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Dr. Ronaldo Bastos Francini
Labbor, Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

Dr. Renato Rogner Ramos
Labbor, Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

Ingo Grantsau
Secretaria do Meio Ambiente, Prefeitura Municipal de Santos André, Santo André, São Paulo, Brazil

Corresponding Author:
Dr. Ronaldo Bastos Francini
Labbor, Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

Higher and hotter: the continuous use of a territorial area by males of the threatened butterfly, *Actinote zikani* (Nymphalidae: Heliconiinae: Acraeini) in a Serra do Mar hilltop

Dr. Ronaldo Bastos Francini, Dr. Renato Rogner Ramos and Ingo Grantsau

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Abstract
This study describes the main territorial area in hilltop of Serra do Mar above Paranapiacaba village, Santo Andre municipality, Sao Paulo, SE Brazil, in relation to topographic and microclimate conditions which are analyzed using hemispherical photographs.

Keywords: Atlantic forest, hilltopping behavior, hemispherical photography, thermoregulation, *Mikania biformis*

Introduction
Actinote zikani D'Almeida 1950 (Nymphalidae: Heliconiinae: Acraeini) presents peculiar behaviors and strategies to maintain their territories and defend their encounters with females. The species is included in the Brazilian list of species threatened to extinction [1-3] and mentioned by IUCN as one of the 100 most threatened species on the planet [4].
Until now, only one population is known in the summit of Serra do Mar near the Paranapiacaba Village (Santo André Municipality, São Paulo, Brazil) which was discovery in 1991 [5]. However, since this date, amidst disappearances and reappearances, several records and preliminary tests were conducted showing decreases, expansions, and displacements of population along the hilltop of Paranapiacaba until the 2016 [6-7].
The hilltopping behavior in butterflies happens when individuals of both sexes search for landscape highest points to mate [8]. The first record of hilltopping in Acraeini butterflies was done in Africa [9] but, a most complete study on hilltopping Acraeini was conducted in Africa showing that at least 16 *Acraea* species could aggregate in the hilltop of Radio Mast Hill in Mabira Forest (Uganda) [10].
Additionally, butterflies are ectothermic organisms and need continuous control of their body temperature, and the thermoregulatory behavior is intrinsically connected to their flight activity [11-13]. Unlike its other sister species in the region, the *Actinote zikani* has a dark coloration that allows it to warm up, start quickly and maintain its activities during the cold mornings and fog periods on the tops of the Serra do Mar.
The objective of this study is to describe the main territorial area in the summit above Paranapiacaba village in relation to topographic and microclimate conditions. Male territorial and mating behaviors will be described in other papers.

Methodology
Study area and territory characteristics
Study area encompassed the surroundings of the village of Paranapiacaba, Santo Andre, Sao Paulo, Brazil [5]. Territory description was done using drone aerial photographs. The main behavioral observations were done along the Bela Vista trail, between last TOWERS (Figure 1A; TOWER 2 and TOWER 3) which is practically on the watershed of this stretch of Serra do Mar. Nowadays this area is part of the Parque Natural Municipal Nascentes de Paranapiacaba (Paranapiacaba Municipality Springs Natural Park).

Approximate schematic profile (30° → 210°) with 40 km in length showing the isolated position of the study area (T1) in Serra do Mar in relation to the Sao Paulo plateau and the coastal plain (Figure 1B).

