December 2023

TROPICAL LEPIDOPTERA Research

VOL. 33 NO. 2

TROPICAL LEPIDOPTERA
RESEARCH

VOLUME 33 NUMBER 2 December 2023

CONTENTS

Front Cover Photo - *Celaenorrhinus dhanada* (Hesperiidae), Nepal, Gandaki Province. Photo by Sajan KC.
Inside Front Cover Photo - *Adelpha iphiclus* (Nymphalidae), Ecuador, Sucumbíos. Photo by Keith Willmott.
Back Cover Photo - *Nacaduba pavana georgi* Small Four-line Blue (Lycaenidae), Philippines, Negros Island. Photo by Jade Aster T. Badon.

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Abdominal microbiome composition and diversity of two *Heliconius* species (Lepidoptera: Nymphalidae) in the Colombian Andes

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Date of issue online: 7 April 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.7809269.

Abstract: Internal insect microbial communities, that is microbe taxa that live within an organism, play important roles in digestion, protection from pathogens, and fitness of their insect hosts. Recent expansion of research in this field has highlighted the importance of endosymbiotic communities to their hosts and elucidated microbial community patterns based on host life history. Here, we document the bacterial microbiome of two species of the butterfly genus *Heliconius* (Nymphalidae), each from two fragmented populations, by sequencing the V4 region of the 16S rRNA gene. We used 14 individual adult butterflies from two species, *Heliconius cydno* (n = 10) and *H. clysonymus* (n = 4), from two forest reserves in the Central Colombian Andes. *Commensalibacter* (Acetobacteraceae) was the most common bacterial genus across all samples, although relative abundance varied across groups. Notably, we also observed the bacterial genera *Spiroplasma* (Spiroplasmataceae) and *Wolbachia* (Ehrlichiaceae). While we did not find distinct spatial or species-level patterns in bacterial composition of microbiomes, we did find disparate bacterial diversity across the two butterfly species, with *H. cydno* harboring higher diversity than *H. clysonymus*. The microbiome composition of the two butterfly species did not differ, but that of *H. cydno* was distinct from the microbiome composition of environmental/butterfly trap bait samples. These findings contribute to the documented diversity of insect microbiomes and inform future experimental and sampling efforts.

Keywords: butterfly, Central Cordillera, Colombia, *Heliconius clysonymus*, *Heliconius cydno*, *Spiroplasma*, *Wolbachia*.

Resumen: La microbiota que reside dentro de los insectos contribuye a la digestión, protección contra patógenos, e influye en la aptitud darwiniana del huésped. Estudios recientes han destacado la importancia que tienen las comunidades bacterianas dentro sus hospederos, además de dilucidar sus patrones de diversidad y cómo estas están relacionadas a la historia natural del organismo que las alberga. En este trabajo, exploramos y documentamos las comunidades de bacterias presentes en mariposas del género *Heliconius*, mediante la secuenciación de la región V4 del gen ARNr 16S. En total se capturaron 14 individuos que corresponden a las especies, *Heliconius cydno* (n=10) y *Heliconius clysonymus* (n=4), presentes en dos reservas naturales (poblaciones) de la Cordillera Central de los Andes colombianos. *Commensalibacter* fue el género de bacteria más abundante en todas las muestras, pero su abundancia relativa fue variable entre los grupos. Adicionalmente, observamos la presencia de otras bacterias como los simbiontes, *Spiroplasma* y *Wolbachia.* Aunque no encontramos un patrón particular en cuanto a la composición bacteriana en los microbiomas a nivel de especie ni sitio, la diversidad de bacterias de los microbiomas entre ambas especies de mariposa fue distinta - *H. cydno* tenía más diversidad que *H. clysonymus.* Las composiciones de los microbiomas de las dos especies de mariposa fueron muy parecidas, aunque la de *H. cydno* fue distinta de la composición bacteriana del microbioma de muestreos del ambiente/cebo de trampa. Esta investigación contribuye a la diversidad documentada de los microbiomas en insectos, además de informar futuros experimentos y métodos de muestreo.

Palabras Claves: Colombia, Cordillera central, *Heliconius clysonymus*, *Heliconius cydno*, mariposa, *Spiroplasma*, *Wolbachia*.

INTRODUCTION

Host-associated microbiomes are the characteristic microbial community associated with an organism (Whipps *et al.*, 1988) as well as that community's dynamic function in time and space (Berg *et al.*, 2020). Microbiomes, specifically bacterial communities, play important roles in the ecology, life history, digestion, and behavior of their hosts (Majumder, 2019; Krishnan *et al.*, 2014; Zytynska & Meyer, 2018). Attention to insect microbiomes has increased in recent years, generating research documenting the diversity of insect-associated microbes, studying effects of diet on microbiomes, and tying insect development to the microbiome across distinct life stages (e.g., Chandler, 2011; Hammer, 2019; van Schooten *et* *al.*, 2018). Microbiomes can also directly affect host fitness, by, for example, altering sex ratios (Anbutsu & Fukatsu, 2011; Saridaki & Bourtzis, 2010).

Lepidoptera can be an ideal group with which to examine microbiomes because of their ties to food plants, and diversity in form and function. Butterfly microbiomes have been studied, for example, within the context of metamorphosis, demonstrating that the microbiome transforms in congruence with the dietary and morphological changes associated with metamorphosis (Hammer *et al.*, 2014). Long-wing butterflies, members of the genus *Heliconius* Kluk (Lepidoptera: Nymphalidae), are a well-studied insect group with a high degree of wing pattern divergence (Joron *et al.*, 2006), including 48 described species at present (Jiggins, 2017). *Heliconius* butterflies are famed for their diversity of wing variants, ability to collect and use pollen as a protein source as adults (Gilbert, 1972), and larval fidelity to plants of Passifloraceae. While recent studies (e.g., van Schooten *et al.*, 2018; Hammer *et al.*, 2020; Ravenscraft *et al.*, 2019) have increased our understanding of how microbiomes vary across several *Heliconius* species, much detailed information is biased to specific locations (primarily Gamboa, Panama), and species.

In this study we document the bacterial microbial diversity of two *Heliconius* species from two populations in Colombia: *Heliconius cydno* (Doubleday, 1847), with nine described subspecies in that country, and *Heliconius clysonymus* Latreille, 1817, which has two subspecies within Colombia and displays little wing color pattern variation (Holzinger & Holzinger, 1970). These two species are sympatric and likely overlap significantly in their trophic niches (Young & Montgomery, 2020). Here we add to the body of knowledge on insect microbiomes and to the geographic extent of sequenced *Heliconius* microbiomes. We do so by examining the structure and variation of bacterial microbiomes across *Heliconius cydno cydnides* Staudinger, 1885 and *H. clysonymus clysonymus* from two geographically isolated forest fragments in the Colombian Central Andes.

MATERIALS AND METHODS

Microbiome sample collection

We sampled adult *Heliconius cydno cydnides* and *H. clysonymus clysonymus* using entomological nets and baited traps in two forested sites in Colombia: a fragment called El Águila in Manizales, Caldas (5.10655 N, 75.50636 W), and Bremen Reserve in Filandia, Quindío (4.672131 N, 75.64066 W; Figure 1). Trap baits consisted of one cup containing fermented fruit mixed with urine and one cup containing blended shrimp, fish, and urine. Traps were deployed for 10 days at each site and butterflies were collected from traps every 24-48 hours. Both sites are in the Colombian Central Andean mountain range at approximately 1,800 m above sea level. We collected 10 *H. cydno* (five from both El Águila and Bremen) and 4 *H. clysonymus* (two from both El Águila and Bremen) in May and June of 2019. Butterflies were collected under permits issued to C.S. The small sample size is a byproduct of logistical constraints of the field research team and cost constraints for sequencing.

We excised abdomens of each butterfly under sterile conditions and preserved them in RNA*later* (ThermoFisher) in Eppendorf tubes in a conventional freezer at approximately -15°C. Butterfly samples therefore included all components of the gastro-intestinal tract (save for the foregut component present in the head and thorax), genitalia, and cuticle. We collected samples of the environment and/or trap bait to document background bacteria and control for effects of bait on butterfly microbiome diversity. In other words, samples collected in traps could be compared with the bacterial content of the bait, and those caught with a hand net could be compared with environmental bacteria samples collected passively. Control sample collection for trapped butterflies involved stirring bait, adding a tiny drop of bait to the RNA*later*, and placing a tube with RNA*later* open next to the bait for at least 15 minutes. Samples that served as controls for butterflies caught with a net were collected by placing a tube with RNA*later*

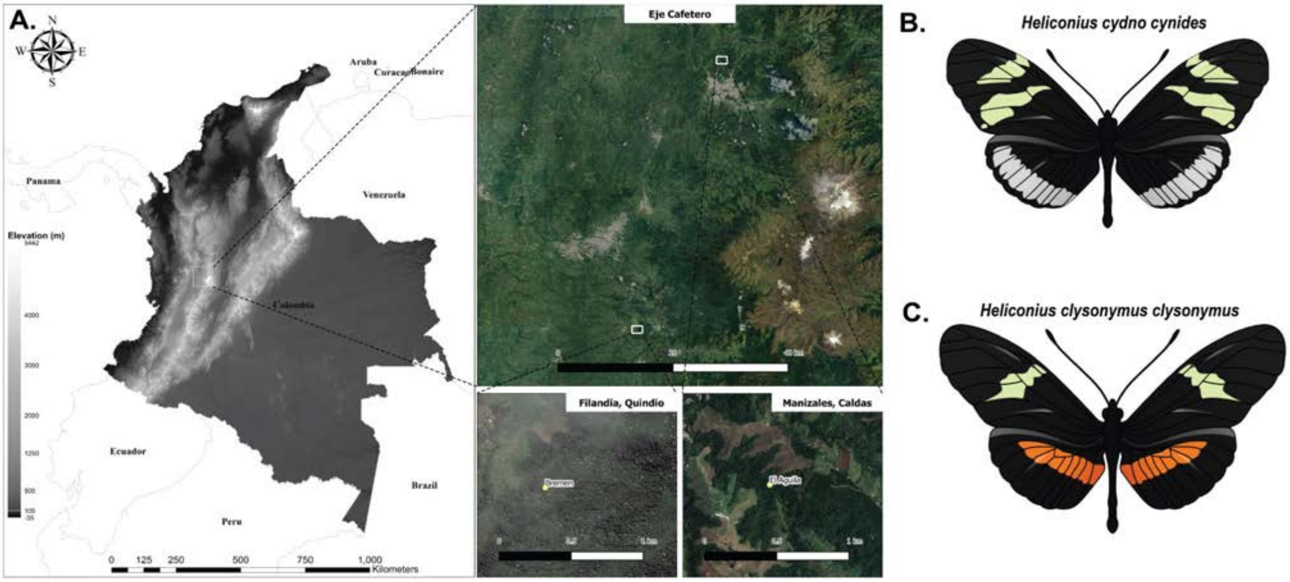

Figure 1. A. Butterfly sampling locations in the central cordillera of the Colombian Andes. Forest fragments Bremen and El Águila are in the departments of Quindío and Caldas, respectively. B. *Heliconius cydno cydnides*, and C. *Heliconius clysonymus clysonymus*.

open in the environment for at least 15 minutes. We collected control samples for each of the sampling events represented by butterflies, i.e., if two samples were collected from one trap, the sample from that trap's bait was the control associated with both butterflies.

DNA extraction, 16S rRNA gene amplification and sequencing

Before DNA extraction of microbiome bacteria, we vertically cut each abdomen, leaving half as a voucher specimen. Similarly, we processed half of each control sample. We performed extractions using the DNeasy PowerSoil Extraction Kit (Qiagen, Germantown, MD) for soil bacteria following the manufacturer's protocol. We quantified the concentration and purity of the DNA with a Nanodrop 2000 (ThermoScientific) and assessed the presence of a band using a 1% agarose gels with SYBR Safe dye (ThermoFisher).

We sequenced the 16S rRNA gene from controls (environmental/bait samples, n = 3) and butterfly samples (n = 14), following the Earth Microbiome Protocol (Gilbert *et al.*, 2014; Meyer *et al.*, 2019). For each PCR reaction, we used 1.25 µL of each 5 µM of the V4 Earth Microbiome primers 515F (GTGYCAGCMGCCGCGGTAA) and 806RB (GGACTACNVGGGTWTCTAAT) (Gilbert *et al.*, 2014; Apprill *et al.*, 2015), 2 µL of DNA, 0.75 µL of dimethyl sulfoxide and 12.5 µL Phusion High-fidelity Master Mix (New England BioLabs, Ipswich, MA), and water, for a 25 µL reaction. These universal primers were used in part for comparison across different species as well as with other studies with butterflies (e.g., Hammer *et al.*, 2020; van Schooten *et al.*, 2018). PCR conditions were: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 1 min, 72 °C for 90 s, and a final elongation step at 72 °C for 10 min. Each sample was amplified in triplicate. Negative controls (no PCR template) were run on a 1% agarose gel with Ethidium bromide to ensure no contamination but were not sequenced. The triplicate PCR products were combined, purified and concentrated with the MinElute PCR purification kit (QIAGEN) and purified products were quantified with a Denovix (Denovix,Wilmington, DE) before pooling the library. A final amplicon pool of 240 ng of each sample was submitted to the University of Florida, Gainesville for 150bp paired-end sequencing on an Illumina MiSeq. Raw sequences are in the NCBI SRA database with accession numbers SAMN28093385 - SAMN28093402.

Quality Control and ASVs generation

Using raw reads, we removed primers and adapters using cutadapt v. 1.8.1 (Martin, 2011) and then we used the DADA2 v. 1.14.1 pipeline (Callahan *et al.*, 2016) for filtering and combining the sequences into ASVs (Amplicon Sequence Variants). Reads were quality filtered and trimmed using the default parameters in dada2: *filterAndTrim (fnFs, filtFs, fnRs, filtRs, truncLen=c(150,150), maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE, compress=TRUE, multithread=TRUE)*. We then estimated error rates for the forward and reverse reads and merged forward and reverse reads into ASVs. Following merging, we removed chimeras (PCR artifacts not associated with the 16S region). We then assigned taxonomy using the SILVA rRNA database (v. 138.1, Quast *et al.*, 2013) to the genus level. We used phyloseq for further data processing and analysis (v 1.30.0, McMurdie & Holmes, 2013), where we also removed chloroplasts, mitochondria, and eukaryotes from our dataset. To further classify taxa, we used NCBI BLASTn to identify sequences to species at their highest percent sequence identity (>99.5%).

Bacterial diversity analyses

To compare alpha diversity of all microbiome bacteria samples, we created rarefaction curves using observed ASV richness and used ASV richness to estimate Shannon diversity in phyloseq. Due to the uneven and non-normal nature of our data, we evaluated differences in alpha diversity indices between the controls and butterfly species, and between the two butterfly species, using Kruskal-Wallis tests followed by Dunn's *post hoc* tests.

We visualized the bacterial community composition between control samples and butterfly samples; between both butterfly species; and between sampling locations using principal coordinates analysis (PCoA) using Bray-Curtis distances of the ASV relative abundances. We accompanied ordinations with Permutational Multivariate Analysis of Variance (PERMANOVA) to statistically test for differences between groups. We tested for differences between butterfly species and butterfly species versus controls in two separate PERMANOVAs. Multivariate analyses were performed using the vegan package in R (Oksanen *et al.*, 2014; R version 3.6.1, R Core Team, 2019). We then followed this analysis by pairwise PERMANOVA analyses using the pairwise.adonis package in R (Martinez Arbizu, 2020) to elucidate which groups most differed in composition from the others. To further investigate patterns in microbiome composition, we used the top_taxa function in the package phyloseq (McMurdie & Holmes, 2013) to examine patterns of relative abundance in the top 15 most abundant bacterial ASVs across groups.

Table 1. Total read counts per sample.

SampleID	**Site**	**Species**	**Sequences per sample**
BBCntl22519	BB	Control	43633
BBJCntrl-261518	BB	Control	70257
BBJCntrl-281519	BB	Control	9
EABCont322019	EAB	Control	86297
DNA0528	BB	*Heliconius clysonymus*	116385
DNA0688	BB	*Heliconius clysonymus*	155774
ESK0781	EAB	*Heliconius clysonymus*	47860
DNA8797	EAB	*Heliconius clysonymus*	140219
ESK0571	BB	*Heliconius cydno*	122837
ESK0659	BB	*Heliconius cydno*	93743
ESK0666	BB	*Heliconius cydno*	74710
ESK0667	BB	*Heliconius cydno*	110715
ESK0702	BB	*Heliconius cydno*	114668
ESK0777	EAB	*Heliconius cydno*	112826
ESK0785	EAB	*Heliconius cydno*	126914
ESK0796	EAB	*Heliconius cydno*	129417
ESK0918	EAB	*Heliconius cydno*	98509
DNA0937	EAB	*Heliconius cydno*	70971
Total			1715744

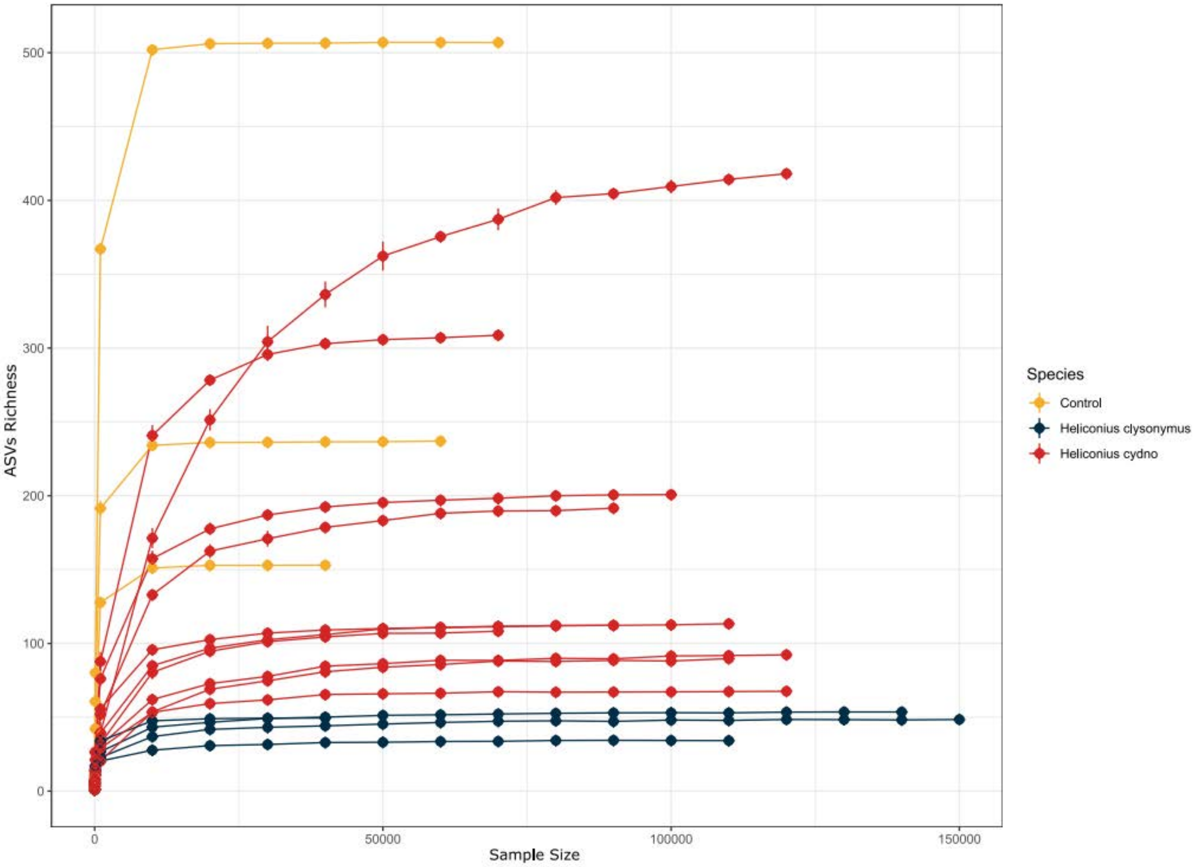

Figure 2. Rarefaction curves of observed ASV richness and of Shannon diversity index of microbial communities in *H. cydno*, *H. clysonymus*, and control samples.

RESULTS

Overall, we obtained 1,715,744 good-quality reads with an average of 95,319.11 (±38880.04, sd) reads per sample (Table 1). The asymptotic trend of the rarefaction curves demonstrates that we detected most of the microbial taxonomic richness present in our butterfly and control samples (Figure 2). As such, we used raw sequence abundance to estimate diversity and did not rarefy the data to an equal sequence depth. Observed ASV richness differed between butterfly species (K-W χ^2 = 6.51, p = 0.038) driven by the higher diversity harbored in *Heliconius cydno* (mean = 189.1 ± 39.0, se) compared to *H. clysonymus* (mean = 49.0 ± 4.34, se; Dunn Test Z = -2.406, p = 0.048). Control samples were variable (mean = 353 ± 192, se). The estimated Shannon diversity, however, did not differ among the three groups (Wilcoxon tests: control v. *H. cydno* (p = 0.14), control v. *H. clysonymus* (p=0.34), *H. cydno* v. *H. clysonymus* (p = 0.73)).

Despite differential observed alpha diversity, bacterial community structure did not significantly differ between the two butterfly species (PERMANOVA: F = 0.72, p = 0.86), nor by site (F = 1.07, p = 0.37; Figures 3, 4), a discrepancy likely stemming from our small sample size. Differences between control and butterfly microbiome composition reflected in the PCoA were corroborated by a PERMANOVA which demonstrated significant differences between the microbiome composition of the three groups (F = 1.62, p = 0.004; Figures 3, 4). Pairwise analyses showed the strongest community differentiation between *H. cydno* and environmental bacterial communities (F = 1.18; adjusted p = 0.012), while there was less distinction between *H. clysonymus* and environmental samples (F=1.23; adjusted p = 0.26). Pairwise PERMANOVA between the microbiome composition of the two butterfly species corroborated the pattern demonstrated in the ordination, and was non-significant (F=1.09; adjusted p = 0.37).

The discrepancies between microbiome composition are illustrated using the top 15 taxa as extracted by phyloseq (see above; Figure 3). Due to the fact that 16S rRNA datasets can harbor bias with respect to relative abundance, we did not run pairwise comparisons of relative abundance of these taxa; however, Figure 3 illustrates variation in bacterial composition.

We found *Wolbachia* (Hertig, 1936: Ehrlichiaceae). and *Spiroplasma* (Sagilo *et al.*, 1973: Spiroplasmataceae), ecologically relevant bacteria, in several samples. In two individuals, one *H. cydno* and one *H. clysonymus*, we observed those bacterial genera in high relative abundances (Figure 3). *Wolbachia*, matching to the strain *W. pipientis* (Hertig 1936) at 99.3% sequence identity, comprised 74% of the total reads of the *H. cydno* individual from El Águila. The single *H. clysonymus* from Bremen showed *Spiroplasma* in high abundance, 44% of that individual's total microbiome reads. *Spiroplasma* sequences recovered in our data were unable to be matched with any individual strain with certainty.

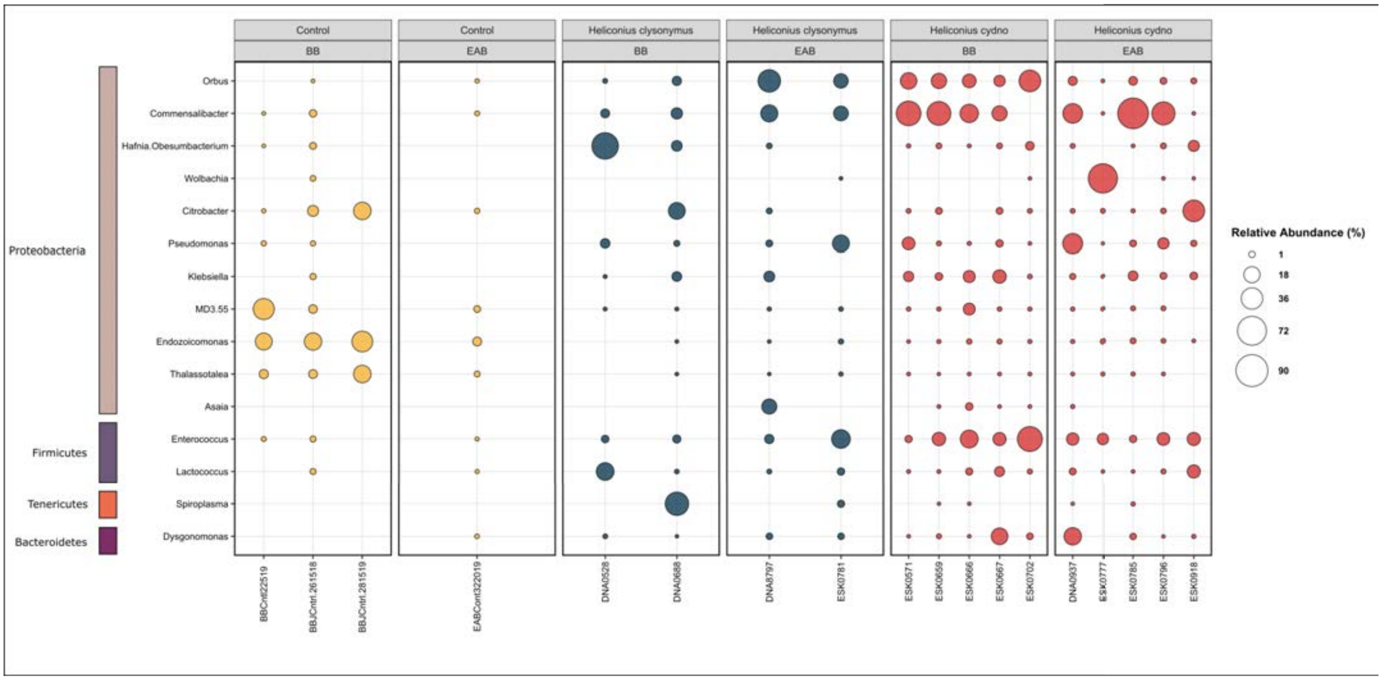

Figure 3. Relative abundance of the 15 most abundant genera of microorganisms found in both butterfly species and control samples at the two sampling sites (EAB = El Águila; BB = Bremen), all samples are represented. The size of the bubble indicates relative abundance. If a bubble is absent, that indicates that the bacterial taxon is not present in the sample.

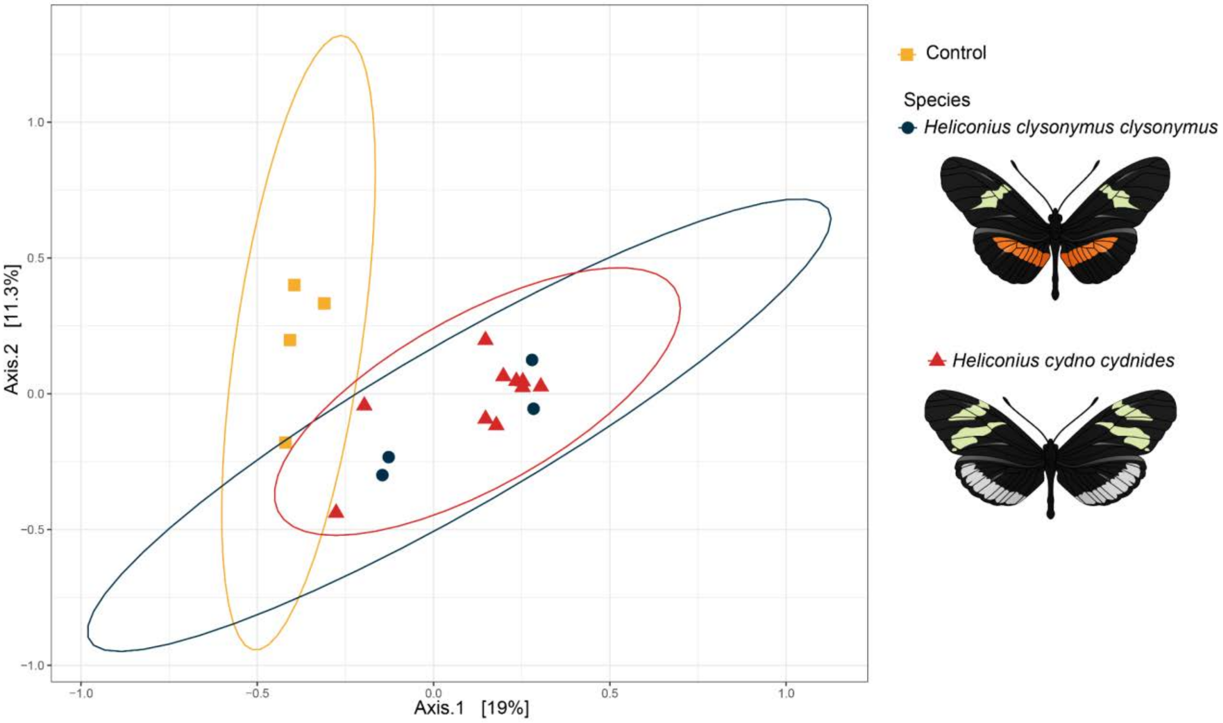

Figure 4. Principal coordinate (PCoA) biplot of all microbiome samples. Butterfly and control microbiomes differed in ASV (taxonomic) composition (PERMANOVA: F = 1.62, p = 0.004).

DISCUSSION

The purpose of this study is to describe the bacterial composition within two species of *Heliconius* butterfly not previously extensively studied, in a location not previously sampled for this purpose. The two focal *Heliconius* species shared similar microbial communities both within and across sampling sites. While we did not document adult behavior or foraging resource use, this similarity is likely due, in part, to overlapping trophic niches of the two butterfly species. Although communities were similar, we detected potentially biologically relevant differences in relative abundances of several bacterial genera between the species. The genus *Commensalibacter* (Rho *et al.*, 2008: Acetobacteraceae) was highly abundant in *H. cydno* but was present in low relative abundances in *H. clysonymus* (Figure 3). *Commensalibacter* has been documented in many insects with sugar-heavy diets like *Drosophila* (Fallén, 1823) (Drosophilidae) and honeybees (*Apis mellifera* Linnaeus, 1758; Apidae) and is known to have an important role in gut immune homeostasis in *Drosophila* (Chandler *et al.*, 2011; Siozios *et al.*, 2019.). The genus *Orbus* (Volkmann *et al.*, 2010) (Gammaproteobacteria) was also present in both species, but its relative abundance was extremely uneven across sites (Figure 3). It was not as prevalent in our two focal species as Hammer *et al.*, (2019) found it to be in *Heliconius erato* (Linneaus 1758) (Nymphalidae) in Panama, or as Ravenscraft *et al.*, (2019) found in fruit- and nectar-feeding butterflies in Costa Rica, suggesting general ubiquity but variability in density across sites. We found significant amounts of Proteobacteria and Firmicutes (see Figure 3), two bacterial phyla that dominate the microbiomes of several other *Heliconius* species (van Schooten *et al.*, 2018). Results from our study and those previously published on *Heliconius* species in other locations imply that the overall bacterial composition of the microbiomes of this group of butterflies is fairly similar despite some species and site variation, perhaps due to the similarities of foraging behaviors across the genus.

Wolbachia, a genus with diverse effects on hosts (Saridaki & Bourtzis, 2010), was found in high relative abundance in one individual of *H. cydno*, and present in low abundances in an additional three individuals. These bacteria, particularly *Wolbachia pipientis*, the strain found in our sample, can play important roles in reproduction of butterflies and other insects (Chandler *et al.*, 2011; Stouthamer *et al.*, 1999). Its effects on *Heliconius* butterflies specifically, however, remain to be examined, as well as how widely associated it is with *H. cydno*. We also found bacteria in the genus *Spiroplasma*, another bacterial group known for fitness effects on insect hosts, in *H. clysonymus*. *Spiroplasma* has been found in other *Heliconius* species (Hammer *et al.*, 2020; van Schooten *et al.*, 2018); this is the first record in microbiome samples of *H. clysonymus*, suggesting that the prevalence of this genus varies among host species and populations. *Spiroplasma* has been documented widely in the insect world, including in other butterflies (e.g., Jiggins *et al.*, 2000), and has been shown to influence survival rates of some *Drosophila* species (Xie *et al.*, 2010). The precise location within the butterfly and effects of these symbionts on *Heliconius* butterflies throughout their life cycle remains to be investigated. Additionally, although high relative abundances do not always translate into high cell counts of these bacteria, their detection in these samples are important to note.

While we found little distinct spatial or species-level patterns in the microbial composition of the individuals studied, perhaps due to the low power resulting from a small sample size, we did find higher observed bacterial diversity in the microbiome of *H. cydno* compared with *H. clysonymus*. The discrepancies in pattern between observed ASV diversity and estimated Shannon ASV richness is likely due to our low sample size. Increased sampling as well as detailed foraging data on the two species is needed to confirm and understand this observed pattern. The trend of higher bacterial diversity in *H. cydno* compared with *H. clysonymus* corresponds with the diversity of wing morphs of the two species; across its range, *H. cydno* has a greater diversity of phenotypes (Jiggins, 2017), which may influence foraging or other behaviors resulting in differential diversity of microbiomes. In this study, however, we only sampled one color morph of each species. The difference in bacterial composition of the microbiomes of *H. cydno* and that of the control samples collected may imply a possible disconnect between the microbiomes of *H. cydno* butterflies and that of their environment, and perhaps food sources. More robust patterns, including distinctions between environmental microbial diversity and butterfly-associated bacteria, may come to light with increased sampling, representation across sexes, species/subspecies, and morphological variants, and with detailed data on diet as collected by observing individuals as well as broad floristic surveys. With increased efforts across taxonomy and space, we will better understand patterns of microbial diversity, including intraspecific variation and geographic patterns. Likewise, more data on butterfly diet and plant inventories may further explain the structure and variability of microbiomes, and the interactions between an individual's diet and microbiome; and how bacteria are transferred via ecological interactions. These data add to the fast-growing library of microbiome sequences of *Heliconius* butterflies and advance the exploration of ecological and evolutionary patterns in insect microbiomes.

ACKNOWLEDGMENTS

We thank Jaime Haggard, Sebastián Durán, Indiana Cristóbal Ríos-Málaver for help with sample collection and DNA extraction. We also thank two reviewers for helpful comments and suggestions. Funding was, in part, provided by the Office of Global Research Engagement of the University of Florida grant awarded to ESK. We thank Julie Meyer at the University of Florida for molecular lab resources, and the John J and Katherine Ewel Fellowship awarded to AB. We also thank the High Performance Computing Service of Universidad del Rosario (CENTAURO), where part of the analyses was run.

LITERATURE CITED

Apprill, A., Mcnally, S., Parsons, R., Weber, L. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology* 75: 129-137.

Anbutsu, H., Fukatsu, T. 2011. *Spiroplasma* as a model insect endosymbiont. *Environmental Microbiology Reports 3*(2): 144-153.

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Xiaoyulong, C., Cocolin, L., Eversole, K., Corral, G H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ruan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G. S., Selvin, J., de Souza, R. S. C., van Oberbeek, L., Singh, B. K., Wagner, M., Walsh, A., Sessitsch, A., Schloter, M. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8(1): 1-22.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7): 581-583.

Chandler, J. A., Lang, J. M., Bhatnagar, S., Eisen, J. A., Kopp, A. 2011. Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLoS Genetics* 7(9), e1002272.

Gilbert, J. A., Jansson, J. K., Knight, R. 2014. The Earth Microbiome project: Successes and aspirations. *BMC Biology* 12: 1-4.

Gilbert, L. E. 1972. Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Sciences* 69(6): 1403-1407.

Hammer, T. J., Dickerson, J. C., McMillan, W. O., Fierer, N. 2020. *Heliconius* butterflies host characteristic and phylogenetically structured adult-stage microbiomes. *Applied and Environmental Microbiology* 86(24).

