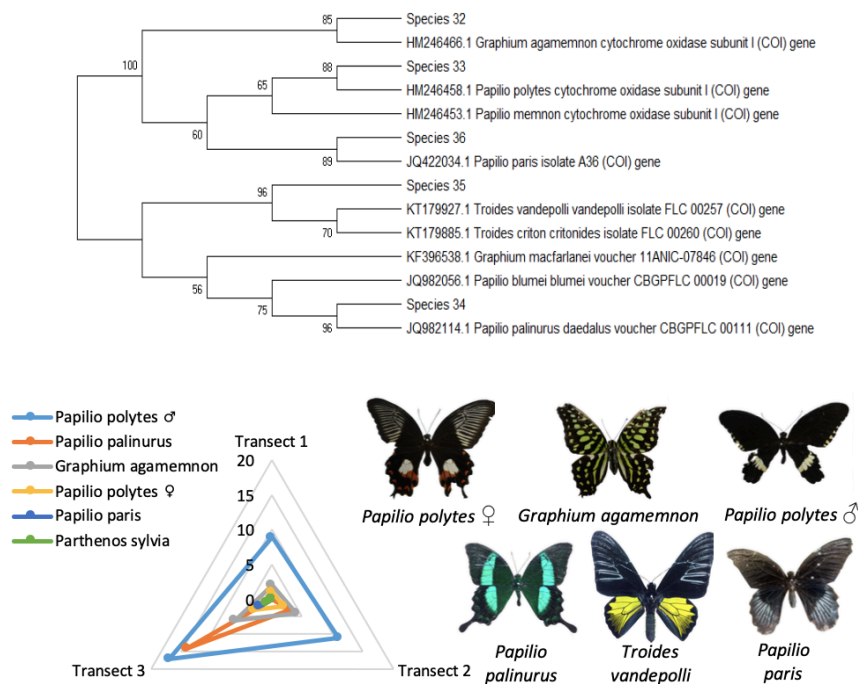
**Figure 4.** Butterfly distribution for family Lycaenidae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Lycaenidae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Lycaenidae along the selected sampling sites. Bottom right: Rhopalocera species under the family Lycaenidae.

Three Rhopalocera species under the Hesperidae family occurred mainly on the third transect, with *Borbo. Cinnara* being the most dominant. Not one Hesperidae species was found in transect 1, which means that these butterflies lack preference to an agroecosystem environment. Hesperidae, also known as skippers, are typically small to medium in size, with wingspans ranging from 30 – 55 mm, their coloration varies from brown, black, white, or gray. Their wings behave differently when they perch, and their upper wings have a sharper shape. They have relatively large eyes, and their antennae is shaped like a hooked club.

Three Rhopalocera species under the Lycaenidae family (Figure 4) occurred mainly on the second and third transect, with *Jamides alecto* being the most dominant. These butterflies were the smallest

butterflies observed with at least 20 mm wingspan and are often brilliantly colored with iridescent blues and whites, sometimes with metallic gloss. They have small dark spots in their lower wings, nearby their short curly tails.

Papilionidae species were mostly observed in the third transect, as how most of them point toward transect 3 in the radar graph. Some of them were also seen on the other transects such as the male *Papilio polytes*, which dominated the family in all selected transects. Although coming from the same species, the female *P. polytes* was not frequently observed. Papilionidae species are medium to large, with wingspans ranging from 80 – 125 mm. Their wings are very varied, having many colors, patterns, and some with tails (Figure 5).



**Figure 5.** Butterfly distribution for family Papilionidae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Papilionidae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Papilionidae along the selected sampling sites. Bottom right: Rhopalocera species under the family Papilionidae.

Five butterflies under the Pieridae family were observed during the sampling sessions (Figure 6). Pieridae species were seen in all transects with *Eurema hecabe* and *Pieris rapae* being the dominant ones, observation counts of Pieridae species are relatively higher in the first transect. This means that Pieridae can inhabit either of the three areas but likely prefer the agroecosystem environment. Butterflies under this family are typically small to medium, with wingspans ranging from 40 – 70 mm, and their wing color is usually white, yellow, or orange, with some black.