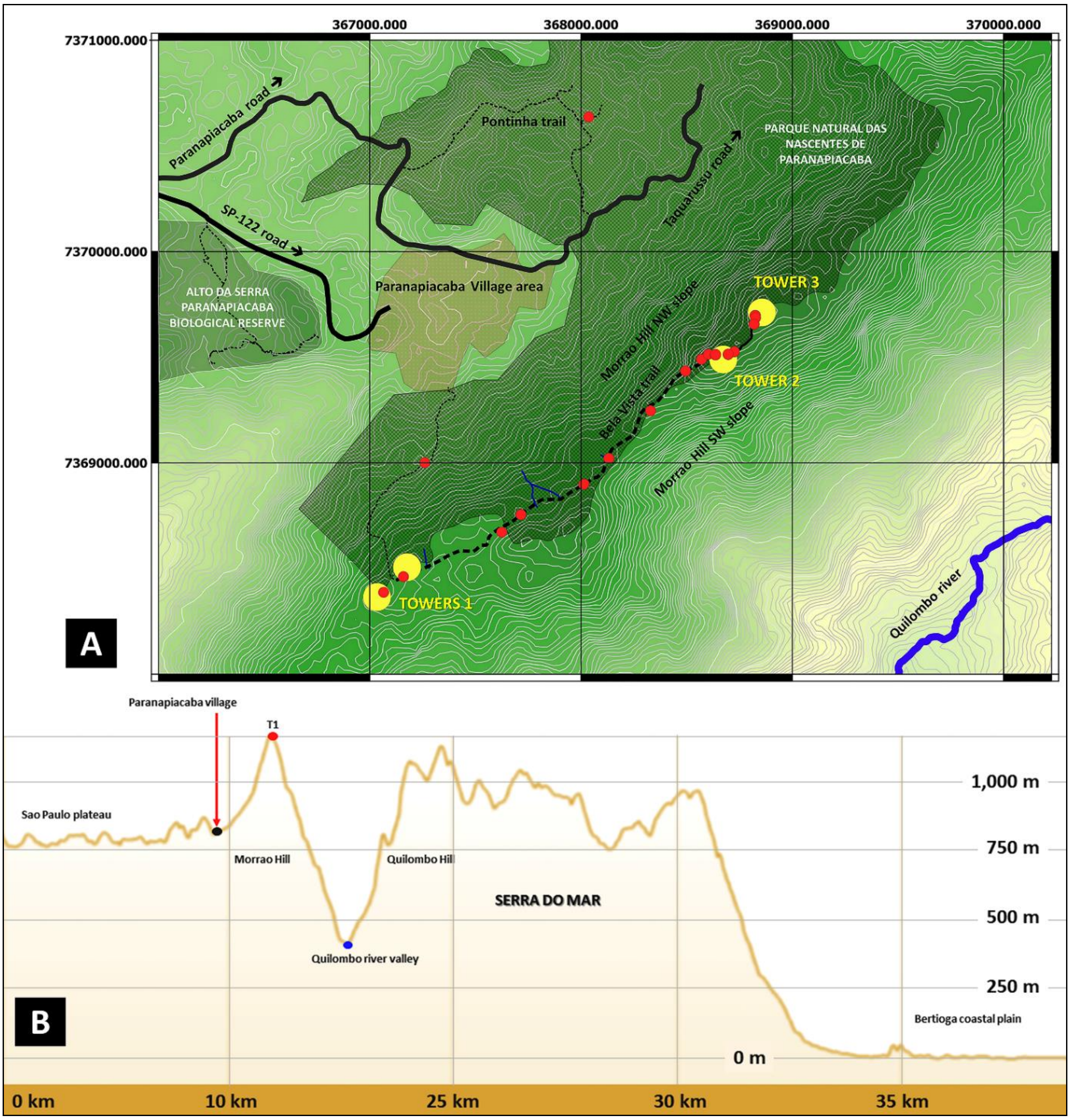

Fig 1: Localization of the study area near Paranapiacaba Village, Santo Andre, Sao Paulo, Brazil. (A) Study area around of Paranapiacaba Village and inside the Paranapiacaba Springs Natural Park (Parque Municipal Natural Nascentes de Paranapiacaba). Individuals of *A. zikani* were recorded mainly along the Bela Vista trail which is about 400 meters above de village and few in flowers in Pontinha trail and in the Paranapiacaba dirty road. More than 90% of the records were done between microwave towers TOWER 2 and TOWER 3 which are the two higher points in the area. UTM squares are 1,000 m wide. Red dots are points where one or more individuals of the *A. zikani* were sighted. (B) Approximate schematic profile (30° → 210°) with 40 km in length showing the position of the study area (T1) in Serra do Mar in relation to the Sao Paulo plateau and the Bertioga coastal plain. Horizontal distances are exaggerated nine times in relation to vertical distances (altitudes).