Hammer, T. J., Dickerson, J. C., McMillan, W. O., Fierer, N. 2019. Pollen-feeding *Heliconius* butterflies host distinctive adult-stage microbiomes. *bioRxiv* 820829.

Hammer, T. J., McMillan, W. O., Fierer, N. 2014. Metamorphosis of a butterfly-associated bacterial community. *PloS One* 9(1): e86995.

Holzinger, H., Holzinger, R. 1970. *Heliconius hygianus fischeri* (Fassl) comb. nov., eine Subspecies aus West-Columbien (Lep. Nymph.). *Zeitschrift der Arbeitsgemeinschaft österreichischer Entomologen* 22(2): 33-41.

Jiggins, C. D. 2017. *The Ecology and Evolution of* Heliconius *Butterflies*. Oxford, Oxford University Press.

Jiggins F. M., Hurst G. D., Jiggins C. D., Schulenburg J. H., Majerus, M. E. 2000. The butterfly *Danaus chrysippus* is infected by a male-killing *Spiroplasma* bacterium. *Parasitology* 120(5):439-446

Joron, M., Jiggins, C. D., Papanicolaou, A., McMillan, W. O. 2006. *Heliconius* wing patterns: an evo-devo model for understanding phenotypic diversity. *Heredity* 97(3): 157-167.

Kim, J. Y., Lee, J., Shin, N. R., Yun, J. H., Whon, T. W., Kim, M. S., Jung, M. J., Roh, S. W., Hyun, D. W., Bae, J. W. 2013. *Orbus sasakiae* sp. nov., a bacterium isolated from the gut of the butterfly *Sasakia charonda*, and emended description of the genus *Orbus. International Journal of Systematic and Evolutionary Microbiology* 63(5): 1766-1770.

Krishnan, M., Bharathiraja, C., Pandiarajan, J., Prasanna, V. A., Rajendhran, J., Gunasekaran, P. 2014. Insect gut microbiome-An unexploited reserve for biotechnological application. *Asian Pacific Journal of Tropical Biomedicine* 4: S16-S21.

Majumder, R., Sutcliffe, B., Taylor, P. W., Chapman, T. A. 2019. Next-Generation Sequencing reveals relationship between the larval microbiome and food substrate in the polyphagous Queensland fruit fly. *Scientific Reports* 9(1): 1-12.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *Advances in Environmental Biology* 7: 2803-2809.

Martinez, A. P. 2020. *pairwiseAdonis: Pairwise multilevel comparison using adonis*. R package version 0.4.

McMurdie, P. J., Holmes, S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*. 8(4): e61217.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, H. H. H., Sxoecs, E., Wagner, H., Barbour, M., BEdward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H. B. H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M. H., Cunha, E. R., Smith, T., Stier, A., Braak, C. J. F. T., Weedon, J. 2019. *vegan: Community Ecology Package. R package version 2.5-6*. https://CRAN.R-project.org/package=vegan.

Quast, C, Pruesse, E , Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41(D1): D590-D596.

Ravenscraft, A., Berry, M., Hammer, T., Peay, K., Boggs, C. 2019. Structure and function of the bacterial and fungal gut microbiota of Neotropical butterflies. *Ecological Monographs* 89(2): e01346.

Saridaki, A., Bourtzis, K. 2010. *Wolbachia*: more than just a bug in insect genitals. *Current Opinion in Microbiology* 13(1): 67-72.

Siozios, S., Moran, J., Chege, M., Hurst, G. D., Paredes, J. C. 2019. Complete reference genome assembly for *Commensalibacter* sp. Strain AMU001, an Acetic Acid Bacterium isolated from the gut of Honey Bees. *Microbiology Resource Announcements* 8(1).

Stouthamer, R., Breeuwer, J. A., Hurst, G. D. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology 53*(1): 71-102.

van Schooten, B., Godoy-Vitorino, F., McMillan, W. O., Papa, R. 2018. Conserved microbiota among young *Heliconius* butterfly species. *PeerJ* 6: e5502.

Whipps, J., Lewis, K., Cooke, R. 1988. *Mycoparasitism and plant disease control*, p. 161-187. *In*: Burge, M. N. (Ed.), *Fungi in Biological Control Systems*. Manchester, Manchester University Press.

Xie, J., Vilchez, I., Mateos, M. 2010. Spiroplasma bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp Leptopilina heterotoma. *PLOS ONE* 5: e12149.

Young, F. J., Montgomery, S.H. 2020. Pollen feeding in *Heliconius* butterflies: the singular evolution of an adaptive suite. *Proceedings of the Royal Society B* 287(1938): 20201304.

Zytynska, S. E., Meyer, S. T. 2019. Effects of biodiversity in agricultural landscapes on the protective microbiome of insects-a review. *Entomologia Experimentalis et Applicata* 167(1): 2-13.

Scientific Note: A new host plant record for the Yellow-tipped Flasher, *Telegonus anausis anausis* Godman & Salvin, 1896 (Lepidoptera: Hesperiidae: Eudaminae), from Cuba

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Date of issue online: 7 April 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.7790101.

Telegonus anausis Godman & Salvin, 1896 (*sensu* Zhang *et al.*, 2020) ranges from Mexico to Argentina, the Greater Antilles and on several of the islands in the Lesser Antilles chain including the Virgin Islands, Montserrat, Guadeloupe, Dominica, Martinique, St Lucia, St Vincent, and Grenada. Of the five subspecies, only *T. a. anausis* occurs in the West Indies (Smith *et al*., 1994). In Cuba it is a generally rare forest insect, though occasionally abundant, but widely ranging with records including western, central, and eastern sections of the main island, and it also inhabits the Isle of Youth (Hernández, 2004). We have observed it to stray sometimes into town gardens and other disturbed man-maintained spots.

Along its Antillean range, this skipper has been reared in Jamaica (Turner & Turland, 2017); there it has been found on *Vigna luteola* (Jacq.) Benth., *Mucuna pruriens* (L.) DC., and *Pachyrhizus tuberosus* (Lam.) Spreng, all Fabaceae. In Cuba the hostplants have remained largely unknown compared with the availability of records from Jamaica, and especially those from continental relatives (Beccaloni *et al*., 2008). The only published account was of a single final instar larva found in a leaf shelter and eating the leaves of *Lablab purpureus* (L.) Sweet (Fabaceae) (Fernández, 2001).

On 3 August 2022, while we were inspecting small shelters on *Mucuna pruriens* leaves along a forested roadside near Villa Soroa, Artemisa province, Yosiel Álvarez discovered a recently moulted final instar larva in a leaf shelter made by spinning two leaflets of the plant together with silk. This individual was then reared with leaves of the same plant until pupation. An

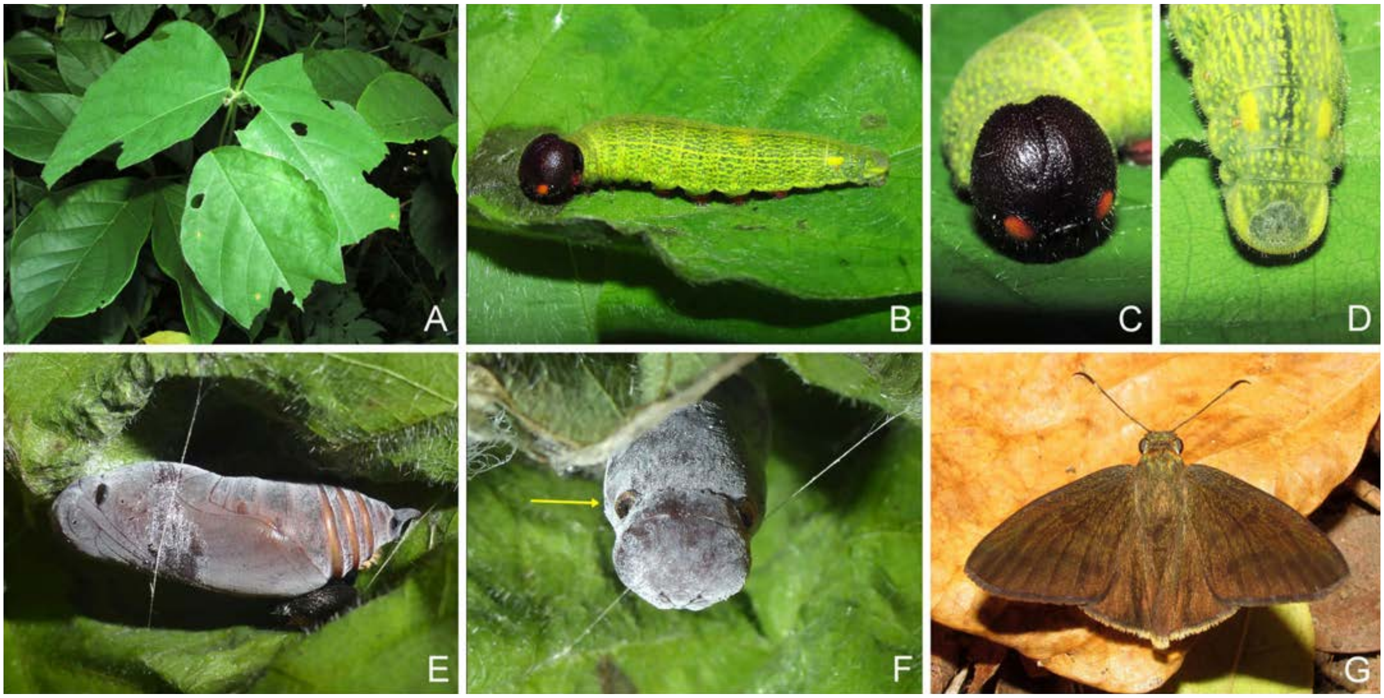

Figure 1. Life history of *Telegonus a. anausis*: **(A)** Shelter of final instar larva and feeding damage on *Mucuna pruriens*; **(B)** Nearly mature larva (lateral view); **(C)** Frontal view of head capsule of mature larva showing shape of head and orange spots; **(D)** Caudal end of mature caterpillar showing larger greenish yellow spots on 8th abdominal segment, and anal plate; **(E)** Pupa of three days (lateral view); **(F)** Pupa (frontal view), the yellow arrow points to the enlarged dark brown prothoracic spiracle which resembles a false eyespot; **(G)** Freshly emerged female (dorsal view).

adult, *Telegonus a. anausis,* emerged after 11 days. This is a new hostplant record for Cuba.

Figure 1 shows the shelter on the foodplant and the life history stages. The immatures have not been photographed before in Cuba. The color and pattern of the mature larva conforms primarily to that described for the insect in Jamaica (Turner & Turland, 2017), but in our larva the ventral prolegs were pinkish red only laterally and the caudal pair greenish. The length of the larva was 4 cm, but wild-raised individuals presumably attain a larger size. The pupa is brown covered in a thin whitish, waxy exudate. The prothoracic spiracles are dark brown and prominent, resembling a pair of false eye-spots. No other markings are present. The length of the pupa was 2.2 cm, but this might also be smaller than in wild-raised individuals, as in the larva. The previously recorded development time for the pupa was 15 days in the dry season (Fernández, 2001), and the difference between this duration and our observed duration might indicate some differences in pupal duration between seasons, a trend that is also observed in many Cuban butterflies.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Rayner Núñez, Zoological Museum Alexander Koenig, Germany, for providing helpful taxonomical information and kindly sending necessary literature. We thank an anonymous reviewer who kindly reviewed and improved the manuscript.

LITERATURE CITED

Beccaloni, G. W., Viloria, Á. L., Hall, S. K, Robinson, G. S. 2008. *Catalogue of the host plants of the Neotropical butterflies. Catálogo de las plantas huésped de las mariposas neotropicales.* Zaragoza, Sociedad Entomológica Aragonesa. (Monografías del Tercer Milenio, Vol. 8). 536 pp.

Fernández, D. M. 2001. New oviposition and larval hostplant records for twenty-three Cuban butterflies, with observations on the biology and distribution of some species. *Caribbean Journal of Science* 37: 122-125.

Hernández, L. R. 2004. *Field Guide of Cuban-West Indies Butterflies*. Maracaibo, Ediluz. 269 pp.

Smith, D. S., Miller, L. D., Miller, J. Y. 1994. *The butterflies of the West Indies and South Florida*. New York, Oxford University Press. 264 pp.

Turner, T. W., Turland, V. A. 2017. *Discovering Jamaican Butterflies and their Relationships around the Caribbean*. Manitoba, Friesens Press, Caribbean Wildlife Publications. 492 pp.

Zhang, J., Cong, Q., Shen, J., Opler, P. A., Grishin, N. V. 2020. Genomic evidence suggests further changes of butterfly names. *The Taxonomic Report of the International Lepidoptera Survey* 8(7): 1-40.

Immature stages of *Mechanitis lysimnia nesaea* (Nymphalidae: Danainae: Ithomiini)

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Date of issue online: 7 April 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.7790105.

Abstract: The immature stages (eggs, larvae and pupae), larval behavior, oviposition and host plant of *Mechanitis lysimnia nesaea* Hübner, [1820] are described for the first time. Eggs are laid in clusters on the upper side of leaves of its solanaceous hostplants. The larvae are gregarious and undergo five instars. The first instar head capsule is black and the body is translucent yellow. From the second instar on, the body changes to bluish gray with yellow stripes, with colors becoming more vivid in the last instar. Initially, the pupa is yellow, turning reflective (silvery/golden) with dark contours on the wing cases and abdomen after the first day. A comparison with other species of *Mechanitis* shows that, despite their similarities, the early stages are informative at the species level, and could help to understand the species limits in the currently non-monophyletic species in this genus.

Key words: host plant, immatures stages, life cycle, Mechanitina, Solanaceae.

Resumo: Os estágios imaturos (ovos, larvas e pupas), comportamento larval, oviposição e planta hospedeira de *Mechanitis lysimnia nesaea* Hübner, [1820] são descritos pela primeira vez. Os ovos são depositados em grupos na parte superior das folhas de suas plantas hospedeiras (solanáceas). As larvas são gregárias e passam por cinco instares. A cápsula cefálica do primeiro instar é preta e o corpo é amarelo translúcido. A partir do segundo instar, o corpo muda para cinza azulado com faixas amarelas, com cores mais vívidas no último instar. A pupa é amarela inicialmente, tornando metálica (prateada/dourada) com contornos escuros na região das asas e no abdome após o primeiro dia. Comparações com outras espécies de *Mechanitis* mostram que, além das semelhanças, os estágios iniciais são informativos em nível de espécie e podem ajudar a entender os limites das espécies não monofiléticas deste gênero.

Palavras-chave: planta hospedeira, estágios imaturos, ciclo de vida, Mechanitina, Solanaceae.

INTRODUCTION

Distributed from Mexico to northern Argentina, the Neotropical tribe Ithomiini (Nymphalidae: Danainae) includes about 390 species occurring in several forested habitats from sea level to 3,000 meters (Brown & Freitas, 1994; Lamas, 2004; Willmott & Freitas, 2006; Brower *et al*., 2014). The adults are all aposematic and unpalatable and are involved in numerous Müllerian mimicry rings (Brown & Benson, 1974; Beccaloni, 1997). Ithomiini larvae are usually very host-specific to plant family and genus, feeding mostly on plants of the family Solanaceae, with a few exceptions using Apocynaceae and Gesneriaceae (Drummond & Brown, 1987; Beccaloni *et al*., 2008).

The tribe Ithomiini is currently divided into ten subtribes supported by morphological and molecular data (Willmott & Freitas, 2006; Brower *et al*., 2006, 2014; Garzon-Orduña *et al*., 2015). The subtribe Mechanitina contains five genera, including *Mechanitis* Fabricius, 1807, a small genus containing five described species and over 50 subspecies distributed throughout the Neotropics (Fox, 1967; Brown, 1977, 1979; Lamas, 1987, 2004; Dasmahapatra *et al*., 2010; Hill *et al*., 2012; Giraldo *et al*., 2014), and with a reputation for being taxonomically 'difficult' (Fox, 1967). Occurring from Mexico to Argentina, *Mechanitis lysimnia* (Fabricius, 1793) is the most widespread species, with at least 14 described subspecies and several unnamed local forms (Brown, 1979; Lamas, 2004; Mota *et al*., 2022). One of these subspecies, *Mechanitis lysimnia nesaea* Hübner, [1820], is common in northeastern Brazil, occurring in well-preserved and secondary forests especially on the coast, but also present in forest patches in semi-arid regions (Caatinga) (Kesselring & Ebert, 1979; Nobre *et al*., 2008; Paluch *et al*., 2011; Zacca & Bravo, 2012; Kerpel *et al*., 2014; Melo *et al*., 2019).

Species of *Mechanitis* are common components of most lowland Neotropical forests, and although the immature stages have been described for one or more populations of most species, only a few subspecies and/or populations have been studied in this respect (Carvalho *et al*., 2019, and references therein). Detailed information on immature stages from different populations could assist in conservation and help to understand species limits and help to identify cryptic taxonomic diversity. Accordingly, the present paper describes in detail the immature stages of *M. lysimnia nesaea*, aiming to improve the understanding of species limits and variation in *Mechanitis* immature stages.

MATERIAL AND METHODS

Study Sites

Adults and immatures of *M. lysimnia nesaea* were studied from August to September 2014, from January to March 2016, and in November 2022, in Parque Estadual Dois Irmãos (08º00'48" S, 34º56'42.9" W; altitude 40-60 m), a forest fragment of 1,157.72 hectares located in the metropolitan region of Recife, Pernambuco, Brazil (Fig. 1A, B). Solanaceous hostplants were searched for eggs and larvae of *M. lysimnia nesaea*, along forest edges, trails and at the margins of streams near weirs, where the host plant is common. Additional data was obtained in August 2006 in Serra Negra District, Bezerros Municipality, Pernambuco, Brazil (08º10'45" S, 35º46'57" W; altitude 800-900 m).

Rearing, Morphology, and Behavior

Descriptions here are mostly based on material from Parque Estadual Dois Irmãos, with larvae reared on *Solanum stramoniifolium* Jacq. (Solanaceae) (Fig. 1B, C). Eggs and larvae were reared in the laboratory in plastic pots under ambient conditions, cleaned daily, and with food available *ad libitum* following Carvalho *et al.* (2019). Leaves were kept fresh by immersing their petioles in small vials with clean water. Data were recorded on behavior and development time for all stages. Dry head capsules and pupal exuviae were retained in glass vials. Measurements were obtained using a Leica MZ7.5 stereomicroscope equipped with a micrometric scale. Egg size is presented as height and diameter, and head capsule size is the distance between the most lateral stemmata (as in Carvalho *et al*., 2019). Maximum length was measured one or two days before each molt (when larvae reach maximum length). Immature stages were fixed in Kahle-Dietrich solution and then preserved in 70% ethanol. The specimens were deposited at the Zoological Collection (ZUEC) of the Museu de Diversidade Biológica, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Adult behavior, including oviposition and the flight activities of butterflies were studied in the field from 08:00 to 16:00 at forest edges and in the forest interior. The taxonomy follows Lamas (2004) modified after Hill *et al*. (2012), where *M. mazaeus* and *M. messenoides* are considered as distinct species (see also Warren *et al*., 2019). Accordingly, data discussed here for the immature stages of *M. messenoides* correspond to a population belonging to "haplogroup A" of Hill *et al*. (2012).

RESULTS

Hostplants, oviposition and larval behavior

Based on field observations, the main host plant of *M. lysimnia nesaea* is *Solanum stramoniifolium*, popularly known as "Jurubeba" in Recife. In addition, four unidentified species of Solanaceae (three *Solanum* and one unidentified genus) were recorded in the field (e.g. Fig. 1D). Eggs are laid in groups of variable size (from five to more than 130 eggs) (Figs. 1E, 2A, B), always on the upper leaf surface near the apex of both young and mature leaves of *Solanum stramoniifolium* (Fig. 1B, C), and on mature leaves of the unidentified Solanaceae from Bezerros (Fig 1D). The total duration of the oviposition process was not recorded, but females were observed to spend up to three hours in the process of laying eggs, until flying away when disturbed by the wind.

Larvae consume the egg corion just after hatching (Fig. 2C) and soon start feeding on the leaf blade, producing small holes nearby the oviposition site. Subsequently, larvae move to the under surface of the leaf where they preferentially stay until the last instar (although some larvae were observed on the upper surface during nocturnal feeding). First instars weave a silken thread, using it as a "platform" to move around among the thorns and trichomes of the hostplant. The first instars feed on almost the entire leaf, avoiding only the trichomes. From the second instar on, larvae start to feed on the leaf edges, consuming also the secondary veins. Only from the third instar on are the thorns are eaten, while the central leaf vein is not consumed in any instar.

Larvae are gregarious in all instars, participating in all the activities such as feeding, resting, and movement between the leaves and molting, at the same time. When handled, the larvae are able to regurgitate recently eaten food. In the field, pupation occurs off the host plants, and pupae were never observed on the host plant, but in the laboratory, pupation can occur on the leaf underside and on the lid of the plastic containers.

Description of the immature stages

Egg (Fig. 2A, B): Diameter 0.64-0.70 mm (mean = 0.66 mm, SD = 0.025, n = 8); height 1.10-1.24 mm (mean = 1.17 mm, SD = 0.063, n = 8). White and elongated with a pointed apex. Eggs become transparent near hatching making it possible to visualize the dark head capsule of larvae (Fig. 2C). Egg surface sculptured with 10-11 horizontal ridges and 12-14 vertical ridges (n = 15). Duration: 5 days (n = 77).

First instar (Fig. 2C, D, E): Head capsule width 0.48-0.50 mm (mean = 0.48 mm, SD = 0.017, n = 11). Head capsule black and smooth, without any projections; body translucent yellow with visible intestinal contents; prothoracic segment with two dorsolateral small protuberances; a pair of short rounded lateral projections from A1 to A8; legs black; prolegs light yellow with a lateral gray plate (Fig. 2D). A triangular dark patch is present in anal plate. Maximum body length 3.5 mm (n =10). Duration: 3-4 days (n = 155).

Second instar (Fig. 2F, G): Head capsule width 0.70-0.76 mm (mean = 0.74 mm, SD = 0.017, n = 14). Head capsule black and smooth; body light green, with a pair of short lateral projections from A1 to A8; prothoracic protuberances and abdominal lateral projections more conspicuous than in first instar; legs black, prolegs light cream; lateral dark plates absent on proleg (Fig. 2F). Maximum body length 9.5 mm (n = 10). Duration: 2-3 days (n = 100).

Third instar (Fig. 2H, I): Head capsule width 1.0-1.12 mm (mean = 1.07 mm, SD = 0.035, n = 16). Similar to previous instar, darker, with lateral projections more developed, pointed and with a yellow patch at base. Prolegs light cream; lateral dark plates absent on proleg (Fig 2H). Maximum body length 15.5 mm (n = 10). Duration: 2-3 days (n = 210).

Fourth instar (Fig. 2J, K): Head capsule width 1.44-1.64 mm (mean = 1.55 mm, SD = 0.068, n = 15). Head capsule black with frons and mouthparts pale; in some individuals head capsule is predominantly light cream or brown (Fig. 2K), with a black patch restricted to the stemmatal region. Body light bluish grey, a dorsal light-yellow stripe from T2 to A8; prothorax white with a pair of short dorsolateral protuberances; lateral abdominal projections longer than in previous instar, white, each yellow at base with a black circled spiracle; legs black; prolegs light cream; lateral dark plates absent on proleg (Fig. 2J). Maximum body length 29.0 mm (n = 10). Duration: 2-3 days (n = 145).

Fifth (last) instar (Fig. 3A-F): Head capsule width 2.0-2.1 mm (mean = 2.04 mm, SD = 0.033, n = 7). Head capsule black with frons and mouthparts

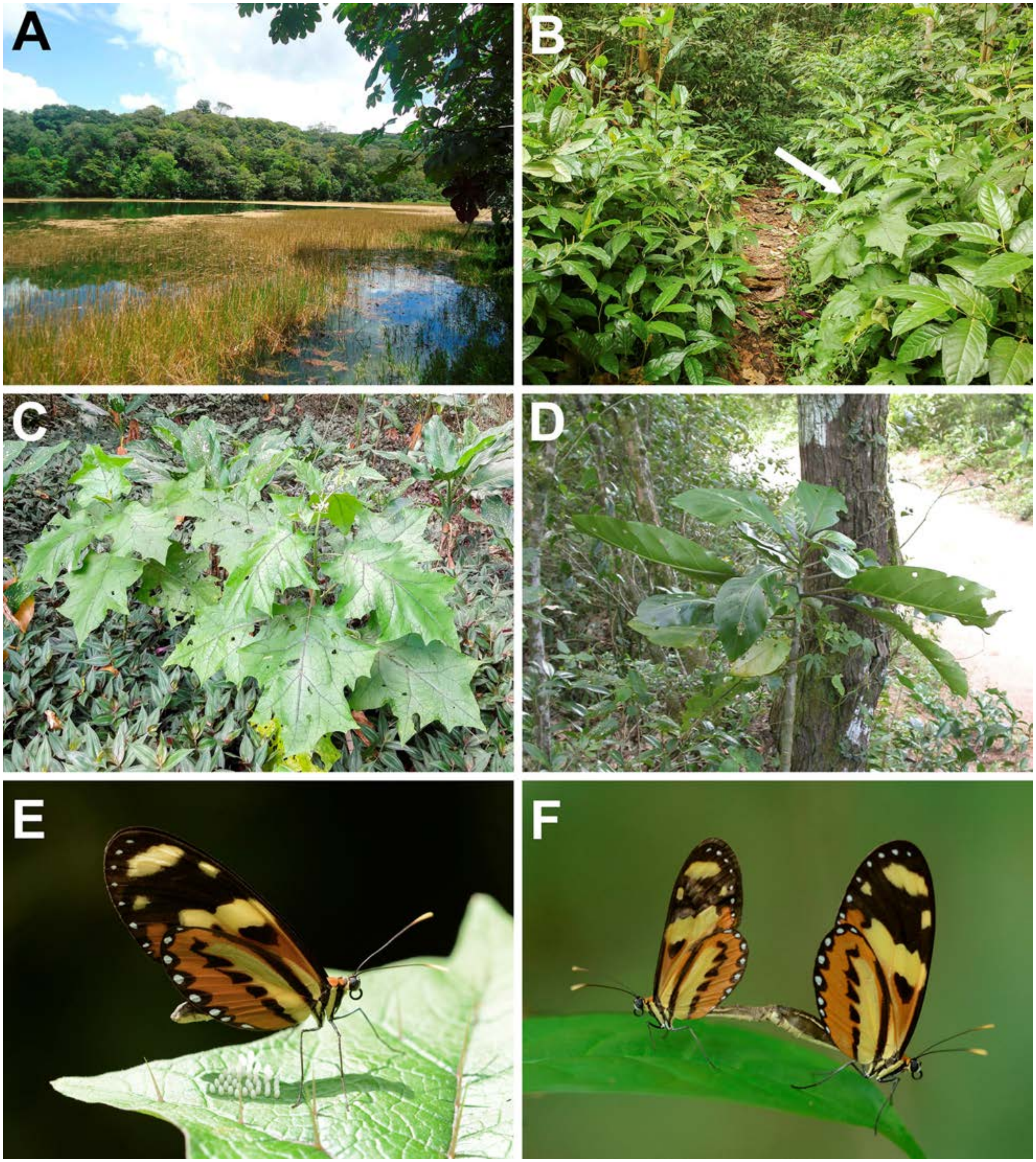

Figure 1. Habitat, host plants and adults of *Mechanitis lysimnia nesaea* in Parque Estadual Dois Irmãos, Recife, Pernambuco, Brazil. **A**. general view of the habitat; **B.** trail inside the area where *Solanum stramoniifolium* (white arrow) occurred; **C.** close view of a shrub of *Solanum stramoniifolium*; **D**. unidentified Solanaceae used as host plant in Bezerros municipality; **E.** Ovipositing female, showing newly laid eggs; **F.** mating pair (male on left).

pale (Fig. 3C, D) or predominantly light cream, with a black patch restricted to the stemmatal region (Fig. 3A, B, E). Just after molt, body similar to previous instar; color then becomes vivid, having a dark bluish green background with three bright yellow stripes, the first dorsal from T2 to A8 and a pair of subdorsal stripes from T2 o A9; prothorax white, with well-developed prothoracic protuberances; lateral abdominal projections very long, yellow, white at distal half and with a conspicuous black circled spiracle near the body insertion. Legs black; prolegs light cream; lateral black plates can be present (Fig. 3B, D) or absent (Fig. 3A, F); anal prolegs lacking lateral dark plates (Fig. 3A, B, D). Larvae change to entire yellow just before pupation (Fig. 3F). Maximum body length 32.0 mm (n = 10). Duration: 3-4 days (n = 90).

Pupa (Fig. 3G-J): Pendant, reflective metallic, general profile elongated. Translucent yellow initially, changing to metallic with dark contours at wing cases and abdomen after 24 h; cremaster black; a slightly protruding dorsal edge on dorsal A3 and short pointed ocular caps. Pupa loses reflections at maturity close to adult emergence revealing the body and wing pattern of adult (Fig. 3J). Maximum length 17.0 mm (n = 10). Duration: 5-7 days (n = 30).

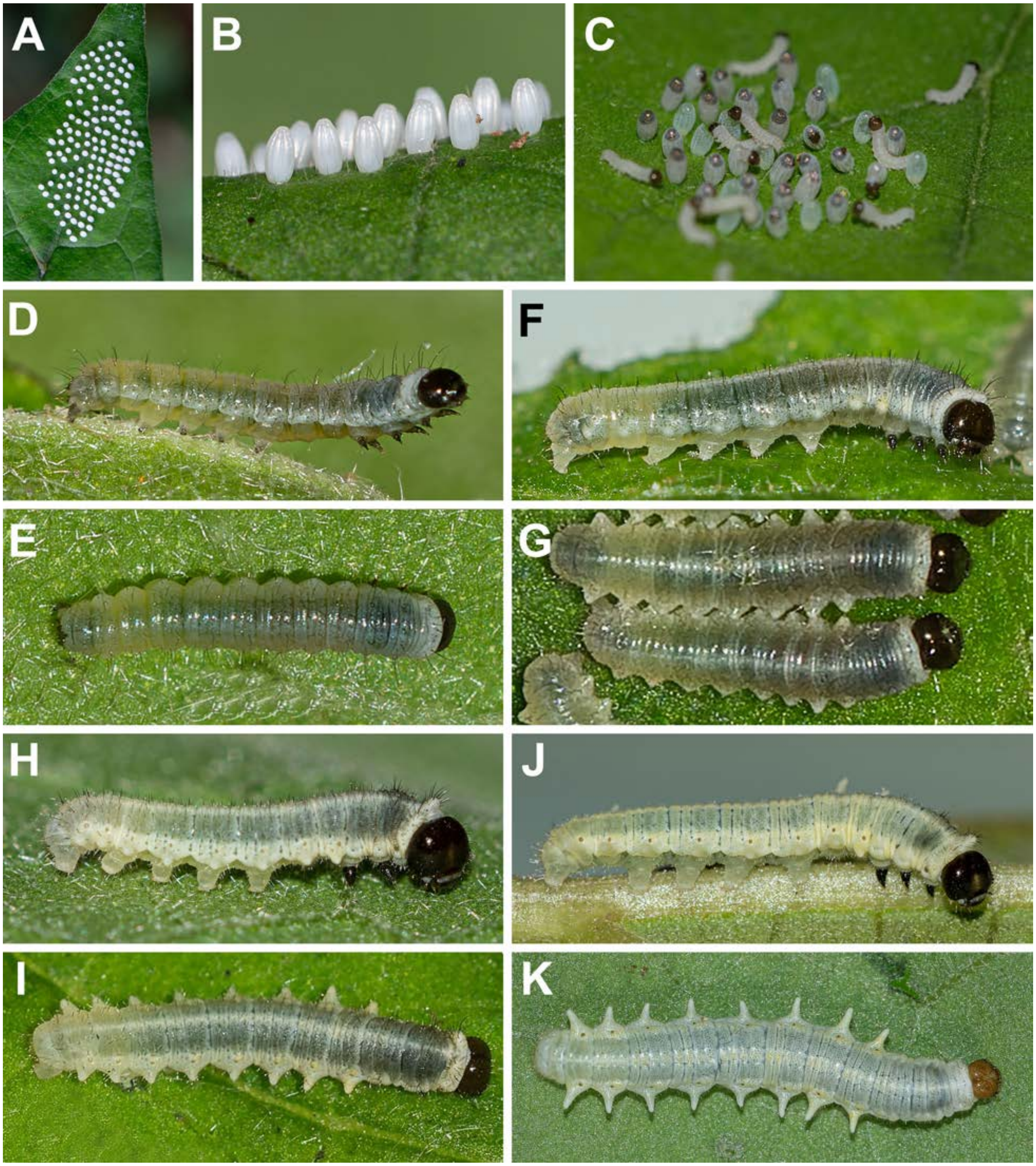

Figure 2. Immature stages of *Mechanitis lysimnia nesaea* from Parque Estadual Dois Irmãos, Recife, Pernambuco, Brazil. **A, B.** oviposition pattern; **C**. a newly hatched clutch of eggs with first instars consuming the eggshells; **D, E.** first instar (lateral, dorsal); **F, G.** second instar (lateral, dorsal); **H, I.** third instar (lateral, dorsal); **J, K.** fourth instar (lateral, dorsal).

Adult behavior

Adults remain in the dark forest interior most of the day. In the early morning, males can be observed flying in sunny clearings and forest edges visiting flowers, and females search these same open habitats in warm hours of the day for host plants (Fig. 1E). Adults fly low, near the ground from 30 cm to 1.5 m. They can be locally abundant, forming large groups in wet areas inside the forest described as "ithomiine pockets", which usually contain other sympatric tiger-patterned ithomiine co-mimics such as *Hypothyris ninonia daetina* (Weymer, 1899) and *Hypothyris euclea laphria* (E. Doubleday, 1847). Larger co-mimics in the pockets include the nymphalids *Lycorea halia discreta* Haensch, 1909 (Danainae, Danaini), *Heliconius ethilla flavomaculatus* Weymer, 1894 and *Eueides isabella dianasa* (Hübner, 1806) (Heliconiinae, Heliconiini). *Mechanitis lysimnia nesaea* is multivoltine, with adults flying all year round. Mating pairs (Fig. 1F) were only observed in the forest interior.

