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The Heliconiinae butterfly assemblage (Lepidoptera: Papilionoidea: Nymphalidae) in a typical ruderal environment in Southeastern Brazil

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Abstract
The Acraeini and Heliconiini butterflies are widespread through the Atlantic Forest, an endangered environment considered as a hotspot for its biodiversity and high levels of endemism. Using the antagonistic life strategies of these two butterfly tribes, this study used a protocol to monitor, from winter 2016 to autumn 2017, the structure of the Heliconiinae assemblage, as well as their dynamics throughout space, in response to some abiotic and biotic factors, along a dirt road in the Atlantic Forest in the Quilombo River valley, in the continental area of the municipality of Santos, São Paulo. Climate of this area is type Af with average annual rainfall of 2550 mm and average annual temperature of 22.0°C. Original vegetation is Submontane Ombrophilous Forest currently occupied by anthropogenic ruderal vegetation. Samplings encompassed all seasons and 17 species of 26 potentially present were recorded. Heliconiini and Acraeini larval food plants, all Passifloraceae and Asteraceae, respectively were recorded. The most important flowering plant was *Bidens alba*, present in all seasons and used by all species. Assemblage richness was correlated with mean solar radiation of road segments. The total frequency of *Heliconius* species could be used to indicate a gradient of anthropogenic landscape modification. The presence of *Heliconius numata robigus* could be used as an indicator of less impacted environments and frequency of other species in this community, such as *H. sara apseudes*, *H. erato phyllis*, *H. ethila narcaea* and *A. pellenea pellenea*, could be used as an indicator of a gradient of anthropogenic impact.

Keywords: road ecology, environmental conditions, butterfly resources

1. Introduction

The Acraeini and Heliconiini groups are widespread through the Atlantic Forest, an endangered environment considered as a hotspot for its biodiversity and high levels of endemism with only 12.6% of native forest remaining [1, 2]. Many of these vegetation fragments have distinct levels of impacts and with different environmental gradients, thus can select and limit the diversity to some butterfly groups [3]. Circa 83% of the remaining Atlantic Forest fragments are smaller than 50 ha; even so, the Metropolitan Region of Baixada Santista, Sao Paulo, houses part of the largest continuous forest fragment which represents 7% of the total left, extending from the coast of São Paulo state to the north part of the Rio de Janeiro state [4].

Butterflies' assemblage structure and diversity are dependent of the organization and succession of forest systems which can be dependent to abiotic factors as climate and weather [5], beyond the biotic factors as availability of larval and adult resources and the trophic interaction with predators and competitors [6]. The Acraeini and Heliconiini larvae do not compete for the same foodplants [7-9]. *Actinote* species have greater tolerance and affinity for colder climates being almost absent in the Amazon region while *Heliconius'* affinity is for warmer climates [5, 10]. The *Heliconius* butterflies have a further ability, they can understand the surrounding environment and make daily updates about general conditions throughout the home range [11-12]. Conversely, *Actinote* butterflies are more sessile staying in the neighborhood of their larval foodplant [8]. Monitoring the behavior ecology, moreover the interactions involving communities, can be easy and would show intervening ecological process which can be used to evaluate the status of tropical forests and their management [7, 13].

Although some butterflies are known for their site fidelity, others will migrate in response to changes in the seasons and its consequences in the plants' accessibility. This susceptibility to environmental conditions, specificity in resources use and relatively well-known taxonomy and systematics make them suitable indicators [14-16].

Alterations in the abundance of plant resources can then affect the distribution of phytophagous insects, having impact on community dynamics, ecosystem functioning and species diversity, hence habitat fragmentation is now one of the greatest threats to global biodiversity [14, 17-19]. Roads which create edges in forested systems will allow the development of unstable and aggressive ruderal environments. Thus, species that colonize these areas have adapted to disturbances caused mainly by the traffic of people and vehicles, and management activities as weeding, burning and pollution by chemical herbicides [20-22]. Such environments may present great phytophysiognomic variations throughout the year and between microhabitats, serving as important refuges of biodiversity in the urban scenario, creating resources for various groups of animals. Together, climate, weather and anthropogenic pressures can influence the spatial distribution of insects that use this environment [23-26].

Using the antagonistic life strategies of these two butterfly tribes, this study used a protocol to monitor the structure of the Heliconiinae assemblage, as well as their dynamics throughout space, in response to some abiotic and biotic factors, along a dirt road in the Atlantic Forest.

2. Material and Methods

2.1 Study area

The study area covered the entire length of the dirt road that runs along the right bank of the Quilombo River, in the continental area of the municipality of Santos, São Paulo (Figures 1 A-C).

This road is 8.6 km long and runs from the Conego Domenico Rangoni Highway to a water catchment area belonging to USIMINAS (geographical coordinates: 23.858503°S and 46.352081°W to 23.817125°S and 46.301656°W; see [27] for more details). To allow a spatial comparison, the road was divided into segments of 1 km length (Figure 1D).

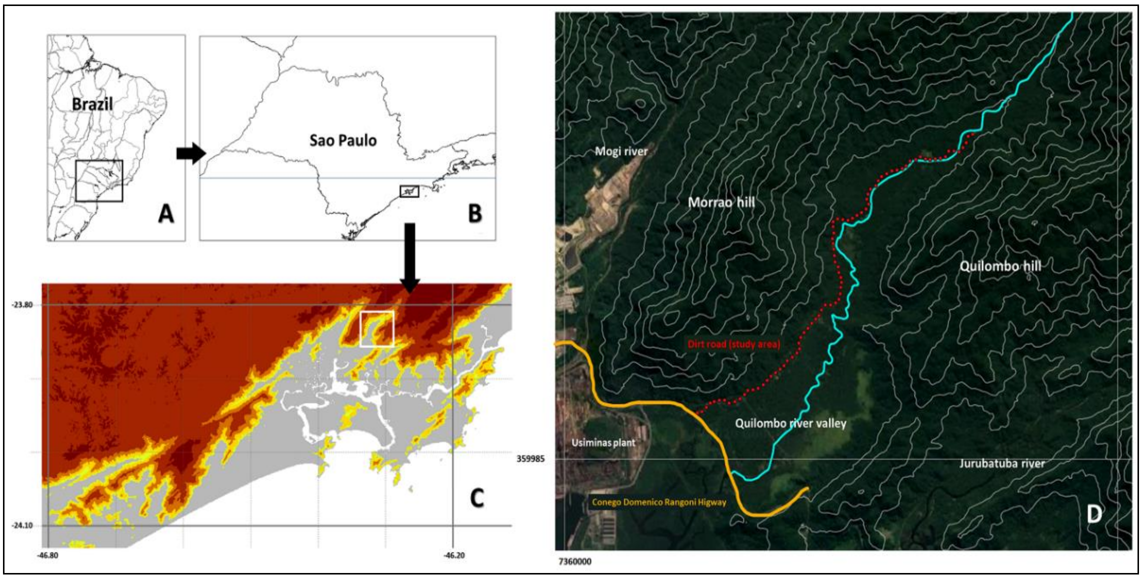

Fig 1: Localization of the study area (A) in Brazil, (B) in Sao Paulo, (C) in the Metropolitan Region of Santos. The white square at top right indicates the study area in the Quilombo river valley. (D) Details of the study area showing the dirt road (red points) where the study was done. The width of the Quilombo river valley where it reaches the Conego Domenico Rangoni highway is 2.7 km e and only 0.5 km in the end of dirt road (considering 100m contour of both Morrao hill and Quilombo hill). Therefore, there is a bottleneck as the dirt road moves towards the end of the Quilombo river valley.

2.2 Climate and vegetation of the study area

The normal climate diagram followed the standards [28], being constructed with data from 30 years of observations from 1970 to 2000, based on Worldclim data [29-31]. This data indicates that the study area has an Af type climate in the Köppen classification [32, 33] with average annual rainfall of 2550 mm and average annual temperature of 22.0 °C. The rainiest month was February (331 mm), in the summer and the driest July (100 mm), in the winter.

The predominant vegetation in the studied area was originally composed by Submontane Ombrophilous Forest [34], but currently, many parts of dirt roadside are occupied by anthropogenic ruderal vegetation.