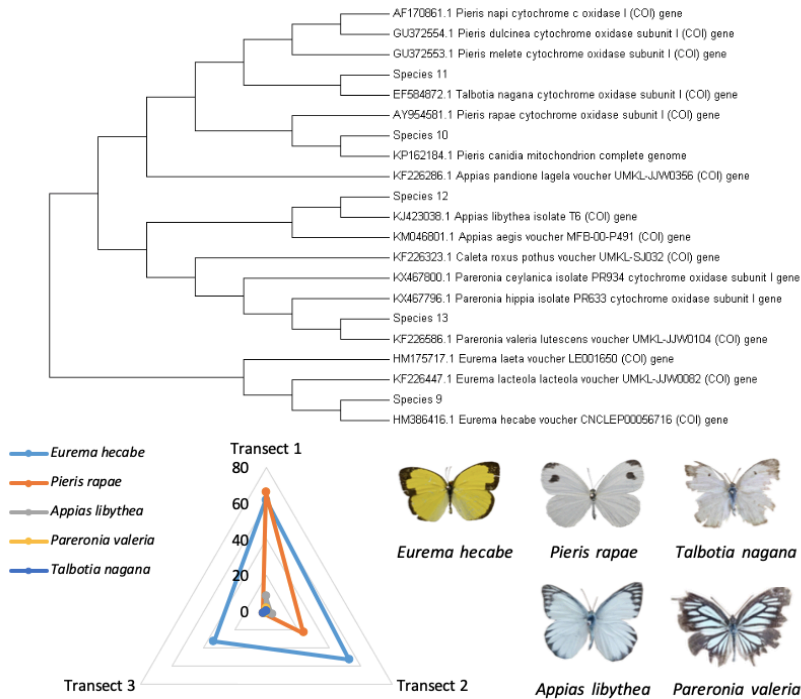
Only two Rhopalocera species under the Riodinidae family were seen during the sampling session. No Riodinidae species were observed in the third transect

since *Melanis smithae* occurred in both transects 1 and 2 only, and *P. phereclus* occurred in transect 2. Riodinidae, also called skippers, are typically small to medium, with wingspans ranging from 30 – 55 mm, and are usually brown, black, white, or gray. Their wings behave differently when they perch, and their upper wings have a sharper shape. Their eyes are relatively large, and their antennae is shaped like a hooked club. Figure 7 shows the distribution of Family Riodinidae.

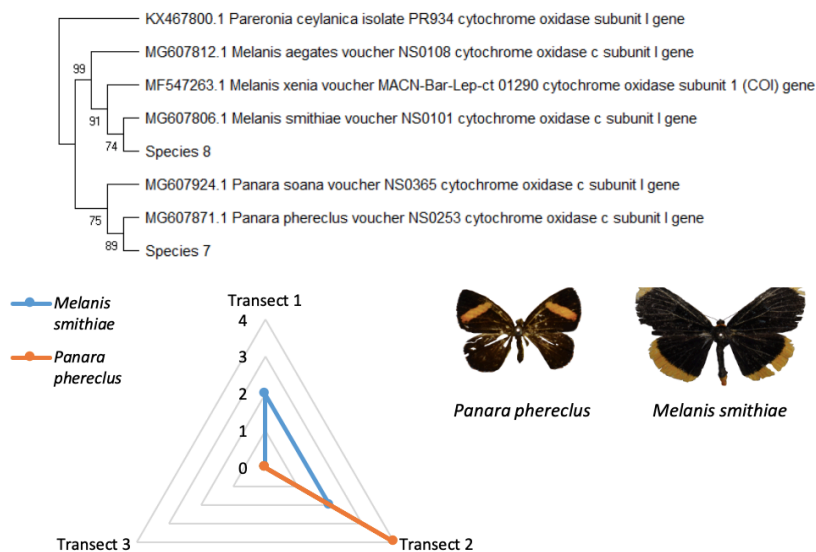
Family Nymphalidae was found to have the highest species richness observed with a total of 16 different species (shown in Figure 8). These butterflies were seen in all transects with *Y. pandocus* and *A. leuce* dominating both the Transects 2 and 3. Butterflies

under this family are typically small to large, with wingspans ranging from 40 – 125 mm. Their wing shapes have many variations, and their colors are usually brown, yellow, and black, but some have rare iridescent colors such as purples and blues when looking at certain angles.