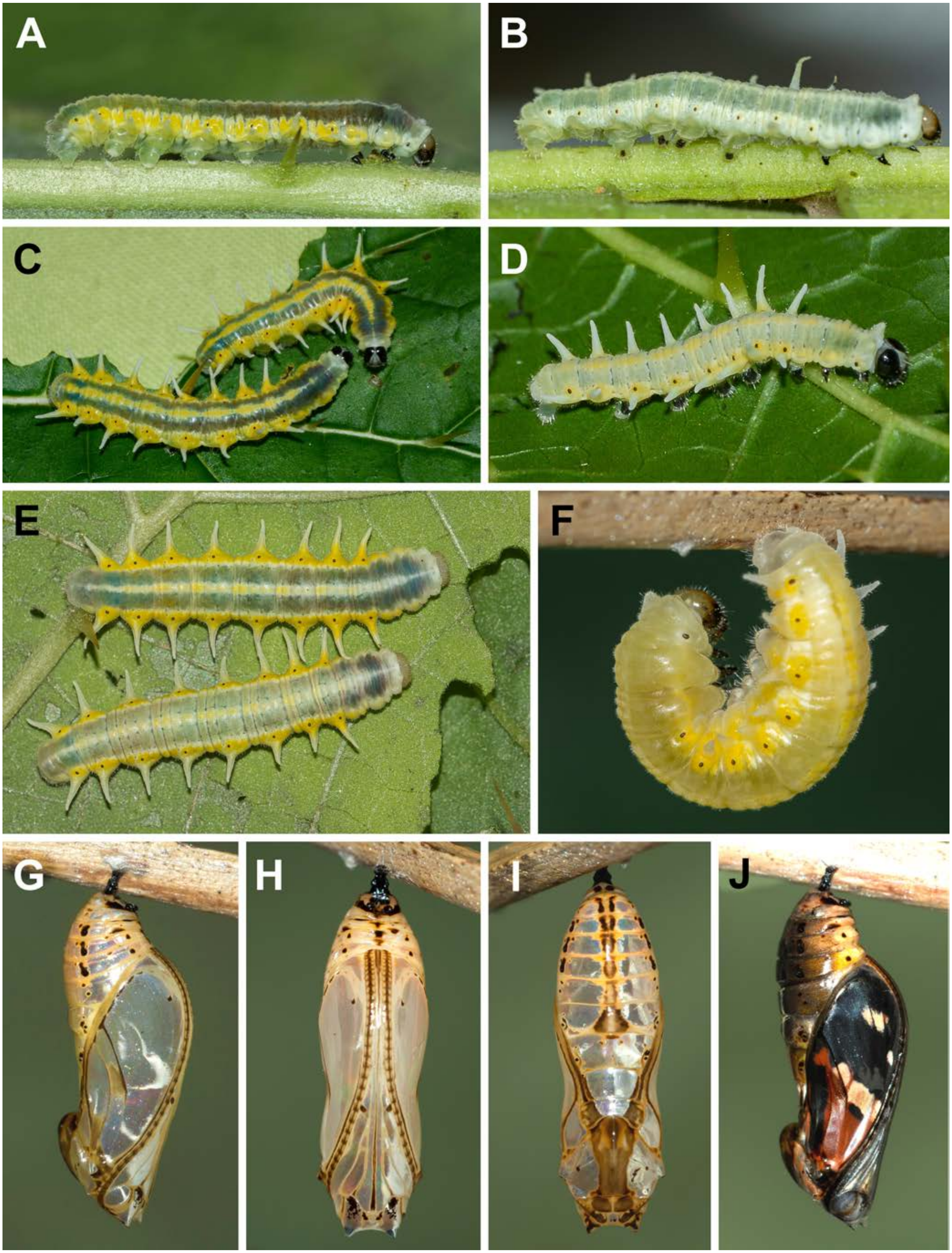

Figure 3. Immature stages of *Mechanitis lysimnia nesaea* from Parque Estadual Dois Irmãos, Recife, Pernambuco, Brazil. **A, B.** fifth (last) instars, lateral view; **C, D, E.** Fifth (last) instars, dorsal view; **F.** pre pupa; **G, H, I.** pupa (lateral, ventral, dorsal, respectively); **J.** pupa near eclosion.

DISCUSSION

All life stages of *M. lysimnia nesaea* are similar to those of other known *Mechanitis*, including the tall, ellipsoid eggs, laid in clusters on the upper leaf surface of the host plants, the larvae being gregarious in all instars, the pair of short dorsolateral protuberances on the prothorax and the presence of long fleshy lateral projections on the abdominal segments (Brown & Freitas, 1994; Motta, 1989; Giraldo & Uribe, 2010a,b, 2012; Anteparra *et al*., 2011; Hill *et al*., 2012; Giraldo *et al*., 2014; Ruiz *et al*., 2015; Carvalho *et al*., 2019; Santacruz *et al.*, 2019). The lateral abdominal projections are one of the clear synapomorphies of the subtribe Mechanitina (Willmott & Freitas, 2006), but only in *Mechanitis* are they long and pointed in the last instar; they are short in *Thyridia* Hübner, 1816, *Sais* Hübner, 1816, and *Scada* Kirby, 1871, and can be short or absent in *Forbestra* R. Fox, 1967 (Brown & Freitas, 1994; Freitas & Brown, 2002; Brévignon, 2003; Hill, 2006; Carvalho *et al*. 2019; Freitas *et al*., 2020). The paired, short, dorsolateral protuberances on the prothorax are present in all known *Mechanitis* and in *Forbestra olivencia* and can be considered a synapomorphy of the clade *Forbestra* + *Mechanitis*, with a possible loss in *F. equicola* (Brévignon, 2003). However, these structures are relatively shorter in *M. lysimnia nesaea* and *M. lysimnia lysimnia* compared to other species of *Mechanitis* (see references above).

The eggs of *M. lysimnia nesaea* have 10-11 horizontal ridges, fewer than *M. polymnia casabranca* (13-16 ridges, Carvalho *et al*., 2019; Motta, 1989), *M. polymnia caucaensis* (about 14 ridges, Giraldo & Uribe, 2010a), and *M. polymnia* ca. *dorissides* (11-13 ridges in a population from Acre, north Brazil, AVLF pers. obs.). The number is lower than that observed in *M. lysimnia lysimnia* in southeastern Brazil (15-16 ridges, Motta, 1989; AVLF pers. obs.) and *Mechanitis lysimnia elisa* (18 ridges, Ajmat-de Toledo & Terán, 1970). Concerning the vertical ridges, the pattern is not the same: these comprise 12-14 in *M. lysimnia nesaea* (present study), fewer than *M. lysimnia elisa* (15 ridges, Ajmat-de Toledo & Terán, 1970) and *M. menapis* (13-15 ridges, Giraldo & Uribe, 2010b; Santacruz *et al.* 2019). However, in this case the number of vertical ridges is similar to that reported for *M. lysimnia lysimnia* (13-14 ridges; AVLF pers. obs.), *M. polymnia casabranca* (13-14 ridges, Carvalho *et al*., 2019; Motta, 1989), and higher than reported for *M. polymnia* ca. *dorissides* (11-13 ridges, AVLF pers. obs.), *M. mazaeus* and *M. messenoides* (10-14 ridges for both species, with averages of 11.7 and 12.0 ridges, respectively; R. Hill, pers. comm.).

Comparison of known *Mechanitis* immature stages clarifies similarities and highlights differences. Compared to other species and populations of *Mechanitis*, fifth instars of *M. lysimnia nesaea* are very distinct from those of *Mechanitis polymnia* (Linnaeus, 1758), whose larvae present a light gray body and brown head capsule (Giraldo & Uribe, 2010a; Anteparra *et al*., 2011; Ruiz *et al*., 2015; Carvalho *et al*., 2019). Conversely, in these two respects, fifth instars of *M. lysimnia nesaea* are more similar to those of *Mechanitis menapis* Hewitson, [1856], *Mechanitis mazaeus* Hewitson, 1860, *Mechanitis messenoides* C. Felder & R. Felder, 1865, and the nominal subspecies, *M. lysimnia lysimnia* (Fabricius, 1793), with the body more richly colored and head capsules predominantly black or cream with black areas (D'Almeida, 1922; Giraldo & Uribe, 2010b, 2012; Giraldo *et al*., 2014; Hill *et al*., 2012; Santacruz *et al*., 2019; AVLF unpublished). However, the variation in the color of the head capsule in the fourth and fifth instar of *M. lysimnia nesaea* is interesting and distinguishes it from the nominal subspecies, where such variation has never been reported (D'Almeida, 1922; AVLF, unpublished). These differences are equivalent to the species-level differences reported among other species of *Mechanitis*, and this is an interesting topic for future research. The lateral abdominal projections also vary in relative size in the different species of *Mechanitis*, being longer in *M. menapis* (Giraldo *et al*., 2010b; Santacruz *et al.*, 2019) and comparatively short in *M. mazaeus* (Hill *et al*., 2012), whereas in *M. polymnia*, and *M. messenoides*, *M. lysimnia lysimnia* and *M. lysimnia nesaea* they are more intermediate in length (Giraldo & Uribe, 2010a; Hill *et al*., 2012; Carvalho *et al*., 2019, present study and AVLF unpublished). The paired short dorsolateral protuberances on the prothorax are relatively shorter in *M. lysimnia nesaea* and *M. lysimnia lysimnia* compared to other species of *Mechanitis* (see references above).

As indicated with the addition of *M. lysimnia nesaea* here and as previously mentioned by Carvalho *et al*. (2019), comparative studies of immature stages could gain in robustness as more species, subspecies, and populations are described in detail. Previous studies have shown that the ultrastructure of eggs and first instar larvae, for example, are broadly informative at the species level in Danainae (Motta, 1989, 2003; Kitching, 1984, 1985), and other aspects of immature stages have been informative for clarifying species differences (Hill *et al.*, 2012). For example, study of the immature stages of sympatric *Mechanitis* revealed differences in clutch size, larval host plant use, and fifth instar morphology in *M. messenoides* and *M. mazaeus*, clarifying their species status. Thus, knowledge about additional subspecies and populations could help to understand the species limits within the several non-monophyletic species of *Mechanitis* revealed by molecular evidence, as shown by Hill *et al*. (2012) and Giraldo & Uribe (2012). Accordingly, the detailed description of life stages of more subspecies and populations of *Mechanitis*, especially the nominal subspecies, could provide valuable data to better understand the taxonomy and species limits of this iconic ithomiine genus.

ACKNOWLEDGMENTS

We are indebted to all the staff of the "Parque Estadual Dois Irmãos" for allowing research in the area. AVLF thanks the CNPq (process304291/2020-0) and the National Science Foundation (DEB-1256742). Carlos E. Giraldo and Ryan Hill read the manuscript and made valuable suggestions that improved the final text. This publication is part of the RedeLep 'Rede Nacional de Pesquisa e Conservação de Lepidópteros' SISBIOTABrasil/ CNPq (563332/2010-7), and of the BIOTA-FAPESP Program (grants 2011/50225-3 and 2021/03868-8). The present study is registered at the SISGEN (A59BC58).

LITERATURE CITED

Ajmat de Toledo, Z. D., Terán, H. 1970. Fauna del noroeste argentino - contribución al conocimiento de los lepidópteros Argentinos I. *Mechanitis lysimnia elisa* (Guérin-Ménéville) (Rhopalocera - Ithomiidae). *Acta Zoologica Lilloana* 26: 33-48.

Anteparra, M. E., Ayvar, J. C., Granados, L. B. 2011. Algunos aspectos sobre la biología de *Mechanitis polymnia proceriformis* Bryk, 1953 (Lepidoptera: Nymphalidae) asociado con la cocona (Solanum sessiliflorum Dunal) en Tingo María. *Aporte Santiaguino* 4(1): 64-74.

Beccaloni, G. W. 1997. Ecology, natural history and behaviour of ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). *Tropical Lepidoptera* 8: 103-124.

Beccaloni, G. W., Viloria, Á. L., Hall, S. K, Robinson, G. S. 2008. *Catalogue of the host plants of the Neotropical butterflies. Catálogo de las plantas huésped de las mariposas neotropicales.* Zaragoza, Sociedad Entomológica Aragonesa. (Monografías del Tercer Milenio, Vol. 8). 536 pp.

Brévignon, C. 2003. Inventaire des Ithomiinae de Guyane Française (Lepidoptera, Nymphalidae). *Lambillionea* 103: 41-58.

Brower, A. V. Z., Freitas, A. V. L., Lee, M. -M., Silva-Brandão, K. L., Whinnett, A., Willmott, K. R. 2006. Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. *Systematic Entomology* 31: 288-301.

Brower, A. V. Z., Willmott, K. R., Silva-Brandão, K. L., Garzón-Orduña, I. J., Freitas, A. V. L. 2014. Phylogenetic relationships of ithomiine butterflies (Lepidoptera: Nymphalidae: Danainae) as implied by combined morphological and molecular data. *Systematics and Biodiversity* 12(2): 133-147.

Brown Jr., K. S. 1977. Geographical patterns of evolution in Neotropical Lepidoptera: differentiation of the species of *Melinaea* and *Mechanitis* (Nymphalidae: Ithomiinae). *Systematic Entomology* 2: 161-197.

Brown Jr., K. S. 1979. *Ecologia Geográfica e Evolução nas Florestas Neotropicais.* PhD Thesis. Campinas, Universidade Estadual de Campinas. xxxi + 385 p.

Brown Jr., K. S., Benson, W. W. 1974. Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata* (Lepid. Nymph.). *Biotropica* 6: 205-228.

Brown Jr., K. S., Freitas, A. V. L. 1994. Juvenile stages of Ithomiinae: overview and systematics. *Tropical Lepidoptera* 5(1): 9-20.

Carvalho, M. R. M., Barbosa, E. P., Freitas, A. V. L. 2019. Immature stages of *Mechanitis polymnia casabranca* (Nymphalidae, Danainae). *Tropical Lepidoptera Research* 19(1): 12-18.

D'Almeida, R. F. 1922. *Mélanges lépidoptérologiques. Études sur les lépidoptères du Brésil*. Berlin, R. Friedländer & Sohn. viii + 226 pp.

Dasmahapatra, K. K., Elias, M. E., Hill, R., Hoffman, J. I., Mallet, J. 2010. Mitochondrial DNA barcoding detects some species that are real, and some that are not. *Molecular Ecology Resources* 10: 264-273.

Drummond, B. A., Brown Jr., K. S. 1987. Ithomiinae (Lepidoptera: Nymphalidae): summary of known larval food plants. *Annals of the Missouri Botanical Garden* 74: 341-358.

Fox, R. M. 1967. A monograph of the Ithomiidae (Lepidoptera) Part III The tribe Mechanitini Fox. *Memoirs of the American Entomological Society* 22: 1-190.

Freitas, A. V. L., Brown Jr., K. S. 2002. Immature stages of *Sais rosalia* (Nymphalidae, Ithomiinae). *Journal of the Lepidopterists' Society* 56: 104-106.

Freitas, A. V. L., Rosa, A. H. B., Nobre, C. E. B., Melo, D. H. A., Mota, L. L., Silva-Brandão, K. L., Machado, P. A., Carreira, J. Y. O. 2020. Immature stages, natural history, systematics and conservation of an endangered Neotropical butterfly: the case of *Scada karschina delicata* (Nymphalidae: Ithomiini). *Neotropical Entomology* 49: 685-695.

Garzón-Orduña, I. J., Silva-Brandão, K. L., Willmott, K. R., Freitas, A. V. L., Brower, A. V. Z. 2015. Incompatible ages for clearwing butterflies based on alternative secondary calibrations. *Systematic Biology* 64(5): 752-767

Giraldo, C. E., Uribe, S. I. 2010a. Registro de *Mechanitis polymnia* (Lepidoptera: Ithomiinae) en *Solanum jamaicense* y ciclo de vida en laboratorio. *Revista Colombiana de Entomología* 36(1): 165-168.

Giraldo, C. E., Uribe, S. I. 2010b. *Solanum hirtum* as a host plant for *Mechanitis menapis menapis* (Lepidoptera: Ithomiinae) in Colombia. *Revista Colombiana de Entomología* 36(1): 169-171.

Giraldo, C. E., Uribe, S. I. 2012. Taxonomy of *Mechanitis* (F.) (Lepidoptera, Nymphalidae) from the west Colombian Andes: an integrative approach. *Neotropical Entomology* 41: 472-484.

Giraldo, C. E., Ramírez-Restrepo, L., Escobar, F., Uribe, S. I. 2014. Life cycle and host plants of *Mechanitis menapis doryssus* (Danainae: Ithomiini) in Mexico. *Southwestern Entomologist* 39(4): 761-771.

Hill, R. I. 2006. Life history and biology of *Forbestra olivencia* (Bates, 1862) (Nymphalidae, Ithomiinae). *Journal of the Lepidopterists' Society* 60: 203-210.

Hill, R. I., Elias, M., Dasmahapatra, K. K., Jiggins, C. D., Koong, V., Willmott, K. R., Mallet, J. 2012. Ecologically relevant cryptic species in the highly polymorphic Amazonian butterfly *Mechanitis mazaeus s.l.* (Lepidoptera: Nymphalidae; Ithomiini). *Biological Journal of the Linnean Society* 106: 540-560.

Kerpel, S., Zacca, T., Nobre, C. E. B., Júnior, A. F., Xavier, M., Fonseca, A. 2014. *Borboletas do Semiárido, conhecimento atual e contribuições do PPBio*, pp. 245-275. *In*: Bravo, F., Calor, A. (Eds.), *Artrópodes do Semi-Árido: biodiversidade e conservação*. Feira de Santana, Printmídia.

Kesselring, J., Ebert, H. 1979. Relação das borboletas encontradas na "Mata do Buraquinho", João Pessoa, Estado da Paraíba, Brasil. *Revista Nordestina de Biologia* 2: 105-118.

Kitching, I. J. 1984. The use of larval chaetotaxy in butterfly systematics, with special reference to the Danaini (Lepidoptera: Nymphalidae). *Systematic Entomology* 9: 49-61.

Kitching, I. J. 1985. Early stages and classification of the milkweed butterflies (Lepidoptera: Danainae). *Zoological Journal of the Linnean Society* 85: 1-97.

Lamas, G. 1987. The identity of *Mechanitis doryssus* Bates and *M. saturata* Godman (Lepidoptera: Nymphalidae; Ithomiinae). *Revista Peruana de Entomología* 30: 48-50.

Lamas, G. (Ed.) 2004. *Ithomiinae*, pp. 172-191. *In*: Lamas, G. (Ed.), *Checklist: Part 4A. Hesperioidea - Papilionoidea*. *In*: Heppner, J. B. (Ed.), *Atlas of Neotropical Lepidoptera. Volume 5A*. Gainesville, Association for Tropical Lepidoptera/Scientific Publishers.

Melo, D. H. A., M. Duarte, O. H. H. Mielke, R. K. Robbins, Freitas, A. V. L. 2019. Butterflies (Lepidoptera: Papilionoidea) of an urban park in northeastern Brazil. *Biota Neotropica* 19(1): e20180614.

Mota, L. L., Vasconcellos, L. R., Willmott, K. R., Freitas, A. V. L. 2022. A new subspecies of *Mechanitis lysimnia* from southern Amazonia (Nymphalidae: Danainae: Ithomiini). *Tropical Lepidoptera Research* 32(1): 47-51.

Motta, P. C. 1989. *Análise filogenética de Ithomiinae (Lep.: Nymphalidae) com base nos ovos: Relação com plantas hospedeiras*. MSc Dissertation. Campinas, Universidade Estadual de Campinas.

Motta, P. C. 2003. *Phylogenetic relationships of Ithomiinae based on first-instar larvae*, pp. 409-429. *In*: Boggs, C., Ehrlich, P., Watt, W.B. (Eds.), *Butterflies: Ecology and Evolution Taking Flight.* Chicago, Chicago University Press.

Nobre, C. E. B., Schlindwein, C., Mielke, O. H. H. 2008. The butterflies (Lepidoptera: Papilionoidea and Hesperioidea) of the Catimbau National Park, Pernambuco, Brazil. *Zootaxa* 1751: 35-45.

Paluch, M., Mielke, O. H. H., Nobre, C. E. B., Casagrande, M. M., Melo, D. H. A., Freitas, A. V. L. 2011. Butterflies (Lepidoptera: Papilionoidea and Hesperioidea) of the Parque Ecológico João Vasconcelos Sobrinho, Caruaru, Pernambuco, Brazil. *Biota Neotropica* 11(4): 229-238.

Ruiz, E., Vásquez, J., Zárate, R., Pinedo, J. 2015. Aspectos biológicos de *Morpho helenor theodorus* (Fruhstorfer) (Lepidoptera: Nymphalidae; Morphinae) y *Mechanitis polymnia* (Linnaeus), (Lepidoptera: Nymphalidae; Ithomiinae), en la Amazonía baja del Perú. *Folia Amazónica* 24(1): 45-54.

Santacruz, P. G., Despand, E., Giraldo, C. E. 2019. Ciclo de vida y enemigos naturales de *Mechanitis menapis* (Lepidoptera: Ithomiini)**.** *Revista de Biología Tropical* 67(6): 1488-1504.

Warren, A. D., Davis, K. J., Stangeland, E. M., Pelham, J. P., Grishin, N. V. 2019. *Illustrated Lists of American Butterflies.* http://www.butterfliesofamerica.com. Accessed November 2022.

Willmott, K. R., Freitas, A. V. L. 2006. Higher-level phylogeny of the Ithomiinae (Lepidoptera: Nymphalidae): classification, patterns of larval hostplant colonization and diversification. *Cladistics* 22(4): 297-368.

Zacca, T., Bravo, F. 2012. Borboletas (Lepidoptera: Papilionoidea e Hesperioidea) da porção norte da Chapada Diamantina, Bahia, Brasil. *Biota Neotropica* 12(2). https://doi.org/10.1590/S1676-06032012000200012.

A new *Prosotas* (Lepidoptera, Lycaenidae) from the islands of Milne Bay Province, Papua New Guinea

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Date of issue online: 16 August 2023
Zoobank Registered: urn:lsid:zoobank.org:pub:E753AF9D-C607-4128-BE33-046B33AF71E7
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8140734

Abstract: A new polyommatine lycaenid butterfly, *Prosotas cloe* **sp. n.**, is described from the Trobriand Islands and eastern Louisiades, Papua New Guinea. The new species bears some superficial similarity to congeners *P. dubiosa* Semper, 1879, *P. nora* Felder, 1860, and *P. patricae* Tennent, 2003, with which it is compared.

Keywords: Lepidoptera; Lycaenidae; Milne Bay Province islands; new taxon; Papua New Guinea; Polyommatini; *Prosotas.*

INTRODUCTION

The polyommatine lycaenid genus *Prosotas* Druce, 1891 comprises *ca.* 20 small or very small species occurring from India and Sri Lanka to Taiwan and eastwards through the southeast Asian islands to New Guinea, Australia, the Solomon Islands, Vanuatu and Fiji. Species may be tailed or tailless. Six *Prosotas* species were reported from Papua New Guinea by Parsons (1998); of these, *P. atra* Tite, 1963, *P. talasea* Tite, 1963, and *P. papuana* Tite, 1963, all have slender hindwing tails. *Prosotas nora* Felder, 1860 is unusual, in that nominotypical *nora* is tailless, but *P. n. caliginosa* Druce, 1891, once considered a distinct species but treated as a subspecies of *P. nora* by Hirowatari (1992: 30), has a filamentous tail. The two remaining Papua New Guinea species, *P. gracilis* Röber, 1886 and *P. dubiosa* Semper, 1879, are tailless.

Two additional tailless species were described by the author (Tennent, 2003) from the New Hebrides Archipelago: *P. russelli*, from Vanuatu (TL: Maewo) and islands of the Santa Cruz group (Solomon Islands), and *P. patricae*, from the island of Futuna, one of the most southerly islands of Vanuatu. During fieldwork on the islands of Milne Bay Province between 2010 and 2016, the author collected 1♂ and 2♀♀ specimens of an undescribed *Prosotas* species at low elevation on the Trobriands and the Calvados chain in the eastern Louisiades, a distribution that suggests it is likely to be widespread. The species bears some superficial similarity to *P. dubiosa*, with which it is compared; all comparisons with other *Prosotas* species are with specimens from the Milne Bay islands.

It is noted that no other *Prosotas* species is recorded from either of the two islands where *P. cloe* is known to occur, but these islands are small and under-recorded and it is quite possible that sympatry with other *Prosotas* species occurs. The new species has been compared with many hundreds of *Prosotas* specimens collected by the author on the islands of Milne Bay, Vanuatu and the Solomon Islands over almost three decades, as well as with the extensive collections of the Natural History Museum, London, United Kingdom.

Prosotas cloe **sp. n.**
(Figs. 1a-d, 2a-c, 8a, 9a)

Diagnosis: Some groups of Pacific polyommatine lycaenids present difficulties in identification (e.g. *Jamides* Hübner, [1819]; *Nacaduba* Moore, 1881). Identification of many, including *Prosotas*, is based on often subtle differences in the arrangement of transverse lines on the under surface. *Prosotas* species are small or very small in overall size, with angular forewings (in comparison to *Jamides* and *Nacaduba*) and prominent marginal markings that are generally diagnostic. This new species is similar to other *Prosotas* species, except as follows. *Prosotas cloe* **sp. n.** is tailless (compared with *P. atra*, *P. talasea*, *P. papuana* and *P. nora caliginosa*); male upperside appears plain dark brown, but close examination under oblique lighting reveals that both fore- and hindwings have a loose scattering of pale blue and pinkish-violet scales, broken by veins (Fig. 1c; unknown in any other *Prosotas* species); broad dark border; underside markings distinctive; hindwing submarginal markings rounded, almost linear (saggitate in associated *Prosotas* species); tornal black spot with associated iridescent scales vestigial or absent (iridescent scales generally more prominent in other *Prosotas* species).

Description: small, in size comparable to *P. patricae*; forewing length 8 mm; antennae with prominent white bands; clubs with area of distinct creamy-white hairs; eyes bordered white; body gray-white dorsally; legs mottled brown/white.

Male upperside appears uniformly dark brown, but viewing obliquely or under a microscope reveals a broad dark border and a loose scattering of pale blue scales, pinkish violet on

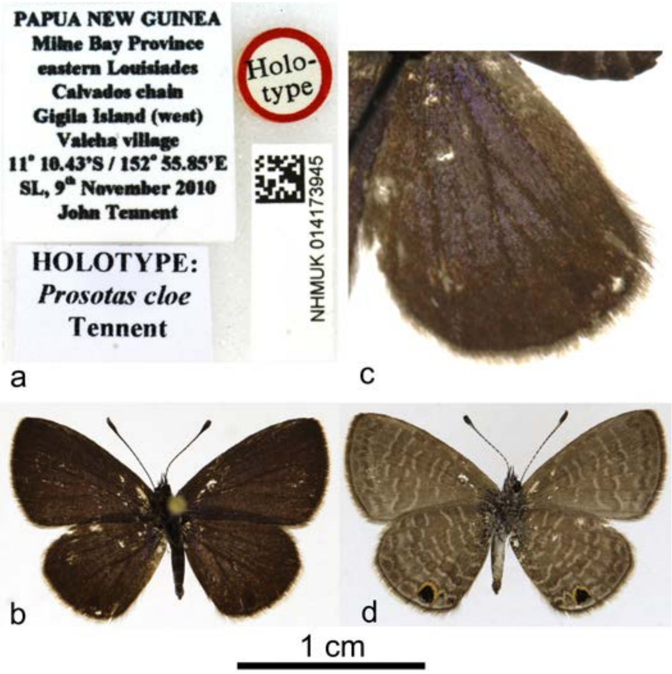

Figure 1. *Prosotas cloe* **sp. n**. ♂ holotype. **a,** labels; **b,** upper surface; **c,** section of hindwing showing blue/violet scales; **d,** under surface.

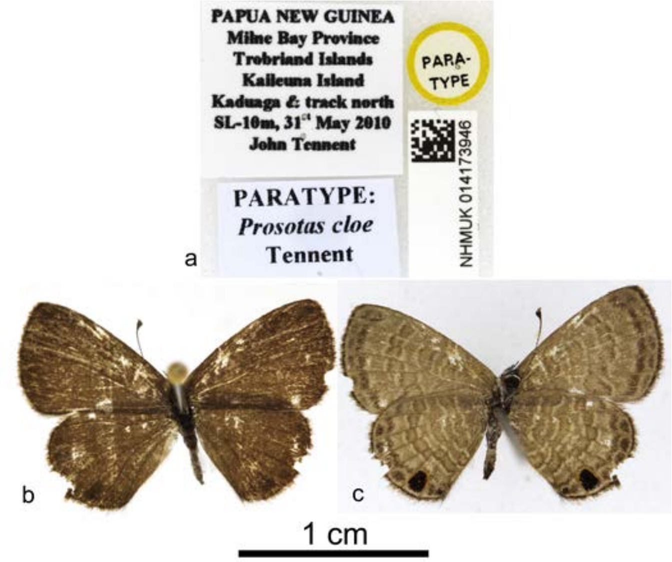

Figure 2. *Prosotas cloe* **sp. n.** ♀ paratype. **a**, labels; **b,** upper surface; **c,** under surface.

hindwing near inner margin (upperside blue in *dubiosa*; dark purple-blue, appearing almost black, with dark border, in *patricae*); underside like *dubiosa*; ground color dark brown; forewing with usual *Prosotas* median and postbasal paired markings, irregular, broad, dark brown, outwardly bordered pale brown (narrower, more regular in *dubiosa*; markings sparse, completely absent from median area above inner margin in *patricae*); submarginal band narrow, indistinct (larger, more prominent in *dubiosa* and *nora*; more spaced, absent towards tornus in *patricae*); hindwing with usual *Prosotas* median and basal markings with irregular but continuous areas of ground color between bands (ground color largely overwhelmed by bands in *dubiosa*; paler, more contrasting in *nora*; bands

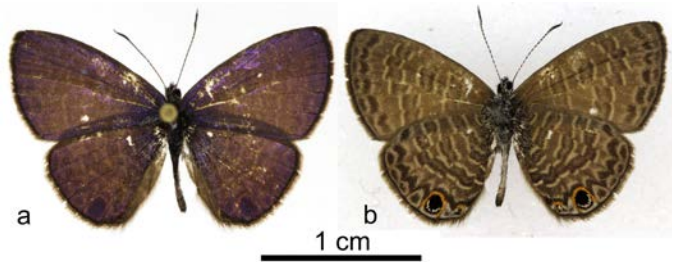

Figure 3. *Prosotas dubiosa* ♂. **a,** upper surface; **b,** under surface.

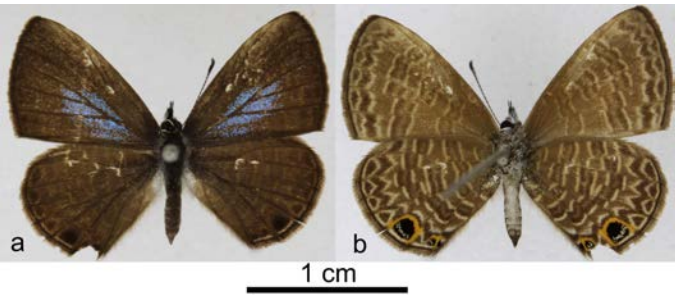

Figure 4. *Prosotas dubiosa* ♀. **a,** upper surface; **b,** under surface.

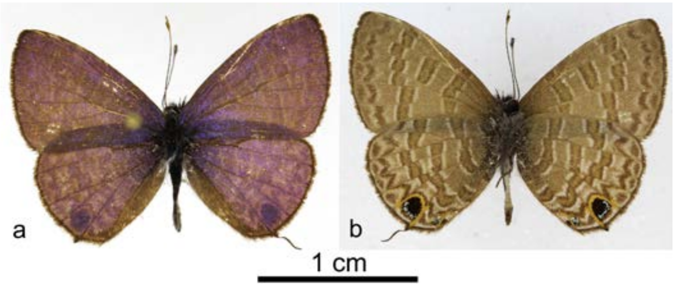

Figure 5. *Prosotas nora* ♂. **a,** upper surface; **b,** under surface.

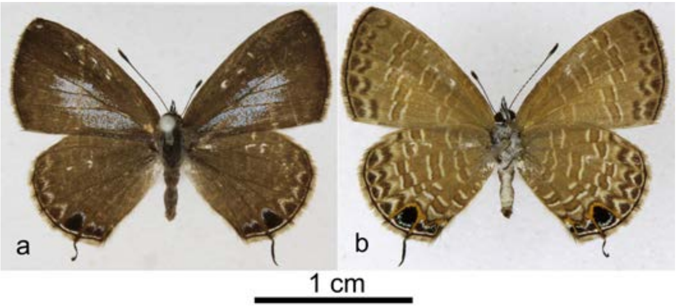

Figure 6. *Prosotas nora* ♀. **a,** upper surface; **b,** under surface.

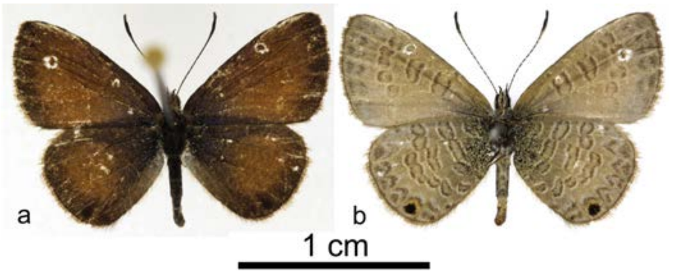

Figure 7. *Prosotas patricae* ♂ paratype. a, upper surface; b, under surface.

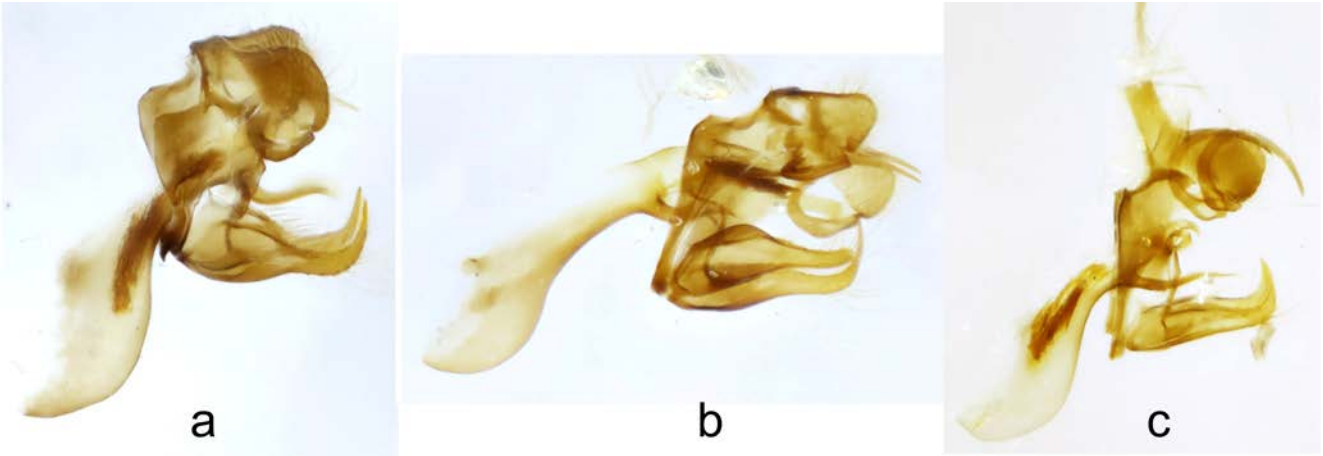

Figure 8. *Prosotas* ♂ genitalia, lateral view. **a,** *Prosotas cloe* **sp. n.**; **b,** *Prosotas dubiosa* (tip of one valve missing); **c,** *Prosotas nora* (vinculum broken).

Figure 9. *Prosotas* ♂ genitalia, frontal view. **a,** *Prosotas cloe* **sp. n.**; **b,** *Prosotas dubiosa*; **c,** *Prosotas nora*.

significantly reduced in *patricae*); subtornal black spot large, weakly edged orange basad, with iridescent green scales distad vestigial or absent (spot larger, orange border and iridescent scales more extensive; often with smaller, secondary tornal spot in *dubiosa* and *nora*; tornal spot small, orange border indistinct, iridescent scales absent in *patricae*); submarginal series of dark brown, slightly oval spots, bordered pale brown, with irregular dark brown slightly curved line edged paler distad (spots pyramidal, dark brown line deeply saggitate in *dubiosa* and *nora*; pyramidal, saggitate, often weakly so in *patricae*).

Female similar to male; upperside plain dark brown; underside gray-brown, markings less cluttered than other *Prosotas* species, leaving larger areas of ground color; hindwing underside marginal markings rounded, submarginal line almost linear, segments weakly rounded (deeply sagittate in other *Prosotas* species).

Male genitalia similar to *P. dubiosa* and *P. nora*; valva broad, terminating in slender, but relatively short, inwardly curving blunt point (valva less broad, terminus longer, more slender in *dubiosa*); seen from lateral view, valva slender, terminus short, blunt in *nora*); labides (uncus) sharply stepped (less so in *dubiosa* and *nora*); from frontal view, valvae separated to approximately half length (deeply separated in *dubiosa*; slightly less so in *nora*). *Prosotas cloe* aedeagus with well-defined, prominent area of cornuti along ventral margin (cornuti elongated, at apex of aedeagus in *P. dubiosa*; cornuti less extensive, situated on dorsal margin in *P. nora*). Male genitalia of *P. patricae* dissimilar (see Tennent, 2003: 42).