2.3 Samplings

Samplings encompassed all seasons. Samplings totalized 15 days (3386 min), from June 2016 to May 2017 and accomplished the presence of species in each segment of 1 km length (resolution = 100 m) permitting a more precise differentiation between sectors. Sampling was done using a motor vehicle traveling between 10 and 20 km/h which was stopped whenever a butterfly was spotted. Whenever a higher concentration of butterflies was observed due to the presence of resources, stops of several minutes were made until all the species present were identified or collected. Data was collected using digital photographic equipment with camera lenses up to 2000 mm focal length which allowed the identification of an individual of a species that was resting at a distance of up to 50m. Even though most days had good weather and clear skies without clouds, cloudy or even rainy days were also sampled. Sampling effort usually covered the morning period (08:00h to 16:00h). The observations were made in both directions of the road, that is, along the 8.6 km long transection. During this work all butterfly species of the Heliconiinae assemblage were studied.

2.4 Data analysis

Data was analyzed using R software v. 3.6.3 [35] and RStudio interface v. 1.3.959 [36] with packages vegan [37], biodiversityR [38], ggplot2 [39], FactoMineR, v. 2.3 [40, 41], factoextra, v. 1.0.7 [42], and mgcv [43]. Jaccard index was used to compare the assemblage's number of species and Morisita index to compare their frequencies. Data normality was tested by the Shapiro Wilk test. Relationships between variables were tested using Generalized additive (mixed) models (GAM) [44]. The independent variables were tested in different combinations and the choice of the best model was made using the one with the lowest value of the Akaike Information Criterion (AIC). Principal component analysis (PCA) was used to show the distribution of environmental characteristics in the study area. All variables are Ln + 0.01 transformed. Two variables are of geographic nature: DISMOR (mean distance in meters from Morrao Hill - straight line distance in meters from 500m contour from Morrao Hill in E-W Direction) and DIRIV (mean distance in meters from Quilombo River W-E). One variable is biological, QFLOWE (number of flowering plants with nectar available to butterflies). The quantification of floral resources was based on the availability of plants along the edges of the road, observing exclusively the species on which Heliconiinae adults feed. Values from 1 (absent), 2 (few) to 3 (abundant) were assigned, in ascending order of abundance, according to the valuation of the composition of species present in patches of each sampled segment. One variable is connected to light availability to butterflies and plants: RADIAT (mean potential solar radiation (W/m^2, using canopy photography at each 100m). Two variables are of anthropogenic nature: SETTLE (number of human settlements) and DISHIG (Mean distance in meters from Conego Domenico Rangoni highway). In the final analysis, 1 km data were grouped in three sectors. The first (SECTOR1; up to km 3) was considered more impacted due to the presence of many human settlements on the edges of the road. The second (SECTOR2; from km 3 to 5.5), with intermediary impact and the last (SECTOR3; from km 5.5 to 8.6) representing the less impacted segment.

3. Results and Discussion

The width of the Quilombo River valley (distance between 100 m contour from Morrao Hill and Quilombo hill) is 2.7 km in the section that it is cut by the Cônego Domênico Rangoni highway and decreases to 0.5 km when the it reaches km 8.6 of the study area (Figure 1 D).

Along the 8.6 kilometers of the dirt road (study area) the distance from Quilombo river, the distance from 500m contour of Morrao Hill and the number of settlements were variable. Throughout the first 500m, the forest was replaced by ruderal vegetation due to the presence of underground pipelines for the transportation of chemical products from Petrobras. In some points the roadside is more open and receives more solar radiation and in other it is more stretched (Figure 2 A-C).

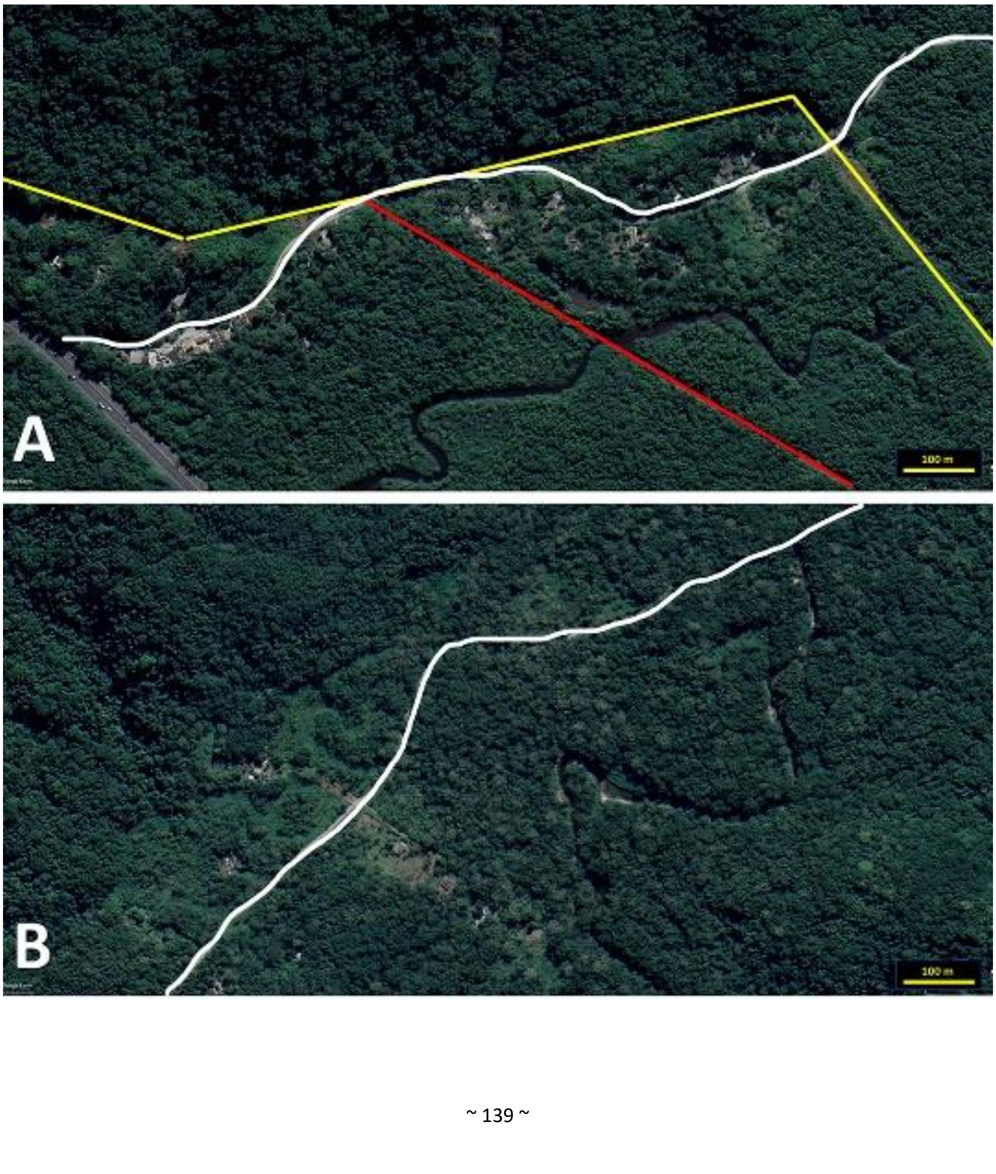

Fig 2: White line in photographs indicates the dirt road path along the right bank of Quilombo river. (A) Aerial view of the of the km 1 segment of the study area. The yellow line indicates a high voltage line and the red line the underground pipeline for the transportation of chemical products from Petrobras. (B) Aerial view of the road near km 5 where the number of settlements begin diminishing. (C) Aerial view of the km 7-8 showing the last settlement (banana plantation) and the point where road surpasses the Quilombo river at right. Source: Google Earth.

Cluster analysis using bray-curtis distance and complete clustering using ln-transformed environmental variables of road segments showed the formation of three clusters (Figure 3 A). One grouping the kilometers 1 to 3 and other the kilometers 4 and 5. The clusters are formed by similar conditions in the kilometers 1-3, and 4-5, and 6-7. The last segment, kilometer 8 was enough different to stay isolated. Principal component analysis of same data showed that the first two dimensions of analysis expressed 77.36% of the total dataset inertia being greater than the reference value therefore the results explained by the first dimension are significant. Variables QFLOWE and DISRIV appeared as strong in the grouping the road segments being followed by SETTLE, DISMOR, and DISHIG (Figure 3 B). These results permitted to cluster the eight segments in three sectors as explained in the methodology. Integrated solar radiation (RADIAT) appears as a weak variable here because the low variability but opposed to km 7 which have the lower value.

