

## **Project Update: March 2024**

We carried out four field campaigns every 3 months throughout 2023 to cover different periods of the year. Each campaign lasted approximately 1 month, totalling 113 field days. We had the help of three field assistants, Jovelino and Ricardo from the Belmonte region and Adson from the Una region. Additionally, we engaged in the fieldwork seven undergraduate students of biological sciences and seven postgraduate students of ecology and biodiversity conservation from the Universidade Estadual de Santa Cruz.

During each campaign, we walked along pre-existing trails in 18 Atlantic Forest fragments between 9 am and 3 pm. During these walks, we used an entomological net to collect the observed butterflies. Each butterfly was immediately sacrificed and preserved in entomological envelopes for later identification in the laboratory. In total, 2,552 butterflies were collected.

We also collected hourly data on temperature, humidity, and weather. Additionally, we took canopy photographs in each of the 18 forest fragments during each campaign to evaluate canopy openness and to characterise the local habitat. We also recorded all flowering plants along the trails and at the edges of the fragments to assist us on plant identification to build the butterfly-plant interaction networks.

During our fieldwork, we observed few flowers in the fragments, which made it impractical to build interaction networks by recording the flowers with cameras, as originally proposed. Therefore, in addition to collecting butterflies, we also collected flowers found in the understorey along the trails. We collected plant material for later identification and flower buds for pollen collection to build up a reference pollen collection for the project area.

In the laboratory, we will remove the pollen from the butterflies using a stereoscope with Kisser's glycerine gelatine cubes [1]. This material, as well as the material extracted from the floral anthers, will be placed on a histological slide for observation under the microscope. The butterfly pollen will be identified by comparing it with the pollen from local flowers and databases, and with the help of specialists.

This procedure will allow us to reconstruct butterfly-flower interaction networks from the pollen found on the collected butterflies. Similar methodologies have been employed in other studies to reconstruct interaction networks by identifying pollen found in animal bodies, for example for bats and moths [2], bees [3], and butterflies [4]. Manincor et al. (2020) found that collecting data on insect visitation activities does not change the network structure compared to analysing data from pollen present on insects. The interaction network reconstructed from the pollen found on the body of flower visitors can increase the number of connections recorded compared to visit observation, as demonstrated for bees by Manincor et al. (2020).

## References

1. Kearns CA, Inouye DW. Techniques for pollination biologists. Niwot, Colorado: University Press of Colorado; 1993.
2. Queiroz JA, Diniz UM, Vázquez DP, Quirino ZM, Santos FAR, Mello MAR, et al. Bats and hawkmoths form mixed modules with flowering plants in a nocturnal interaction network. *Biotropica*. 2021;53: 596–607. doi:10.1111/btp.12902
3. Manincor N de, Hautekèete N, Mazoyer C, Moreau P, Piquot Y, Schatz B, et al. How biased is our perception of plant-pollinator networks? A comparison of visit- and pollen-based representations of the same networks. *Acta Oecologica*. 2020;105: 103551. doi:10.1016/j.actao.2020.103551
4. Balmaki B, Christensen T, Dyer LA. Reconstructing butterfly-pollen interaction networks through periods of anthropogenic drought in the Great Basin (USA) over the past century. *Anthropocene*. 2022;37. doi:10.1016/j.ancene.2022.100325