Type material (1♂, 2♀♀): HOLOTYPE ♂, (1) typed “Papua New Guinea, Milne Bay Province, eastern Louisiades, Calvados chain, Gigila Island (west), Valeha village, 11° 10.43' S / 152° 55.85' E, SL, 9th November 2010, John Tennent”; (2) typed “Holotype: *Prosotas cloe* Tennent”; (3) circular, red-bordered, typed “Holotype”;

PARATYPES: 1♀ labelled (1) typed “Papua New Guinea, Milne Bay Province, Trobriand Islands, Kulawa Island, SL, 29th May 2010, John Tennent; (2) typed “Paratype: *Prosotas cloe* Tennent”; (3) circular, yellow-bordered, typed “paratype”; 1♀ labelled (1) typed “Papua New Guinea, Milne Bay Province, Trobriand Islands, Kaileuna Island, Kaduaga & track north, SL-10m, 30th May 2010, John Tennent; (2) typed “Paratype: *Prosotas cloe* Tennent”; (3) circular, yellow-bordered, typed “Paratype”.

Holotype and paratypes all in the Natural History Museum (NHM), London, United Kingdom.

Distribution: Papua New Guinea (Milne Bay Province): islands of The Trobriands and Louisiades.

Etymology: *Prosotas cloe* is named for Mlle Claudine Lozach, who very kindly made photographic records of butterflies on several remote Pacific islands in pursuance of the authors planned update of a Pacific butterfly checklist (Tennent, 2006).

DISCUSSION

Parsons (1998: 435-437) recognized the occurrence of six *Prosotas* species in Papua New Guinea and believed all but one of them were generally rare (*P. papuana* was said to congregate in thousands under favorable conditions). The concept of rarity is of limited value in a tropical environment, particularly when related to small, inconspicuous butterflies that fly extremely swiftly and spend significant time in the canopy. It is noted that *P. patricae*, first discovered on the Vanuatu island of Futuna, was extremely fast-flying and only collected eventually at one flowering shrub which individual butterflies visited regularly but very briefly from adjacent high trees (Tennent, 2003). The species was subsequently discovered to be present on New Caledonia (Tennent, 2006) and it is likely that the butterfly is more widespread. The same applies to *P. cloe*, which is highly unlikely to be restricted in distribution to the Trobriands and the eastern Louisiades, on islands several hundred kilometres apart. It is probable that the species may occur on the islands of the D'Entrecasteaux, the western Louisiades and possibly the eastern New Guinea mainland. It is also probable that further species of this genus of small and fast-flying butterflies await discovery.

There is significant diversity, in particular among the Lycaenidae, to be found among the smaller butterfly species in tropical southeast Asia and it is clear from the number of new lycaenid taxa described from New Guinea and associated islands in recent years that the process of discovery, identification and description of new taxa is set to continue for some time to come. In these days of habitat destruction at an alarming rate, all faunae are desperately in need of taxonomic recognition and recording.

ACKNOWLEDGMENTS

The author is grateful to the National Research Institute, the Department of Environment and Conservation, Papua New Guinea Government, and the Provincial Research Committee, Milne Bay Provincial Government, for supporting the author's butterfly research in Milne Bay Province.

The length of time spent in Milne Bay and the large number of islands visited was enabled through the collective generosity of organisations and individuals who provided funding: National Geographic, Washington D.C. (CRE Grant # 8319-07 and GEFNE Grant # 6-11); The Linnean Society, London (Appleyard Fund); The Percy Sladen Exploration Fund, London (Linnean Society); the Natural History Museum, London (Special Funds); the Royal Entomological Society, St Albans (formerly London), the British Ecological Society, London (SEPG No 3651/4516) and, in particular, Martin Jacoby, Somerset, UK.

Alessandro Giusti, NHM London, kindly prepared and photographed genitalia of *P. dubiosa* and *P. nora* (Figs. 8b,c, 9b, c), and two reviewers provided comments on the manuscript.

LITERATURE CITED

Hirowatari, T. 1992. A generic classification of the tribe Polyommatini of the Oriental and Australian Regions (Lepidoptera, Lycaenidae, Polyommatinae). *Bulletin of the University of Osaka Prefecture* (B) 44 (Supplement): 1-102.

Parsons, M. J. 1998. *The Butterflies of Papua New Guinea: their Systematics and Biology*. London, Academic Press. 736 pp., xxvi + 104 pls.

Tennent, W. J. 2003. Two elusive new *Prosotas* Druce, 1891 (Lepidoptera, Lycaenidae) from the New Hebrides archipelago (Solomon Islands and Vanuatu). *Butterflies* 36: 39-44.

Tennent, W. J. 2006. A checklist of the butterflies of Melanesia, Micronesia, Polynesia and some adjacent areas. *Zootaxa* 1178: 1-209.

Ecological and biological aspects of a leaf miner on *Erythroxylum tortuosum* (Mart.) (Erythroxylaceae) in a Cerrado fragment in Southeastern Brazil

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Date of issue online: 16 August 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8140746

Abstract: Ecological and biological features of a lepidopterous leaf miner and its host plant, *Erythroxylum tortuosum* (Erythroxylaceae), were studied in a Brazilian Cerrado fragment. Foraging traits, the temporal emergence pattern of the leaf miners and the morphology and anatomy of the mines were described. The leaf miner was identified as an *Agnippe* (Chambers) (Gelechiidae) species; the mines were characterized as blotch mines because the larva moved forward and backward in all directions within the mines while feeding. The pupal stage remained in a darkened region of the leaf, under which a pair of semicircular openings were observed on the abaxial external leaf surface. The openings consisted of a resistant, thick layer of silk. A delicate network of silk spun by the larvae was also observed within the mines. On average, the area of mines and the percentage of mined area of leaves reached maximums of 83.174 mm^2 and 2.750%, respectively. The caterpillars consumed the palisade parenchyma and some small veins. Cells with phenolic compounds near mine cavities were also observed. The mined and unmined leaves had a single-layered epidermis, with mucilaginous cells on the adaxial surface, and papillary cells and stomata on the abaxial surface. Finally, the temporal emergence of the leaf miner and its parasitoids (and their identification) are presented.

Key words: Erythroxylaceae; insect-plant interaction; mine anatomy; mine morphology; parasitoids.

INTRODUCTION

It has been argued that among herbivorous insects the leaf miners are one of the most neglected groups (Sinclair & Hughes, 2010), probably because their immature stages are very cryptic. Larvae of leaf miners are endophagous, feeding and living within leaf tissue and producing channels or mines (thus the popular name) (Hespenheide, 1991). Therefore, studies focused on the ecology and general biology of this insect group have been numerically less representative when compared to studies considering many other ectophagous insects (Sinclair & Hughes, 2010), particularly also because of their small size and because the adults are often difficult to identify. Approximately 10,000 species of leaf miners have been described, distributed over 60 holometabolous families (Eiseman, 2022). Leaf miners occur in Lepidoptera, Diptera, Coleoptera and Hymenoptera (Hering, 1951; Hespenheide, 1991; Sinclair & Hughes, 2008; Elb *et al*., 2010).

At first glance, it seems that leaf miners experience several disadvantages while feeding, because most of them live within a single leaf which makes them quite dependent on resource choice (e.g., leaf quality and quantity) (Ishino *et al.,* 2011), and vulnerable to natural enemies such as predators and parasitoids (Hawkins *et al*., 1997). However, there are many species of leaf miners. They probably appeared 275-250 million years ago (Rozefelds, 1988; Labandeira, 1998, 2002), and environmental regulation, predator/parasitoid avoidance and avoidance of plant defenses, appear as potential hypotheses to explain the adaptive importance of the leaf-mining habit (see Sinclair & Hughes (2010) for details).

Despite their great diversity, leaf miners are one of the least studied groups within Lepidoptera (Davis *et al*., 2002). In addition, very little is known about the host plants that are inhabited by leaf miners. So far, most of the studies done on leaf miners have been conducted in the northern hemisphere, and, even so, our knowledge of the host plants of many species is far from complete (Sinclair & Hughes, 2008). In the Neotropics, most research attention has been focused on species that have some sort of applied relevance (e.g., crop pests or weed biological control agents) (e.g., Mc Kay *et al*., 2009; Pantoja-Gomez *et al*., 2019), which clearly reveals the importance of further studies with leaf miners, especially in tropical regions where very little is known about their fundamental ecology, biology and diversity.

Study system and goals: The Cerrado biome, which comprises various typically Brazilian savanna vegetation formations, is considered one of the world's richest ecosystems (Felfili & Silva Júnior, 2001). However, this important biome has suffered severe fragmentation due to anthropogenic activities. This system is also included in the world list of "hotspots" of biodiversity (Seligmann *et al.,* 2007).

Erythroxylaceae has a wide geographical distribution, occurring in subtropical and pantropical regions, but only one genus occurs in the Neotropics, namely *Erythroxylum* P.

Browne (Wanderley *et al.,* 2002). This plant genus encompasses approximately 180 species, with 130 occurring in Brazil; many species are arboreal and shrubby, distributed across forest and Cerrado areas (Ribeiro *et al.,* 1999). *Erythroxylum tortuosum* (Mart.) is typical of the Cerrado, being a deciduous shrub-arboreal species (Amaral Jr., 1973). There is currently little information available about the ecology and biology of organisms that use *E. tortuosum* as a host plant (specific information can be found in the Natural History Museum's HOSTS database (Robinson *et al.* 2010)), and even less is known about the natural enemies of the herbivores that feed on *E. tortuosum*. One of the few insects associated with *E. tortuosum* that has been reported is a univoltine species of micro-moth whose larvae are leaf miners (Ishino *et al.,* 2011; De Sibio & Rossi, 2012).

Here, we studied some ecological and biological features of the leaf miner mentioned above and its host plant, *E. tortuosum*, in a Brazilian Cerrado fragment. Specifically, we describe the foraging traits and the temporal emergence pattern of the leaf miners as well as the morphology (including mine size throughout the season) and the anatomy of the mines. Finally, the parasitoids of the leaf miner were identified and their temporal emergence pattern is defined.

MATERIALS AND METHODS

Study area: The study was conducted in a Cerrado fragment (176.7 ha) situated in the Palmeira-da-Serra Farm, municipality of Pratânia, State of São Paulo, Brazil (22°48'50''S, 48°44'35''W). Dry summers and wet winters characterize the region (mesothermic climate), where the average annual rainfall and temperature are, respectively, 1,534 mm and 20.3°C; in the rainy and dry seasons the average annual daytime relative humidity is around 80% and 55%, respectively. In the dry season the daily minimum relative humidity can reach 15% (Rodrigues & Machado, 2008). Crops such as soybean, corn, *Eucalyptus*, *Pinus*, and sugarcane have been cultivated around the study area, suggesting that disturbance is a common process near the fragment (Ishino *et al.,* 2012). Four transects (100 m apart) were established, each 20 m wide and 150 m long (from the edge to the interior of the fragment), where all *E. tortuosum* plants were located. Numbered metal labels (5 x 5 cm) were used to mark 54 *E. tortuosum* plants.

Assessing mine morphology and anatomy, and leaf miner foraging: Mined leaves were collected at fifteen-day intervals from November 2005 until June 2006; 15 mined leaves were randomly collected from each of 16 plants distributed over transects, totaling 316 leaves. Under a stereomicroscope (Nikon SMZ 800 – 40X of magnification), these mines were observed externally and internally after dissection and some external and internal structures were described on both abaxial and adaxial mine surfaces.

To measure the area of the mines during leaf collections, a total of 366 mined leaves were randomly collected from 35 *E. tortuosum* individuals distributed over the transects. As above, leaves were collected at fifteen-day intervals from November 2005 until June 2006, and the number of plants used for leaf collections was reduced during the season in order to guarantee the availability of plant material during the whole period of mine growth (Table 1). After collection, leaves were photographed in the laboratory at a standard distance of 20 cm using a digital camera (resolution: 2048 x 1536 pixels; Canon Powershot A400), and a transparent plate was placed over the leaves to flatten them and minimize distortions (Sinclair & Hoffman, 2003; Ishino *et al.,* 2011; De Sibio & Rossi, 2012). Mined areas of leaves (mm^2) were measured on digital photographs using image analysis software (MetaVue, Version 6.3r4). The area of each leaf was also measured and the percentage of leaf area that was mined was calculated.

To study mine anatomy, three plants were randomly selected, and five mined and five unmined leaves were collected at random from each plant. The leaves were taken to the laboratory and fixed in a FAA 50 solution (Johansen, 1940), and then kept in 70% ethanol. After that, the leaves were dehydrated in an ethyl series and infiltrated in methacrylate according to the manufacturer's protocol. The leaves were then sectioned by using a rotating microtome (8μm thickness) and stained with blue toluidine (0.05%; pH 4.7) (O'Brien *et al.,* 1964). Permanent slides were mounted in synthetic resin (Permount), analyzed under light microscopy and photographed with a photomicroscope. The photos were used to characterize the main anatomical features of mined and unmined leaves.

Table 1. Mean values (± standard deviation) of mined areas (mm^2), and the percentage of leaf area mined, observed on leaves collected throughout the growing season. N_P = Number of plants used per collection; N_L = Number of leaves collected.

Months	Mined area	% of leaf mined	N_P	N_L
Nov-Dec	4.246 (± 1.671)	0.143 (± 0.052)	17	129
Jan	31.778 (± 9.483)	1.010 (± 0.467)	17	79
Feb	47.431 (± 9.991)	1.703 (± 0.756)	11	60
Mar	72.086 (± 18.262)	2.750 (± 1.318)	10	38
Apr	78.898 (± 41.825)	2.225 (± 1.024)	6	16
May	78.763 (± 35.631)	2.606 (± 2.008)	8	26
Jun	83.174 (± 20.592)	2.449 (± 0.685)	5	18

Assessing the emergence of the leaf miner and its parasitoids: In order to verify the emergence of leaf miners and their parasitoids, we used the same 366 leaves collected for mine area measurements (see previous section). Collected leaves were first photographed for mine area measurement, and then kept in Petri dishes for insect emergence. To determine the period of leaf miner oviposition, both sides of the leaf surface were carefully examined during collections in a search for leaf miner eggs. Assessment of leaf miner oviposition started in October 2005, one month before the first collection of leaves.

Leaves were individually kept in Petri dishes with a piece of moist cotton attached to the leaf petiole. The emergences of the leaf miner and its parasitoids were recorded monthly. Leaf miners were mounted with wings extended on small triangles fixed with entomological pins and placed in a small cardboard box. The parasitoids were placed in small plastic tubes filled with alcohol (70%). All insects were then sent to specialists, containing all collection information (e.g., collection location, date, collector), as follows: Klaus Sattler (Department of Entomology, Natural History Museum, UK) identified the micro-Lepidoptera; Michael Sharkey (Department of Entomology, University of Kentucky, USA) and Zuleide A. Ramiro (Experimental Center of the Biological Institute, Campinas, SP, Brazil) identified the braconid wasps; Christer Hansson (Biological Museum, Lund University, Sweden) and Valmir A. Costa (Experimental Center of the Biological Institute, Campinas, SP, Brazil) identified the Eulophidae individuals; Valmir A. Costa also identified Eupelmidae parasitoids; John S. Noyes (Department of Life Sciences, Natural History Museum, UK) identified the Encyrtidae. The leaf miner specimens were deposited in the Lepidoptera Collection of the Natural History Museum, London, UK, and the parasitoid specimens were deposited in the Department of Botany, São Paulo State University (Unesp), Botucatu-SP, Brazil.

RESULTS AND DISCUSSION

Mine morphology and anatomy, and leaf miner foraging: Only one species of leaf miner emerged from the leaves and was identified as an undescribed species of the genus *Agnippe* Chambers, 1872 (Lepidoptera, Gelechiidae) (Fig. 1), as already reported in two other studies (Ishino *et al.,* 2011; De Sibio and Rossi, 2012). Table 2 shows the known *Agnippe* species (including junior synonyms *Evippe* Chambers, 1873, *Phaetusa* Chambers, 1875, and *Tholerostola* Meyrick, 1917), including this undescribed species occurring on *E. tortuosum* (hereafter *Agnippe* sp.2; Ishino *et al.,* 2011), with their regions of occurrence and host plants.

Leaf damage caused by *Agnippe* sp.2 was typical of leaf miners, which by definition are larvae that feed internally, in the mesophyll between the upper and lower epidermis (Fig. 2A), where they form galleries (Jolivet, 1998; Labandeira, 2002). Depending on the species, leaf miner larvae can consume tissues like parenchyma, epidermis (leaving the cuticle intact) and veins of different thicknesses (Hering, 1951; Jolivet, 1998; Melo-de-Pinna *et al.,* 2002; Elb *et al*., 2010; Pereira

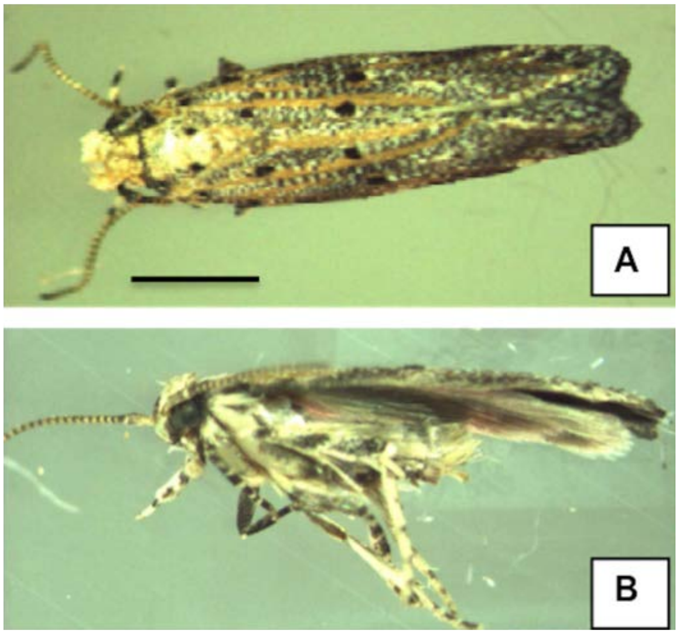

Figure 1. Adult individuals of *Agnippe* sp.2: **(A)** dorsal view; **(B)** lateral view. Scale (1.0 mm) is represented by the black bar in (A).

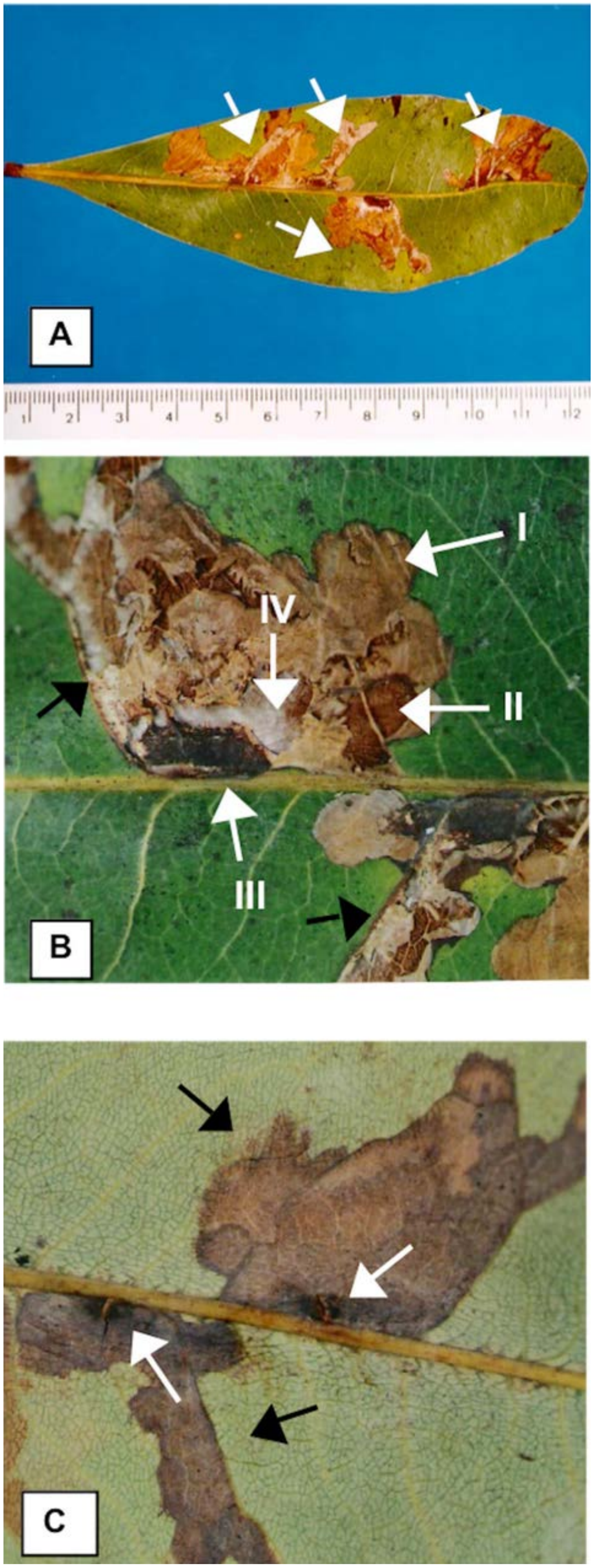

Figure 2. (A) Mines (arrows) of *Agnippe* sp.2 on *Erythroxylum tortuosum* (adaxial surface); **(B)** two mines on the adaxial surface (black arrows) with augmented view: (I) epidermis after larval damage; (II) remains of leaf nervure; (III) darkened region; (IV) thin silk network made by the larva; **(C)** two mines (black arrows) of *Agnippe* sp.2 on *E. tortuosum* (abaxial surface): white arrows show a pair of semicircular openings, indicating the exact location where the first instar larva penetrated the leaf.

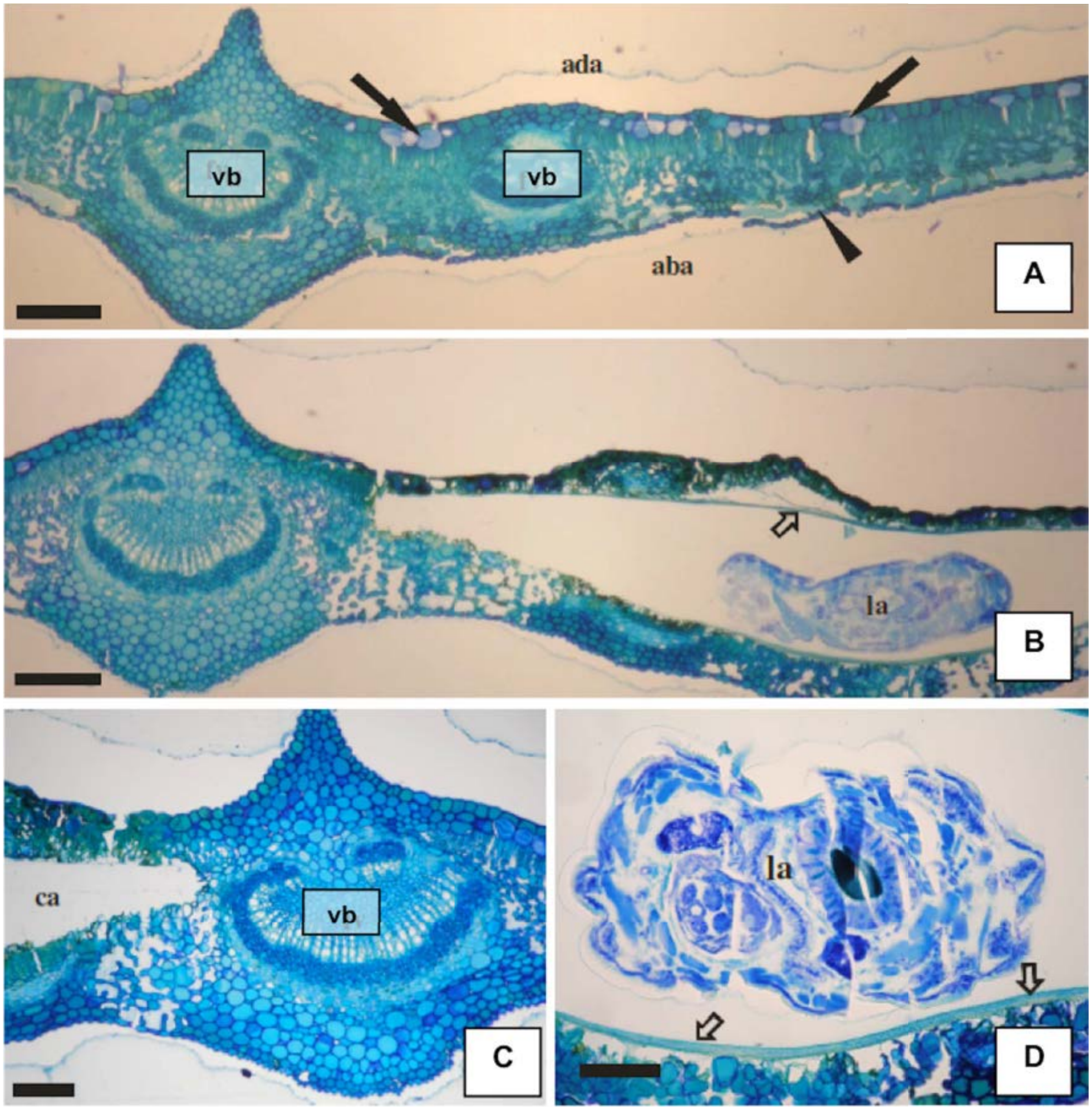

Figure 3. Cross-section in the midrib and intervein regions of *Erythroxylum tortuosum* leaves. **(A)** Unmined leaf (without mine cavity) showing vascular bundles, the adaxial epidermis with mucilaginous cells (arrows above) and the abaxial epidermis with papillary cells (arrow below). **(B)-(D)** Leaves with mine cavity: **(B)** a small leaf miner caterpillar within the mine and a soft membrane (empty arrow) right above it; **(C)** presence of the mine cavity and the vascular bundle; **(D)** detail of the mine cavity showing the leaf miner caterpillar and the soft membrane inside the cavity (empty arrows). ada = adaxial; aba = abaxial; vb = vascular bundle; la = larva (caterpillar); ca = cavity. Bars: (A) and (B) = 250µm; (C) = 150µm; (D) = 100µm.

et al., 2018), although some leaf miners do not eat leaf veins (Almeida-Cortez & Melo-de–Pinna, 2006). Most *Agnippe* sp.2 mines were characterized as “blotch mines” because elongated and bending corridors, which characterize “serpentine mines”, were rarely seen (Elb *et al.*, 2010). The larvae, which had protruding heads, formed blotch mines because they moved forward and backward in all directions within the mines while feeding. Serpentine mines were seen only for new growing larvae (i.e., young larvae from first instars).

On the adaxial surface, each mine presented a rigid darkened region, longitudinally situated right beside the central vein (Fig. 2B). In the laboratory, it was verified that the pupae of the leaf miners remained in the darkened region, and the prepupal stage usually started in June. Because mine expansion was almost complete by April (Table 1), it is possible that larvae spend their last two months of development deepening the area already mined, or that leaf consumption was drastically reduced due to low humidity at this time of the year (winter season), when the leaves are drier. Under the darkened region, a pair of semicircular openings was observed on the abaxial external leaf surface, indicating the exact location where the first instar larva penetrated the leaf (Fig. 2C). The openings consisted of a resistant and thick layer of silk and were also used by the larvae to eject faeces after foraging and by the adult moths to emerge from the mine. A delicate network of silk spun by the larvae was also observed within the mines, mainly on the inferior surface (Fig. 2B); the openings and silk network formed a single structure. Mines never crossed the leaf midrib,

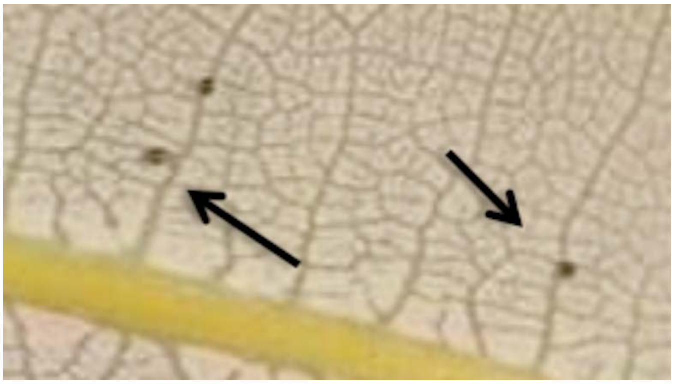

Figure 4. Eggs of *Evippe* sp.2 (indicated by the arrows) deposited on the abaxial leaf surface of *Erythroxylum tortuosum*.

and unification of two mines into one occurred in about 5% of the observed mines. The area of mines and the percentage of leaf area mined reached, on average, a maximum of 83.174 mm^2 and 2.750%, respectively (Table 1).

Cross-sections in the mine region showed that the caterpillars consumed the palisade parenchyma and some small veins (third or fourth order), leaving the epidermis intact (Fochezato *et al*., 2018). Cells with phenolic compounds near mine cavities were also observed, which had a soft membrane around the interior (Fig. 3B,D). The mined and unmined leaves had a single-layered epidermis, with mucilaginous cells on the adaxial surface, and papillary cells and stomata on the abaxial surface, as previously noted by Beiras & Sajo (2004) (Fig. 3A). Figure 3C shows the detail of the vascular bundle of the midrib that remained intact. In the mined leaves it was possible to observe a caterpillar within the mine cavity (Fig. 3B,D). As mentioned above, *Agnippe* sp.2 caterpillars consume parenchymal tissue and the smaller veins (see Melo-de-Pinna *et al.,* 2002 for another leaf miner species) and a soft membrane was observed inside the mine cavity. It is possible that this membrane was formed by silk threads woven by the larva; however, more studies are necessary to confirm the nature of this membrane.

Emergence of the leaf miner and its parasitoids: It has been argued that plant phenology is an important selective force for endophytic parasites such as leaf miners (e.g., Mopper *et al.,* 2000). For example, leaf miner oviposition and larval development must be strictly linked with the production of leaves and leaf abscission (Faeth *et al.,* 1981; Crawley & Akhteruzzaman, 1988; Hunter, 1992; Komatsu & Akimoto, 1995). *Agnippe* sp.2 females oviposited on the abaxial leaf surface, from October until April, and most eggs were laid in November when leaves were young, presented tender tissues and were generally expanded (Fig. 4 shows some eggs deposited on a leaf); this enables the first instar larva to reach the mesophyll. In the laboratory, six adult leaf miners emerged from August to October (Table 3), indicating that adult emergence and oviposition were synchronized with the peak of production of young leaves (see plant phenology details in Ishino *et al.,* 2012). Because five adult leaf miners emerged in April and May (Table 3), it is possible that the life cycle of some individuals was shortened due to stress after removing the leaves from the plants. On the other hand, as we did not observe the emergence of adults under field conditions, it is also possible that the life cycle of those individuals that emerged from August to October was lengthened due to suboptimal conditions. However, this second hypothesis is less likely, since many caterpillars in the intermediate stage of development died inside the leaves during March and April (we dissected the leaves when adult leaf miners did not emerge), indicating that the most likely period of emergence is from August to October. We also cannot neglect the possibility that a few leaf miners may emerge before the expected time in the field, resulting in a small partial second generation. Pupation occurred within the mine and adult moths emerged from semicircular openings on the abaxial external leaf surface (see "Mine morphology and anatomy, and leaf miner foraging" section). As leaf abscission occurs from August to September (Ishino *et al.,* 2012), many pupae may drop to the ground within the abscised leaves, from which most moths may emerge.

Because the leaves dried quickly after collection, reducing larval survival, the emergence of adult leaf miners was very low. However, mortality imposed by parasitoids may also explain why few adults emerged (Table 3). Although mines may confer some protection against predators and parasitoids, many parasitoids prefer to attack larvae that are either chemically or physically (e.g. galls and mines) protected against predators (Jolivet, 1998; Gentry & Dyer, 2002). Parasitoid wasps of the families Braconidae, Encyrtidae and Eulophidae emerged from *Agnippe* sp.2 larvae (Table 3). As above, fewer parasitoids emerged, probably due to reduced larval survival (Table 3).

The parasitoids *Bracon* sp. and *Bassus* sp. (Braconidae) emerged mostly in March and April, and only one individual was observed in August. *Closterocerus coffeellae* Ihering, 1914 (Eulophidae), *Copidosoma* sp. (Encyrtidae) and *Chelonus* (*Microchelonus*) sp. (Braconidae) emerged mostly in August and September; however, one individual of *Chelonus* (*Microchelonus*) sp. was also observed emerging in April (Table 3). *Horismenus aeneicollis* Ashmead, 1904 (Eulophidae) presented less seasonal distribution than the other species, emerging in May, July, August and October, and *C. coffeellae* was the most abundant parasitoid (Table 3). Although two other parasitoids emerged, *Brasema* sp. (Eupelmidae) and *Chrysocharis* sp. (Eulophidae), the emergence dates could not be precisely determined.

Conclusion: Although it is a common consensus today that the Brazilian Cerrado has a great biological diversity, there is a scarcity of insect species described. Therefore, many leaf miners and other moth species remain to be discovered as well as their parasitoids. To improve the knowledge about the diversity of small Lepidoptera in this very important ecosystem, biological and ecological information about a Cerrado leaf miner was therefore provided here.

ACKNOWLEDGMENTS

We thank Dr Klaus Sattler (Department of Entomology, Natural History Museum, UK) for the micro-Lepidoptera identification, and Drs Michael Sharkey (Department of

Table 2. Known species of the genus *Agnippe* Chambers, 1872, their regions of occurrence and host plants. Information about the focal species of this study is shown in bold. Data provided by Klaus Sattler (Department of Entomology, Natural History Museum, UK).

Species of *Agnippe*	Author	Year	Region of occurrence	Host plant
abdita	Braun	1925	Nearctic	*Cercocarpus* (Rosaceae)
aequorea	Meyrick	1917	Neotropical (Peru)	-
albidorsella	Snellen	1884	East Palaearctic	*Lespedeza* (Leguminosae)
aulonota	Meyrick	1917	Neotropical (Equator)	-
Biscolorella (type species of *Agnippe*)	Chambers	1872	Nearctic	*Gleditsia* (Leguminosae)
conjugella	Caradja	1920	Palaearctic	-
crinella	Keifer	1927	Nearctic	-
dichotoma	Li	1993	Palaearctic	-
echinulata	Li	1993	Palaearctic	-
evippeella	Busck	1906	Nearctic	-
evippella	Forbes	1931	Neotropical (Porto Rico)	*Aeschynomene* (Leguminosae)
fuscopulvella	Chambers	1872	Nearctic	-
haberlandi	Amsel	1961	Palaearctic	*Alhagi* (Leguminosae)
kuznetzovi	Lvovsky and Piskunov	1989	Palaearctic	-
laudatella	Walsingham	1907	Nearctic	*Quercus* (Fagaceae)
leuconota, Syn. *plutella* Chambers, 1875	Zeller	1873	Nearctic	-
lunaki	Rebel	1940	Palaearctic	-
miniscula	Li	1993	Palaearctic	-
novisyrictis	Li	1993	Palaearctic	-
omphalopa	Meyrick	1917	Neotropical (Ecuador)	-
penicillata	Amsel	1961	Palearctic	-
plumata	Meyrick	1917	Neotropical (Guyana)	-
postpallescens	Walsingham	1897	Neotropical (West Indies)	-
prunifoliella	Chambers	1873	Nearctic	*Prunus* (Rosaceae)
pseudolella, Syn. *cephalella* Caradja, 1920	Christoph	1888	Palaearctic	-
scoteropis	Meyrick	1931	Neotropical (Paraguay)	-
syrictis	Meyrick	1936	Palaearctic	*Prunus* (Rosaceae)
yongdengensis	Li	1993	Palaearctic	-
zhengi	Wang and Li	1994	Palaearctic	-
zhouzhiensis	Li	1993	Palaearctic	-
sp.1 (introduced into Australia (1998-2000) for biological control of *Prosopis* spp.)	-	-	Neotropical (Argentina)	*Prosopis* (Leguminosae)
sp.2	**-**	**-**	**Neotropical (Brazil)**	***Erythroxylum tortuosum* (Erythroxylaceae)**

Table 3. Monthly emergence of adult leaf miners and parasitoids (n° of individuals) from *Erythroxylum tortuosum* leaves throughout the year 2006.