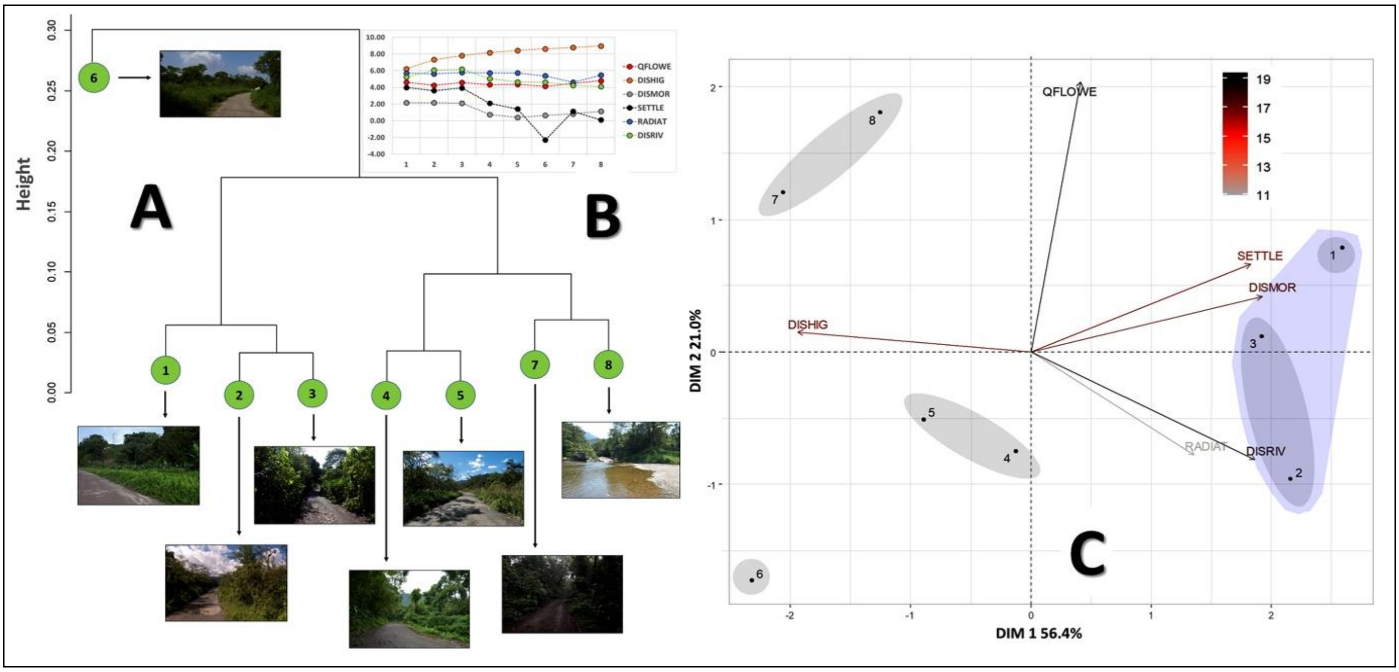

Fig 3: (A) Cluster analysis using bray-curtis distance and complete clustering using ln-transformed environmental variables of road segments. The clusters are formed by similar conditions in the kilometers 1-3, and 4-5, and 6-7. The last segment, kilometer 8 was enough different to stay isolated. Table inside dendrogram shows values of the six variables used. Variable codes: (QFLOWE) number of flowering plants with nectar available to butterflies. (DISHIG) distance from the Conego Domenico Rangoni Highway. (DISMOR) distance from the Morrao Hill 100m contour. (SETTLE) number of human settlements. (RADIAT) mean solar radiation. (DISRIV) mean distance from Quilombo River. See methodology for units of the variables. (B) Ln transformed values of the environmental variables showing their variation along the road. (C) Principal component analysis of same data showing the strength of each environmental variable. Variables QFLOWE and DISRIV appeared as strong in the grouping the road segments being followed by SETTLE, DISMOR, and DISHIG. Permanova ($F = 40.67$; $p < 0.0001$).

3.1 The Heliconiinae assemblage

The assemblage of Heliconiinae in the study area had been observed since 1968 [45], and 26 Heliconiinae species could potentially be present in the study area, including the eventual migrant *Dione moneta moneta* (Hübner, [1825]). However, only 17 (65.38%) were observed during this study (Table 1).

Table 1: Heliconiinae species found during this study.

Species	Author	CODE
Actinote pellenea pellenea	Hübner, [1821]	PEL
Actinote brylla	Oberthür, 1917	BRY
Actinote pyrrha pyrrha	(Fabricius, 1775)	PYR
Actinote melanisans	Oberthür, 1917	MEL
Actinote parapheles	Jordan, 1913	PAR
Actinote discrepans	D'Almeida, 1958	DIS
Agraulis vanillae maculosa	(Stichel, [1908])	VAN
Dione juno juno	(Cramer, 1779)	DIO
Dryadula phaetusa	(Linnaeus, 1758)	PHA
Dryas iulia alcionea	(Cramer, 1779)	DRY
Philaethria wernickei	(Röber, 1906)	PHI
Eueides aliphera aliphera	(Godart, 1819)	ALI
Eueides isabella dianasa	(Hübner, [1806])	ISA
Eueides pavana	Ménétriés, 1857	PAV
Heliconius besckei	Ménétriés, 1857	BES
Heliconius erato phyllis	(Fabricius, 1775)	ERA
Heliconius ethilla narcaea	Godart, 1819	ETI
Heliconius numata robigus	Weymer, 1875	NUM
Heliconius sara apseudes	(Hübner, [1813])	SAR

Four species presented percentages (relative frequency) above 15%: *Heliconius erato phyllis*, *Heliconius sara apseudes*, *Dryas iulia alcionea* and *Actinote pellenea pellenea* (Figure 4 A-D). Eleven species presented percentages lower than 5% (Figure 4 E).

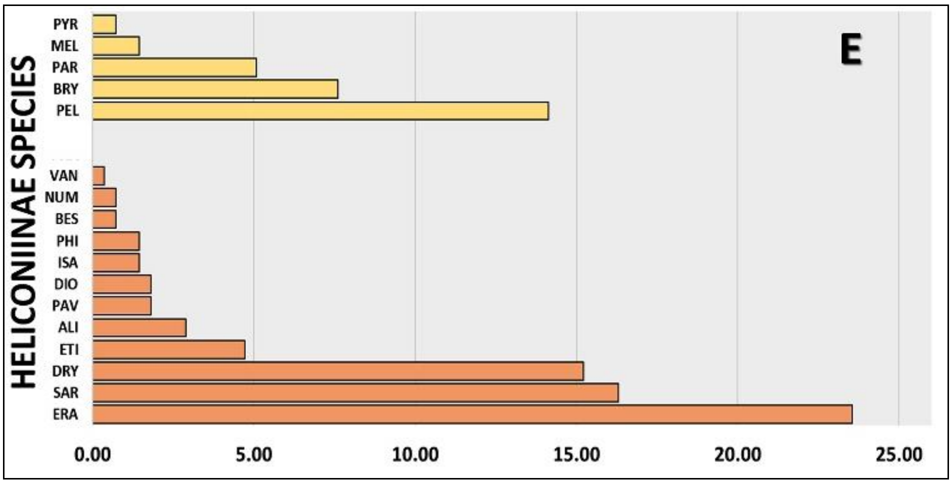

Fig 4: (A) *Actinote pellenea pellenea* feeding on flowers of *Bidens alba*. (B) *Dryas iulia alcyonea* feeding on flowers of *Bidens alba*. (C) *Heliconius sara apseudes* feeding on flowers of *Mikania micrantha.* (D) *Heliconius erato phyllis* feeding on flowers of *Chromolaena laevigata.* (E) Percentage of Heliconiinae species in the study area in samplings 2016-17. Species codes: (ERA) *Heliconius erato phyllis*. (ETI) *Heliconius ethilla narcaea*. (BES) *Heliconius besckey*. (NUM) *Heliconius numata robigus*. (SAR) *Heliconius sara apseudes*. (PHI) *Philaethria wernickey wernickey*. (DRY) *Dryas iulia alcyonea*. (AGR) *Agraulis vanillae maculosa*. (DIO) *Dione juno juno*. (PHA) *Dryadula phaetusa*. (ISA) *Eueides isabella dianasa*. (ALI) *Eueides aliphera aliphera*. (PAV) *Eueides pavana*. (PEL) *Actinote pellenea pellenea*. (BRY) *Actinote brylla*. (PYR) *Actinote pyrrha pyrrha*. (MEL) *Actinote melanisans*. (PAR) *Actinote parapheles*. (DIS) *Actinote discrepans*.