## Pictures

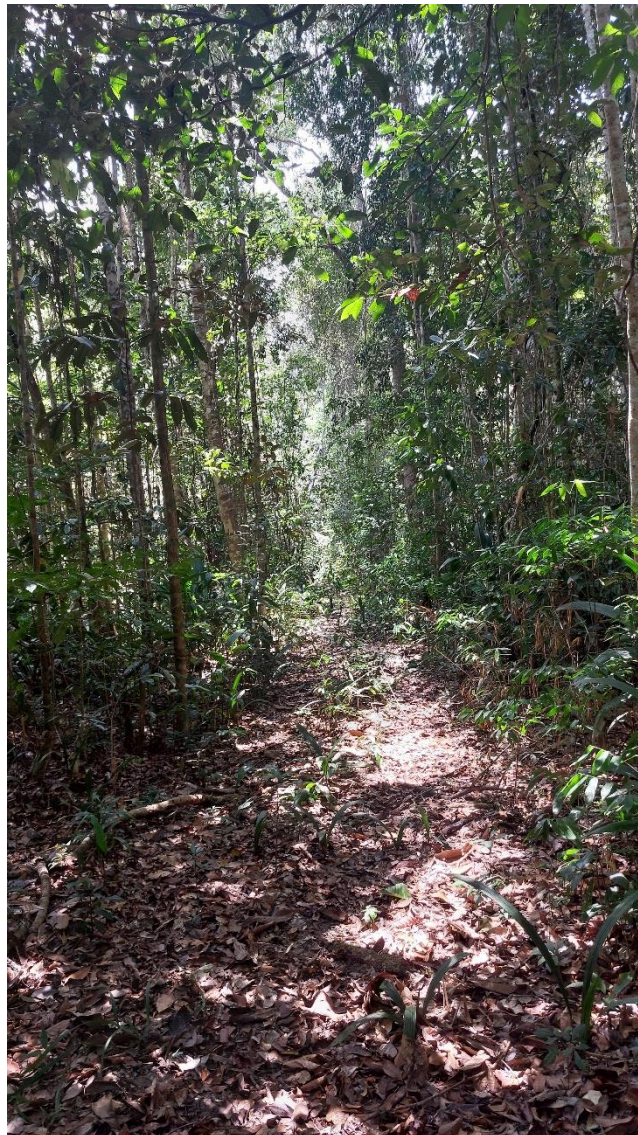


Figure 1: Trail in a fragment of the Atlantic Forest © Geanne Pereira.



Figure 2: Butterfly sampling © Geanne Pereira.





Figure 3: Butterfly sampling © Geanne Pereira.



Figure 4: Butterfly sampling © Geanne Pereira.





Figure 5: Butterfly sampling © Geanne Pereira.





Figure 6: Collecting temperature and humidity with a portable weather station  
© Ana Flávia do Nascimento.





Figure 7: Taking canopy hemispherical photos using a fisheye lens © Ana Flávia do Nascimento.



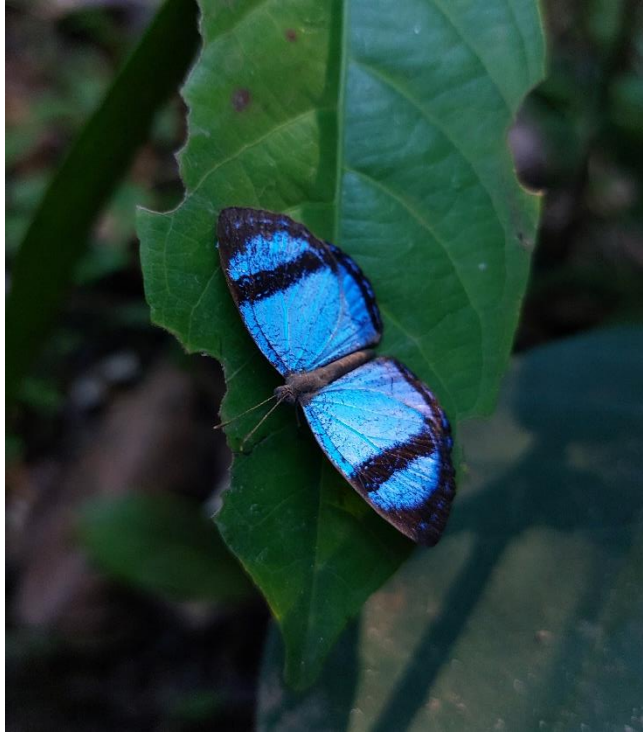


Figure 8: *Semomesia geminus* © Geanne Pereira.



Figure 9: *Anartia amathea* © Geanne Pereira.

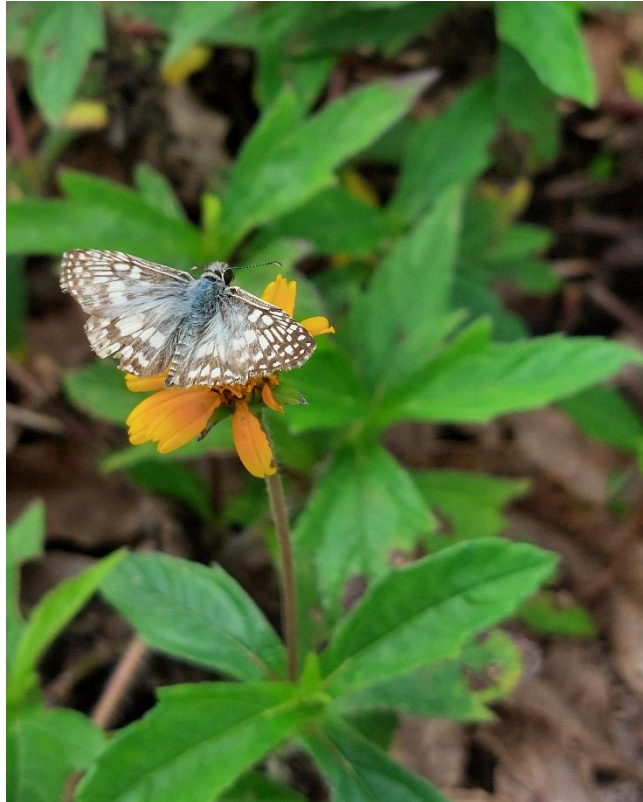


Figure 10: *Burnsius orcus* © Geanne Pereira.