	Months of emergence									
Leaf miner and its parasitoids	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Agnippe sp.2	0	0	0	2	2	0	0	2	2	1
Bracon and *Bassus*	0	0	3	1	0	0	0	1	0	0
Microchelonus sp.	0	0	0	1	0	0	0	2	2	0
Closterocerus coffeellae	0	0	0	0	0	0	0	5	3	0
Horismenus aeneicollis	0	0	0	0	1	0	1	1	0	2
Copidosoma sp.	0	0	0	0	0	0	0	1	1	0

Entomology, University of Kentucky, USA), John S. Noyes (Department of Life Sciences, Natural History Museum, UK), Christer Hansson (Biological Museum, Lund University, Sweden), Zuleide A. Ramiro (Experimental Center of the Biological Institute, Campinas, SP, Brazil) and Valmir A. Costa (Experimental Center of the Biological Institute, Campinas, SP, Brazil) for the identification of the parasitoids. M.N. Ishino and M.N. Rossi are particularly grateful to Fapesp (Fundação de Amparo à Pesquisa do Estado de São Paulo – process number 05/52569-0), and P. R. De Sibio acknowledges Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), for financial support.

LITERATURE CITED

Almeida-Cortez, J. S., Melo-de–Pinna, G. F. A. 2006. Morphology and anatomy of a leaf mine in *Vismia guianensis* (AUBL.) Choisy (Clusiaceae) in a fragment of Brazilian Atlantic Forest. *Brazilian Journal of Biology* 66: 759-763.

Amaral Jr, A. 1973. *O G*ênero Erythroxylum *no Município de Botucatu, São Paulo*. Botucatu, São Paulo, School of Medical and Biological Science of Botucatu.

Beiras, A. C., Sajo, M. G. 2004. Anatomia foliar de *Erythroxylum* P. Browne (Erythroxylaceae) do Cerrado do estado de São Paulo, Brasil. *Acta Botanica Brasilica* 18: 601-612.

Crawley, M. J., Akhteruzzaman, M. 1988. Individual variation in the phenology of oak trees and its consequences for herbivorous insects. *Functional Ecology* 2: 409-415.

Davis, D. R., Landry, B., Roque-Albelo, L. 2002. Two new Neotropical species of *Bucculatrix* leaf miners (Lepidoptera: Brucculatricidae) reared from *Cordia* (Boraginaceae). *Revue Suisse de Zoologie* 109: 277-294.

De Sibio, P. R., Rossi, M. N. 2012. Oviposition of a leaf-miner on *Erythroxylum tortuosum* (Erythroxylaceae) leaves: hierarchical variation of physical leaf traits. *Australian Journal of Botany* 60: 136-142.

Eiseman, C. 2022. *Leafminers of North America*. 2nd. Edition. E-book, available at http://charleyeiseman.com/leafminers/.

Elb, P. M., Melo-de-Pinna, G. F., Menezes, N. L. 2010. Morphology and anatomy of leaf miners in two species of Commelinaceae (*Commelina diffusa* Burm. f. and *Floscopa glabrata* (Kunth) Hassk). *Acta Botanica Brasilica* 24: 283-287.

Faeth, S. H., Connor, E. F., Simberloff, D. 1981. Early leaf abscission: a neglected source of mortality for folivores. *American Naturalist* 117: 409-415.

Felfili, J. M., Silva Júnior, M. C. 2001. *Biogeografia do Bioma Cerrado: Estudo Fitofisionômico da Chapada do Espigão Mestre do São Francisco*. Brasilia, Department of Forest Engineering, School of Technology, University of Brasília.

Fochezato, J., Brito, R., Isaias, R. M. S., Gonçalves, G. L., Moreira, G. R. P. 2018. *Phyllocnistis hemera* sp. nov. (Lepidoptera: Gracillariidae): a new species of leaf-miner associated with *Daphnopsis fasciculata* (Thymelaeaceae) in the Atlantic Forest. *Revista Brasileira de Entomologia* 62: 57-65.

Gentry, G. L., Dyer, L. A. 2002. On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology* 83: 3108-3119.

Hawkins, B. A., Cornell, H. V., Hochberg, M. E. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology* 78: 2145-2152.

Hering, E. M. 1951. *Biology of the Leaf Miners*. Dordrecht, Springer.

Hespenheide, H. A. 1991. Bionomics of leaf-mining insects. *Annual Review of Entomology* 36: 535-560.

Hunter, M. D. 1992. A variable insect–plant interaction: the relationship between tree budburst phenology and population levels of insect herbivores among trees. *Ecological Entomology* 16: 91-95.

Ishino, M. N., De Sibio, P. R., Rossi, M. N. 2011. Leaf trait variation on *Erythroxylum tortuosum* (Erythroxylaceae) and its relationship with oviposition preference and stress by a host-specific leaf miner. *Austral Ecology* 36: 203-211.

Ishino, M. N., De Sibio, P. R., Rossi, M. N. 2012. Edge effect and phenology in *Erythroxylum tortuosum* (Erythroxylaceae), a typical plant of the Brazilian Cerrado. *Brazilian Journal of Biology* 72: 587-594.

Jolivet, P. 1998. *Interrelationship Between Insects and Plants*. Boca Raton (FL), CRC Press.

Komatsu, T., Akimoto, S. 1995. Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica. Ecological Entomology* 20: 33-42.

Labandeira, C. C. 1998. Early history of arthropod and vascular plant associations. *Review of Earth and Planetary Sciences* 26: 329-377.

Labandeira, C. C. 2002. *The History of Associations Between Plants and Animals*, pp. 26-74. *In:* Herrera, C. M., Pellmyr, O. (Eds.), *Plant-Animal Interactions: An Evolutionary Approach*. Oxford (UK), Blackwell Science.

Mc Kay, F., Oleiro, M., Walsh, G. C., Gandolfo, D., Cuda, J. P., Wheeler, G. S. 2009. Natural enemies of Brazilian peppertree (Sapindales: Anacardiaceae) from Argentina: their possible use for biological control in the USA. *Florida Entomologist* 92: 292-303.

Melo-de-Pinna, G. F. A., Kraus, J. E., Menezes, N. L. 2002. Morphology and anatomy of leaf mine in *Richterago riparia* Roque (Asteraceae) in the Campos Rupestres of Serra do Cipó, Brazil. *Brazilian Journal of Biology* 62: 179-185.

Mopper, S., Stiling, P., Landau, K., Simberloff, D., Van Zandt, P. 2000. Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology* 81: 1577-1587.

Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni, G. W., Hernández, L. M. 2010*. HOSTS: a database of the world's Lepidopteran hostplants. Natural History Museum, London.* https://data.nhm.ac.uk/dataset/hosts. Accessed 15 February 2023.

O'Brien, T. P., Feder, N., McCully, M. E. 1964. Polychromatic staining of plant cell walls by toluidine blue. *O Protoplasma* 59: 368-373.

Pantoja-Gomez, L. M., Corrêa, A. S., Oliveira, L. O., Guedes, R. N. C. 2019. Common origin of Brazilian and Colombian populations of the Neotropical coffee leaf miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). *Journal of Economic Entomology* 112: 924-931.

Pereira, C. M., Silva, D. S., Vargas, H. A., Moreira, G. R. P. 2018. Description of immature stages and natural history of *Stigmella schinivora* (Lepidoptera: Nepticulidae), a leaf-miner associated with the Brazilian peppertree. *Zoologia* 35: e24485.

Ribeiro, J. E. L. da S., Hopkins, M. J. G., Vicentini, A., Sothers, C. A., Costa, M. A. da S., de Brito, J. M., de Souza, M. A. D., Martins, L. H. P., Lohmann, L. G., Assunção, P. A. C. L., Pereira, E. da C., da Silva, C. F., Mesquita, M. R., Procópio, L. C. 1999. *Flora da Reserva Ducke: Guia de Identificação das Plantas Vasculares de uma Floresta de Terra Firme na Amazônia Central.* Manaus, IMPA.

Rodrigues, T. M., Machado, S. R. 2008. Pulvinus functional traits in relation to leaf movements: a light and transmission electron microscopy study of the vascular system. *Micron* 39: 7-16.

Rozefelds, A. C. 1988. Insect leaf miners from the Eocene Anglesea locality, Victoria, Australia. *Alcheringa* 12: 1-6.

Seligmann, P., Mittermeier, R. A., da Fonseca, G. A. B., Gascon, C., Crone, N., da Silva, J. M. C., Famolare, L., Bensted-Smith, R., Rajaobelina, L., Beehler, B. 2007. *Centers for Biodiversity Conservation: Bringing Together Science, Partnerships, and Human Well-Being to Scale Up Conservation Outcomes*. Arlington (VA), Conservation International.

Sinclair, C., Hoffmann, A. A. 2003. Monitoring salt stress in grapevines: are measures of plant trait variability useful? *Journal of Applied Ecology* 40: 928-937.

Sinclair, R. J., Hughes, L. 2008. Leaf mining in the Myrtaceae. *Ecological Entomology* 33: 623-630.

Sinclair, R. J., Hughes, L. 2010. Leaf miners: the hidden herbivores. *Austral Ecology* 35: 300-313.

Wanderley, M. G. L., Shepherd, G. J., Giulietti, A. M. 2002. *Flora Fanerogâmica do Estado de São Paulo*. São Paulo (Brazil), Hucitec/ Fapesp.

Filling the distributional gap of *Celaenorrhinus pyrrha* de Nicéville, 1889 (Lepidoptera: Hesperiidae: Tagiadinae) in the Himalayas

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Date of issue online: 16 August 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8140750

Abstract: *Celaenorrhinus pyrrha* de Nicéville, 1889 is recorded for the first time from central Nepal. Three individuals were recorded in March and November, 2021 from 800–930 meters elevation in Pokhara, Kaski District. The species was recorded previously from Uttarakhand in the West Himalayas and Sikkim to Yunnan in the East Himalayas. Its distribution record, however, was missing from the central Himalayas. This new record is proposed to fill the distributional gap for this species in the central Himalayas. The identification is based on external morphology and the morphology of male genitalia.

Key words: Double Spotted Flat; Main Spotted Flat; Nepal butterflies; new distribution; spread winged skipper.

INTRODUCTION

The genus *Celaenorrhinus* Hübner, [1819] is distributed across Asia, Africa, and North and South America (Watson, 1893; Evans, 1952) with the occurrence of over 100 species (Evans, 1949; Yuan *et al.*, 2015), out of which 25 have been recorded on the Indian subcontinent (Gasse, 2018), wherein *Celaenorrhinus pyrrha* de Nicéville, 1889 is recorded from East Uttarakhand (India) in the West Himalayas to Arunachal Pradesh through Sikkim, NW Bengal and Bhutan, and also in other parts of NE India such as Meghalaya, Nagaland, and Manipur from 1200–2700 meters elevation (Varshney & Smetacek, 2015; Kehimkar, 2016; Gasse, 2018). *Celaenorrhinus pyrrha* is also recorded from China, Myanmar, Thailand, Laos, Vietnam, and Malaysia (Evans, 1949; Eliot, 1959; Osada *et al.*, 1999; Kimura *et al.*, 2011; Xue *et al.*, 2020; Inayoshi, 2022). Smith (2010, 2011a) made no mentioning of this taxon from Nepal. While stating the distributional range of *C. pyrrha* on the Indian subcontinent, Gasse (2013) reported that it occurs from Kumaon to NE India. Kehimkar (2016) listed Nepal under the distribution range of this species. Gasse (2018) did not include Nepal under the distribution range of *C. pyrrha*, but mentioned that it “doubtless” occurs in Nepal. Sondhi & Kunte (2018) mentioned that there were no recent published records of this taxon from Nepal. KC & Sapkota (2022) reported *C. pyrrha* for the first time from Dhankuta, Nepal, in the East Himalayas.

Pokhara is a popular town in Kaski District of Nepal (Fig. 1). The elevation ranges from about 700–1,700 meters. Lakeside, the discovery area, is a popular lake town in Pokhara with an average elevation of 850 meters, with evergreen forests flanking the town; Raniban Forest lies across the lake and Methlang Forest lies in the proximal part of the downtown.

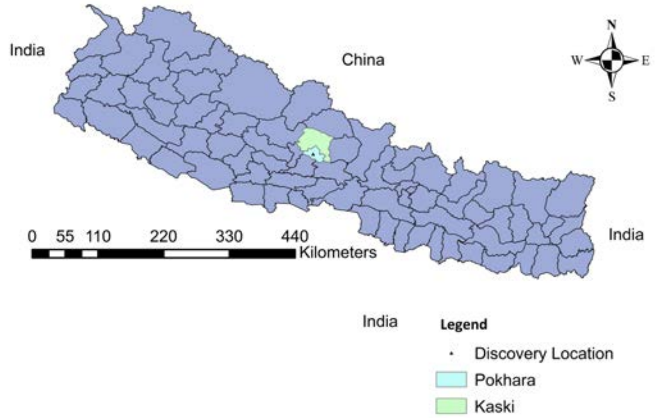

Figure 1. Map of Nepal with the study area.

Figure 2. Fewa Lake from Methlang Forest, Lakeside, Pokhara.

Both forests overlook the beautiful Fewa Lake below (Fig. 2) and the giant Himalayas toward the north. The discovery of *C. pyrrha* was made in the latter forest, i.e., Methlang. Both are community forests and thus well protected despite the growing urbanization in Lakeside. The Methlang Forest reaches an elevation of 1,000 meters and ends up near Sarangkot, a popular hill station of about 1,700 meters. This forest is home to many species of butterflies including some seldom seen species such as *Aeromachus dubius* Elwes & Edwards, 1897, *A. jhora* (de Nicéville, 1885), *A. pygmaeus* (Fabricius, 1775), *Burara anadi* (de Nicéville, [1884]), *B. jaina* (Moore, [1866]), *B. oedipodea* (Swainson, 1820), *Coladenia agnioides* Elwes & Edwards, 1897, *Liphyra brassolis* Westwood, 1864, *Matapa purpurascens* Elwes & Edwards, 1897, *Pithauria murdava* (Moore, [1866]), etc.; the forest also shelters some other *Celaenorrhinus* species such as *C. dhanada* (Moore, [1866]), *C. leucocera* (Kollar, [1844]), *C. nigricans* (de Nicéville, 1885), and *C. patula* de Nicéville, 1889 (Smith, 2011b; KC, 2020; Smith, C., 2020, personal communication; KC & Sapkota, 2022; KC, 2022).

The prominent vegetation in the forest includes *Castanopsis indica* (Fagaceae), *Schima wallichii* (Theaceae), *Senegalia catechu* (Fabaceae), *Ageratina adenophora* (Asteraceae), *Lantana camara* (Verbenaceae), *Rubus* spp. (Rosaceae), and several species of undergrowth most of which might act as larval host plants to various species of butterflies. The dicotyledon weeds, for example, host the larvae of several pyrgine/tagiadine species (Evans, 1952; Cock, 1983; Janzen *et al.*, 1998).

MATERIALS AND METHODS

Butterflies were observed during opportunistic surveys by the author in 2021. They were photographed using a “Canon 7D Mark II” camera coupled with a “100 mm f/2.8L Macro IS USM” lens (Figs. 3A-3D) and captured using an aerial net whenever required or possible (Figs. 3E-3F). The captured specimens were euthanized in killing jars charged with ethyl acetate. The specimens were spread and mounted. The length of the forewing was measured using the method used by Evans (1932), i.e., measurement was taken from the center of the thorax to the tip of forewing apex. If genitalia examination was deemed necessary, the abdomen of the specimen was cut off and relaxed using 10% KOH solution while leaving it as such overnight. Dissection was performed in glycerin using a “LEICA MZ9.5” stereoscope. Photographs of the genital parts were taken by placing them in glycerin and using an “Axiocam 105 color” camera attached to a “ZEISS SteREO Discovery. V20” stereoscope. Multiple images were taken using Zeiss “ZEN” software and stacked with “CombineZP” software. The contrast was adjusted using “IrfanView 64” software. The map of the study area (Fig. 1) was made by using “ArcMap 10.8.2” software. Evans (1949), Kehimkar (2016), Smetacek (2016), Xue *et al.* (2020), and Inayoshi (2022) were used as references for the identification. The morphological traits, such as wing venation and genitalia, are described based on the terminologies used by Evans (1949).

RESULTS

Hesperiidae Latreille, 1809
Tagiadinae Mabille, 1878
Celaenorrhini Swinhoe, 1912
Genus *Celaenorrhinus* Hübner, [1819]

Celaenorrhinus pyrrha de Nicéville, 1889

Material Examined: 1♂; Central Nepal, Gandaki Province, Kaski District, Pokhara, Lakeside, Methlang Forest, 28°12’59” N, 83°57’49” E, 800 m a.s.l., 5.xi.2021, 14:23 hrs. (GMT +5:45), coll. Sajan KC.
Forewing length: 25 mm

Diagnosis: *Celaenorrhinus pyrrha* can be distinguished from the other congeners due to the presence of double pale spots in space 1b on the underside of forewing beyond double discal spots, except two species i.e., *C. ambareesa* (Moore, 1865) which is found in the Western Ghats and the Eastern Ghats of India (Gasse, 2018), and *C. consaguinea* Leech, 1891 which is found in China (Sichuan, Tali) (Evans, 1949) and Taiwan (Huang, 2003). *Celaenorrhinus pyrrha* has checkered cilia on hindwing, while *C. ambareesa* and *C. consaguinea* have checkered cilia on both wings (Evans, 1949). Evans (1949) also mentioned that *C. pyrrha* has the basal spot of forewing upper side space 1b very small or obsolete, and the antennae in both sexes are white only at the club bases and not at the shafts or the apiculi. This basal spot on forewing upper side space 1b is, however, variable as evident in Sondhi & Kunte (2016) and Inayoshi (2022).

Observation and taxonomic notes: The first individual was seen by the author in the Methlang Forest (28°13’2.32’’ N, 83°57’52.63’’ E, 930 meters) on 10.v.2021 at 14:17 hrs. (GMT +5:45 hrs.) (Fig. 3A). It was feeding on nectar from the flowers of *Lantana camara* (Verbenaceae) on a hilltop. Only the upper side was photographed, the specimen was not captured. Although the underside is also required to identify *C. pyrrha* conclusively, the author was convinced from the photographs of the upper side alone regarding its identification and continued to look around for it in the following months as well. Another sighting was made on 04.xi.2021 at 15:47 hrs. (GMT +5:45 hrs.) (28°12’59” N, 83°57’49” E, 800 meters) when the author was on an opportunistic survey in the same forest. The individual (Figs. 3B-3C) was sipping nectar from a wild flower by a forest stream. However, the author was unable to capture the specimen and only took some photographs, including underside. The next day, on 05.xi.2021, at 14:23 hrs. (GMT +5:45 hrs.), near the same place, the author came upon a partly worn individual which was sitting under a leaf of an unknown plant next to the same water source (Fig. 3D). This individual was photographed and then successfully collected.

The collected individual was thought to be a species new to science for three reasons: 1. The basal spot in space 1b of forewing upper side was prominent and white, while that of *C. pyrrha* is typically obscure (Evans, 1949). 2. The hind tibia of the collected individual had a prominent tuft of yellow scales

Figure 3. *Celaenorrhinus pyrrha* de Nicéville, 1889 from Nepal. **A**: Live individual, upper side; 10.v.2021, from Pokhara (930 m). **B**: Live individual, upper side; 04.xi.2021, from Pokhara (800 m). **C**: Ditto, showing underside. **D**: Live individual, upper side; 05.xi.2021, from Pokhara (800 m). **E**: Specimen, upper side; collected on 05.xi.2021, from Pokhara (800 m). **F**: Ditto, underside.

which the author had never noticed before in *Celaenorrhinus* of the Indian subcontinent. This tibial tuft acts as secondary sexual character of this group (Austin & Steinhauser, 1996). 3. The male genitalia (Fig. 4), especially the distal end of the valva (cuiller) (Fig. 4A) and cornutus of the aedeagus (Figs. 4D-4F), were different from those shown in Evans (1949) and Xue *et al.* (2020).

Regarding the above confusion, the author contacted Hao Huang (China) and asked him if he had come across any such specimens of *C. pyrrha*. Upon request, Huang dissected a specimen from Hekou, Yunnan-Vietnam border (Fig. 5). The genitalia were similar to that of the author's specimen, but there was still some variation among them, i.e., the specimen examined by the author, the specimen examined by Huang, and the photographs of Xue *et al.* (2020). The shapes of the tegumen, uncus, and gnathos were the same while the shapes of the cuiller of valva, and cornutus of the aedeagus, showed some prominent variation. The cuiller is short and blunt in Xue *et al.*

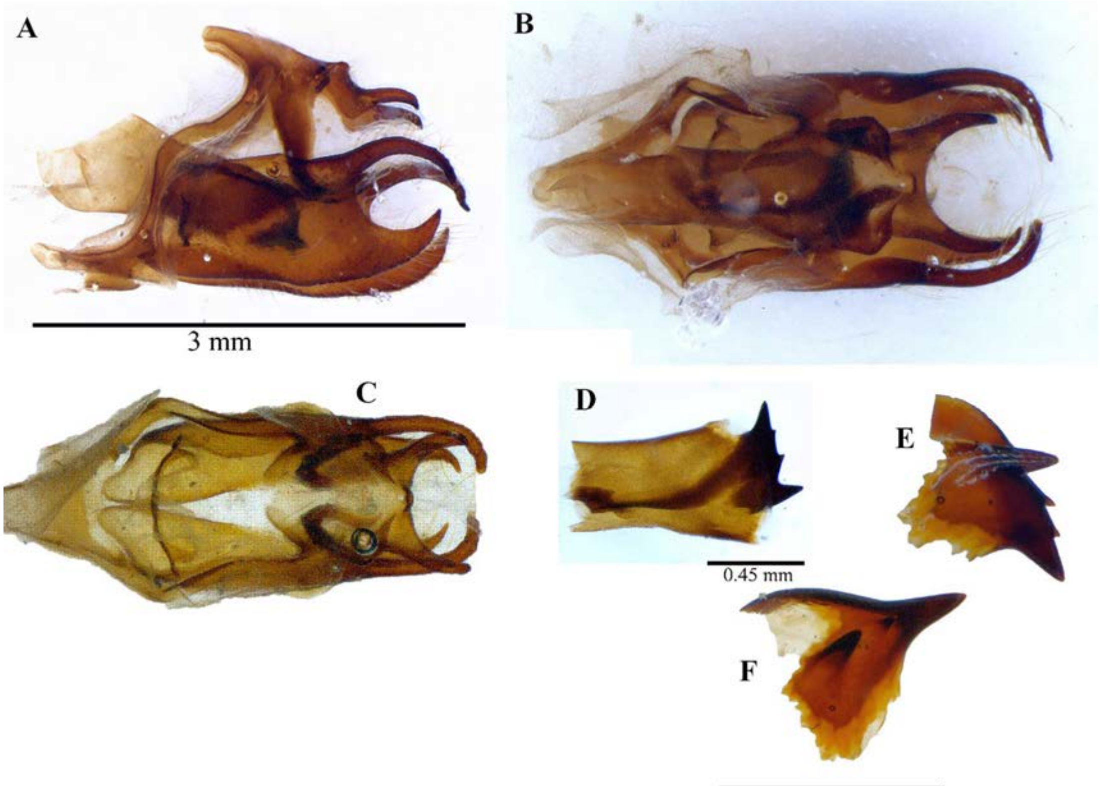

Figure 4. *Celaenorrhinus pyrrha* de Nicéville, 1889 male genitalia of Figure 1D individual. **A**: Genitalia capsule, lateral view. **B**: Genitalia capsule, dorsal view. **C**: Genitalia capsule, dorsal view with aedeagus removed. **D**: Aedeagus portion dorsal view with cornutus **E**: Cornutus lateral view. **F**: Cornutus ridge view.

(2020) while it is longer in the examined specimen (Fig. 4A), and its tip is straight after the curve in Huang's specimen (Fig. 5C). The cornutus is anchor-shaped in Xue *et al.* (2020), while it is serrated in the examined specimen (Fig. 4D) and Huang's specimen (Fig. 5D), although the pattern of serration is different. This could also represent subspecific variation, while Xue *et al.* (2020) considered such anomalies as individual variations. For now, the author considers this to be intraspecific variation in male genitalia, which does not seem unusual in skippers. In terms of external morphology, the examined specimen and the specimen examined by Huang had the first discal spot in space 1b missing on the upper side of the forewing and present on the underside of the forewing. The prominent yellow hair pencil on the hind tibia (Fig. 3F) is assumed to be present in all individuals, but it is probably often overlooked by taxonomists. Huang's specimen also seemed to have some tuft, but not as exposed as in the author's specimen (Huang, H., 2022, personal communication). The examined specimen is deposited at the Annapurna Natural History Museum (ANHM), Pokhara.

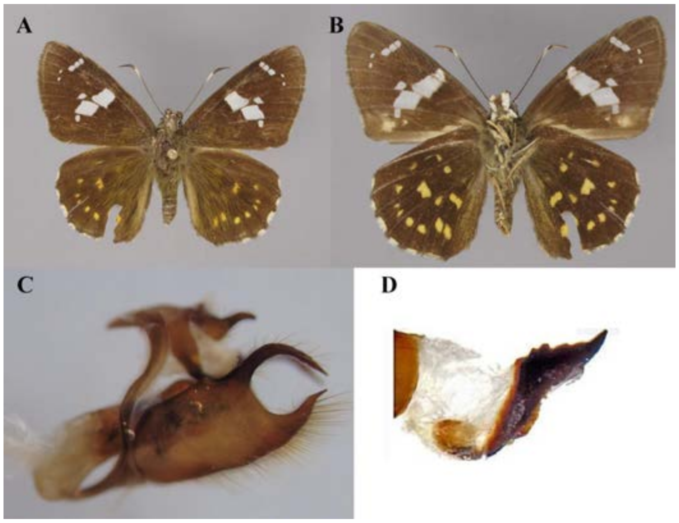

Figure 5 (Images copyright: Hao Huang). *Celaenorrhinus pyrrha* de Nicéville, 1889 from Hekou (Yunnan-Vietnam border). **A**: Spread specimen, upper side **B**: Ditto, underside. **C**: Ditto, Male genitalia capsule, lateral view **D**: Cornutus lateral view.

DISCUSSION

Gasse (2018) was correct about his prediction on the occurrence of *C. pyrrha* in Nepal. Herein, it is recorded from East as well as central Nepal, it is only a matter of time before someone will record it from West Nepal as well. In the Himalayas, it has now been recorded from the West to East Himalayas. A similar result was obtained regarding the record of *Burara anadi* which Gasse (2018) predicted would occur in Nepal and was recorded by KC (2020) from central Nepal, and *Seseria sambara* (Moore, [1866]) which was recorded by KC & Sapkota (2022) from East Nepal. Gasse (2018) made such predictions for other species such as *Celaenorrhinus pulomaya* (Moore, 1865) and *Pyrgus cashmirensis* Moore, 1874 which are found in the West and the East Himalayas and are yet to be recorded from Nepal or the central Himalayas. They should eventually be recorded from these neighboring places if their habitats and host plants are available.

ACKNOWLEDGMENTS

The author is grateful to Hao Huang (China) for kindly checking his specimens along with the images of the author's specimen, and providing the required information to the author while also allowing to use his images as a reference. The author would like to thank Mr. Piet van der Poel (Netherlands) and Mr. Surendra Pariyar (Annapurna Natural History Museum, Pokhara) for their kind support. He is thankful to Dr. Darren A. Pollock (Eastern New Mexico University, NM, USA), Dr. Joe Schelling (Albuquerque, NM, USA), and Mr. Steve J. Cary (Santa Fe, NM, USA) for proofreading the manuscript and offering their valuable comments, and to the anonymous reviewers.

LITERATURE CITED

Austin, G. T., Steinhauser, S. R. 1996. Hesperiidae of central Rondônia, Brazil: *Celaenorrhinus* Hübner (Lepidoptera: Pyrginae), with descriptions of three new species and taxonomic comments. *Insecta Mundi* 10(1-4): 25-44.

Cock, M. J. 1983. The skipper butterflies (Hesperiidae) of Trinidad Part 3 Pyrginae (first section). *Living World, Journal of the Trinidad and Tobago Field Naturalists' Club* 1984: 38-42.

Eliot, J. N. 1959. New or little known butterflies from Malaya. *Bulletin of the British Museum (Natural History) Entomology* 7(8): 371-391.

Evans, W. H. 1932. *The Identification of Indian Butterflies. (Second Edition Revised).* Madras, Bombay Natural History Society. x + 454 pp., 32 pl.

Evans, W. H. 1949. *A Catalogue of the Hesperiidae from Europe, Asia and Australia in the British Museum (Natural History)*. London, British Museum (Natural History). xix + 502 pp., 53 pl.

Evans, W. H. 1952. *A Catalogue of the American Hesperiidae in the British Museum (Natural History). Part II. Pyrginae Section 1.* London, British Museum (Natural History). v + 178pp., 25 pl.

Gasse, P. van. 2013. *Butterflies of the Indian Subcontinent – Annotated Checklist*. https://dokumen.tips/documents/butterflies-of-india-annotated-web-view2013-08-21butterflies-of-india-.html. Accessed 4 November 2022.

Gasse, P. van. 2018. *Butterflies of the Indian Subcontinent – Annotated Checklist*. https://www.biodiversityofindia.org/images/2/2c/Butterflies_of_India.pdf%20. Accessed 28 October 2022.

Huang, H. 2003. A list of butterflies collected from Nujiang (Lou Tse Kiang) and Dulongiang, China with descriptions of new species, and revisional notes. *Neue Entomologische Nachrichten* 55: 3-114.

Inayoshi, Y. 2022. *A check list of butterflies in Indo-china, chiefly from Thailand, Laos & Vietnam*. http://yutaka.it-n.jp/hes/90430001.html. Accessed 29 October 2022.

Janzen, D. H., Sharkey, M. J., Burns, J. M. 1998. Parasitization biology of a new species of Braconidae (Hymenoptera) feeding on larvae of Costa Rican dry forest skippers (Lepidoptera: Hesperiidae: Pyrginae). *Tropical Lepidoptera Research* 9(suppl. 2): 33-41.

KC, S. 2020. Some new distribution records of hesperiid butterflies in Nepal. *Bionotes* 22(3): 190-194.

KC, S. 2022. Rediscovery of Moth Butterfly *Liphyra brassolis* Westwood, 1864 (Lepidoptera: Lycaenidae) from Nepal. *Journal of the Bombay Natural History Society* 119:52-54.

KC, S., Sapkota, A. 2022. Additional distribution records of butterflies (Lepidoptera: Rhopalocera) with seven species new to Nepal. *Biodiversitas Journal of Biological Diversity* 23(5): 2711-2738.

Kehimkar, I. 2016. *BNHS Field Guides, Butterflies of India*. Bombay Natural History Society. Mumbai, Oxford University Press. 506 pp.

Kimura, Y., Aoki, T., Yamaguchi, S., Uémura, Y., Saito, T. 2011. *The Butterflies of Thailand. Based on Yunosuke KIMURA Collection Vol. 1. Hesperiidae, Papilionidae, Pieridae.* Mokuyosha, Tokyo. 220 pp.

Osada, S., Uémura, Y., Uehara, J. 1999. *An Illustrated Checklist of the Butterflies of Laos P.D.R*. Mokuyo-sha, Tokyo. 240 pp.

Smetacek, P. 2016. *A Naturalist's Guide to the Butterflies of India, Pakistan, Nepal, Bhutan, Bangladesh and Sri Lanka*. United Kingdom, John Beaufoy Publishing Ltd. 176 pp.

Smith, C. P. 2010. *Lepidoptera of Nepal*. Kathmandu, Himalayan Nature, Sigma General Press. iv + 184 pp.

Smith, C. P. 2011a. *Illustrated Checklist of Nepal's Butterflies, 3rd edition*. Lashkar, Kathmandu. ii + 129 pp.

Smith, C. P. 2011b. *A Photographic Pocket Guide to Butterflies of Nepal*. Kathmandu, Himalayan Map House. 144 pp.

Sondhi, S., Kunte, K. 2016. Butterflies (Lepidoptera) of the Kameng Protected Area Complex, western Arunachal Pradesh, India. *Journal of Threatened Taxa* 8(8): 9053-9124.

Sondhi, S., Kunte, K. 2018. *Butterflies of Uttarakhand - A Field Guide*. Dehradun: Bishen Singh Mahendra Pal Singh; Titli Trust; Bengaluru: National Centre for Biological Sciences; Indian Foundation of Butterflies. x + 310 pp.

Varshney, R. K., Smetacek, P. 2015. *A Synoptic Catalogue of the Butterflies of India*. New Delhi, Butterfly Research Centre, Bhimtal & Indinov Publishing. ii + 261 pp., 8 pl.

Watson, E. Y. 1893. A proposed classification of the Hesperiidae, with a revision of the genera. *Proceedings of the Zoological Society of London* 1893: 3-132.

Xue, G. X., Inayoshi, Y., Li, M., Zhang, F. M., Lai, D. K., Tian, H. Y. 2020. New records of *Celaenorrhinus pyrrha* de Nicéville, 1889 and *C. munda* (Moore, 1884) from China (Lepidoptera, Hesperiidae). *ZooKeys* 985: 61-70.

Yuan, F., Yuan, X. Q., Xue G. X. 2015. *Fauna Sinica. (Insecta Vol.55. Lepidoptera, Hesperiidae)*. China, Science Press. 754 pp., 15 pl.

Taxonomic and distributional notes on *Bolla tepeca* (Bell, 1942), new combination (Lepidoptera: Hesperiidae: Pyrginae)

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Date of issue online: 16 August 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8140763

Abstract: We propose a new generic combination for the Mexican skipper *Pholisora tepeca* Bell, 1942, transferred into the genus *Bolla* Mabille, 1903, based on morphological and molecular data. In addition, taxonomic notes on this species, including a redescription of the male and the first illustrations of the female genitalia, a taxonomic catalogue, and a distributional map, are provided. Finally, we show that *Bolla tepeca* **new comb.** is closely related to *Bolla saletas* (Godman & Salvin, 1896).

Key words: Carcharodini; Mexico; Neotropical; skipper; *Staphylus*.

Resumen: Nosotros proponemos una nueva combinación genérica para la mariposa mexicana *Pholisora tepeca* Bell 1942, transferida para el género *Bolla* Mabille, 1903, basados en datos morfológicos y moleculares. Adicionalmente, notas taxonómicas para esta especie, incluyendo la re-descripción del macho y las primeras ilustraciones de la genitália femenina, un catálogo taxonómico, e un mapa de distribución son presentados. Finalmente, es demostrado que *Bolla tepeca* **new comb.** es relacionada con *Bolla saletas* (Godman & Salvin, 1896).

Palabras claves: Carcharodini; mariposa; Mexico; Neotropical; *Staphylus*.