Vegetation and climate of the study area

The vegetation of the study area in Atlantic Forest was described in [5-6]. We used Worldclim data [14] with spatial resolution of 30 seconds (~ 0.86 km^2 at the equator) for the period 1970-2000 for the cell that encompasses the Bela Vista trail. The bioclimatic characteristics are: average annual temperature = 16.1°C; average annual rainfall = 2,235 mm (Figure 2A); minimum temperature of coldest month = 7.6°C; and maximum temperature of warmest month = 23.5°C; temperature annual range = 15.9°C; precipitation of wettest month = 314 mm; precipitation of driest month = 82 mm. Therefore, the climate of the study area is Cfa type, humid subtropical, with hot, humid summers, and mild winters [15]. Rainfall data from the period of study (Figure 2B) was obtained from the Cemaden automatic station (geographical coordinates: 23°46'40.80"S and 46°18'10.80"W [16]) located approximately 1,600 m from the *A. zikani* colony studied on the Bela Vista trail. Unfortunately, it stopped to work on February 2018.

Important condition in the study area, was the presence of frequent fog [17], however, personal communications from elder former residents in the Paranapiacaba village indicate that it is decreasing (Figure 2C).

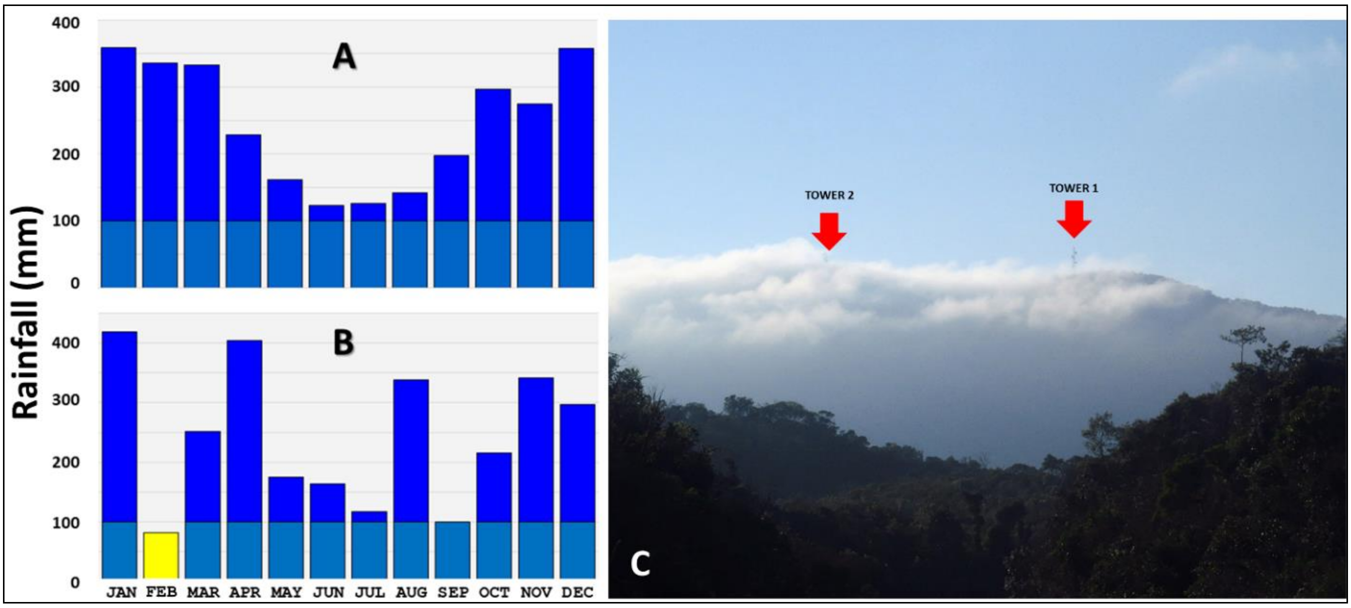

Fig 2: (A) Mean (normal) climate diagram of the study area [14]. (B) Rainfall during 2017 [16]. The comparison with the average values shows great differences. The months of February, March, September, October, and December are much drier, and the months of April and August are much more humid. (C) View of the top of the NW slope of the Morrao Hill showing the accumulation of clouds on April 4, 2017 (a sunny day) which causes a mist environment in the study area. Red arrows indicate the position of main territory T1 near TOWER 2 but only TOWER 1 is visible in the photography.

Territory microclimate estimation

The level of solar radiation of territories T1 to T4 was estimated by using hemispherical photographs at points five meters apart. The raw 24-bit photographs (8 bits per channel) were treated with Photoshop software and analyzed in the Gap Light Analyzer program, v. 2.0 [18]. Parameters used are: (1) local magnetic declination = -021.48°W ± 0.39° which changes -00.12°/year at the geographical coordinates: -23.780289° and -46.288899° [19]; (2) magnetic north and polar projection; (3) site orientation horizontal without topographic shading; (4) resolution with solar time steps of one minute; (5) number of azimuth regions of 90; (6) number of zenith regions of 45; (7) radiation model parameters used solar constant of 1367 W/m^2, cloudiness index of 0.5, spectral fraction of 0.5 with UOC (Universal Overcast Sky) model.