The collectors' curve for sampling period showed that the asymptote was reached at sample 14 on April 24, 2017 (Figure 5 A). The Whittaker plot showed a very smooth drop due to the frequency distribution showing a high diversity (Figure 5 B) as expressed by the Simpson Index (0.86).

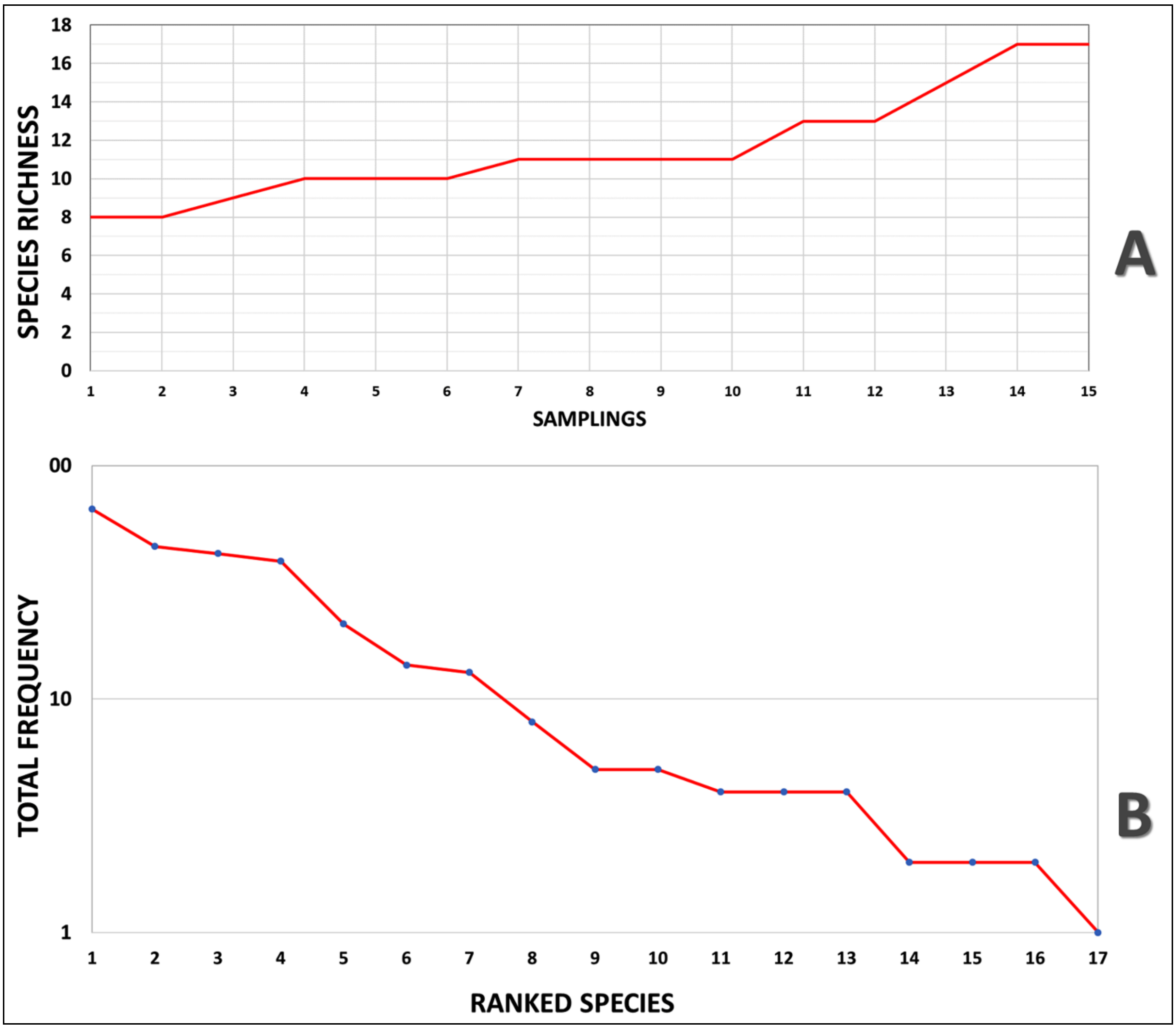

Fig 5: (A) Collector' curve of Heliconiinae assemblage in the study area during series 2016-17. (B) Whittaker plot of Heliconiinae assemblage in the study area.

The peak of Heliconiinae species richness was during autumn (April to June), with 15 species. Despite this, the species percentage (relative frequencies) along the seasons was extremely variable (Table 2).

Table 2: Season phenology of Heliconiinae butterflies in the study area during sampling periods of 2016-17.

	AUTUMN 16	WINTER 16	SPRING 16	SUMMER 17	AUTUMN 17
VAN	0.0	0.0	100.0	0.0	0.0
ALI	12.5	0.0	0.0	0.0	87.5
ISA	25.0	50.0	0.0	0.0	25.0
PAV	40.0	0.0	0.0	60.0	0.0
DIO	0.0	0.0	0.0	60.0	40.0
DRY	26.2	11.9	11.9	21.4	28.6
BES		50.0			50.0
ERA	33.8	10.8	12.3	15.4	27.7
ETI	23.1	7.7	7.7	15.4	46.2
NUM					100.0
SAR	15.6	4.4	0.0	20.0	60.0
PHI	25.0				75.0
PEL		5.1	17.9	7.7	69.2
BRY				9.5	90.5
PYR					100.0
MEL					100.0
PAR					100.0

CODE
0%
1-25%
26-59%
> 59%

3.2 Larval foodplants

Potentially, at least 23 *Passiflora* species may have been used by Heliconiini larvae in the study area (Table 3), being some domesticated species, particularly *P. alata* and *P. edulis.* In 41 farms of the local community (48.8%) the owners cultivate plants of maracuja (passionflower) [46].

Table 3: Species of larval food plants used by Heliconiinae butterflies in the study area. All Heliconiini larvae eat exclusively *Passiflora* (Passifloraceae). All Acraeini larvae eat only species of Asteraceae (genera *Mikania*, *Austroeupatorium* and *Vernonanthura*). Species codes: (ERA) *Heliconius erato phyllis*. (ETI) *Heliconius ethilla narcaea*. (BES) *Heliconius besckey*. (NUM) *Heliconius numata robigus*. (SAR) *Heliconius sara apseudes*. (PHI) *Philaethria wernickey wernickey*. (DRY) *Dryas iulia alcyonea*. (AGR) *Agraulis vanillae maculosa*. (DIO) *Dione juno juno*. (PHA) *Dryadula phaetusa*. (ISA) *Eueides isabella dianasa*. (ALI) *Eueides aliphera aliphera*. (PAV) *Eueides pavana*. (PEL) *Actinote pellenea pellenea*. (BRY) *Actinote brylla*. (PYR) *Actinote pyrrha pyrrha*. (MEL) *Actinote melanisans*. (PAR) *Actinote parapheles*. (DIS) *Actinote discrepans*. TOTAL1 = potential number of plants available to butterfly species; TOTAL2 = number of potential butterfly species using the plant. (*).