Resumo: É proposto uma nova combinação genérica para a borboleta mexicana *Pholisora tepeca* Bell, 1942, transferida para o gênero *Bolla* Mabille, 1903, baseado em dados morfológicos e moleculares. Além disso, notas taxonômicas para essa espécie, incluindo a redescrição do macho e as primeiras ilustrações da genitália feminina, um catálogo taxonômico, e um mapa de distribuição são apresentados. Finalmente, é demonstrado que *Bolla tepeca* **new comb.** é fortemente relacionada à *Bolla saletas* (Godman & Salvin, 1896).

Palavras chaves: borboleta; Carcharodini; México; Neotropical; *Staphylus*.

INTRODUCTION

Staphylus Godman & Salvin, 1896 and *Bolla* Mabille, 1903 are two genera of small to medium-sized brown skippers that are notoriously difficult to identify. With very few markings on their wings, the specific identification of most of the species depends on the analysis of the male genitalia (Evans, 1953; Steinhauser, 1989). For females, identification is even more difficult, as only a few species have had their female genitalia illustrated (Mielke, 1975, 1980; Steinhauser, 1989, 1991; Steinhauser & Austin, 1993; Austin, 1997; Austin & Warren, 2002). Moreover, the morphological characters defining both genera are poorly circumscribed (Steinhauser, 1989).

According to Evans (1953), *Bolla* species are larger, the nudum of the antennal club has 13 segments, and the forewing and hindwing outer margins are more even. As for *Staphylus*, the antennal club is shorter, more or less flattened and hollowed, with a nudum of only 10 to 11 segments, and the outer wing margins are usually more undulated. Taxonomical studies are in progress to better establish the diagnostic characters of the two above-cited genera (Lemes *et al*., in prep.).

Recent systematic studies on Hesperiidae using genomic scale approaches have demonstrated that several genera of Carcharodini are not monophyletic (Cong *et al.*, 2019; Zhang *et al.*, 2022). Accordingly, some species previously classified under *Staphylus* and *Bolla* were transferred to the genera *Clytius* Grishin, 2019, *Incisus* Grishin, 2019, *Perus* Grishin, 2019 and *Pholisora* Scudder, 1872.

Although there have been advances towards a more robust classification of skippers in order to maintain the monophyly of genera, these studies have only accessed a limited number of species to date. The inclusion of more species in such studies are likely to result in new taxonomical arrangements. This is the case for *Staphylus tepeca* (Bell, 1942), popularly known as the Grizzled Sootywing (Warren *et al.*, 2017) or the Checkered Scallopwing (iNaturalist).

Bell (1942) described *Pholisora tepeca*, currently in the genus *Staphylus*, from a single male specimen collected by Roberto Müller in Lomas de Chapultepec, now a part of greater Mexico City. This species, endemic to Mexico, is poorly represented in most museum collections, and has a highly restricted geographic distribution. It has most often been encountered in and near the Valley of México, Mexico, although it has been found at several sites in Oaxaca in recent decades and once in Puebla (first record for the state, see results section).

In this study, an integrative approach using morphological and molecular data demonstrated that *Staphylus tepeca* actually belongs in the genus *Bolla*. Therefore, a new combination is proposed as *Bolla tepeca* **new comb**. Moreover, a morphological redescription of the male is provided, including detailed images of the genitalia, as well as the first illustrations of the female genitalia. In addition, a taxonomical catalogue and a distributional map with all known records for *Bolla tepeca* **new comb.** are also provided.

MATERIALS AND METHODS

Specimens deposited in the following collections were used for the molecular and comparative morphological studies: **DZUP** - Coleção Entomológica Padre Jesus de Santiago Moure, Universidade Federal do Paraná, Curitiba, Paraná, Brazil; **JJGD** - Private collection of José de Jesús García-Díaz, Tehuacán, Puebla, Mexico; **MGCL -** McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA; and **OM** - Olaf Hermann Hendrik Mielke Collection, Curitiba, Paraná, Brazil.

For the molecular analysis, legs from two specimens of *Bolla tepeca* **new comb.** were detached and sent to the Canadian Center for DNA Barcoding (CCDB) for DNA extraction, amplification, and sequencing of the mitochondrial cytochrome oxidase subunit 1 (COI) using standardized methods (Hebert *et al.*, 2003). These sequences were included in GenBank with the accession numbers OQ789933-OQ789934 (NCBI, 2020). Sequences of 39 additional closely related Carcharodini species available in GenBank were also used (Table 1). The molecular alignment of COI sequences was performed using the software Muscle in MEGA7 (Kumar *et al.*, 2016). Optimal models of

Table 1. Sequences of mitochondrial cytochrome oxidase subunit 1 (COI) used in this study. Sequences of *Bolla tepeca* **new comb.** in bold. The depository collections of the specimens used in this study and their respective vouchers are also cited.

GenBank accession numbers	Specimen identification	Locality	Reference	Collection - Voucher
MW807713	*Bolla cupreiceps*	Bolivia, La Paz	Earl *et al.* (2021)	
MW807689	*Bolla eusebius*	Guatemala, Sacatepequez	Earl *et al.* (2021)	
JF751716	*Bolla evippe*	Costa Rica, Guanacaste	Janzen *et al.* (2011)	
MW807749	*Bolla imbras*	Mexico, Veracruz	Earl *et al.* (2021)	
OQ789929	*Bolla mancoi*	Brazil, Acre	Lemes *et al.* in press.	
MW807738	*Bolla orsines*	Guatemala, Sacatepequez	Earl *et al.* (2021)	
JF752485	*Bolla zorilla*	Costa Rica, Alajuela	Janzen *et al.* (2011)	
MW807712	*Bolla saletas*	Guatemala, Sololá	Earl *et al.* (2021)	
OQ789933	***Bolla tepeca* new comb.**	**Mexico, Oaxaca**	**This study**	**MGCL - MGCL 1106807**
OQ789934	***Bolla tepeca* new comb.**	**Mexico, Oaxaca**	**This study**	**MGCL - MGCL/FLMNH 37251**
MW807751	*Clytius clytius*	Mexico, Veracruz	Earl *et al.* (2021)	
MW982428	*Gorgopas c. chlorocephala*	Peru, Cusco	Santos *et al.* (2022)	
MZ335173	*Gorgopas trochilus*	Argentina, Jujuy	Attiná *et al.* (2021)	
KP895747	*Hesperopsis alpheus*	United States, California	Pratt *et al.* (2015)	
KP895759	*Hesperopsis gracielae*	United States, Nevada	Pratt *et al.* (2015)	
KP895739	*Hesperopsis libya*	United States, California	Pratt *et al.* (2015)	
OQ789917	*Incisus fasciatus*	Brazil, Paraná	Lemes *et al.* in press.	
OQ789918	*Incisus incisus*	Brazil, Paraná	Lemes *et al.* in press.	
MZ335256	*Perus minor*	Argentina, Salta	Attiná *et al.* (2021)	
MH310833	*Pholisora catullus*	Mexico, Sonora	Pfeiler (2018)	
OQ793590	*Staphylus ascalaphus*	Panama, Colón	Lemes *et al.* in press.	
OQ789924	*Staphylus ascalon*	Brazil, Minas Gerais	Lemes *et al.* in press.	
OQ792217	*Staphylus azteca*	Panama, Panama	Lemes *et al.* in press.	
OQ793589	*Staphylus caribbea*	Costa Rica, Limón	Lemes *et al.* in press.	
OQ789911	*Staphylus hayhurstii*	United States, Oklahoma	Lemes *et al.* in press.	
OQ789923	*Staphylus kayei*	Colombia, Meta	Lemes *et al.* in press.	
OQ789915	*Staphylus lenis*	Guatemala, Petén	Lemes *et al.* in press.	
OQ789921	*Staphylus lizeri lizeri*	Colombia, Meta	Lemes *et al.* in press.	
OQ793588	*Staphylus mazans*	Mexico, Tampa	Lemes *et al.* in press.	
OQ789925	*Staphylus melaina*	Bolivia, Santa Cruz	Lemes *et al.* in press.	
OQ789919	*Staphylus m. melangon*	Brazil, Minas Gerais	Lemes *et al.* in press.	
OQ789913	*Staphylus melius*	Argentina, Salta	Lemes *et al.* in press.	
MF546431	*Staphylus musculus*	Argentina, Entre Rios	Lavinia *et al.* (2017)	
OQ789912	*Staphylus oeta*	Argentina, Jujuy	Lemes *et al.* in press.	
OQ789914	*Staphylus perforata*	Bolivia, La Paz	Lemes *et al.* in press.	
OQ801568	*Staphylus perna*	Brazil, Pernambuco	Lemes *et al.* in press.	
OQ789916	*Staphylus tierra*	Mexico, Jalisco	Lemes *et al.* in press.	
OQ789920	*Staphylus tingo*	Peru, San Martin	Lemes *et al.* in press.	
OQ789922	*Staphylus tucumanus*	Paraguay, Presidente Hayes	Lemes *et al.* in press.	
JQ578308	*Staphylus vulgata*	Costa Rica, Guanacaste	Janzen *et al.* (2011)	
MF547398	*Viola minor*	Argentina: Buenos Ayres	Lavinia *et al.* (2017)	

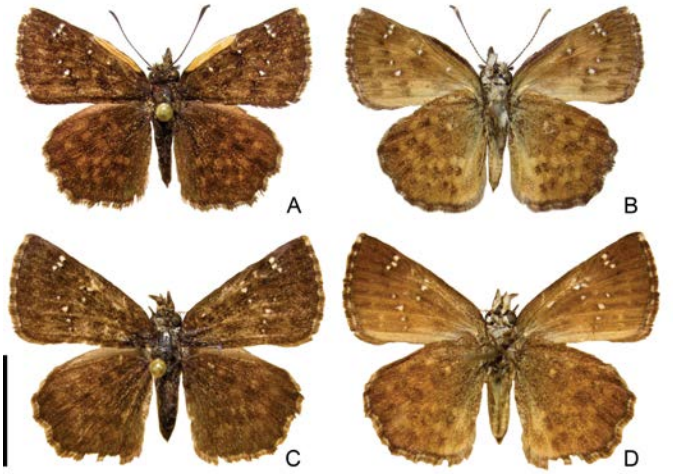

Figure 1. *Bolla tepeca* (Bell, 1942) **new comb.** in dorsal and ventral views: **(A-B)** male (Mexico, Oaxaca, La Cumbre, El Punto, 22.IV.1989, MGCL 1092164), **(C-D)** female (Mexico, Oaxaca, Sierra Madre del Sur, El Guajolote, 14.VI.1989, MGCL/FLMNH 37251). Scale bar = 1 cm.

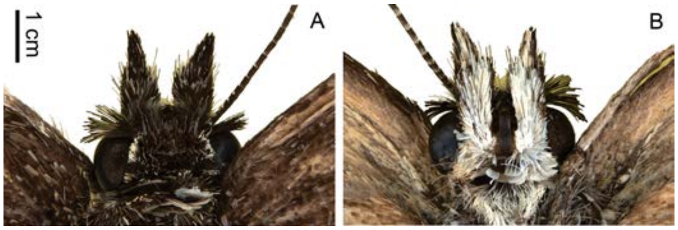

Figure 2. Male head of *Bolla tepeca* (Bell, 1942) **new comb.** in dorsal **(A)** and ventral **(B)** views (Panama, Chiriquí, Alto Lino, 6.IV.1989, MGCL 1106966).

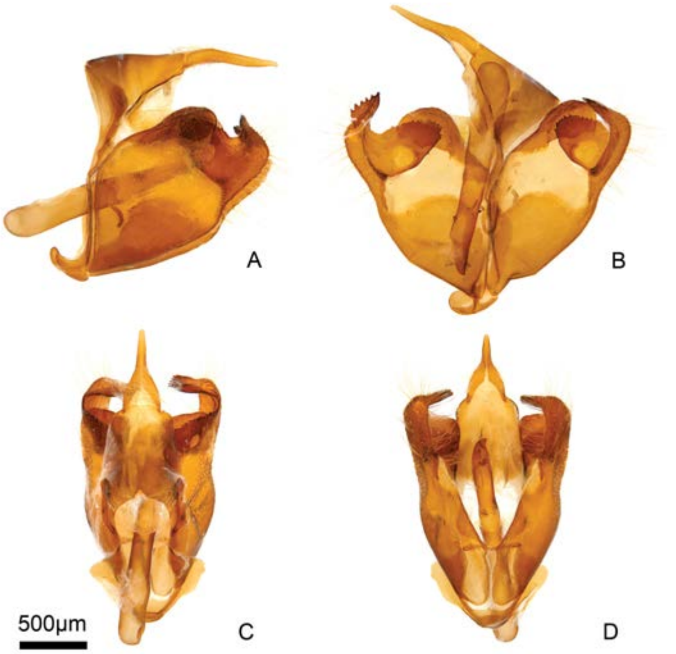

Figure 3. Male genitalia of *Bolla tepeca* (Bell, 1942) **new comb.** in lateral **(A)**, internal **(B)**, dorsal **(C)** and ventral **(D)** views (Mexico, Oaxaca, La Cumbre-El Punto, 22.IV.1989, MGCL 1112110).

Figure 4. Sterigma (female genitalia) of *Bolla tepeca* (Bell, 1942) **new comb.** in ventral **(A)** and lateral **(B)** views (Mexico, Oaxaca, Sierra Madre del Sur, El Guajolote, 14.VI.1989, MGCL/FLMNH 37251).

nucleotide evolution were selected through a partition model by ModelFinder (Kalyaanamoorthy *et al.*, 2017) using the AICc criterion, resulting in TIM3+F+I+G4 for the subsets 1 and 2 and GTR+F+G4 for the subset 3. Maximum likelihood analysis was conducted using the software IQ-Tree v. 2.2.0 (Minh *et al.*, 2020). For the maximum likelihood analysis, the ultra-fast bootstrap approximation method - UFBoot (5,000 bootstrap repetitions) was used (Minh *et al.*, 2013), together with the SH-aLRT test (1,000 repetitions) (Guindon *et al.*, 2010), and with the Bayesian-like transformation of aLRT (aBayes) (Anisimova *et al.*, 2011). The tree obtained was visualized and initially edited in the software FigTree v.1.4.3 (Rambaut, 2006-2009), and later in the software Inkscape 1.0.1 (Harrington *et al.*, 2004-2005). The tree was rooted with *Viola minor* (Hayward, 1933). Branches were considered supported when SH-aLRT ≥ 80, aBayes ≥ 0.5 and UFBoot ≥ 95.

For the morphological analysis, specimens had their abdomen detached and soaked in a heated 10% potassium hydroxide solution (KOH) for 5-10 minutes, and subsequently dissected for the study of the genitalia. Dissected genitalia were stored in vials with glycerin, labeled accordingly, and photographed using a photo stacking process (Leica Application Suite X software) associated with a Leica M205C microscope and a Leica DMC 5400 camera. The general terminology follows Evans (1953), and the genitalia morphology follows Carneiro *et al.* (2013). The following abbreviations were used throughout the paper: DW (dorsal wing), FW (forewing), DFW (dorsal forewing), VFW (ventral forewing), HW (hindwing), DHW (dorsal hindwing), and VHW (ventral hindwing).

A taxonomical catalogue including all publications mentioning the species is provided below, updated from Mielke (2005). The distributional map was produced with SimpleMappr (Shorthouse, 2010) from the label data of the specimens analyzed, iNaturalist (iNaturalist, 2022), and literature records. In the "examined material" section, the symbol "*" indicates that the specimen had its genitalia dissected and studied.

RESULTS

Bolla tepeca (Bell, 1942), **new combination**

Figs. 1-5

Pholisora tepeca Bell, 1942. **An. Esc. Nac. Cienc. Biol.**, Mexico, **2**(4): 458, fig. 2 (male gen.); holotype male, Lomas de Chapultepec, Mexico D.F.,

Figure 5. Live male adults of *Bolla tepeca* (Bell, 1942) **new comb.** photographed by John Kemner in Oaxaca, Mexico (approximately 2150 m): **(A)** 7 km. NE San Miguel Etla, 27.V.2021; **(B)** Dist. Etla, Mpo. San Juan Bautista Guelache, stream 5 km. NE San Miguel Etla, 1.V.2017; **(C)** 5 km. NE San Miguel Etla, 12.V.2017; **(D)** Dist. Miahuatlán, 1 km. N. San Sebastián Río Hondo, 16.III.2017.

Mexico, R. Müller *leg.*, nº 15.192; collection Escuela Nacional Ciencias Biologicas, Mexico.- Beutelspacher, 1980. **Marip. diurn. Valle Mexico**, p. 99, pl. 14, fig. 9 (male d).- Hernández Baz, 1993. **La Cienc. y Hombre 14**: 78.

Staphylus tepeca; Evans, 1953. **Cat. Amer. Hesp. 3**, p. 89, pl. 37 (male gen.).- Okano, 1981. **Tokurana 1**: 80.- Bridges, 1983. **Lep. Hesp. 1**, p. 117; **2**, p. 34.- Bridges, 1988. **Cat. Hesp. 1**, p. 185; **2**, p. 57.- Llorente-Bousquets *et al*., 1990. **Publ. esp. Mus. Zool.**, Mexico, **1**: 30.- J. de la Maza-Elvira, White & R. G. de la Maza-Elvira, 1991. **Rev. Soc. Mex. Lep. 14**(1): 19.- Bridges, 1994. **Cat. Fam.-Group, Gen.-Group, Sp. Group Nam. Hesp. (Lep.) World 8**, p. 222; **9**, p. 64.- Llorente-Bousquets *et al*., 1996. **Dugesiana 3**(2): 5.- Warren *et al*., 1998. **Jour. Lep. Soc. 52**(1): 46, 51.- Warren, 2000, *in* Llorente, *et al*. (eds). **Biodiv., Tax., Biogeogr. Art. Mex. 2**, p. 552.- Luis-Martínez *et al*., 2003. **Proc. Ent. Soc. Wash. 105**(1): 222.- O. Mielke, 2004. Hesperioidea, p. 56, *in* Lamas (ed.). **Checklist: Part 4A, Hesperioidea-Papilionoidea**, *in* Heppner (ed.). **Atlas Neotrop. Lep. 5A**.- Luis-Martínez ***et al***., 2004, *in* García-Mendoza *et al*., **Biodiv. Oaxaca**, p. 353. - Michán *et al*., 2004, in Llorente-B *et al*., **Biod., Tax. y Biog. Artrop. México 4**, p. 41.- O. Mielke, 2005. **Cat. Amer. Hesperioidea 3**, p. 735.- Salinas-Gutiérrez *et al*., 2005. **Folia Entomol. Mex. 44**(3): 315.- Glassberg, 2007. **Swift Guide Butt. Mexico and C. America**, p. 188, figs (male, female d).- Díaz-Batres & Llorente-Bousquets, 2011. **Mariposas de Chapultapec**, Mexico, p. 126, figs. (male, d, v).- Garwood & Lehman, 2013. **Butt. C. Amer. 3, Hesp**., p. 107, figs (d, v).- Luis-Martínez *et al*., 2016. **Southw. Ent. 41**(1): 191.- Vargas-Fernández *et al.* 2016, Mariposas Diurnas (Rhopalocera), apêndice 1, p. 186, *in* Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. (Conabio). **La Biodiversidad en Colima**. Estudio de Estado. Mexico.

(no genus) *tepeca*; Beattie, 1976. **Rhop. Direct**., p. 271.

Taxonomic history. Bell (1942) described *Pholisora tepeca*. Evans (1953) placed the species in the genus *Staphylus*; this combination has been followed by almost all subsequent authors that have mentioned this species in taxonomic comments, faunistic studies, and catalogs.

Type. *Pholisora tepeca* Bell, 1942 was described from a male holotype from Lomas de Chapultepec, [DF] (now Mexico City), Mexico, collected by Roberto Müller, said to be deposited in the collection of the Escuela Nacional de Ciencias Biológicas, Mexico. The holotype of *Pholisora tepeca* is currently deposited in the AMNH with the following labels: / HOLOTYPE [red label]/ Lomas de Chapultepec S. F. V./ R. Müller Coll. 15192/ slide 15192./ G1991/ Holotype ♂ *Pholisora tepeca* Bell/ (Warren *et al*., 2023).

Diagnosis (Figs. 1-5). *Bolla tepeca* can be distinguished from other species of *Bolla* by its single or double hyaline spot in the forewing discal cell, reddish-brown dorsal and yellow reddish-brown ventral ground color of the wings and the darker brown rounded patches on the discal and postdiscal area of VHW. The male genitalia are similar to those of *Bolla saletas* (Godman & Salvin, 1896) (Fig. 9), with valva longer than wide and somewhat rectangle-shaped, sacculus broad, rounded, and harpe exceeding ampulla and bearing a serrated dorsal margin. However, the harpe on *B. tepeca* **new comb.** possesses a strongly curved process that transposes the ampulla, while *B. saletas* lacks such a process. Moreover, the aedeagus of *B. saletas* is distally bifid, while *B. tepeca* **new comb.** has a cylindrical aedeagus. The female genitalia of *B. tepeca* **new comb.** possesses a lamella antivaginalis developed as two large, separate triangular, sclerotized plates, distinct from other known species of *Bolla*.

Male redescription (Figs. 1A-B, 2, 3, 5)**.** ***Head:*** Brown, with some yellow scales dorsally; pure white with some black and yellow hair-like scales ventrally,

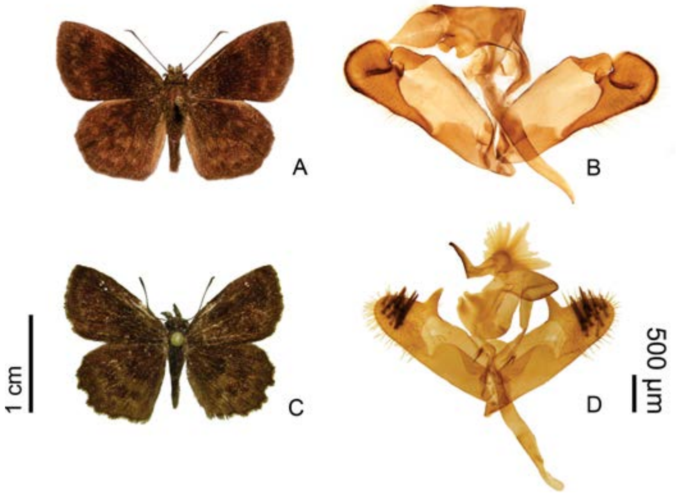

Figure 6. Type species of *Bolla* and *Staphylus* in dorsal views and male genitalia in internal view. **(A-B)** *Bolla imbras* (Godman & Salvin, 1896), **(A)** El Salvador, Santa Tecla, 19.VIII.1972 (MGCL); **(B)** Guatemala, Retalhuleu Province, NN Nuevo San Carlos, 22.XII.2002, Ulrich Reber leg., MGCL 1112131 (MGCL). **(C-D)** *Staphylus ascalaphus* (Staudinger, 1876), **(C)** Guatemala, Zacapa, La Unión, 3.X.1981, Welling leg., DZ 45.176 (DZUP); **(D)** Mexico, Chiapas, San Jerónimo, 17.VII.1978, Welling leg., OM 44.713 (OM).

except the third segment of the palpus which is brown. Antenna brown dorsally, ventrally with small yellow dots at the joints on the side. Nudum with 13 segments (n = 5). ***Thorax:*** Brown with yellow hair-like scales dorsally, brown with white hair-like scales ventrally. Legs brown with yellow scales and white hair-like scales. ***FW length:*** 1.3 cm (n = 2). ***FW shape:*** Outer margin rounded. ***DFW:*** Reddish brown. Two distinct transverse bands with paler pattern in the discal and postdiscal areas, which gives the wings a maculated aspect; few sparse paler scales present across wing. Costal fold present. Three small white dots in the subapical area. One white dot in the upper distal end of the discal cell (some specimens with an additional white dot in the lower distal end of the discal cell), and the other two between M$_3$-CuA$_1$ and CuA$_1$-CuA$_2$. Fringe bicolored, paler and darker brown. ***VFW:*** Yellow reddish-brown, much paler than DFW. Spots as on DFW but surrounded by darker brown scales. Fringe as on DFW, but darker. ***HW shape:*** Outer margin slightly undulated. ***DHW:*** Reddish brown. Two distinct transverse bands with paler pattern in the postdiscal and central area, which gives the wings a maculated aspect. Some pale scales and long, thin, brown hair-like scales present in basal area. Fringe as on FW. ***VHW:*** Yellow reddish-brown, much paler than DHW. Three transverse lines of small darker brown rounded patches present on the sub-basal, discal and postdiscal areas. Paler scales sparsely distributed, predominantly on the internal margin. ***Abdomen*****:** Brown dorsally and ventrally with scattered paler scales. ***Genitalia*****:** Tegumen slightly longer than wide, except the proximal margin that connects with the ventral arms of the tegumen, which is convex. Ventral arms of the tegumen narrow and fused with dorsal projection of saccus, assuming that the boundaries between these structures are at the angle between them. Saccus triangular, short, rounded proximally. Uncus longer than wide, enlarged at the base, ventrally with a large concavity that becomes a longitudinal opening towards the narrower apex, bearing some short setae dorsally. Gnathos slightly sclerotized, as two lateral plates connected ventrally. Valva longer than wide in lateral view; sacculus broad, rounded; harpe bifid, the ventrodistal lobe strongly serrated distally and the dorsal subterminal lobe rounded, weakly serrated at the distal margin and reaching the center of ampulla; ampulla longer than wide, rounded at the dorsal-distal margin, fitting in with the harpe's process; costa not well defined. Aedeagus cylindrical, about the same length as valva, insertion of manica in the middle of aedeagus; vesica without cornuti. Fultura inferior developed, thin, moon-shaped in anterior view.

Female description (Figs. 1C-D, 4)**.** The female differs from the male as follows: ***FW length:*** 1.4 cm (n = 1). The FW has five white dots: two (one above the other) in the discal cell, one at the base of R$_2$-R$_3$, just below the lower discal cell spot, and two in CuA$_2$ and 2A. ***Genitalia.*** Corpus bursae membranous, globular, about 1/3 the length of the ductus bursae; ductus bursae membranous. Lamella antevaginalis forming two large, triangular, sclerotized plates, which project bellow the lamella postvaginalis. Lamella postvaginalis as a sclerotized plate with fine microtrichia, rounded at the proximal margin and with the distal margin nearly straight, with a subtle central concavity on the ventral margin that is produced into a subtle center carina; laterally expanded as a triangular projection. Papilla analis semi-rounded, covered by setae; posterior apophysis slightly curved, about the same length as papilla analis.

Comments (Fig. 6). *Bolla tepeca* **new comb.** shows some similarities in the male genitalia when compared with *B. imbras* (Godman & Salvin, 1896) (the type species of the genus). The uncus, in ventral view, has a proximal rounded opening that turns into a slit towards the distal portion. In *Staphylus ascalaphus*, the type species of *Staphylus*, the slit opening is

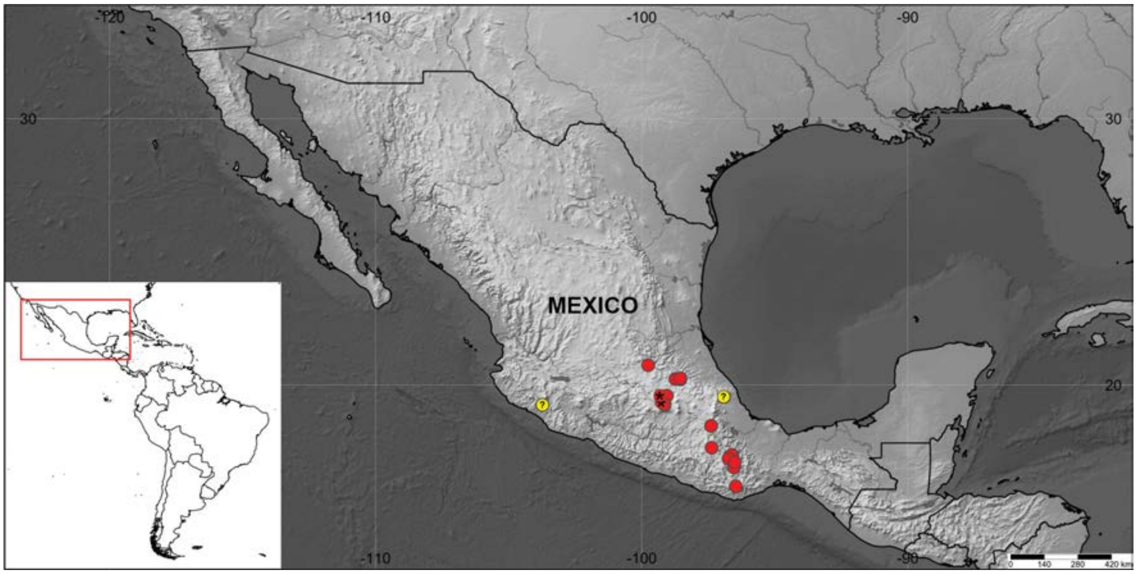

Figure 7. Distributional map of *Bolla tepeca* (Bell, 1942) **new comb.** The symbol "*" indicates literature records, and the yellow circles with "?" indicate questionable literature records.

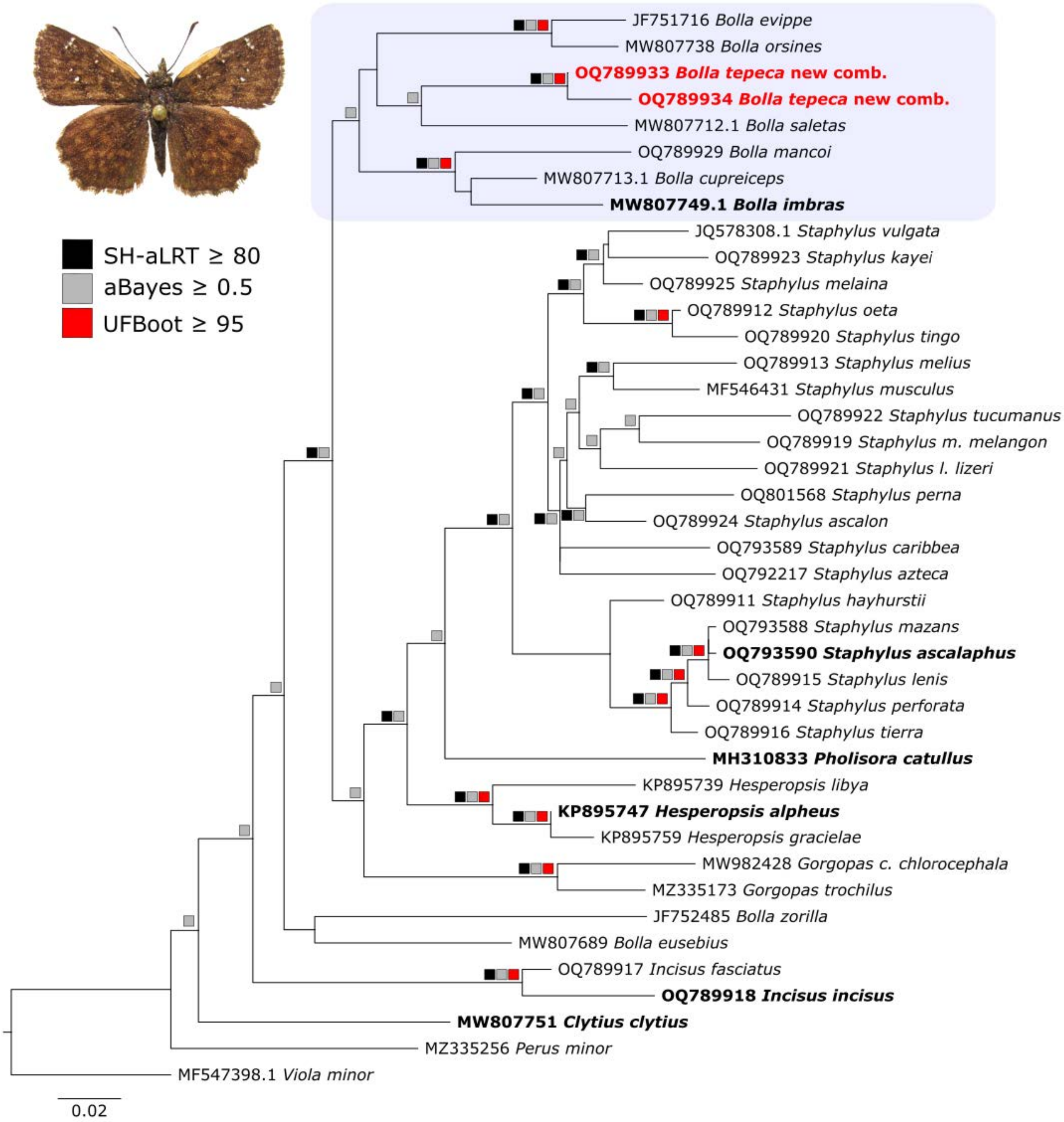

Figure 8. Relationships among *Bolla tepeca* (Bell, 1942) **new comb.** and other species of Carcharodini based on DNA COI sequences obtained by Maximum likelihood inferred with IQTREE. The blue rectangle represents the genus *Bolla*. The type species of different genera used in the analysis are bold highlighted. Support values were considered sufficient when SH-aLRT ≥ 80, aBayes ≥ 0.5 and UFBoot ≥ 95.

absent. Besides that, the uncus in lateral view of *S. ascalaphus* bends in a somewhat right-angle at the center and bears dorsally a prominent tuft of long-sized hairs proximally, which are absent in *B. tepeca* **new comb.** and *B. imbras*. Another difference is the presence in *S. ascalaphus* of a set of prominent socketed spines directed posteriorly at lateral margins of the fultura superior, absent in *B. tepeca* **new comb.** and *B. imbras*. The valva of *S. ascalaphus* presents the ampulla fused with harpe, which externally bears a set of stout socketed spines, while in *B. tepeca* **new comb.** and *B. imbras* the ampulla is clearly separated from harpe, and the harpe externally lacks the spines.

Distribution (Fig. 7)**.** *Bolla tepeca* **new comb.** is endemic to Mexico, with confirmed records from Distrito Federal (now Ciudad de México) (Beutelspacher, 1980), in the northern region of the Tehuacán-Cuicatlán Valley in Puebla (herein), Oaxaca (Luis-Martínez et al. (2016); Warren *et al.*, 2017; iNaturalist), Hidalgo and Querétaro (iNaturalist), and with questionable records from Veracruz (Hernández-Baz, 1993) and Colima (Llorente-Bousquets *et. al.*, 1996). Sites where *B. tepeca* **new comb.** has been found range from 2011-2500m elevation. Hernández-Baz (1993) reported *Pholisora tepeca* from Xalapa, Veracruz, from the month of May. We have not

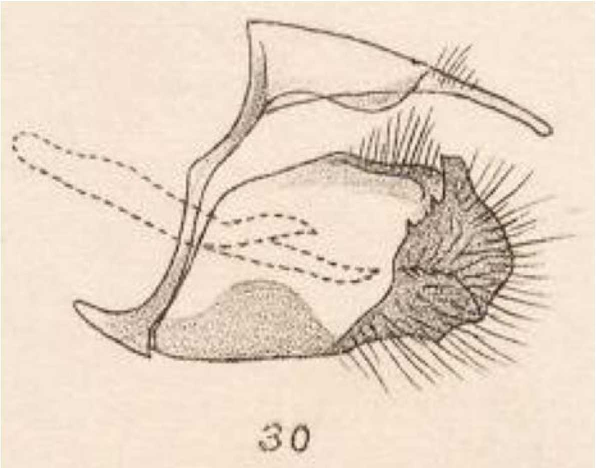

Figure 9. Illustration of the male genitalia of *Bolla saletas* (Godman & Salvin, 1896) from Godman & Salvin (1896, volume 3, plate 89, fig. 30).

examined material upon which this record is based, but feel the possibility of a misidentification exists, given the low elevation (~1190-1520m) and tropical climate of the Xalapa area. As noted by Warren *et al.* (1998), the record of *B. tepeca* **new comb.** from Colima is based on material in the AMNH simply labeled "Colima", without specific locality data. Given that no additional specimens of *B. tepeca* **new comb.** have been found in Colima or other western Mexican states, we suspect that the AMNH material is likely to be mislabeled, and probably from the Valley of México.