Hemispherical photographs technique estimates the potential level of solar radiation in a geographical point using astronomical equations based in the sun path along a day. The actual value of radiation is dependent of cloud cover. However, as estimates are used to make relative comparisons between very close points or the same point at different times of the year, they are perfectly acceptable. Photos were done with a SONY Alpha 6500 mirrorless camera with objective Canon 18-55/4.0-5.6 with a Raynox fish-eye adapter. The camera was fixed on a tripod at 140 cm above substrate with objective oriented to zenith. A magnetic compass and bubble level of Iphone5 were used for camera leveling and to found magnetic north. Camera height was chosen to mimic the average heights of *A. zikani* flights. Analysis of hemispherical photographs furnished three variables: sky openness (%), potential solar radiation transmitted total (MJ/m^2/day), and sunfleck duration (minutes of direct sun) during a chosen period.

Sampling effort and statistical analysis

From October 28, 2016, to November 22, 2019, 69 fieldtrips were done to collect data in an accumulated total of 197 hours of observation. In 46 samplings (66.7%) at least two researchers were present, working in different points to maximize data. Fifty-eight samplings (84.0%) were done during flying seasons in Bela Vista trail and 11 in Pontinha trail and Paranapiacaba road to record larval food plant data and search for adults. Territorial behavior of males and/or copulations were observed in 60 dates (56.7%) (Table 1).

Data were analyzed using R software v. 3.6.3 [20] and RStudio interface v. 1.3.959 [21].

Results

Territory characteristics

Drone aerial photographs show the topographic position of territories between towers TOWER 2 and TOWER 3 (Figure 3A; TOWER 2 and TOWER 3). The main territories, T1 to T4, are between TOWER 2 and TOWER 3 (Figure 3A-3B) and are isolated on the watershed with the São Paulo plateau to the north and the coast to the south (Figure 1A). After the landslide which occurred on March 2019, a new territorial area, territory T5, apparently became active (Figure 3B).

The territory characteristics were studied during the spring season of 2016 and both flight seasons of 2017-2019 (summer-autumn, and spring). Male territorial behavior was observed in different ranges, indicated by red dots, along the Bela Vista trail (Figure 1A). However, the work was concentrated between TOWER 2 and TOWER 3 (Figures 1A, 3A) where most of the male's air strikes occurred, and the four copulations were recorded. Aerial view of these area (Figure 3A) shows that T1-T2 are at altitudes of 1,160 m, and T3 at altitudes of 1,155 m, both plain; T2, is partially inclined. There, the trail has 5 m width and is recovered by shrubs of the bamboo *Chusquea* sp., and *Hedychium coronarium* and trees of different species with predominance of the *Pleroma mutabilis* (Melastomataceae; Figure 3B). Bela Vista's roadside vegetation is managed whenever necessary to maintain the power supply to the towers and allow access for service vehicles. After cleaning, the road had a low cover of herbaceous grasses, but this vegetation grows quickly and soon recovers the road. Territories T1 and T2 (Figure 4A-B), have a length of 30m, being oriented to 85° and were used during the five consecutive seasons (2017-2019). Territories T3 and T4 were less used.