The eight sample collecting sessions along the established transects in PC Hills resulted in a total of 35 voucher specimen caught with about 737 total individuals observed (Figure 9). Through morphological analyses, the butterflies were classified into six families: Nymphalidae, Lycaenidae, Hesperidae, Riodinidae, Papilionidae, and Pieridae.

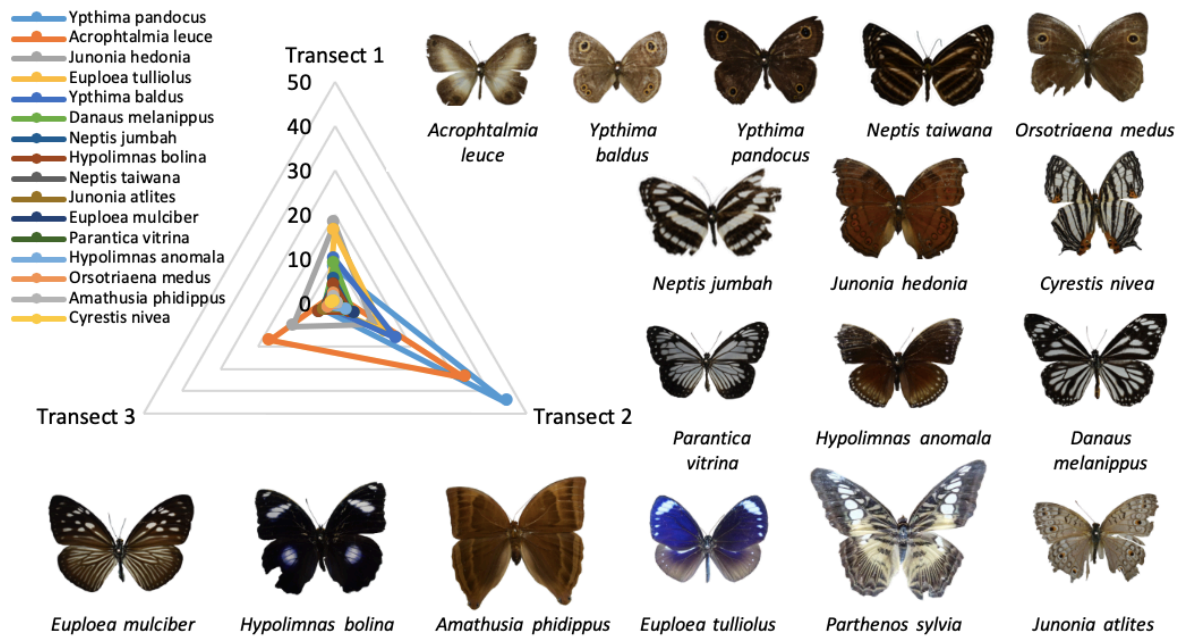
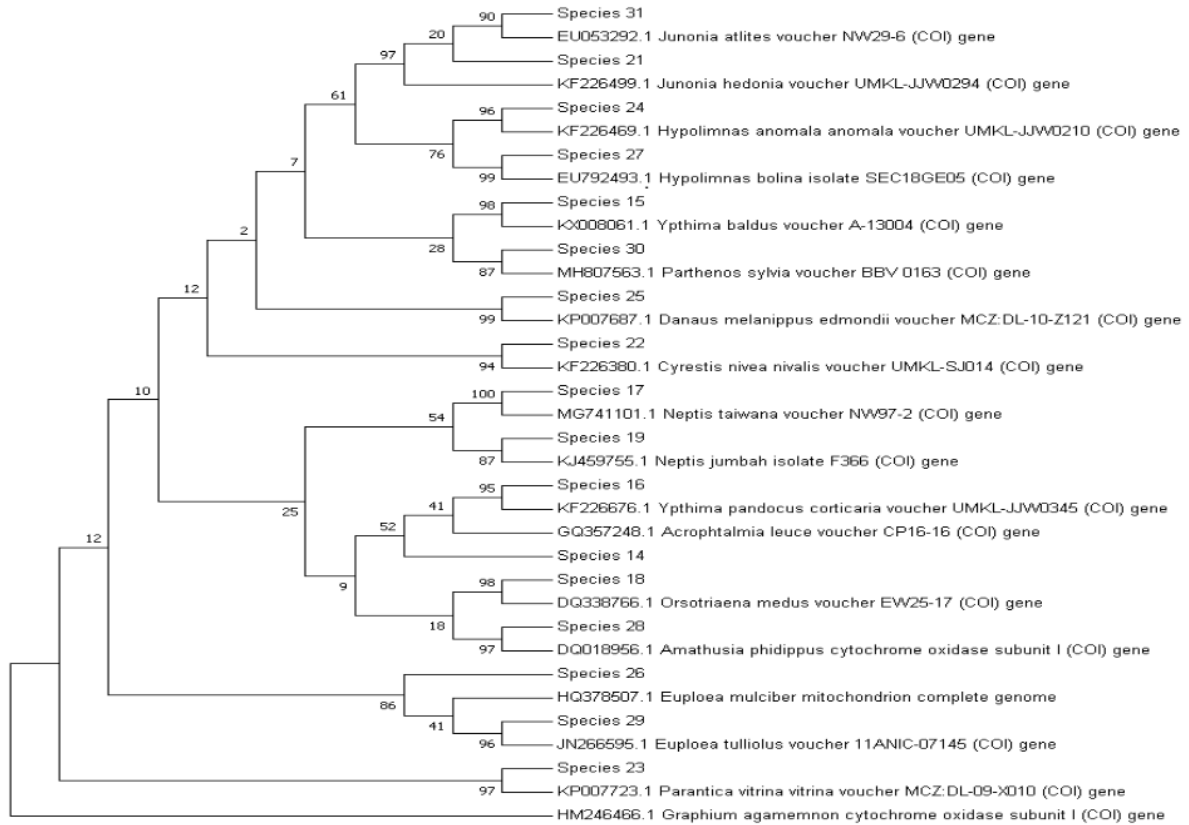