Biology. While poorly represented in most collections, *Bolla tepeca* **new comb.** may be locally abundant in the Valley of México (Díaz-Batres & Llorente-Bousquets, 2011). As noted by Beutelspacher (1980) and Díaz-Batres & Llorente-Bousquets (2011), *B. tepeca* **new comb.** flies from April to September in the Valley of México. No information on the larval foodplant of *Bolla tepeca* **new comb.** has been reported.

Etymology. Although not mentioned in the original description, the epithet *tepeca* is clearly in reference to the type locality, Lomas de Chapultepec (Mexico City). As noted by Díaz-Batres & Llorente-Bousquets (2011), *Bolla tepeca* **new comb.** remains common within the Bosque de Chapultepec, a protected natural area adjacent to the Lomas de Chapultepec neighborhood, surrounded by urban development of greater Mexico City.

Material examined. Mexico – ***Puebla*****: Tehuacán,** 1.VIII.2012, 1♂, J. J. García-Díaz *leg.* (JJGD). ***Oaxaca*****: La Cumbre-El Punto**, 13.IV.1989, 1♂, John Kemner *leg*., Allyn Museum Photo No. 930921-5,6. Genitalia Vial SRS-4464* Allyn Museum Acc. 1989-7, DNA Voucher JRAL-COI-07 José R. A. Lemes, MGCL 1106807 (MGCL); 22.IV.1989, 3♂, John Kemner *leg*., G. T. Austin colln. MGLC Accession #2004-5, MGCL 1092164, Gen. Prep. Lemes 2021-45*, MGCL 1112110 (MGCL). **Sierra Madre del Sur**, **El Guajolote**, 14.VI.1989, 1♀, John Kemner *leg*., G. T. Austin colln. MGCL Acc. 2004-5, DNA Voucher JRAL-COI-08 José R. A. Lemes, MGCL/FLMNH 37251* (MGCL**)**. **San Miguel**, 10 miles S., 8500 ft., 6.IV.1989, 1♂, John Kemner *leg*., Allyn Museum Acc. 1989-7. DNA voucher LEP-79278, MGCL 1106966 (MGCL). Additional specimens from the Valley of México (Mexico City) and Oaxaca, examined by ADW without recording details, are deposited at the Colección Entomológica Alfredo Barrera del Museo de Historia Natural y Cultura Ambiental de la Ciudad de México (MHNCM), and at the Museo de Zoología, Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC), respectively.

DISCUSSION

Evans' (1953) classification of new world Pyrginae, while comprehensive, was limited in that he only studied the species that were available in the Natural History Museum (NHMUK), London, United Kingdom. Therefore, while placing species into his generic framework, for many species the only information Evans had available was the original descriptions. This was the case with *Bolla tepeca* **new comb.**, as Evans (1953, p. 89) cites the absence of this species in the NHMUK. According to Evans (1953), the forewings of *Bolla* species never have a hyaline discal cell spot. *Bolla tepeca* **new comb.**, however, has a hyaline spot in the upper (and sometimes lower) part of the discal cell. This was likely one of the reasons that Evans included this species in *Staphylus*. Additionally, in the original description of *Pholisora tepeca*, Bell (1942) compared the new taxon only to *Pholisora* (now *Staphylus*) *mazans* (Reakirt, [1867]), implying a close relationship between the two.

Our COI analysis recovered *Bolla* as a paraphyletic taxon, with a separated clade including *B. eusebius* (Plötz, 1884) and *B. zorilla* (Plötz, 1886). Although a taxonomical revision of *Bolla* is not the purpose of this manuscript, the authors are studying the genus to better elucidate its taxonomical issues (Lemes *et al.* in prep.). Despite that, *Bolla tepeca* **new comb.** was recovered within the clade of the type species of *Bolla*, *B. imbras*. Moreover, our analysis demonstrated that *Bolla tepeca* **new comb.** is the sister-taxon of *Bolla saletas* (Godman & Salvin, 1896) (Fig. 8). In fact, *B. saletas* (adult photos available in Warren *et al.* (2023)) shares many morphological similarities with *B. tepeca* **new comb.**, including checkered wing fringes, patches of reddish-brown scales on the dorsal wings, presence of a costal fold, palpi with yellowish scales dorsally and whitish scales ventrally, male genitalia (Fig. 9) with a tubular uncus, gnathos formed by two lateral plates, valva with sacculus broad and rounded, and harpe strongly developed and ornamented with serrated dorsal margins.

Bolla tepeca **new comb.** is one of the few species of *Bolla* that can be identified without examining the genitalia. While most species of *Bolla* and other Carcharodini (*e.g.*, *Staphylus*) have brown wings without many conspicuous markings, *B. tepeca* **new comb.** has a very characteristic wing maculation. The checkered wing fringes, grizzled aspect dorsally, and the single or double spot in the forewing discal cell separate *B. tepeca* **new comb**. from all other species of *Bolla*.

With the removal of *Bolla tepeca* **new comb.**, 47 species are now included in *Staphylus*, while 29 species are in *Bolla* (Mielke, 2004, 2005; Warren *et al.*, 2023; Cong *et al.*, 2019; Zhang *et al.*, 2022a; Zhang *et al.*, 2022b). Additional taxonomic studies on these two genera are in progress by the authors, including descriptions of new species, in an effort to better understand the boundaries between these two similar genera of skippers (Lemes *et al.*, in prep.).

ACKNOWLEDGMENTS

JRAL thanks the Society of Systematic Biologists for the grant “Graduate Student Research Award (GSRA)” used for COI sequencing; a special thanks to Lyle Buss, Department of Entomology and Nematology, University of Florida, for providing access to the laboratory and equipment used for stacking photos, and to Deborah Matthews Lott, McGuire Center for Lepidoptera and Biodiversity, University of Florida, for assistance; thanks to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - 88882.382399/2019-01), Programa Institucional de Internacionalização (CAPES-PRINT 88887.474486/2020-00) for research fellowships. RRS thanks the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 2020/09595-0) for the research fellowship. MMC and OHHM thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 302084/2017-7 and CNPq - 304849/2019-7) for research fellowships. ADW thanks Jorge Llorente-Bousquets, Armando Luis-Martínez, Isabel Vargas-Fernández (MZFC) and María Eugenia Díaz-Batres (MHNCM) for access to the Lepidoptera collections under their care. The authors thank the Canadian Center for DNA Barcoding (CCDB) for molecular data, José de Jesús García-Díaz (Tehuacán, Puebla, Mexico) (JJGD) for access to specimens under his care, and John Kemner for providing live photos of *Bolla tepeca* **new comb.** The authors also thank Diego Rodrigo Dolibaina, Fernando Maia Silva Dias, Eduardo Carneiro dos Santos, Mônica Piovesan, and especially José de Jesús García Díaz and Shinichi Nakahara for the valuable suggestions made on this manuscript.

LITERATURE CITED

Anisimova, M., Gil, M., Dufayard, J.-F., Dessimoz, C., Gascuel, O. 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology* 60(5): 685-699.

Attiná, N., Núñez Bustos, E. O., Lijtmaer, D. A., Hebert, P. D. N., Tubaro, P. L., Lavinia, P. D. 2021. Genetic variation in neotropical butterflies is associated with sampling scale, species distributions, and historical forest dynamics. *Molecular Ecology Resources* 21(7): 2333-2349.

Austin, G. T. 1997. Notes on Hesperiidae in northern Guatemala, with descriptions of new taxa. *Journal of the Lepidopterists’ Society* 51(4): 316-332.

Austin, G. T., Warren, A. D. 2002. Taxonomic notes on some Neotropical skippers (Lepidoptera: Hesperiidae): Pyrrhopyginae and Pyrginae. *Dugesiana* 9(2): 15-49.

Beattie, J. R. 1976. *The Rhopalocera Directory. Insecta Directory*. Berkeley, JB Indexes. 1: xiv + 365 pp.

Bell, E. L. 1942. New records and new species of Hesperiidae from Mexico. (Lepidoptera: Hesperiidae). *Anales de la Escuela Nacional de Ciencias Biológicas* (México) 2(4): 455-468.

Beutelspacher, C. R. 1980. *Mariposas diurnas del Valle de México*. México, Ediciones Científicas, La Prensa Médica Mexicana. [viii] + 134pp.

Bridges, C. A. 1983. *Lepidoptera: Hesperiidae. Notes on species-group names*. Urbana, Author. [2] + ii + 129 + 41 + 62 + 30 + 13 pp.

Bridges, C. A. 1988. *Catalogue of Family-Group And Genus-Group Names* (*Lepidoptera*: *Rhopalocera*). Urbana, Author. vi + ii + 8 + ii + 3 + ii + 4 + ii + 130 + ii + 33 + ii + 67 + ii + 18 + ii + 59 + ii + 20 + ii + 8 pp.

Bridges, C. A. 1994. *Catalogue of the Family-Group, Genus-Group and Species-Group Names of the Hesperioidea (Lepidoptera) of the World*. Urbana, Author. [xiv] + xxxii + 598 pp.

Carneiro, E., Mielke, O. H. H., Casagrande, M. M. 2013. Thorax and abdomen morphology of some Neotropical Hesperiidae (Lepidoptera). *Insecta Mundi* 327: 1-47.

Cong, Q., Zhang, J., Shen, J., Grishin, N. V. 2019. Fifty new genera of Hesperiidae (Lepidoptera). *Insecta Mundi* 731: 1-56.

Díaz-Batres, M. E. E., Llorente-Bousquets, J. 2011. *Mariposas de Chapultepec. Guía Visual*. Cospapalotl, Mexico City. 156 pp.

Earl, C., Belitz, M. W, Laffan, S. W., Barve, V., Barve, N., Soltis, D. E., Allen, J. M., Soltis, P. S., Mishler, B. D., Kawahara, A. Y., Guralnick, R. 2021. Spatial phylogenetics of butterflies in relation to environmental drivers and angiosperm diversity across North America. *IScience* 24(4): 1-18.

Evans, W. H. 1953. *A Catalogue of the American Hesperiidae Indicating the Classification and Nomenclature Adopted in The British Museum (Natural History). Part III. (Groups E, F, G) Pyrginae. Section 2.* London, British Museum. v + 246 pp.

Garwood, K., Lehman, R. 2013. *Butterflies of Central America. A Photographic Checklist of Common Species. Volume 3: Hesperiidae, the Skippers*. McAllen, RiCalé Publishing. xii + 288 pp.

Glassberg, J. 2007. *A Swift Guide To The Butterflies of Mexico and Central America*. Morristown, Sunstreak Books, Inc. [vi] + 266 pp.

Godman, F. D., Salvin, O. 1896. *Biologia Centrali-Americana. Insecta. Lepidoptera-Rhopalocera.* Volume 2. London, Taylor & Francis. 1269 pp.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307-321.

Harrington, B., Gould, T., Hurst, N., MenTaLgu, Y. 2004-2005. Inkscape. http://www.inkscape.org/.

Hayward, K. J. 1933. Lepidopteros Argentinos. Familia Hesperiidae III. *Revista de la Sociedad Entomológica Argentina* 5(24): 219-275.

Hebert, P. D. N., Cywinska, A., Ball, S. L., deWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 313-321.

Hernández-Baz, F. 1993. La fauna de mariposas (Lepidoptera: Rhopalocera) de Xalapa, Veracruz, México. *La Ciencia y Hombre* 14: 1-87.

iNaturalist. https://www.inaturalist.org. Accessed 11 March 2022.

Janzen, D. H., Hallwachs, W., Burns, J. M., Hajibabaei, M., Bertrand, C., Hebert, P. D. N. 2011. Reading the complex skipper butterfly fauna of one tropical place. *Plos One* 6(8): e19874.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., Jermiin, L. S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587-589.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33, 1870-1874.

Lavinia, P. D., Núñez-Bustos, E. O., Kopuchian, C., Lijtmaer, D. A., García, N. C., Hebert, P. D. N., Tubaro, P. L. 2017. Barcoding the butterflies of southern South America: Species delimitation efficacy, cryptic diversity and geographic patterns of divergence. *Plos One* 12(10): e0186845.

Lemes, J. R. A., Siewert, R. R., Mielke, O. H. H., Casagrande, M. M., Warren, A. D. *in press.* Description of *Uniphylus* gen. nov., a new genus of Carcharodini (Lepidoptera, Hesperiidae, Pyrginae) for *Staphylus evemerus* Godman & Salvin, 1896. *Anais da Academia Brasileira de Ciências* in press.

Llorente-Bosquets, J. E., Luis-Martinez, A., Vargas-Fernandez, I. 1990. Catalogo sistemático de los Hesperioidea de Mexico. *Publicaciones especiales del Museo de Zoología. Facultad de Ciencias. Universidad nacional autónoma de México* 1: 1-70.

Llorente-Bosquets, J. E., Warren, A. D., Vargas, I., Armando Luis, M. 1996. Mariposas diurnas de Colima. *Dugesiana* 3(2): 1-18

Llorente-Bousquets, J., Warren, A. D., Vargas-Fernández, I. & Luis-Martínez, A. 1996. Mariposas diurnas de Colima. *Dugesiana* 3(2): 1-18.

Luis-Martínez, A., Llorente-Bousquets, J. E., Vargas Fernández, I., Warren, A. D. 2003. Biodiversity and biogeography of Mexican butterflies (Lepidoptera: Papilionoidea and Hesperioidea*). Proceedings of the Entomological Society of Washington* 105 (1): 209-224.

Luis-Martínez, A., Llorente-Bousquets, J., Warren, A. D., Vargas-Fernández, I. 2004. *Lepidópteros: Papilionoideos y Hesperioideos*, pp. 335-355. *In*. García-Mendoza, A. J., Ordóñez, M. J., Briones-Salas, M. (Eds.), *Biodiversidad de Oaxaca*. México, D. F., Instituto de Biología, UNAM, Fondo Oaxaqueño para la Conservación de la Naturaleza, World Wildlife Fundation.

Luis-Marínez, A., Hernández-Mejía, B., Trujano-Ortega, M., Warren, A., Salinas-Gutiérrez, J., Ávalos-Hernández, O., Vargas-Fernández, I., Llorente-Bousquets, J. 2016. Avances faunísticos en los Papilioidea (Lepidoptera) *sensu lato* de Oaxaca, México. *Southwestern Entomologist* 41(1): 171-224.

Mabille, P. 1903-4. *Lepidoptera Rhopalocera Fam. Hesperidae* [sic]. *In:* Wystman, P. A. G. (Ed.), *Genera Insectorum* 17: [ii] + 210 pp.

Maza, J. de la, White, A., Maza, R. G. de la. 1991. La fauna de mariposas de México. Parte II. Hesperioidea (Lepidoptera: Rhopalocera). *Revista de la Sociedad mexicana de Lepidopterología* 14(1): 3-44.

Michán, L.; Llorente Bousquets, J., Luis Martínez, A., Castro, D. J. 2004. *Breve história de la taxonomía de Lepidoptera em México durante el Siglo XX*, pp. 5-42. *In*: Llorente Bousquets, J., Morrone, J. J., Ordóñez, O. Y., Fernández, I. V. (Eds.), *Biodiversidad, Taxonomía y Biogeografía de Artrópodos de México: Hacia una síntesis de su conocimiento*, v 4.

Mielke, O. H. H. 1975. Sobre algumas espécies de *Staphylus* Godman & Salvin (Lepidoptera: Hesperiidae). *Acta biologica paranaense* 4(1/2): 25-34.

Mielke, O. H. H. 1980. Contribuição ao estudo faunístico de Hesperiidae americanos. V. Nota suplementar às espécies de Pyrrhopyginae e Pyrginae do Rio Grande do Sul, Brasil (Lepidoptera). *Acta biologica paranaense* 8/9: 7-17.

Mielke, O. H. H. 2004. *Hesperiidae*, pp. 25-86. *In*: Lamas, G. (Ed.), *Checklist: Part 4A. Hesperioidea - Papilionoidea*. *In*: Heppner, J. B. (Ed.), *Atlas of Neotropical Lepidoptera. Volume 5A*. Gainesville, Association for Tropical Lepidoptera; Scientific Publishers.

Mielke, O. H. H. 2005. *Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera)*. Curitiba, Sociedade Brasileira de Zoologia. 1: xiii + 125 pp.; 2: [ii] + 127-410; 3: [ii] + 411-771; 4: [ii] + 773-1055; 5: [ii] + 1057-1383; 6: [ii] + 1385-1536

Minh, B. Q., Nguyen, M. A. T., von Haeseler, A. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution* 30: 1188-1195.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., Lanfearm, R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530-1539.

National Center for Biotechnology Information (NCBI). 2020. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] - [28 Nov. 2020]. https://www.ncbi.nlm.nih.gov/.

Okano, K. 1981. Studies on the Mexican butterflies (1). Additions and revisions to the Hoffmann's "Catálogo sistemático y zoogeográfico de los lepidópteros mexicanos." Part 1. Hesperiidae (Lepidoptera: Rhopalocera). *Tokurana* 1: 23-139.

Pfeiler, E. 2018. DNA barcoding and taxonomic challenges in describing new putative species: examples from Sootywing and Cloudywing Butterflies (Lepidoptera: Hesperiidae). *Diversity* 10: 1-14.

Plötz, C. 1884. Die Hesperiinen-Gruppe der Achlyoden. *Jahrbücher des nassauischen Vereins für Naturkunde* 37: 1-55.

Plötz, C. 1886. Nachtrag und Berichtigungen zu den Hesperiinen. *Stettiner entomologische Zeitung* 47: 83-117.

Pratt, G. F, Rugman-Jones, P. F., Stouthamer, R. 2015. Genetic characterization of North American Sootywings (Lepidoptera: Hesperidae) using nuclear and mitochondrial loci validates specific status of MacNeill's Sootywing, *Hesperopsis gracielae* (MacNeill). *Proceedings of the Entomological Society of Washington* 117(2): 194-202.

Rambaut, A. 2006-2009. *FigTree v1.4.3*. Edinburgh, Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/.

Salinas-Gutiérrez, J. L., Warren, A. D., Martínez, A. L. 2005. Hesperioidea (Lepidoptera: Rhopalocera) del occidente de México. *Folia Entomológica Mexicana* 44(3): 305-320.

Santos, B., Miller, M., Miklasevskaja, M., McKeown, J., Redmond, N., Coddington, J., Bird, J., Miller, S., Smith, A., Brady, S., Buffington, M., Chamorro, M. L., Dikow, T., Gates, M., Goldstein, P., Konstantinov, A., Kula, R., Silverson, N., Solis, M. A., deWaard, S., Naik, S., Nikolova, N., Pentinsaari, M., Prosser, S., Sones, J., Zakharov, E., deWaard, J. 2022 Enhancing DNA barcode reference libraries by harvesting terrestrial arthropods at the National Museum of Natural History. *Arpha Preprints*: 1-21.

Shorthouse, D. P. 2010. *SimpleMappr, an online tool to produce publication-quality point maps*. http://www.simplemappr.net. Accessed September 2022.

Steinhauser, S. R., Austin, G. T. 1993. New species of Hesperiidae from Costa Rica. *Tropical Lepidoptera* 4(Supplement 2): 12-20.

Steinhauser, S. R. 1989. Taxonomic notes and descriptions of new taxa in the Neotropical Hesperiidae. Part I. Pyrginae. *Bulletin of the Allyn Museum* 127: 1-70.

Steinhauser, S. R. 1991. Six new species of Skippers from Mexico (Lepidoptera: Hesperiidae: Pyrginae and Heteropterinae. *Insecta Mundi* 5(1): 25-43.

Vargas-Fernández, I., Warren, A. D., Luis-Martínez, M. A., Llorente-Bousquets, J. E. 2016. *Mariposas diurnas (Rhopalocera)*, pp. 366-375. *In*: *La biodiversidad en Colima. Estudio de estado.* México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad,.

Warren, A. D., Vargas, I., Armando Luis, M., Llorente, J. E. 1998. Butterflies of the state of Colima, Mexico. *Journal of the Lepidopterists' Society* 52(1): 40-72.

Warren, A. D., Davis, K. J., Stangeland, E. M., Pelham, J. P., Willmott, K. R., Grishin, N. V. 2023. *Illustrated Lists of American Butterflies*. http://www.butterfliesofamerica.com. Accessed June 2023.

Warren, A. D., Vargas-Fernández, I., Luis-Martínez, A., Llorente-Bousquets, J. 1998. Butterflies of the State of Colima, Mexico. *Journal of the Lepidopterists' Society* 52(1):40-72.

Warren, A. D. 2000. Hesperioidea (Lepidoptera), p. 535-580. *In*: Llorente-Bousquets, J. E., González Soriano, E., Papavero, N. (Eds), *Biodiversidad, taxonomía y biogeografía de artrópodos de México: hacia una síntesis de su conocimiento.* México, Universidad Nacional Autónoma de México, vol. 2, xvi + 676p.

Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W. X., Wang, G. T. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* 20: 348-355.

Zhang, J., Cong, Q., Shen, J., Grishin, N. V. 2022a. Taxonomic changes suggested by the genomic analysis of Hesperiidae (Lepidoptera). *Insecta Mundi* 0921: 1-135.

Zhang, J., Cong, Q., Shen, J., Song, L., Grishin, N. V. 2022b. Genomic DNA sequencing reveals two new North American species of *Staphylus* (Hesperiidae: Pyrginae: Carcharodini). *The Taxonomic Report* 10(4): 1-14.

Behavioral observations of ant-butterfly symbioses in the Pantanal wetlands of west-central Brazil

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Date of issue online: 16 August 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8140774

Abstract: In this paper we present for the first-time behavioral observations of symbiotic interactions between butterfly caterpillars and ants in the Pantanal wetlands of west-central Brazil (Mato Grosso and Mato Grosso do Sul states). Four species were found: *Aricoris propitia* (Stichel, 1910), *Nymphidium olinda* H. Bates, 1865, *Synargis calyce* (C. Felder & R. Felder, 1862) (Riodinidae), and *Strymon mulucha* (Hewitson, 1867) (Lycaenidae). In the Pantanal, these widely distributed species live in association with host plants and tending ants adapted to flooding. Except for *A. propitia*, which has an obligate association with *Solenopsis saevissima* (Smith, 1855) complex ants, the other butterfly species are facultatively myrmecophilous. A possible local adaptation was observed in *A. propitia* caterpillars, which pupated on the host plant instead of underground. Adaptations to flooding observed in plants, ants, and caterpillars, in addition to the existence of endemic species such as *Synargis rasqueada* Dolibaina, Dias, Mielke & Casagrande, 2013, suggest that wetland-specialized myrmecophiles may have evolved in the Pantanal.

Resumo: Neste artigo nós apresentamos pela primeira vez observações comportamentais de interações simbióticas entre lagartas de borboletas e formigas no Pantanal, Centro-Oeste do Brasil (Mato Grosso e Mato Grosso do Sul). Foram encontradas quatro espécies: *Aricoris propitia* (Stichel, 1910), *Nymphidium olinda* H. Bates, 1865, *Synargis calyce* (C. Felder & R. Felder, 1862) (Riodinidae), e *Strymon mulucha* (Hewitson, 1867) (Lycaenidae). No Pantanal, estas espécies amplamente distribuídas vivem associadas com plantas hospedeiras e formigas atendentes adaptadas ao alagamento. Com exceção de *A. propitia,* que possui associação obrigatória com formigas do complexo *Solenopsis saevissima* (Smith, 1855), as demais espécies são mirmecófilas facultativas. Foi observado uma possível adaptação local em *A. propitia* que empupou sobre a planta hospedeira ao invés do solo. As adaptações para o alagamento observadas em plantas, formigas e lagartas somada ao endemismo, como *Synargis rasqueada* Dolibaina, Dias, Mielke & Casagrande, 2013, sugerem que mirmecófilos especializados de áreas alagadas podem ter evoluído no pantanal.

Key words: *Camponotus*; *Dorymyrmex*; extrafloral nectaries; fire ants; floodplains; florivory; inundation.

Palavras-chave: *Camponotus*; *Dorymyrmex*; florivoria; formiga-lava-pés; inundação; nectário extrafloral; várzea.

INTRODUCTION

Symbiotic associations with ants (Hymenoptera: Formicidae) can provide substantial advantages because these ecologically dominant organisms can offer food resources and enemy-free space (Kaminski *et al*., 2010; Parker & Kronauer, 2021). Many arthropod taxa have developed associations with ants (myrmecophily), including Lepidoptera adults and immatures (reviewed in Pierce & Dankowicz, 2022). Recent studies indicate that this behavior arose independently in the sister butterfly families Riodinidae and Lycaenidae (Papilionoidea), which are the primary myrmecophilous families of Lepidoptera, containing 70% and 20% of the myrmecophilous species, respectively (DeVries, 1991; Espeland *et al*., 2018; Pierce & Dankowicz, 2022).

Ant-butterfly associations can be classified as obligatory, when the caterpillar needs ants to complete its life cycle, or facultative, when the caterpillar can be found without ants (Pierce & Dankowicz, 2022). However, these interactions can vary both spatially and temporally, and investigations of different ecosystems can advance our understanding of how different ecological pressures influence myrmecophilous behavior and its evolution.

Wetlands are ecosystems marked by the influence of aquatic and terrestrial environments, it may be permanently or periodically subjected to flood-pulsing with long terrestrial phases (Junk *et al*., 2014). The Pantanal of west-central Brazil is a periodically flooded savanna and one of the largest wetlands in the world, with diverse habitats and high species richness (Brown, 1987; Alho, 2008; Nunes da Cunha & Junk, 2009). Here, we present behavioral observations of symbiotic interactions between butterfly caterpillars and ants for the

Pantanal, the first data for a Neotropical wetland. In addition, we discuss possible adaptations to living in this environment.

MATERIAL AND METHODS

Adults and immature stages of *Aricoris propitia* (Stichel, 1910), *Nymphidium olinda* H. Bates, 1865, and *Strymon mulucha* (Hewitson, 1867) were studied in the Pirizal region (16°21’48” S, 56°17’32” W), Poconé (Fig. 1), Mato Grosso, Brazil, in November-December of 2019 and 2020. This area consists of a variety of different forested and open habitats, including seasonally flooded evergreen “cambarazal” forest dominated by *Vochysia divergens* Pohl. (Vochysiaceae), and seasonally flooded grass-wood savannas dominated by *Ipomoea carnea* Jacq. (Nunes da Cunha *et al*., 2006; Nunes da Cunha & Junk, 2009). Adults and immature stages of *Synargis calyce* (C. Felder & R. Felder, 1862) were studied near Base de Estudos do Pantanal (19°34’37” S, 57°00’42” W) of the Universidade Federal de Mato Grosso do Sul (UFMS), in the Miranda subregion (*sensu* Silva & Abdon, 1998), Corumbá, Mato Grosso do Sul, Brazil, in September 2013. This area includes portions of riparian forest along the Miranda River (Figs. 1, 4A) (see Wittmann *et al*. (2008) for details of this study site and its vegetation).

At these sites, potential host plants were checked by visually scanning the vegetation for the presence of ant-caterpillar symbioses (as in Kaminski *et al.*, 2013). Behavioral observations between lepidopterans (adults and immatures) and ants were made *ad libitum* (*sensu* Altmann, 1974). Plants with caterpillars were collected for identification, as were the tending ants. We also recorded the presence of food sources that might promote ant visitation on the plants, such as extrafloral nectaries (EFNs) and/or honeydew-producing hemipterans (HPHs). Vouchers of immature stages and adults of the studied species were deposited at the Centro de Pesquisas em Coleções Zoológicas, Universidade Federal do Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil, and the Zoological Collection of the Museu de Diversidade Biológica (ZUEC), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

RESULTS

Behavior and natural history of *Aricoris propitia* (Riodinidae) Adults were observed in open, seasonally flooded areas visiting small white flowers of *Richardia* sp. L. (Rubiaceae). In November and December 2019, mostly fourth and fifth (last) instar caterpillars were found during the day on *Senna* sp. Mill. (Fabaceae) (n = 2), *Ipomoea carnea* Jacq. (Convolvulaceae) (n = 8), and two unidentified plant species (n = 5) tended by several workers of *Solenopsis saevissima* (Smith, 1855) complex (Myrmicinae) (Fig. 2A). In December 2020, last instars and pupae were found on top of leaves of *I. carnea*, also being tended by *S. saevissima* ants (Fig. 2B). A freshly emerged adult (Fig. 2C) was observed being carefully antennated by tending ants with no signs of aggression (supplementary video available at https://doi.org/10.5281/zenodo.7821789).

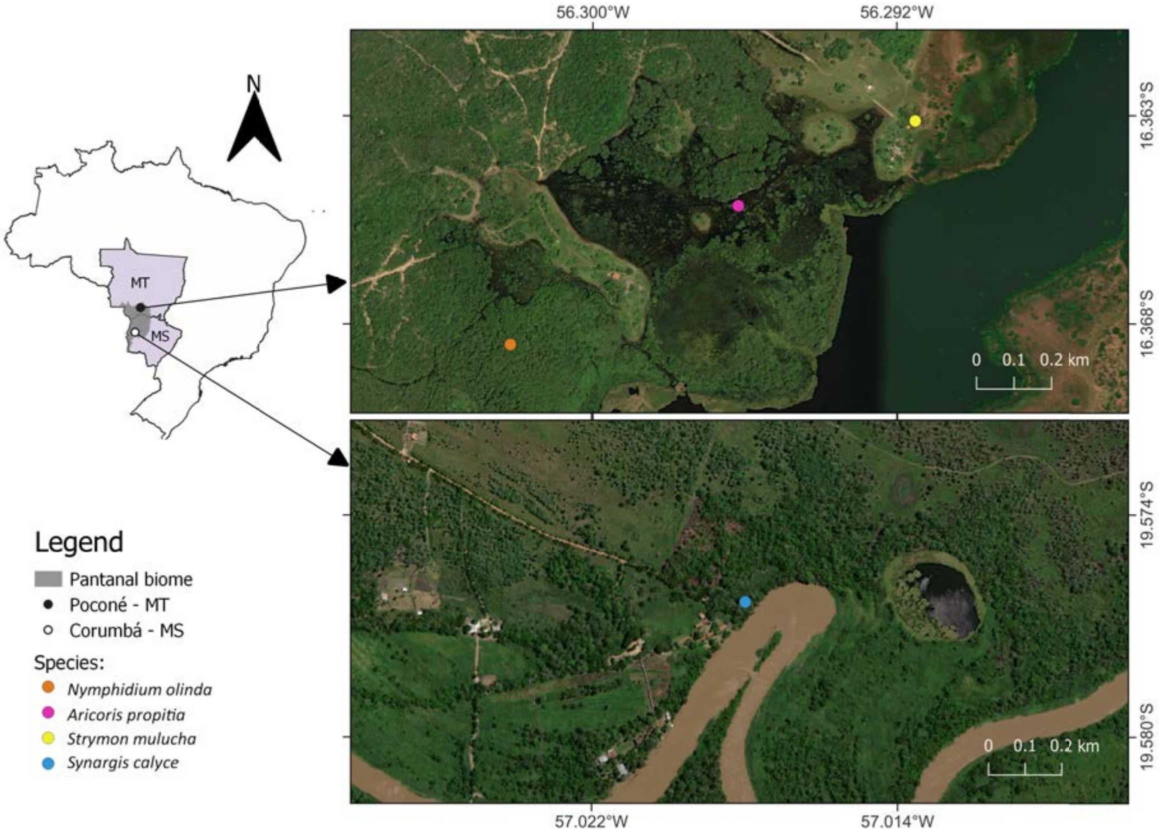

Figure. 1. Satellite imagery of the study areas near Poconé and Corumbá in southern Brazil’s Pantanal region (dark gray patch on the map of Mato Grosso and Mato Grosso do Sul states). Colored dots indicate the four locations where butterfly-ant associations were recorded.

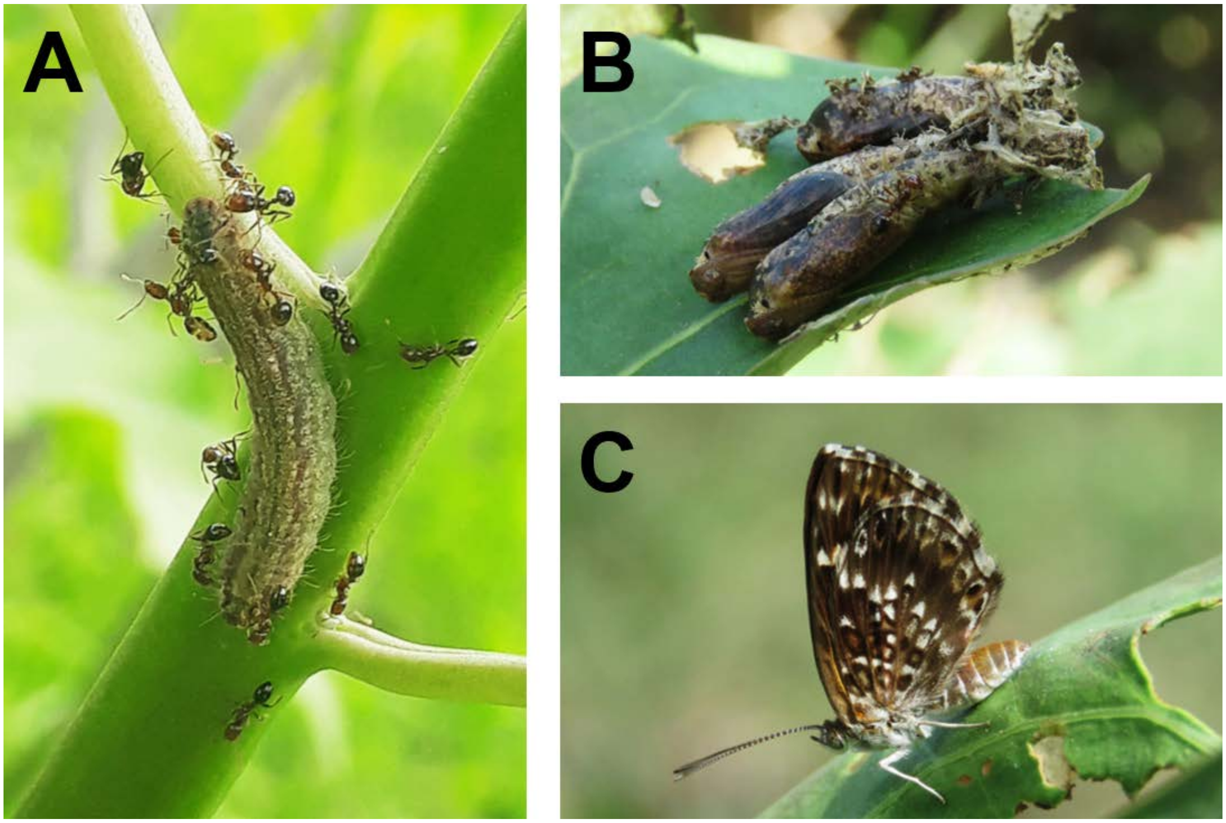

Figure. 2. Life stages of *Aricoris propitia* from Pirizal, Poconé, Mato Grosso, Brazil. **A.** Fifth (last) instar caterpillar on *Ipomoea carnea* tended by *S. saevissima* ants; **B.** Pupae on the host plant tended by ants; **C.** Freshly emerged adult female.