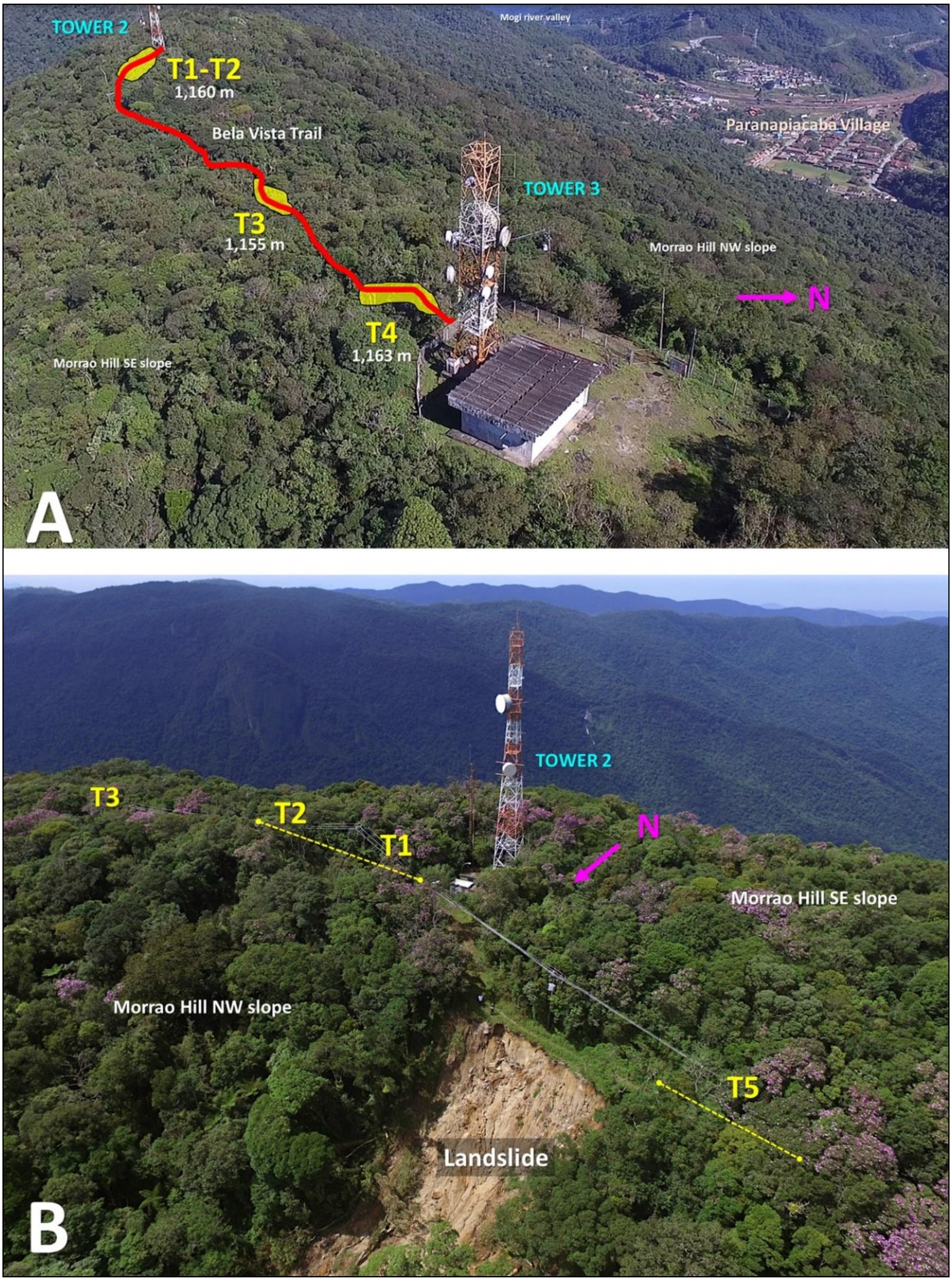

Fig 3: (A) Aerial drone view of main territorial areas along Bela Vista trail (red line). (A) Territories, T1-T2, near TOWER 2, and secondary territories T3 and T4, near TOWER 3. Altitudes indicated (m) are indicated for each territorial area. At top right, the Paranapiacaba Village at altitude of 800 m at base of Morrao Hill SW slope. (B) Aerial drone view of TOWER 2, showing main territorial area T1-T2, and T3 secondary. In the foreground, the landslide in the Bela Vista trail occurred after the heavy rains of March 2019. A new territorial area, T5, became being used by males. Also note the great density of *Pleroma mutabilis* (Melastomataceae) trees, indicated by the pink color of the canopy flowers. Approximate geographical north indicated by magenta arrows in both pictures.