**Figure 6.** Butterfly distribution for family Pieridae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Pieridae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Pieridae along the selected sampling sites. Bottom right: Rhopalocera species under the family Pieridae.



**Figure 7.** Butterfly distribution for family Riodinidae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Riodinidae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Riodinidae along the selected sampling sites. Bottom right: Rhopalocera species under the family Riodinidae.





**Figure 8.** Butterfly distribution for family Nymphalidae. Top: Phylogenetic tree which includes the Rhopalocera species under the family Nymphalidae. Bottom left: Radar graph which shows the species distribution of Rhopalocera species under family Nymphalidae along the selected sampling sites. Bottom right: Rhopalocera species under the family Nymphalidae.

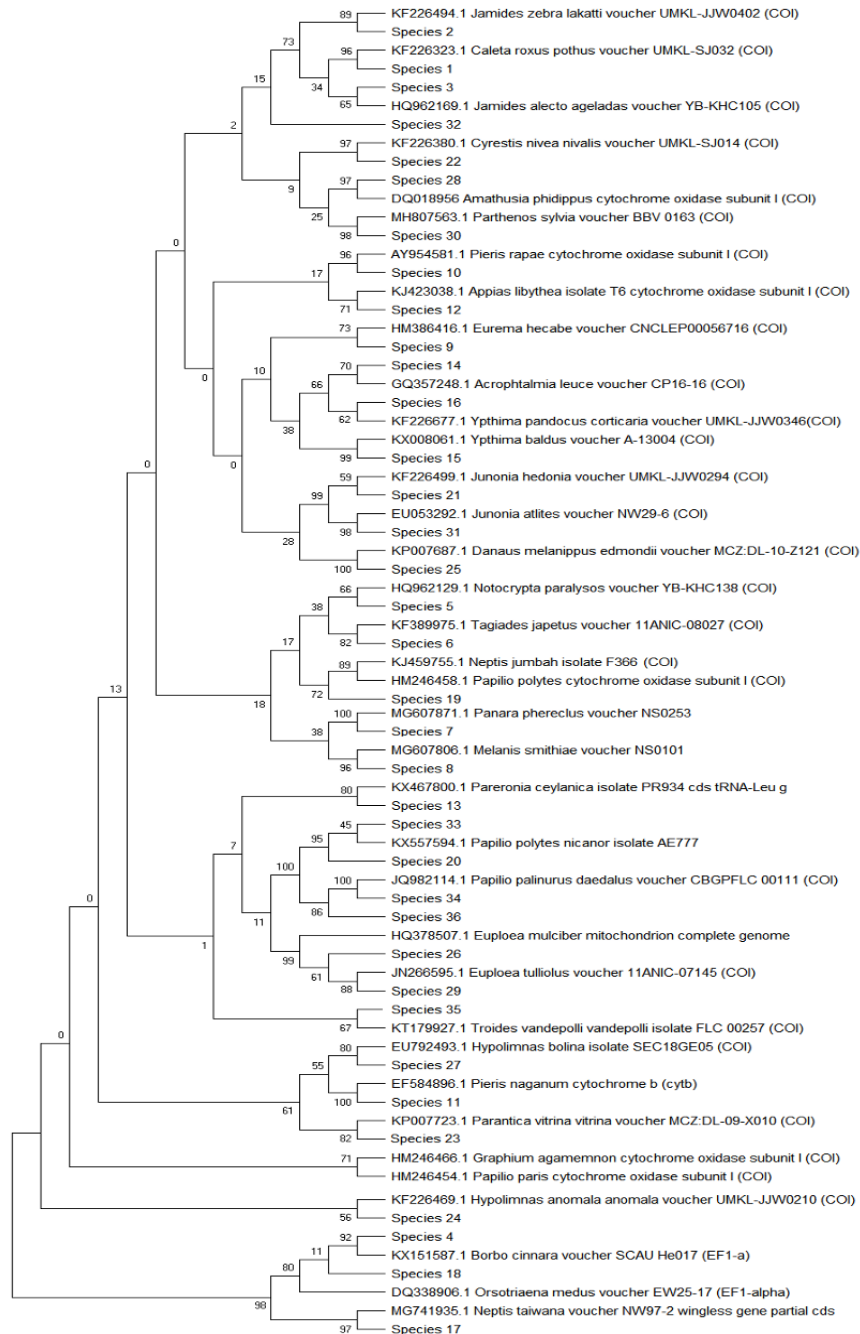
### 3.4. 3.4. Butterfly status

Local assessment of the butterflies in PC Hills showed that 25.71% are very rare, 28.57% are rare, 20% are common, and 25.71% are very common. Of the 35 species of butterflies, only *Notocrypta paralysos volux*

had an International Union for Conservation of Nature (IUCN) conservation status categorised as Vulnerable [19]. A taxon is Vulnerable when the best available evidence indicates that it meets any of the criteria stated by the IUCN [20] and it is therefore considered to be

facing a high risk of extinction in the wild. The comprehensiveness of IUCN's Red List assessments has yielded better results for the state and distribution of larger taxa. However, using IUCN system for butterfly status analyses will only become possible if populations are assessed extensively and supplemented with a compendium of both local and national butterfly studies [21, 11]. The rarity of *Jamides alecto manilana*

in its ecological status as reported by Treadaway and Schröder [17] in the Revised Checklist of the Butterflies of the Philippine Islands, is noted as a very common species in the locality. Also, the sighting of a member of the family Nymphalidae, *Orsotriaena medus*, classified as a rare butterfly species is also noted in the locality of PC Hills.



**Figure 9.** Phylogenetic tree which includes all the Rhopalocera species caught. This tree was generated from the maximum likelihood analysis using MEGA.

All samples of butterfly species used in this study were collected from the PC Hills, Leyte (Philippines). There are no endangered or protected species, and all

the other samples are common Rhopalocera species not included in the “List of Threatened Species in the Philippines” and other related lists of animal species

protection in the world. Thus, the sampling in this study did not violate any law, rule or regulation in the Philippines and all around the world, requiring no ethical or institutional approval.

Further butterfly sampling sessions of PC Hills for future researchers is recommended for a more reliable diversity trend and to discover more butterfly species. Trying other sampling techniques is also suggested to

determine the best way to assess butterfly diversity and also to note the fastest and easiest way to catch butterflies depending on various situations. Expanding the research by assessing the Rhopalocera diversity in other locations should be considered along with studying other factors that affect butterfly species composition and diversity other than vegetation types.