FOODPLANT	ERA	ETI	BES	NUM	SAR	PHI	DRY	AGR	DIO	PHA	ISA	ALI	PAV	TOTAL1
P. actinia Hook. *	1	1	1	1	0	1	1	0	1	0	0	0	0	**7**
P. alata Curtis *	0	1	0	1	0	0	0	1	1	0	1	0	0	**5**
P. amethystina J.C.Mikan	0	1	0	0	0	0	1	1	0	0	1	1	0	**5**
P. caerulea L. *	1	1	1	0	0	1	1	1	1	1	1	1	0	**10**
P. capsularis L.	1	0	0	0	0	0	1	1	1	1	0	1	0	**6**
P. deidamioides Harms	0	0	0	0	0	0	0	1	0	0	0	0	0	**1**
P. edulis Sims *	1	1	1	1	1	0	1	1	1	0	1	0	0	**9**
P. elegans Mast.	1	1	0	0	0	0	0	0	0	0	0	0	0	**2**
P. jilekii Wawra	1	1	0	1	0	1	1	1	0	1	1	1	0	**9**
P. miersii Mart.	1	1	0	0	0	0	1	1	0	0	0	0	0	**4**
P. misera Kunth	1	0	0	0	0	0	1	1	1	1	0	1	0	**6**
P. mucronata Lam.	1	0	0	0	1	1	0	1	1	1	1	0	0	**7**
P. porophylla Vell.	1	0	1	0	0	0	1	0	0	1	0	0	0	**4**
P. ovalis Vell. ex M.Roem.	1	1	0	1	1	0	0	1	0	0	0	0	0	**5**
P. pohlii Mast.	1	0	0	0	0	0	1	0	0	0	0	0	0	**2**
P. quadrangularis L. *	0	0	0	1	0	1	1	1	1	0	1	0	0	**6**
P. racemosa Brot.	0	1	0	0	0	0	0	0	0	0	0	0	0	**1**
P. rhamnifolia Mast.	1	1	1	1	1	1	1	1	0	0	0	0	1	**9**
P. sidifolia M.Roem.	1	1	1	1	1	1	1	1	1	1	1	1	1	**13**
P. suberosa L.Sp.Pl.	1	0	1	0	1	0	1	1	1	1	0	0	0	**7**
P. truncata Regel	1	0	0	0	1	0	1	0	0	0	0	0	0	**3**
P. vellozii Gardner	0	1	1	1	0	0	0	0	0	0	0	0	0	**3**
P. villosa Vell.	0	0	1	0	0	0	0	1	0	0	0	0	0	**2**
TOTAL2	**16**	**13**	**9**	**9**	**7**	**7**	**15**	**16**	**10**	**8**	**8**	**6**	**2**	

	PEL	BRY	PYR	MEL	PAR	DIS	**TOTAL3**
Austroeupatorium inulaefolium (Kunth) R.M.King & H.Rob	1	0	0	0	0	0	1
Mikania micrantha Kunth	1	0	0	0	0	0	1
M. cordifolia (L.f.) Willd.	1	0	0	1	0	0	2
Mikania lundiana DC	0	1	1	0	0	0	2
Mikania sericea Hook. & Am.	0	0	0	0	1	0	1
Vernonathra beyrichii (Less.) H.Rob.	0	0	1	0	0	0	1
TOTAL4	3	1	2	1	1	0	

On the other hand, the larvae of the three *Actinote* species residents in the study area eat leaves of asterace species whose flowers are also used by butterflies (Tables 3-4). Larvae of *Actinote pellenea pellenea* can use three asterace species of two genera, *Austroeupatorium* and *Mikania*, however, species of these genera grow spontaneously on the roadsides.

Table 4: Season flowering phenology of plants whose flowers were used by Heliconiinae butterflies in the study area during 2016-17 showing the frequency of each species by season. Codes: Summer (SUM); Autumn (AUT); Winter (WIN); Spring (SPR).

PLANT SPECIES	FAMILY	SUM	AUT	WIN	SPR
Sanchesia speciosa Leonard	Acanthaceae	1	1	1	1
Asclepias curassavica L.	Apocynaceae	1	1	1	1
Austroeupatorium inulaefolium (Kunth) R.M.King & H.Rob.	Asteraceae	0	1	0	0
Bidens alba (L.) DC	Asteraceae	1	1	1	1
Chromolaena laevigata (Lam.) R. M. King & H. Rob.	Asteraceae	0	1	0	0
Mikania lundiana DC	Asteraceae	1	1	1	1
Mikania micrantha Kunth	Asteraceae	0	1	0	1
Mikania glomerata Spreng.	Asteraceae	0	0	1	0
Vernonanthura beyrichii (Less.) H.Rob.	Asteraceae	0	1	0	0
Malvaviscus arboreus Cav.	Malvaceae	1	1	1	1
Lantana camara L.	Verbenaceae	0	1	1	0
Stachytarpheta cayennensis (LC. Rich.) Vahl	Verbenaceae	1	1	1	0
	TOTAL	6	11	8	6

Despite the importance of larval food plants for the species of the assembly studied in this study, no quantification was made of them. However, it is very likely that the vegetative growth and the availability of leaves have a reasonable relationship with the amount of rainfall.

3.3 Flowering plants

At least 13 plant species were used by adult butterflies in the area during the study with flowering concentrated in autumn (Table 4). Five species, *Bidens alba*, *Asclepias curassavica*, *Mikania lundiana*, *Sanchesia speciosa* and *Malvaviscus arboreus* were present in all seasons. Due to its continuous availability, *B. alba* is a key species in the area because it is used by all species of the Heliconiinae assemblage.

Plant habit and its topographic position in the environment are important because they determine its fate when road edges were managed by anthropogenic activities. Therefore, *Vernonanthura beyrichii* is not affected because grows far from the edges as well as *Malvaviscus arboreus* and *Sanchesia speciosa* which are shrubby bushes that form large stands. However, although their continuous flowering, both *Bidens alba* and *Asclepias curassavica* are the most impaired by anthropogenic management.

A survey of selected publications on nectarivorous butterfly communities in south and southeastern Brazil showed that from 23 papers published since 1986 (Appendix 1; available at: https://archive.org/details/heliconiinae-apendix-1), only four quantified other than species richness and / or relate it to environmental conditions or ecological variables. Most were lists of all butterfly species in relatively large areas and covering relatively long periods of time. This does not diminish their importance because they represented the first attempt to describe this very important community of organisms as showed in the pioneering "state of the art and the priority-areas model for research aiming at conservation using butterfly inventories in Brazil" [47].

Thus, this work is the first that used a nectarivore butterfly assemblage to understand the role of some abiotic and one biotic condition in its seasonal dynamics.

3.4 Spatial comparison

The comparison between the three segments (SECTORS 1 to 3) of the study area was done along a gradient of environmental impact. More impacted, from the Cônego Domênico Rangoni Highway to a less impacted, in the water catchment area belonging to USIMINAS. SECTOR1, has three kilometers length and 142 human settlements, SECTOR2, has 12 human settlements and SECTOR3, only four. Relative frequency of flower clusters was higher in both sector1 and SECTOR3. SECTOR2 had more species (14) than SECTOR1 (13) and SECTOR3 (9). Jaccard index and Morisita index showed that the frequencies of Sector1 and Sector3 were most similar in relation to species number and frequencies (Table 5).

Table 5: Distances of Heliconiinae assemblages in the three segments along the study area in 2016-17 sampling series.

Number	SECTOR1
SECTOR1	13
SECTOR2	14
SECTOR3	9

JACCARD	SECTOR1	SECTOR2	SECTOR3
SECTOR1			
SECTOR2	0.48		
SECTOR3	0.67	0.53	

MORISITA	SECTOR1	SECTOR2	SECTOR3
SECTOR1			
SECTOR2	0.06		
SECTOR3	0.34	0.10	

Cluster analysis (Figure 6) of the presence/absence matrix of each species shows their affinity along this gradient.