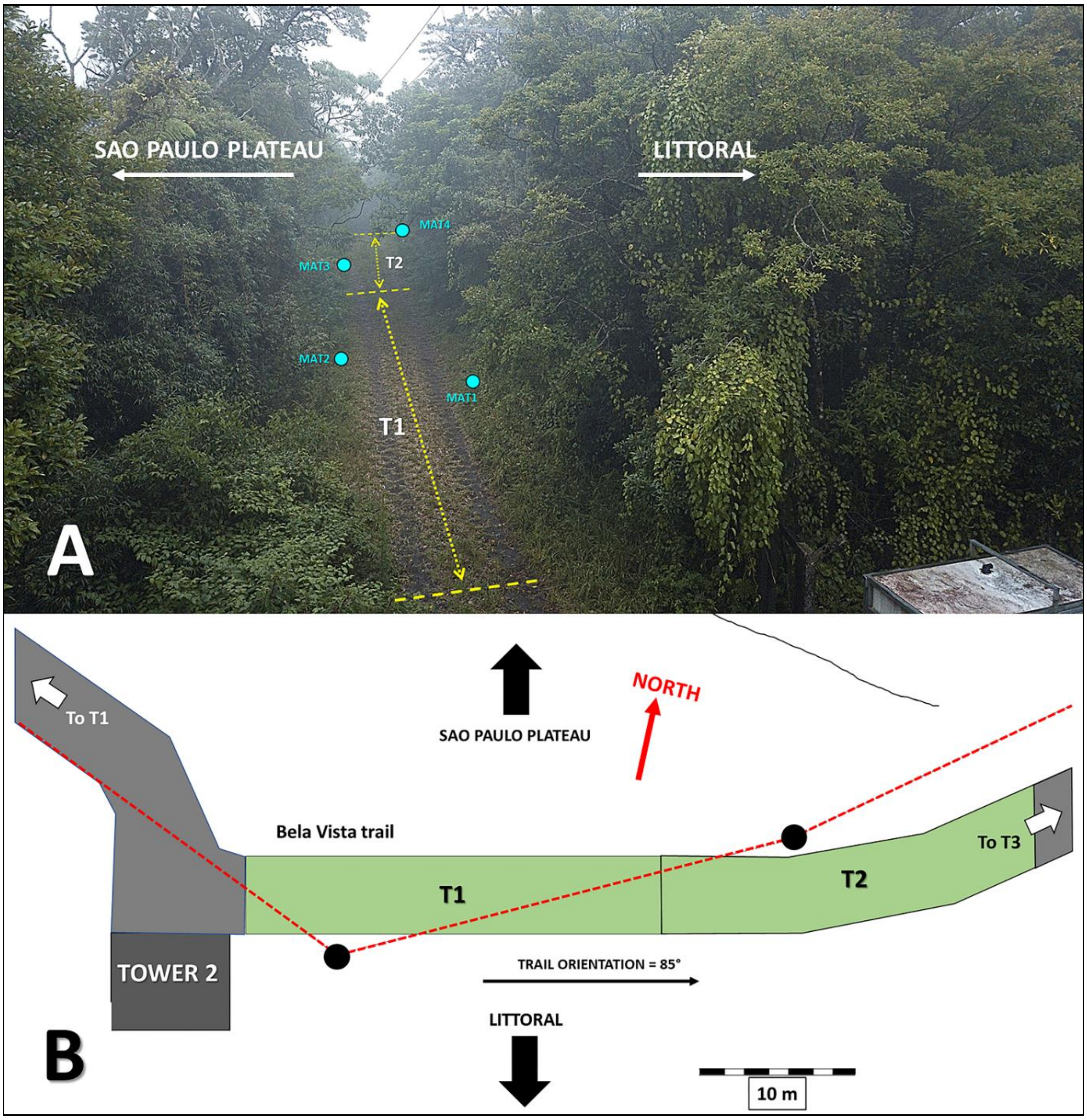

Fig 4: (A) Drone view of main territorial area near TOWER 2 showing the two main territories (T1 and T2). Green dots are point when copulations were observed. White arrows indicate the position of littoral and São Paulo plateau. (B) Croquis of the same area showing 85° orientation of the Bela Vista trail in T1 and part of T2. T3 is near TOWER 3.

The mean percentages of sky openness in the territories T1, T2 and T4 were not significant different ($F_{2,14} = 2.15$; p = 0.16). However, T1 and T2 presented at least six points with higher values than T3 (Figure 5A). We assumed that variation in the sky openness was small and in the same proportion in all territories.

Differently to the sky openness, the total potential solar radiation transmitted could be different between seasons. The statistical analysis of the canopy photographs data done for March and November 15 (autumn and spring seasons of 2018) showed that November value is marginally significantly higher than March values ($F_{5,28} = 2.46$; p = 0.0571). But most important is that at least one point in November is higher than in March in all three territories. Also, the comparison between the three territories in the same season showed that T2 than T1 and T4 although the higher individual point was in T1 (Figure 5B).