**Table 4.** List of Rhopalocera species observed in PC Hills (Philippines) and their status

| Species   | Transect Frequency      |                    |
|---|-------------------------|--------------------|
|   | <sup>1</sup> Ecological | <sup>2</sup> Local |
| <b>Hesperiidae</b>                                  |                         |                    |
| <i>Borbo cinnara</i> Wallace 1866                   | common                  | rare               |
| <i>Notocrypta paralysos volux</i> Mabille 1883      | common                  | very rare          |
| <i>Tagiades japetus titus</i> Plotz 1884            | common                  | very rare          |
| <b>Lycaenidae</b>                                   |                         |                    |
| <i>Caleta roxus angustior</i> Staudinger 1889       |                         | common             |
| <i>Jamides alecto manilana</i> Toxopeus 1930        | rare                    | very common        |
| <i>Jamides zebra</i>                                |                         | very common        |
| <b>Papilionidae</b>                                 |                         |                    |
| <i>Graphium agamemnon</i> Linnaeus 1758             | common                  | common             |
| <i>Papilio polytes</i> (♂)                          | common                  | rare               |
| <i>Papilio palinurus</i>                            |                         | common             |
| <i>Papilio paris</i> Linnaeus 1758                  |                         | very rare          |
| <i>Troides vandepolli</i>                           |                         | very rare          |
| <b>Pieridae</b>                                     |                         |                    |
| <i>Appias libythea</i>                              |                         | common             |
| <i>Eurema hecabe</i> Linnaeus 1758                  | common                  | very common        |
| <i>Pareronia valeria calliparga</i> Fuhstorfer 1910 |                         | very rare          |
| <i>Pieris naganum</i>                               |                         | very rare          |
| <i>Pieris rapae</i>                                 |                         | very common        |
| <b>Riodinae</b>                                     |                         |                    |
| <i>Melanis smithiae</i>                             |                         | rare               |
| <i>Panara phereclus</i>                             |                         | rare               |
| <b>Nymphalidae</b>                                  |                         |                    |
| <i>Amathusia phidippus cebuensis</i> Okano 1986     | common                  | very rare          |
| <i>Acroptalmia leuce</i>                            | common                  | very common        |
| <i>Cyrestis nivea</i>                               | common                  | very rare          |
| <i>Danaus melanippus edmondii</i> Lesson 1837       | common                  | common             |
| <i>Euploea mulciber mindanensis</i> Staudinger 1885 | common                  | rare               |
| <i>Euploea tulliolus pollita</i> Erichson 1834      | common                  | very common        |
| <i>Hypolimnas anomala</i> Wallace 1869              | common                  | rare               |
| <i>Hypolimnas bolina</i> Butler 1874                | common                  | common             |
| <i>Junonia atlites</i> Linnaeus 1758                | common                  | rare               |
| <i>Junonia hedonia</i> Cramer 1775                  | common                  | very common        |
| <i>Neptis jumbah</i>                                |                         | common             |
| <i>Neptis taiwana</i>                               |                         | rare               |
| <i>Orsotriaena medus</i>                            | rare                    | rare               |
| <i>Parantica vitrina</i> Felder & Felder 1861       |                         | rare               |
| <i>Parthenos sylvia</i>                             |                         | very rare          |
| <i>Ypthima baldus selinuntius</i> Fruhstorfer 1911  |                         | very common        |
| <i>Ypthima pandocus</i>                             |                         | very common        |

<sup>1</sup>Ecological Status is derived from the Revised Checklist of the butterflies of the Philippine Islands (Lepidoptera: Rhopalocera) (Treadaway & Schröder, 2012). <sup>2</sup>Local Status: *very rare*: 1-3 occurrences, *rare*: 4-10 occurrences, *common*: 11-20 occurrences, *very common*: 21-above occurrences.

## 4. Conclusions

Butterflies are likely to inhabit dipterocarp and riparian vegetation types than agroecosystem, which influences their species composition and diversity. Moreover, the presence of a river in the riparian vegetation supported its diversity value since butterflies are inclined to visit areas near rivers. Overall, the results suggested that butterflies have the tendency to fly into varying vegetation types which influence their species composition and diversity.

## Conflict of Interest

The authors declare no conflict of interest.