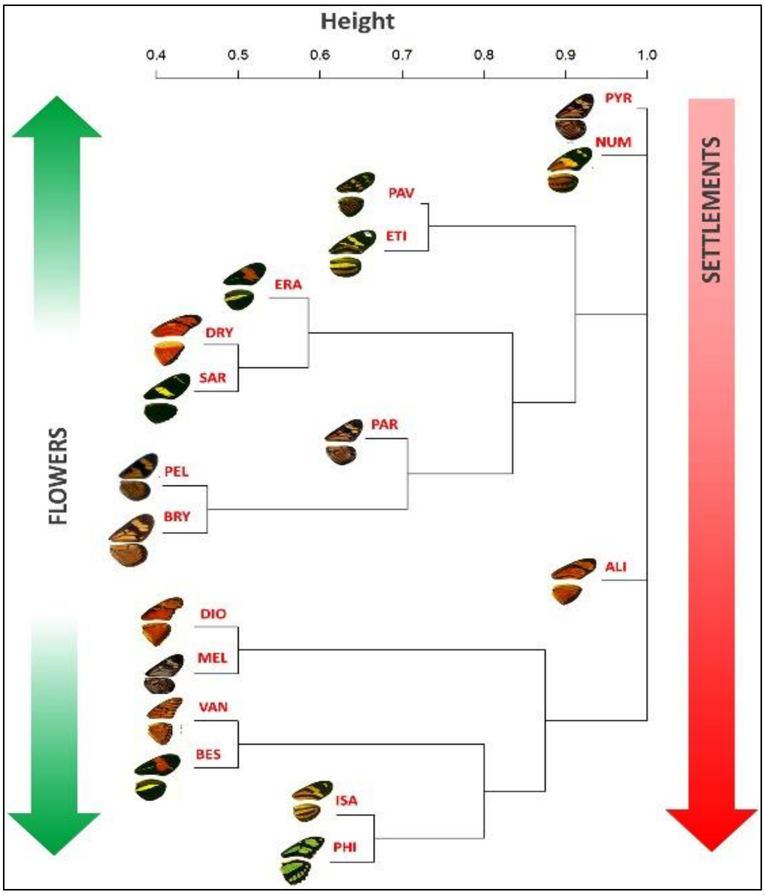

Fig 6: Cluster analysis using Jaccard distance and complete clustering using species presence in road segments. Red arrow shows the direction of most impacted areas with increase of settlements due to Conego Domenico Rangoni highway proximity. Green arrow shows the increase of flower richness in the two limits of the study area.

Modeling of Heliconiinae species richness (RICH) with environmental variables by segments of 1000m using GAM showed that better model with lower AIC was which correlated estimated solar radiation using canopy photography (RADIAT) as smoothing variable (Table 6).

Table 6: Results of the General Additive Model analysis of Heliconiinae assemblage richness (RICH) as a function of the estimated solar radiation using canopy photography (RADIAT). Significance codes: 0 (***); 0.001(**); 0.01(*). AIC (29.66). (See appendix 2 for all models).

MODEL	F	p	deviance explained	r2	AIC
RICH~s(RADIAT, k=3, fx=T)	20.03	**	87.0	0.83	**29.66**
RICH~s(OPPENI, k=3, fx=T)	10.58	*	77.9	0.71	**34.41**
RICH~s(DISHIG, k=3, fx=T)	8.74	*	74.4	0.66	**35.72**
RICH~s(DISRIV, k=3, fx=T)	0.26	**NS**	42.0	0.19	**42.46**
RICH~s(SETTLE, k=3, fx=T)	2.03	**NS**	40.3	0.20	**43.35**
RICH~s(DISQUI, k=3, fx=T)	0.63	**NS**	20.1	-0.19	**45.03**
RICH~s(DISMOR, k=3, fx=T)	0.25	**NS**	8.9	0.27	**46.07**
RICH~s(QFLOWE, k=3, fx=T)	0.43	**NS**	12.5	0.17	**46.80**

Family: gaussian
Link function: identity
Formula: SIMP ~ s(RADIAT, k = 3, fx=T)

(Intercept)	**Estimate SE**	**t value**	**Pr(>|t|)**	
8.11	0.33	24.65	***	
Smooth terms	**edf**	**Ref.df**	**F**	**p**
RADIAT	2	2	20.03	**
Adjusted R^2	**Deviance explained**	**GCV**	**Scale est**	**n**
0.83	87.00%	1.46	0.97	9

Several works done in temperate regions emphasized the importance of solar radiation to butterfly activity [48-52]. In our work, individuals of *Dryas iulia alcionea*, *Heliconius erato phyllis*, *Heliconius sara apseudes* and *Heliconius ethila narcaea* were recorded moving in flight along the road, away from nectarivorous plants as well as couples of *Heliconius sara apseudes*. Males of *Eueides pavana* and *Actinote pellenea pellenea* were also recorded in perches, waiting for mates. All of them in sunnier areas of the road.

The most frequent species by sector was *Actinote pellenea pellenea* in the SECTOR1, *Heliconius erato phyllis*, in the SECTOR2, and both *Heliconius sara apseudes* and *Heliconius erato phyllis* in SECTOR3 (Figure 7).

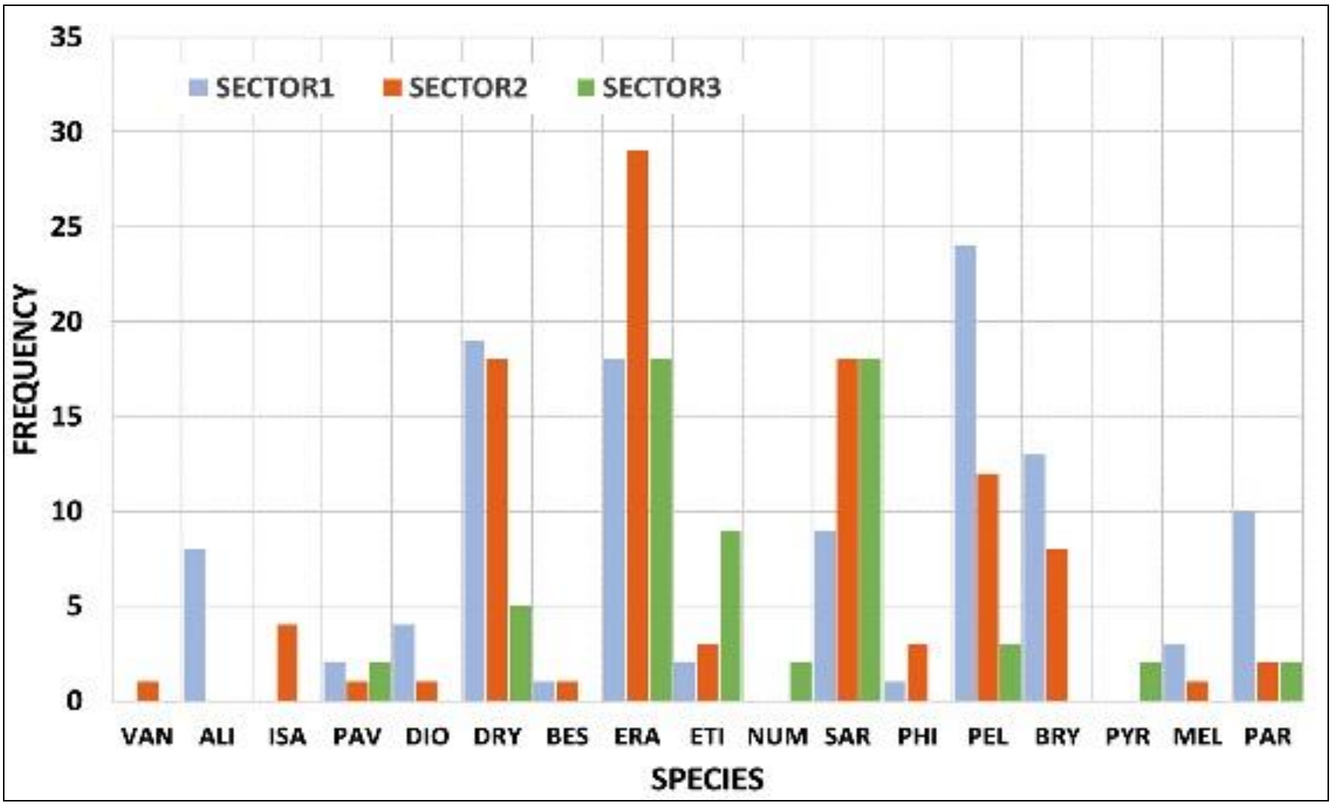

Fig 7: Frequency of Heliconiinae species in the three segments (SECTOR1 to 3) of the study area in samplings 2016-17. Species codes: (ERA) *Heliconius erato phyllis*. (ETI) *Heliconius ethilla narcaea*. (BES) *Heliconius besckey*. (NUM) *Heliconius numata robigus*. (SAR) *Heliconius sara apseudes*. (PHI) *Philaethria wernickey wernickey*. (DRY) *Dryas iulia alcyonea*. (AGR) *Agraulis vanillae maculosa*. (DIO) *Dione juno juno*. (PHA) *Dryadula phaetusa*. (ISA) *Eueides isabella dianasa*. (ALI) *Eueides aliphera aliphera*. (PAV) *Eueides pavana*. (PEL) *Actinote pellenea pellenea*. (BRY) *Actinote brylla*. (PYR) *Actinote pyrrha pyrrha*. (MEL) *Actinote melanisans*. (PAR) *Actinote parapheles*. (DIS) *Actinote discrepans*.