The sky area available to an individual in a point is also dependent of its height above soil and our estimates were based in canopy photos done at 1.4 m (Figure 5D). Therefore, when a butterfly is below or above this height, the available solar radiation is lower or greater than the estimated value.

Also, the minutes of direct sun (sunflecks) in the territory T1 were significant different between autumn and spring seasons (Welch Two Sample t-test = -12.54; df 83.18; $p < 0.0001$; Figure 5C).

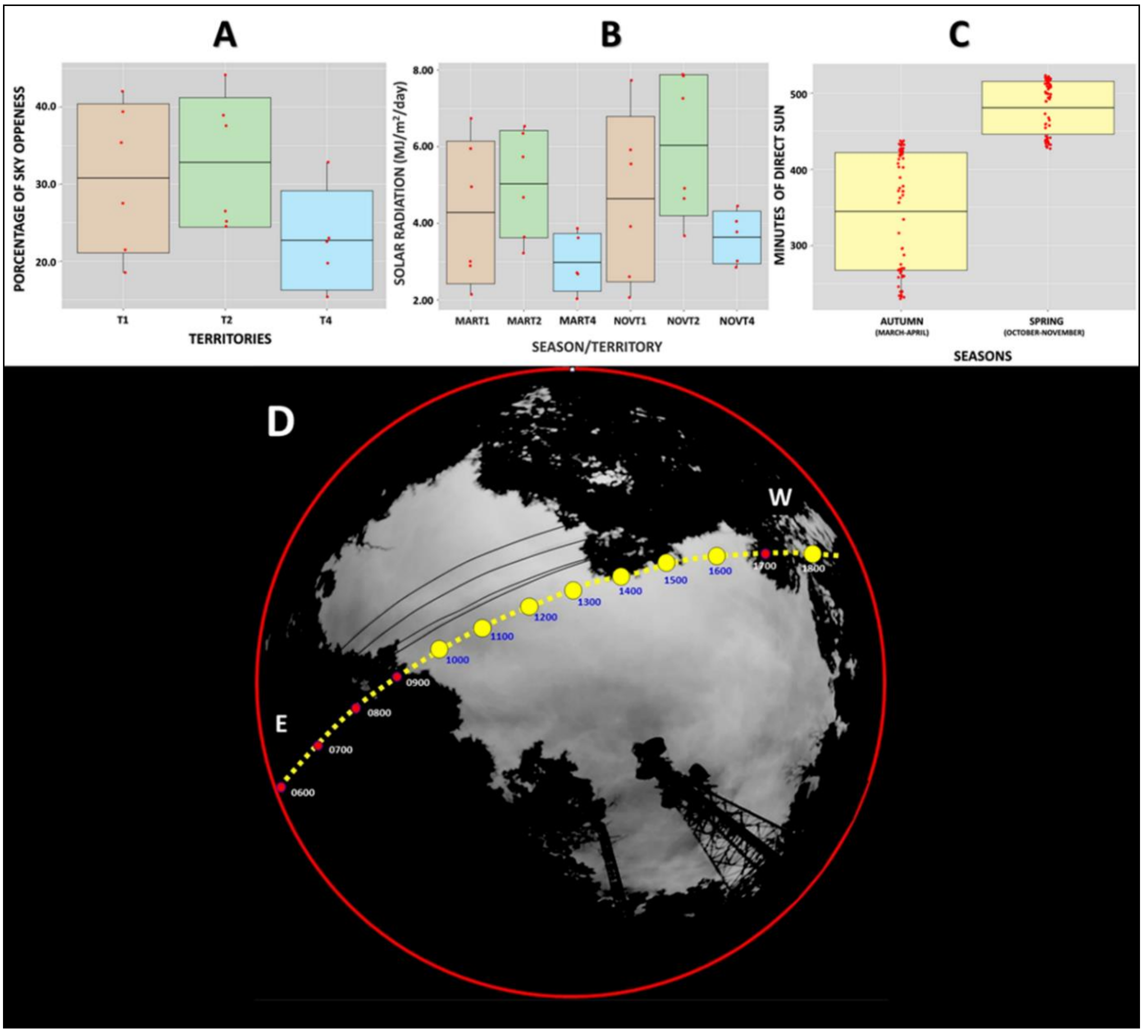

Fig 5: (A) The mean percentages of sky openness in the territories T1, T2 and T4 were not significant different ($F_{2,14} = 2.15$; $p = 0.16$). However, T1 and T2 presented at least six points with higher values than T4. Boxes indicate one standard deviation. (B) Solar radiation transmitted total during March (MAR) and November (NOV) in the three observed territories. November values are marginally significantly higher than March values ($F_{5,28} = 2.46$; $p = 0.0571$) and in one point, the maximum value of November is higher than March in all three territories. Boxes indicate one standard deviation. (C) Minutes of direct sun during autumn (March-April) and spring (October-November) in territory T1 showing that spring values are significantly higher than autumn values. Boxes indicate one standard deviation.

Territory T1 was the most disputed territory by males during both 2017 and 2018 flying seasons, being followed by T2. Territory T3 was used only in March 2017, and territory T4 in all seasons, but individuals did not spend much time there generally flying to territories T1 or T2.

These territorial areas appeared to furnish no food resources to adult butterflies. Few flowering plants were available to adults of *A. zikani*, along Bela Vista trail and, when present, only one female of this species was seen foraging on *Aegiphila* sp. (Verbenaceae).

Finally, differently to other territories, the territory T1 has both lateral sides of Bela Vista trail well protected by the vegetation height against strong winds (mainly SE-NW; 20-30km/h) which are frequent in the study area.

Discussion

The vegetation physiognomy on the banks of Paranapiacaba tracks was different compared to current days [5]. The edges of top road were predominantly composed of shrub vegetation (mainly Asteraceae) with many available flowers which coincided with the peak populations of other species of *Actinote* butterflies. Despite some records of mating behavior, the frenzy caused by the overlapping population peaks and flocks of several butterfly species, the vegetation physiognomy at the time could be a factor that contributed for no territorial behavior involving *Actinote zikani* males during early 90s [5].