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## References

- [1] S. Willis, J. Hill, C. Thomas, D. Roy, R. Fox, D. Blakeley and B. Huntley, "Assisted colonization in a changing climate: a test-study using two U.K. butterflies.," *Conservation Letters*, no. 2, pp. 46-52, 2009.
- [2] K. Nuneza, O. Nuneza and A. Barrion-Dupo, "Species richness of Lepidoptera in bega watershed, Prosperidad, Agusan del Sura, Philippines.," *Bulletin of Environment, Pharmacology, and Life Sciences*, vol. 5, pp. 83-90, 2016.
- [3] P. Nowicki, J. Settele and P. W. M. Henry, "Butterfly monitoring methods: the ideal and the real world.," *Israel Journal of Ecology and Evolution*, vol. 54, pp. 69-88, 2008.
- [4] D. Roy, P. Rothery, D. Moss, E. Pollard and J. Thomas, "Butterfly numbers and weather: Predicting historical trends in abundance and the future effects of climate change.," *Journal of Animal Ecology*, vol. 70, p. 201 – 217, 2008.
- [5] J. Thomas, "Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. Philosophical transactions of the Royal Society of London.," *Series B, Biological sciences*, vol. 360, p. 339–357., 2005.
- [6] A. Caldas and R. Robbins, "Modified Pollard transects for assessing tropical butterfly abundance and diversity.," *Biological Conservation*, vol. 110, p. 211 – 219., 2003.
- [7] A. Mohagan and C. Treadaway, "Diversity and status of butterflies across vegetation types of Mt. Hamiguitan, Davao Oriental, Philippines.," *Asian Journal of Biodiversity*, 2010.
- [8] A. Mohagan, D. Mohagan and A. Tambuli, "Diversity of butterflies in the selected key biodiversity areas of Mindanao, Philippines.," *Asian Journal of Biodiversity*, 2011.
- [9] J. Toledo and A. Mohagan, "Diversity and status of butterflies in Mt. Timpoong and Mt. Hibokhibok, Camiguin Island, Philippines.," *JPAIR Multidisciplinary Research*, 2011.
- [10] P. M. Staff, "Republic Act No. 9147.," 2001. [Online]. Available: <https://www.pms.gov.ph/>. [Accessed May 2020].
- [11] R. Ramirez and A. Mohagan, "Diversity and status of butterflies in Maitum Village, Tandag, Surigao del Sur, Philippines.," *Asian Journal of Biodiversity*, 2012.
- [12] O. H. D. Zoo, "Butterfly: taking science to the backyard.," 2011.
- [13] J. Badon, L. Lahom-Cristobal and A. Talavera, "Illustrated Lists of Philippine Butterflies.," 2013. [Online].
- [14] D. Micklos, G. Freyer and D. Crotty, in *DNA Science*, New York, Cold Spring Harbor Laboratory Press, 2003, p. 575 pp.
- [15] R. Colwell and J. Elsensohn, "EstimateS turns 20: statistical estimation of species richness and shared species from samples, with non-parametric extrapolation.," *Ecography*, vol. 37, p. 609–613., 2014.
- [16] J. R. Sovell, "Butterfly Community Monitoring on City Of Boulder Open Space and Mountain Parks Property: Analysis of the Changes in Species Richness and Diversity from 2002 to 2016.," Colorado Natural Heritage Program, Colorado State University, Fort Collins, Colorado, 2017.
- [17] C. & S. H. Treadaway, "Revised checklist of the butterflies of the Philippine Islands (Lepidoptera: Rhopalocera).," Frankfurt am Main: Entomologischer Verein Apollo, 2012.

- [18] L. Vu and C. Vu, "Diversity Pattern of Butterfly Communities (Lepidoptera, Papilionoidea) in Different Habitat Types in a Tropical Rain Forest of Southern Vietnam," *International Scholarly Research Notices*, 2011.
- [19] F. Danielsen and C. Treadaway, "Priority conservation areas for butterflies (Lepidoptera: Rhopalocera) in the Philippine islands.," *Animal Conservation*, vol. 7, p. 79 – 92. , 2004.
- [20] IUCN, "The IUCN Red List of Threatened Species. Version 2020-1.," 2020. [Online]. Available: <https://www.iucnredlist.org>. [Accessed August 2020].
- [21] A. Rodrigues, J. Pilgrim, J. Lamoreux, M. Hoffmann and T. Brooks, "The value of the IUCN Red List for conservation.," *Trends in Ecology & Evolution*, vol. 21, pp. 71-76, 2006.
- [22] C. Goforth, "Collecting insects: making an insect kill jar.," 2010. [Online]. Available: <https://thedragonflywoman.com/2010/10/05/killjars/> . [Accessed September 2018].