Species found in only one sector were: *Agraulis vanillae maculosa, Eueides aliphera*, *E. isabella dianasa*, *Heliconius numata robigus* and *Actinote pyrrha*. Pooled frequency of Heliconiinae genera in the three sectors showed the increase in the frequency of *Heliconius* species and the decrease in frequency of *Actinote* species. *Heliconius numata robigus* was present only along SECTOR3 (Figure 8).

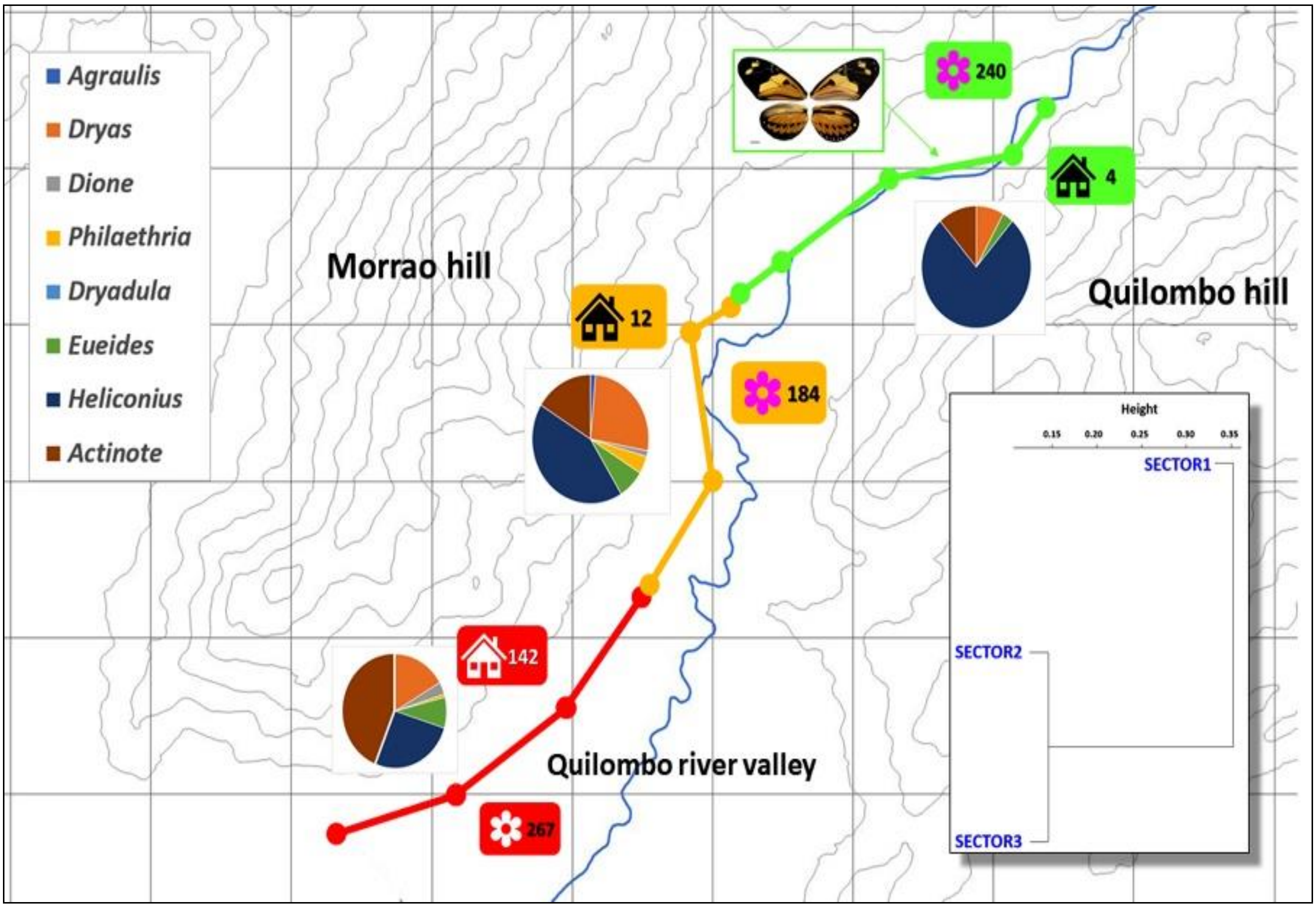

Fig 8: Pooled frequency of Heliconiinae genera in the three segments SECTOR1 (red), SECTOR2 (orange) and SECTOR3 (green) showing the increase in the frequency of *Heliconius* species and the decrease in frequency of *Actinote* species. *Heliconius numata robigus* [showed in the inset] was present only along SECTOR3. Number of human settlements are indicated inside boxes with house symbol. Relative frequency of flower clusters is indicated inside boxes with flower symbol. The inset at right shows the cluster diagram of the three sectors by species presence.

During sampling period, impacts of anthropogenic origin occurred with the intensive cutting of the roadside vegetation. These roadside management impacts were difficult to quantify, but they certainly affected the probability of detecting nectarivorous species. Another factor that could be significant in recording the number of butterflies seen is the traffic of motor vehicles. It usually increases on weekends, holidays and school vacation (January to February and July) and is mainly caused by cars and motorcycles.

4. Conclusions

1. At spatial level, the presence of *Heliconius numata robigus* could be used as an indicator of less impacted environments and frequency of other species in this community, such as *H. sara apseudes*, *H. erato phyllis*, *H. ethila narcaea* and *A. pellenea pellenea*, could be used as an indicator of a gradient of anthropogenic impact.
2. The total frequency of *Heliconius* species could be used to indicate a gradient of anthropogenic landscape modification.
3. The spatial sampling in the 2016-2017 period indicated a difference in the species composition of the Heliconiinae assembly between the three sectors.
4. Even a lower sampling effort allowed detecting all the most frequent species in the study area.

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8. Authors' contribution: RBF designed the project. All authors collected data in the field. RBF wrote primary draft of the manuscript and analyzed the data.

9. References

1. Myers N, Russell AM, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature 2000;403:853.
2. SOS Mata Atlântica. Atlas dos remanescentes florestais da Mata Atlântica período 2018-2019. São Paulo, Fundação SOS Mata Atlântica / Instituto Nacional de