In the last twenty years, the physiognomy of the forest edge undergone profound transformations, shifting to the predominance of arboreal vegetation and few flowering shrubs. This low availability of resources to adults of *A. zikani* butterflies has probably decreased the activities of the population. Thus, due lower frequencies of flowering species occupying the road margins, males of *A. zikani* have risen to claim their territories. Only from this moment on we could visualize the territorial behavior of males.

The territorial behavior of *A. zikani* shows several similarities with data from other authors [22-24], in these cases, the males are probably fighting only for mating areas and not for territories that contain resources used by females. Probably, the main source of adults recruited to main hilltop territories came from NW slopes of the Morrao Hill and that the territory establishment have the only purpose to find mates.

It must be considered that not all hilltopping butterfly species are territorial and differences in population densities may play a role in the evolution of behavioral diversity within this group [25]. The male behavior of certain hilltopping butterfly is also territorial and presents strong similarities [26].

Experimental work with the moth *Arctia virginalis* (Arctiidae) showed that the spatial distribution of its aggregations on hilltops is not random [27].

This unique *A. zikani* population is harmed by low densities of individuals and food resources and the strategy involving a mating point at the highest points of the landscape can mitigate the effects of negative impacts.

An interesting metanalysis on the role of microclimate upon 29 butterfly species showed that the understanding which factors influence the ability of individuals to respond to changing temperatures is fundamental to species conservation under climate change [28].

We need to reinforce that the study area is inserted in cell of the highest amounts of rainfall in Brazil with a small, but significant, tendency of diminishing along the years [29].

Conclusion

The observations of seven generations of *A. zikani*, from 2016 to 2019, showed the continuous use, generation after generation, of the same territorial area between the microwave TOWERS 2 and 3 in the Bela Vista trail. From the environmental parameters collected, the amount of solar radiation and protection against stronger winds were the main characteristics of this area. Even in seasons where few floral resources were available in this area, no individuals of this species were observed foraging on flowers or inflorescences used by this butterfly species during the study.

Activities related to feeding or oviposition were not observed in *A. zikani* main territory, which reinforces the idea of territory as rendezvous meeting place, which may include the need of a hilltopping behavior.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

Author's contribution

RBF designed the project. All authors collected data in the field. RBF analyzed the data and wrote primary draft of the manuscript. RRR and IG done additional suggestions in the final manuscript.

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