Pesquisas Espaciais – INPE. 2020, 61. https://www.sosma.org.br/wp-content/uploads/2019/11/RA_SOSMA_2018_DIGITAL.pdf
3. Iserhard CA, Duarte L, Seraphim N, Freitas AVL. How urbanization affects multiple dimensions of biodiversity in tropical butterfly assemblages. Biodiversity and Conservation 2019;28:621-638.
4. Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological conservation 2009;142:1141-1153.
5. Montejo-Kovacevich G, Martin SH, Meier JI, Bacquet CN, Monllor M, Jiggins CD *et al*. Microclimate buffering and thermal tolerance across elevations in a tropical butterfly. The Journal of Experimental Biology 2020;223: jeb220426.; https://doi.org/10.1242/jeb.220426
6. Castro ÉCP, Zagrobelny M, Cardoso MZ, Bak S. The arms race between heliconiine butterflies and *Passiflora* plants - new insights on an ancient subject: New insights on an ancient subject. Biological Reviews 2018;93:555-573.
7. Brown-Jr KS. The Biology of *Heliconius* and Related Genera. Annual Review of Entomology 1981;26:427-457.
8. Francini RB. Biologia e ecologia das borboletas *Actinote* (Lepidoptera: Nymphalidae, Acraeinae) do sudeste do Brasil. Master dissertation. Universidade Estadual de Campinas - UNICAMP, Campinas, 1989, 236.
9. Francini RB. Ecologia das taxocenoses de *Actinote* (Lepidoptera: Nymphalidae) em Asteraceae (Angiosperma, Magnoliatae) no sudeste do Brasil: subsídios para conservação. PHD Thesis. Universidade Estadual de Campinas - UNICAMP, Campinas 1992, 194.
10. Jordan K. Acraeinae. In: A. Seitz (ed.), GrossSchmetterlinge der Erde. Die amerikanischen Tagfalter, plates 81-83. Alfred Kernen, Stuttgart. 1913;5:358-374,
11. Ramos RR, Freitas AVL. Population biology and wing color variation in *Heliconius erato phyllis* (Nymphalidae). Journal of the Lepidopterists' Society 1999;53:11-21.
12. Merrill RM, Dasmahapatra KK, Davey JW, Dell'Aglio DD, Hanly JJ, Huber B *et al*. The diversification of *Heliconius* butterflies: what have we learned in 150 years? Journal of Evolutionary Biology 2015;28:1417-1438.
13. Freitas AVL, Francini RB, Brown Jr KS. Insetos como indicadores ambientais pp. 125–151. In: Cullen, L., R. Rudran, & C. Valladares-Pádua (Eds.). Métodos de Estudos em Biologia da Conservação e Manejo da Vida Silvestre. Editora da UFPR, Curitiba, Brazil 2003.
14. Laurance WF, Nascimento HEM, Laurance SG, Andrade A, Ewers RM, Harms KE *et al*. Habitat Fragmentation, Variable Edge Effects, and the Landscape-Divergence Hypothesis. PLoS ONE 2007;2(10):e1017.; https://doi.org/10.1371/journal.pone.0001017
15. Bonebrake TC, Ponisio LC, Boggs CL, Ehrlich PR. More than just indicators: A review of tropical butterfly ecology and conservation. Biological Conservation 2010;143:1831-1841.
16. Leidner AK, Haddad NM, Lovejoy TE. Does Tropical Forest Fragmentation Increase Long-Term Variability of Butterfly Communities? PLoS ONE 2010;5:e9534.; https://doi.org/10.1371/journal.pone.0009534
17. Brown-Jr KS. Diversity, disturbance, and sustainable use of Neotropical forests: insects as indicators for conservation monitoring. Journal of Insect Conservation 1997;1:25-42.
18. Maleque MA, Maeto K, Ishii HT. Arthropods as bioindicators of sustainable forest management, with a focus on plantation forests. Applied Entomology and Zoology 2009;44:1-11.
19. Almeida DRA, Stark SC, Schietti J, Camargo JLC, Amazonas NT, Gorgens EB *et al*. Persistent effects of fragmentation on tropical rainforest canopy structure after 20 yr of isolation. Ecological Applications 2019; 29(6).
20. Forman RTT, Alexander LE. Roads and their major ecological effects. Annual Review of Ecology and Systematics 1998;29:207-231.
21. Fahrig L, Rytwinski T. Effects of Roads on Animal Abundance: an Empirical Review and Synthesis. Ecology and Society 2009;14:21.
22. Bernes C, Bullock JM, Jakobsson S, Rundlöf M, Verheyen K, Lindborg R. How are biodiversity and dispersal of species affected by the management of roadsides? A systematic map protocol. Environmental Evidence 2016;5(1):4.
23. Saarinen K, Valtonen A, Jantunen J, Saarnio S. Butterflies and diurnal moths along road verges: Does road type affect diversity and abundance? Biological Conservation 2005;123:403-412.
24. Addo-Fordjour P, Osei BA, Kpontsu EA. Butterfly community assemblages in relation to human disturbance in a tropical upland forest in Ghana, and implications for conservation. Journal of Insect Biodiversity 2015;3(6):1.
25. Valtonen A, Saarinen K, Jantunen J. Effect of different mowing regimes on butterflies and diurnal moths on road verges. Animal Biodiversity and Conservation 2006;29:133-148.
26. Phillips BB, Gaston KJ, Osborne JL. Road verges support pollinators in agricultural landscapes, but are diminished by heavy traffic and summer cutting. Journal of Applied Ecology 2019;56:2316-2327.
27. Francini RB. História natural das borboletas do Vale do Rio Quilombo, Santos, SP, 2nd edition. Editado pelo autor, Santos, São Paulo, 2010, 550. https://archive.org/details/HistoriaNaturalDasBorboletasDoValeDoRioQuilomboSantosSp
28. Walter H. Vegetation und Klimazonen. Grundriss der globalen Ökologie. Eugen Ulmer GmbH & Co., Stutgart. 1984, 382.
29. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 2005;25:1965-1978.
30. Harris I, Jones PD, Osborn TJ, Lister DH. Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. International Journal of Climatology 2014;34:623-642.
31. WORDLCLIM. World Clim - Global Climate Data. Free climate data for ecological modeling and GIS 2019. www.worldclim.org
32. Peel MC, Finlayson BL, McMahon TA. Updated world map of the Köppen-Geiger climate classification.

Hydrology and Earth Systems Sciences 2007;11:1633-1644.
33. Alvares CA, Stape JL, Sentelhas PC. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 2013;22:711-728.
34. Ururahy JC, Collares JER, Santos MM, Barreto RAA. Vegetação. In Projeto RADAMBRASIL. Vol. 32. fls. sf 23-24 (Rio de Janeiro e Vitória). Edit. Ministério das Minas e Energia, Brasília, DF 1987.
35. R Development Core Team. A Language and Environment for Statistical Computing (Version 3.5.2). R Foundation for Statistical Computing, Vienna, Austria. 2019. https://cran.r-project.org
36. RStudio.com. RStudio (Version 1.3.959). RStudio.com. 2020. https://rstudio.com/products/rstudio/download/
37. Oksanen J *et al*. R Package 'vegan'. Community Ecology Package 2017.
38. Kindt R. Package Biodiversity R. R Package for Community Ecology and Suitability Analysis 2019.
39. Wickham H. R Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. V. 3.2.1. 2019.
40. Le S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software 2008;25:1-18.
41. Husson F, Josse J, Le S, Mazet J. R package FactoMineR, Multivariate Exploratory Data Analysis and Data Mining, v. 2.3 2020.
42. Mundt F. R Package "factoextra", Extract and Visualize the Results of Multivariate Data Analyses 2020.
43. Wood S. R Package mgcv. Mixed GAM Computation Vehicle with Automatic Smoothness Estimation 2019.
44. Wood S. Generalized additive models an introduction with R, 2nd edition. Boca Raton, FL, CRC Press 2017.
45. Francini RB, Duarte M, Mielke OHH, Caldas A, Freitas AVL. Butterflies (Lepidoptera, Papilionoidea and Hesperioidea) of the 'Baixada Santista' region, coastal São Paulo, southeastern Brazil. Revista Brasileira de Entomologia 2011;55:55-68.
46. Gondeck ME. Avaliação do uso de borboletas Heliconiinae (Papilionoidea: Nymphalidae) COMO bioindicadores de impactos antrópicos em remanescentes de Mata Atlântica de Santos, São Paulo, Brasil. Master dissertation, Programa Interunidades de pós-graduação Análise Ambiental Integrada, Universidade Federal de São Paulo, Diadema 2017.
47. Carneiro E, Mielke OHH, Casagrande MM. Butterfly inventories in Brazil: the state of the art and the priority-areas model for research aiming at conservation. Natureza & Conservação 2008;6:178-200.
48. Clench HK. Behavioral Thermoregulation in Butterflies. Ecology 1966;47:1021-1034.
49. Douwes P. Activity in *Heodes virgaureae* (Lep., Lycaenidae) in relation to air temperature, solar radiation, and time of day. Oecologia 1976;22:287-298.
50. Turner JRG, Gatehouse CM, Corey CA. Does Solar Energy Control Organic Diversity? Butterflies, Moths and the British Climate. Oikos 1987;48:195.
51. Bryant SR, Thomas CD, Bale JS. The influence of thermal ecology on the distribution of three nymphalid butterflies. Journal of Applied Ecology 2002;39:43-55.
52. Bonebrake TC, Boggs CL, Stamberger JA, Deutsch CA, Ehrlich PR. From global change to a butterfly flapping: biophysics and behaviour affect tropical climate change impacts. Proceedings of the Royal Society B: Biological Sciences 2014;281:1264.