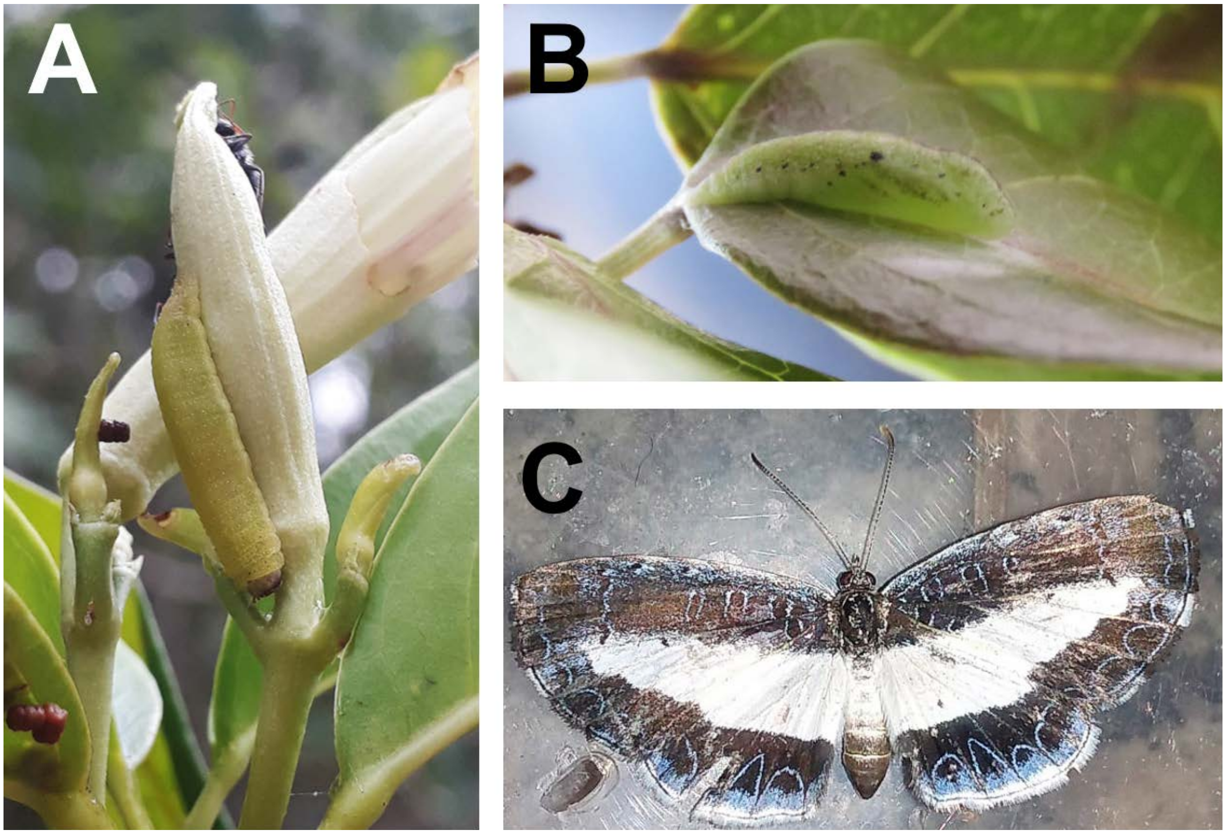

Figure. 3. Life stages of *Nymphidium olinda* from Pirizal, Poconé, Mato Grosso, Brazil. **A.** Last instar caterpillar on *Bignonia corymbosa*, with *Dolichoderus* sp. ant nearby; **B.** Pupa on the leaf base in lateral view; **C.** Adult female.

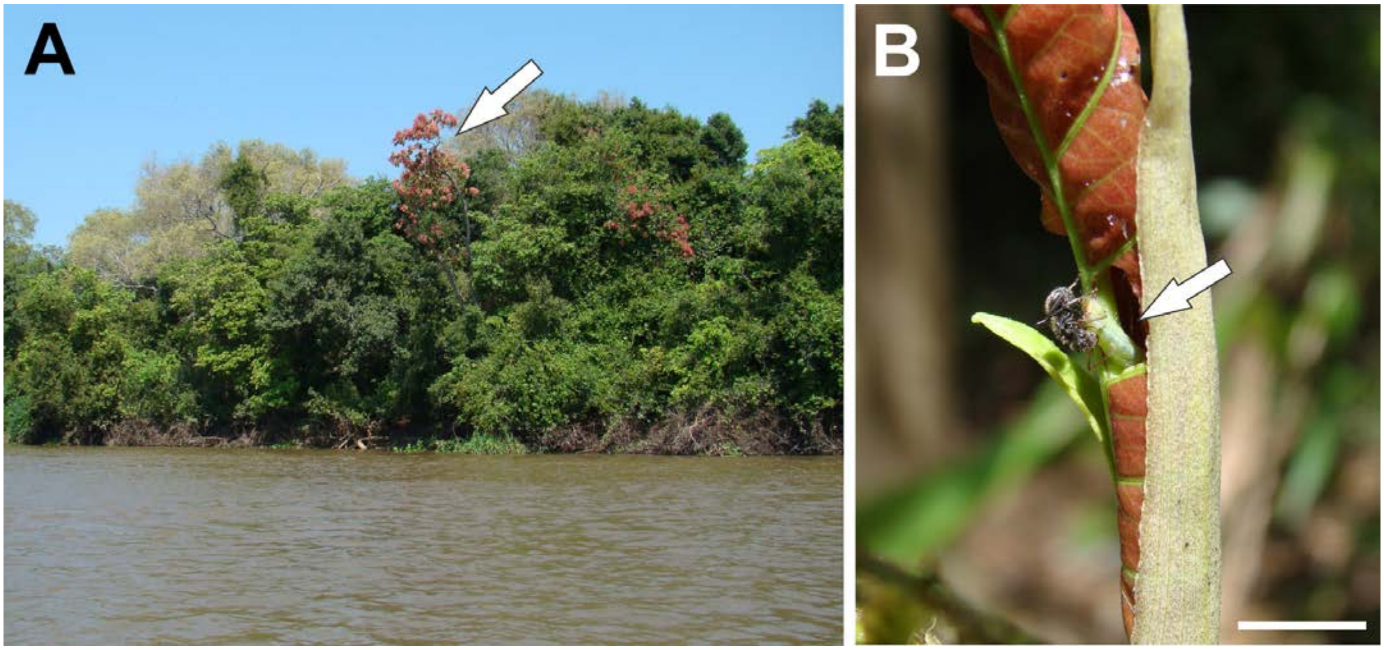

Figure. 4. Habitat and caterpillar of *Synargis calyce* from Corumbá, Mato Grosso do Sul, Brazil. **A.** *Triplaris gardneriana* (arrow) in riparian forest of the Miranda River; **B.** Third instar caterpillar tended by *Camponotus crassus* ant (arrow).

Figure. 5. Habitat and life stages of *Strymon mulucha* from Pirizal, Poconé, Mato Grosso, Brazil. **A.** Adult female ovipositing on *Sida* sp.; **B.** Third instar caterpillar tended by *Dorymyrmex* sp. ant.

Behavior and natural history of *Nymphidium olinda* (Riodinidae)

A single last instar caterpillar (Fig. 3A) was observed on flower buds of *Bignonia corymbosa* (Vent.) L. G. Lohmann (Bignoniaceae) at medium substrate height in seasonally flooded evergreen “cambarazal” forest. This host plant has pericarpal nectaries and as a result it attracted several ant genera. *Dolichoderus* sp. (Dolichoderinae) workers were observed visiting both the caterpillar and the nectaries, although minimal attention was shown to the caterpillar (supplementary video available in https://doi.org/10.5281/zenodo.7821850). The caterpillar has uniform cryptic coloration, with three pairs of tiny brown balloon setae on the prothorax and a pair of tentacle nectary organs on abdominal segment A8. It exhibited minimal movement in the presence of ants and even when manipulated with a stick. When the caterpillar was found, its identity was unknown. It was raised in confinement for two days until it pupated (Fig. 3B), and the adult female emerged 7 days after pupation (Fig. 3C).

Behavior and natural history of *Synargis calyce* (Riodinidae)

A single adult was observed visiting flowers of *Cordia naidophila* I.M. Johnst. (Boraginaceae) at the edge of the forest. Second instar caterpillars (n = 6) were encountered on two nearby seedlings of *Triplaris gardneriana* Wedd. (Polygonaceae) in the understory. The caterpillars were feeding on reddish and not yet fully expanded new leaves, and being tended by *Camponotus crassus* (Formicidae: Formicinae) workers (Fig. 4B). Caterpillars were followed in the field to the third instar, then were collected to confirm identification.

General morphology and duration of immature stages are similar those described by Callaghan (1986).

Behavior and natural history of *Strymon mulucha* (Lycaenidae)

A single female was observed ovipositing on flower buds of *Sida* sp. L. (Malvaceae) (Fig. 5A). The development of this egg was followed in the field, and after six days a yellowish first instar caterpillar was observed. On this day there were *S. saevissima* ants on the plant, but no symbiotic interaction was recorded. After four days, a greenish third instar caterpillar, presumably from the same egg, was found on this plant being tended by *Dorymyrmex* sp. ants (Dolichoderinae) (Fig. 5B; supplementary video available in https://doi.org/10.5281/zenodo.7821875). The host plant was located close to a scrubby, non-seasonally flooded area, with buildings and livestock around. It was not possible to observe the full life cycle of this species because the caterpillar disappeared a few days after the last photos were taken.

DISCUSSION

The butterfly species observed in this work have a wide distribution in South America and are generalists in terms of host plants. These characteristics allow the exploitation of some abundant food resources in the Pantanal, known for the dominance of a few adapted plant species (Pott *et al*., 2011). For example, *Synargis calyce* has polyphagous caterpillars that are facultatively myrmecophilous and interact mainly with *Camponotus* ants (Callaghan, 1986; Alves-Silva *et al*., 2018; Lima *et al*., 2023). Its host plant in the Pantanal, *Triplaris gardneriana,* is a myrmecophytic plant that attracts several species of ants (Souza, 2014). This tree is adapted to flooded environments and is quite abundant in the Pantanal (Pontara *et al*., 2016).

Caterpillars of *Nymphidium olinda* and *Strymon mulucha* were found on flowers, and florivorous myrmecophilous caterpillars typically use several host plant species (see discussion in Kaminski *et al*., 2012). Both butterfly species can be considered facultative myrmecophiles, although more observations are needed to understand the degree of myrmecophily and host repertoire in *N. olinda*. Even though dozens of host plant families have been recorded for *S. mulucha* (Beccaloni *et al*., 2008; Silva *et al*., 2011), there are few records of myrmecophily, and the functionality of the dorsal nectary organ (DNO) has not yet been confirmed (Silva *et al*., 2014; L. A. Kaminski, unpublished data). In flooded environments, a greater probability of encounters between caterpillars and ants on the foliage would be expected, and populations of facultative myrmecophiles may thus face greater selective pressure for association with ants in wetlands, a hypothesis that still needs to be tested.

Caterpillars of *Aricoris propitia,* as well as those of its known congeners, have previously all been reported to pupate in underground shelters built by ants (Kaminski & Carvalho-Filho, 2012; Kaminski *et al*., 2021). In contrast, caterpillars in the Pantanal were found pupating on the host plant. Adaptations to living on vegetation during flooding have been described for terricolous arthropods in the Pantanal, including ant species (Adis *et al*., 2001), with *Solenopsis* ants known for their rafting behavior during floods (Adams *et al*., 2011). Thus, it would be interesting to study the variation in pupation behavior in populations of *Aricoris* Westwood, 1851, in flooded and non-flooded areas to test the hypothesis of local adaptation in the Pantanal.

The caterpillar of *N. olinda* is described here for the first time. Like other members of the genus *Nymphidium* Fabricius, 1807*,* the larvae are tended by ants (DeVries, 1997). Pupation was at the leaf base, in contrast with what has been reported for its sister species *N. mantus* (Cramer, 1775), whose caterpillar rolls the leaf into a semitube and hides inside (DeVries, 1997). Unfortunately, the forest patch in which the caterpillar was found in 2019 caught fire during the dry season of 2020 (*i.e.*, that year it was not found). These forest fires are known to have a serious impact on wildlife (Tomas *et al*., 2021; de Arruda *et al*., 2022), although the impact on the butterfly fauna of the Pantanal is still to be investigated. This also seems to be the first record for *Nymphidium* on Bignoniaceae, extending the established pattern of a highly polyphagous *N. mantus* group (Hall, 2018).

This is the first publication about butterfly-ant interactions from the Pantanal wetlands of Brazil and from a Neotropical wetland in general. Adaptations observed in plants, ants, and caterpillars, in addition to the existence of endemic species such as *Synargis rasqueada* Dolibaina, Dias, Mielke & Casagrande, 2013 (Dolibaina *et al*., 2013), suggest that wetland-specialized myrmecophiles may have evolved in the biome. Confirming this prediction would be of interest in biogeographical terms, since the Pantanal is otherwise recognized as a region with low faunal endemism (Brown, 1987). Further studies are important to better understand the influence of flood dynamics on species' ecology and evolution. There is an urgency to such studies given that the Pantanal has been suffering in recent years from the negative impacts of climate change and human encroachment (Lázaro *et al*., 2020).

ACKNOWLEDGMENTS

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 130443/2019-0) for financial support, Williams Oliveira-Silva for helping with the map in QGIS software and Lúcia G. Lohmann for the identification of *B. corymbosa*. We also thank reviewers for their comments, especially to Jason Hall for English corrections and suggestions. TKG thanks Dona Xuta and Lu, residents of Pirizal who kindly hosted her during the field research; and LAK thanks Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 10/51340-8), and the BEP-UFMS for its Ecologia do Pantanal Field course (2013 edition), logistic support, and the opportunity to get to know the Pantanal. The present study is registered in the SISGEN (A894B46). This publication is part of the RedeLep 'Rede Nacional de Pesquisa e Conservação de Lepidópteros' SISBIOTA-Brasil/CNPq (563332/2010-7).

LITERATURE CITED

Adams, B. J., Hooper-Bùi, L. M., Strecker, R. M., O'Brien, D. M. 2011. Raft formation by the red imported fire ant, *Solenopsis invicta*. *Journal of Insect Science* 11(1): 171.

Adis, J., Marques, M. I., Wantzen, K. M. 2001. First observations on the survival strategies of terricolous arthropods in the northern Pantanal wetland of Brazil. *Andrias* 15: 127-128.

Alho, C. J. R. 2008. Biodiversity of the Pantanal: response to seasonal flooding regime and to environmental degradation. *Brazilian Journal of Biology* 68(4): 957-966.

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behaviour* 49: 227-267.

Alves-Silva, E., Bächtold, A., Del-Claro, K. 2018. Florivorous myrmecophilous caterpillars exploit an ant-plant mutualism and distract ants from extrafloral nectaries. *Austral Ecology* 43(6): 643-650.

Beccaloni, G. W., Viloria, A. L., Hall, S. K., Robinson, G. S. 2008. *Catalogue of the host plants of the neotropical butterflies/Catálogo de las Plantas Huésped de las Mariposas Neotropicales*. *m3m-Monografías Tercer Milenio. Vol. 8*. Zaragoza, Sociedad Entomológica Aragonesa (SEA), 536 pp.

Brown, K. S. 1987. *Zoogeografia da região do Pantanal Mato-grossense*, pp. 137-179. In: *Simpósio sobre recursos naturais e sócio-econômico do Pantanal*. Brazil, Embrapa.

Callaghan, C. J. 1986. Restinga butterflies: Biology of *Synargis brennus* (Stichel) (Riodinidae). *Journal of the Lepidopterists' Society* 40: 93-96.

de Arruda, F. V., Teresa, F. B., Layme, V. M., Vicente, R. E., Camarota, F., Izzo, T. J. 2022. Fire and flood: How the Pantanal ant communities respond to multiple disturbances? *Perspectives in Ecology and Conservation* 20(3): 197-204.

DeVries, P. J. 1991. *Evolutionary and ecological patterns in myrmecophilous riodinid butterflies*, pp. 143-156. *In*: Huxley, C., Cutler, D. F. (Eds.), *Ant-Plant Interactions*. Oxford, Oxford University Press.

DeVries, P. J. 1997. *The Butterflies of Costa Rica and their Natural History, Vol. II: Riodinidae*. New Jersey, Princeton University Press. 368 pp.

Dolibaina, D. R., Dias, F. M. S., Mielke, O. H. H., Casagrande, M. M. 2013. Taxonomy of the '*Synargis axenus* complex' belonging to the '*Synargis regulus*' species group, with a phylogenetic reassessment of the genus *Synargis* Hübner, 1819 (Lepidoptera: Riodinidae: Nymphidiini). *Zoological Journal of the Linnean Society* 168(3): 427-451.

Espeland, M., Breinholt, J., Willmott, K. R., Warren, A. D., Vila, R., Toussaint, E. F. A., Maunsell, S. C., Aduse-Poku, K., Talavera, G., Eastwood, R., Jarzyna, M. A., Guralnick, R., Lohman, D. J., Pierce, N. E., Kawahara, A. K. 2018. A Comprehensive and dated phylogenomic analysis of butterflies. *Current Biology* 28: 770-778.

Hall, J. P. W. 2018. *A Monograph of the Nymphidiina (Lepidoptera: Riodinidae: Nymphidiini): Phylogeny, Taxonomy, Biology, and Biogeography*. Washington, DC, Entomological Society of Washington. 990 pp.

Junk, W. J., Piedade, M. T. F., Lourival, R., Wittmann, F., Kandus, P., Lacerda, L. D., Bozelli, R. L., Esteves, F. A., Nunes da Cunha, C., Maltchik, L., Schöngart, J., Schaeffer-Novelli, Y., Agostinho, A. A. 2014. Brazilian wetlands: their definition, delineation, and classification for research, sustainable management, and protection. *Aquatic Conservation: marine and freshwater ecosystems* 24(1): 5-22.

Kaminski, L. A., Carvalho-Filho, F. S. 2012. Life history of *Aricoris propitia* (Lepidoptera: Riodinidae) - A myrmecophilous butterfly obligately associated with fire ants. *Psyche: A Journal of Entomology* 2012: 1-10.

Kaminski, L. A., Freitas, A. V. L., Oliveira, P. S. 2010. Interaction between mutualisms: Ant-tended butterflies exploit enemy-free space provided by ant-treehopper associations. *American Naturalist* 176(3): 322-334.

Kaminski, L. A., Mota, L. L., Freitas, A. V. L., Moreira, G. R. P. 2013. Two ways to be a myrmecophilous butterfly: natural history and comparative immature-stage morphology of two species of *Theope* (Lepidoptera: Riodinidae). *Biological Journal of the Linnean Society* 108(4): 844-870.

Kaminski, L. A., Rodrigues, D., Freitas, A. V. L. 2012. Immature stages of *Parrhasius polibetes* (Lepidoptera: Lycaenidae): host plants, tending ants, natural enemies and morphology. *Journal of Natural History* 46(11-12): 645-667.

Kaminski, L. A., Volkmann, L., Callaghan, C. J., DeVries, P. J., Vila, R. 2021. The first known riodinid 'cuckoo' butterfly reveals deep-time convergence and parallelism in ant social parasites. *Zoological Journal of the Linnean Society* 193(3): 860-879.

Lázaro, W. L., Oliveira-Júnior, E. S., Silva, C. J., Castrillon, S. K. I., Muniz, C. C. 2020. Climate change reflected in one of the largest wetlands in the world: an overview of the Northern Pantanal water regime. *Acta Limnologica Brasiliensia* 32(104).

Lima, L. D., Ceballos-González, A. V., Prato, A., Kaminski, L. A., Nascimento, F. S. 2023. Plant-treehopper convergence may trick butterflies into trophic oviposition mistakes. *Biotropica* 55: 292-298.

Nunes da Cunha, C., Junk, W. J. 2009. *A preliminary classification of habitats of the Pantanal of Mato Grosso and Mato Grosso do Sul, and its relation to national and international wetland classification systems*, pp. 127-141. *In*: Junk, W. J., Da Silva, C. J., Nunes da Cunha, C., Wantzen, K. M. (Eds.), *The Pantanal: Ecology, Biodiversity and Sustainable Management of a Large Neotropical Seasonal Wetland*. Moscow, Pensoft Publishers.

Nunes da Cunha, C., Rawiel, P., Wantzen, K. M., Junk, W. J., Lemes do Prado, A. 2006. Mapping and characterization of vegetation units by means of Landsat imagery and management recommendations for the Pantanal of Mato Grosso (Brazil), north of Poconé. *Amazoniana* 19(1/2): 1-32.

Parker, J., Kronauer, D. J. 2021. How ants shape biodiversity. *Current Biology* 31(19): R1208-R1214.

Pierce, N. E., Dankowicz, E. 2022. *The natural history of caterpillar-ant associations*, pp. 319-391. *In*: Marquis, R. J., Koptur, S. (Eds.), *Caterpillars in the Middle. Tritrophic Interactions in a Changing World*. Fascinating Life Sciences series. Springer International Publishing.

Pontara, V., Bueno, M. L., Scremin-Dias, E. 2016. Flooding avoidance *Triplaris gardneriana* Wedd. (Polygonaceae): growth and morpho-anatomical aspects. *Acta Scientiarum. Biological Sciences* 38(3): 341-346.

Pott, A., Oliveira, A. K., Damasceno-Junior, G. A., Silva, J. S. V. 2011. Plant diversity of the Pantanal wetland. *Brazilian Journal of Biology* 71: 265-273.

Silva, J. S. V., Abdon, M. M. 1998. Delimitação do pantanal brasileiro e suas sub-regiões. *Pesquisa Agropecuária Brasileira* 33 (Número Especial): 1703-1711.

Silva, N. A. P., Duarte, M., Diniz, I. R., Morais, H. C. 2011. Host plants of Lycaenidae on inflorescences in the central Brazilian cerrado. *The Journal of Research on the Lepidoptera* 44: 95-105.

Silva, N. A. P., Duarte, M., Araújo, E. B., Morais, H. C. 2014. Larval biology of anthophagous Eumaeini (Lepidoptera: Lycaenidae, Theclinae) in the Cerrado of central Brazil. *Journal of Insect Science* 14(184): 2014.

Souza, P. R. D. 2014. *Padrões de distribuição e diversidade de espécies da mirmecofauna (Hymenoptera, Formicidae) de uma savana estépica (chaco) no município de Porto Murtinho, Mato Grosso do Sul, Brasil*. PhD dissertation. Universidade Federal do Mato Grosso do Sul.

Tomas, W. M., Berlinck, C. N., Chiaravalloti, R. M., Faggioni, G. P., Strüssmann, C., Libonati, R., ... & Morato, R. 2021. Distance sampling surveys reveal 17 million vertebrates directly killed by the 2020's wildfires in the Pantanal, Brazil. *Scientific Reports* 11(1): 1-8.

Wittmann, F., Zorzi, B. T., Tizianel, F. A. T., Urquiza, M. V. S., Faria, R. R., Sousa, N. M., Módena, É. D. S., Gamarra, R. M. Rosa, A. L. M. 2008. Tree species composition, structure, and aboveground wood biomass of a riparian forest of the lower Miranda River, Southern Pantanal, Brazil. *Folia Geobotanica* 43(4): 397-411.

A new species of *Concinocordis* Razowski and Brown, 2012 from Kenya (Lepidoptera: Tortricidae)

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Date of issue online: 16 August 2023
Zoobank Registered: urn:lsid:zoobank.org:pub:B071038D-D486-42C1-9FB4-F396F540F1D7
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.8143732

Abstract: *Concinocordis wilsonarum* Razowski and Brown, 2012 was described from seven specimens reared from native fruit (all Sapotaceae) in Kenya. The accumulation of additional specimens and re-analyses of DNA sequence data (COI barcodes) reveals that the species is actually a complex of three distinct entities, each separated from its nearest relative by COI divergences of at least 8%. Differences in forewing maculation provide additional evidence that the three are distinct. *Concinocordis bartocki* Brown, **new species**, is described, but the second new species is not because it is represented by a single male in exceedingly poor condition. A damaged pupal exuvium of *C. wilsonarum* shows marked dissimilarities with the pupae of other tortricids. The enigmatic tribal assignment of *Concinocordis* is discussed briefly.

Key words: COI; DNA barcodes; Enarmoniini; genitalia; pupal morphology; Sapotaceae.

INTRODUCTION

Concinocordis Razowski & Brown, 2012, was described to accommodate the single new species *C. wilsonarum* Razowski & Brown, 2012 based on specimens reared from the fruit of native Sapotaceae in Kenya (Brown *et al.*, 2014). The species is superficially similar to some Enarmoniini (e.g., *Enarmonia* Hübner, *Enarmoniodes* Ghesquière) (e.g., Razowski *et al.*, 2010), the tribe to which it was initially assigned, a few Tortricini (e.g., *Paraccra* Razowski, *Accra* Razowski) (e.g., Razowski, 1981), and at least one Archipini (e.g., *Pseudargyrotoza* Obraztsov) (e.g., Razowski, 2002), sharing small rounded patches of upraised, opalescent scales on the upper surface of the forewing. However, the female genitalia of *Concinocordis* distinguish it from all other genera and are incongruent with its current assignment to Enarmoniini. In particular, the unusual signum with a long, sclerotized tube projecting externally well beyond the outer wall of the corpus bursae is unique in Tortricidae. The absence of the characteristic rows of spines on each abdominal segment of the pupa is also unusual in the family.

Additional molecular and morphological data that have accumulated since the original description of *C. wilsonarum* suggest the name represents a complex of three species. The purpose of this contribution is to described one of the two for which adequate material is available, and briefly discuss the enigmatic tribal assignment of the genus.

MATERIALS AND METHODS

The study sites and methods of fruit-sampling and insect-rearing are described in detail by Copeland *et al.* (2002) and summarized by Brown *et al.* (2014). All but one of the specimens examined (n = 13) were reared from the fruit of Sapotaceae (i.e., *Manilkara butugi* Chiov., *Chrysophyllum albidum* G. Don-Holl., *Chrysophyllum gorungosanum* Engl., *Mimusopos obtusifolia* Lam., and *Synsepalum brevipes* (Baker) T. D. Penn.) during an insect rearing project in Kenya funded by USAID (Copeland *et al.*, 2002); one specimen was field-collected by Todd Gilligan in Kenya. Dissection methods follow those presented in Brown & Powell (1991). Terms for genitalia structures and forewing pattern elements follow Razowski (2002), except "phallus" is used instead of "aedeagus," and "hind margin" is used for the trailing edge of the forewing which frequently is referred to as the "dorsum" in tortricid literature. Images of adults and genitalia were captured using a 65 mm lens attached to a Canon EOS 5D digital SLR camera (Canon U.S.A., Lake Success, NY) mounted on a Visionary Digital BK Lab System (Visionary Digital, Palmyra, VA).

Tissue samples (one leg of an adult moth) were used to amplify the 658-bp region of the mitochondrial gene cytochrome c oxidase subunit 1 (COI), commonly referred to as the DNA "barcode," using standard procedures employed at the Biodiversity Institute of Ontario, University of Guelph (e.g., Craft *et al.*, 2010; Wilson, 2012; Hebert *et al.*, 2013). Sequence data were obtained for nine specimens of *Concinocordis* and one individual each of four outgroup species: *Paraccra mimesa* Razowski (Tortricinae: Tortricini), *Eugnosta percnoptila* (Meyrick) (Tortricinae: Cochylini), *Enarmonia formosana* (Scopoli) (Olethreutinae: Enarmoniini), and *Proteoteras obnigrana* Heinrich (Olethreutinae: Eucosmini). Sequences were trimmed to 654 bp for consistency among all samples. Collection and sequence data are given in Table 1.

The software Geneious Prime 2021.0.3 (Biomatters, Auckland, New Zealand) was used to align sequences with the MUSCLE algorithm (Edgar, 2004). Maximum likelihood (ML) and Bayesian analyses were conducted to determine species distinctness and relationships among them. The best nucleotide substitution models for the dataset were estimated using the software jModelTest2 (Darriba *et al.*, 2012), based on the online version at the Cyberinfrastructure for Phylogenetic Research (CIPRES) website (Miller *et al.*, 2010). According to both the Akaike information criterion (AIC and corrected AIC) and the Bayesian Information Criterion (BIC), the best fit model was TIM2+G. ML analysis was conducted using PhyML (Guindon *et al.*, 2010) with support for nodes estimated by performing 1,000 bootstrap pseudoreplicates. Bayesian analysis was performed using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) with a Markov chain Monte Carlo (MCMC) run for ten million generations using four heated chains and trees sampled every 1,000 generations. Both ML and Bayesian analyses were implemented as Geneious Prime plugins. Average distance within species and distance to the nearest neighbor were calculated as p-distances using algorithms generated by the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007).

Holotypes are deposited in the National Museums of Kenya, Nairobi (NMK). Paratypes are deposited in the latter and in the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM).

RESULTS

Concinocordis wilsonarum Razowski and Brown, 2012

Figs. 1, 2, 7, 9

Concinocordis wilsonarum Razowski and Brown, 2012: 7; Brown *et al.* 2014: 356.

Diagnosis. Superficially, *C. wilsonarum* can be distinguished from *C. bartocki* **new species** by the leaden gray (nearly black), opalescent, raised scale patches of the forewing in both sexes, and by the more extensive orange scaling on the upper surface in the middle of hindwing in the female (compare Figs. 1, 3). In *C. bartocki* the raised scale patches are more opalescent orange (mostly lacking the leaden gray), and the upper surface of the hindwing is nearly uniform brown. The female genitalia of the two species are virtually identical (Figs. 7, 8).

Molecular characterization. There are five barcode sequences for *C. wilsonarum*, four full-length and one short (307-bp), with an infraspecific variability of 0.23%. Together they form a BIN (BOLD:ABW2379) that is 8.68% different from its nearest neighbor.

Specimens examined. Holotype, ♂, Kenya, Western Province, Kakamega Forest, 1600 m, 0°14.38’N, 34°51.86’E, 31 Aug 1999, reared from *Manilkara butugi*, A&M 273, R. S. Copeland (NMK).

Paratypes (1♂, 3♀). Kenya: Western Province: Kakamega Forest: 1600 m, 0°14.38’N, 34°51.86’E, 31 Aug 1999 (1♀), reared from *Manilkara butugi*, R. S. Copeland (USNM); 0°14.16’N, 34°51.82’E, 29 Mar 2000 (1♂, 1♀), reared from *Chrysophyllum albidum*, A&M 573, R. S. Copeland (NMK, USNM); 0°15.16’N, 34°51.68’E, 26 Feb 2000 (1♀), reared from *Synsepalum brevipes*, A&M 540, R. S. Copeland (NMK).

Additional material (2♀). Kenya: Western Province: Kakamega Forest: 0°14.20’N, 34°51.82’E, no date (1♀), reared from *Manilkara butugi*, A&M 309, R. S. Copeland (NMK); 0°13.14’N, 34°53.76’E, 14 Jun 1999 (1♀), reared from *Manilkara butugi*, A&M 311, R. S. Copeland (USNM).

Distribution and biology. *Concinocordis wilsonarum* is recorded only from Kakamega Forest in Western Province, Kenya, at an elevation of about 1600 m. It has been reared from the fruit of *Manilkara butugi* (n = 4), *Chrysophyllum albidum* (n = 2), and *Synsepalum brevipes* (n = 1) (all Sapotaceae). Although the life history is undescribed, we examined a single damaged pupal exuvium.

The pupae of nearly all tortricids are characterized by two rows of spines extending laterally across the dorsum of abdominal segments two (or three) through seven (or eight), with the anterior row shorter and composed of conspicuously larger spines. In contrast, the pupa of *C. wilsonarum* has a broad field of tiny spines across the anterior half of the dorsum of abdominal segments three through seven (Fig. 9). A somewhat similar configuration of pupal spines was previously reported for *Tortrimosaica polypodivora* Brown and Baixeras (Brown *et al.* 2004), which was provisionally assigned to Hilarographini.

Concinocordis bartocki Brown, new species

Figs. 3, 4, 8

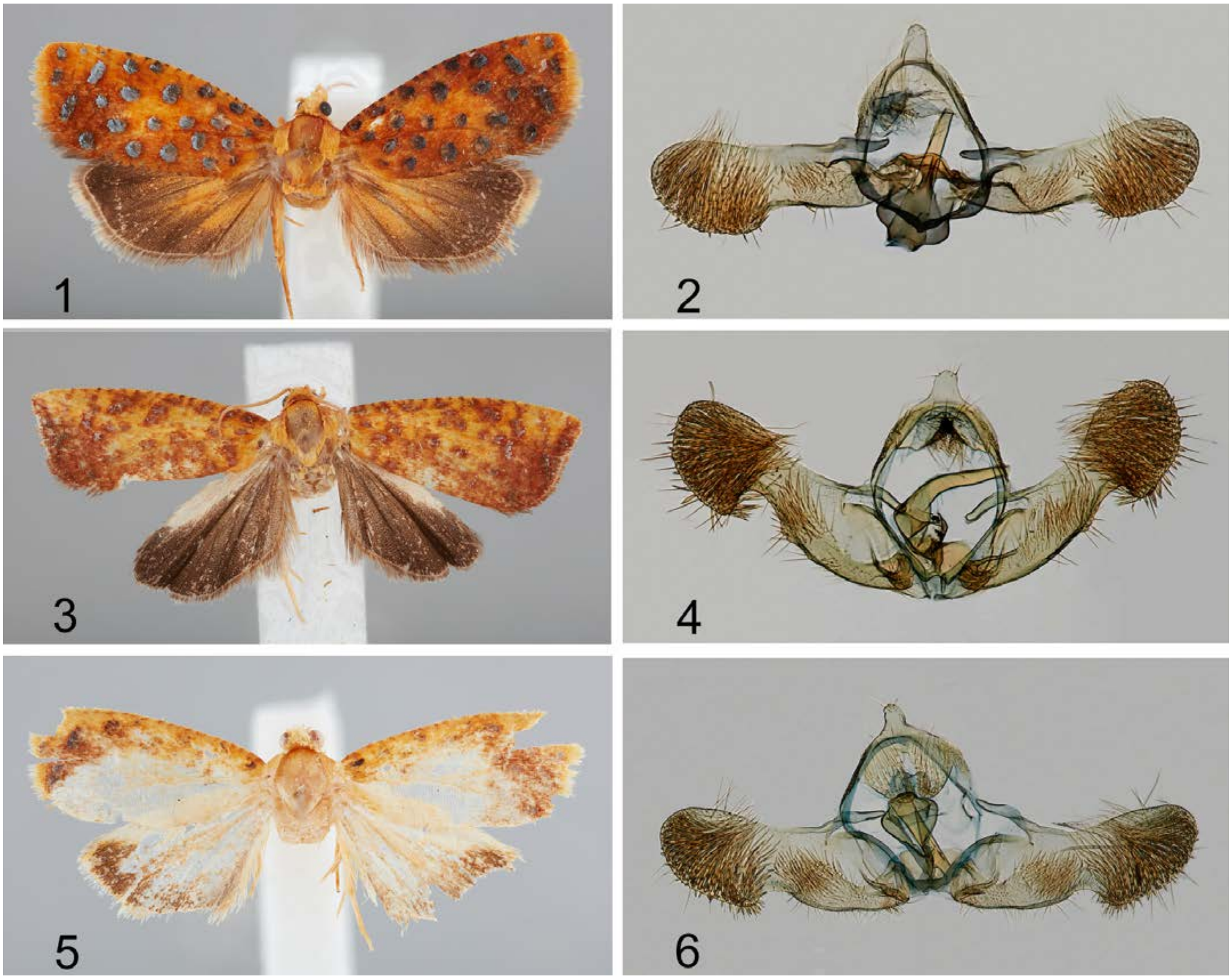
Concinocordis wilsonarum Razowski and Brown, 2012: 7; Brown *et al.* 2014: 356. [In part]

Diagnosis. Superficially, *C. bartocki* can be distinguished from *C. wilsonarum* by the more orange rather than leaden gray, opalescent, raised scale patches of the forewing, and by the more uniform brown hindwing of the female (Figs. 1, 3). Differences between the two species in male genitalia are subtle and unreliable. However, the constriction of the valva near mid-venter (i.e., “neck”) separating the cucullus from the sacculus is slightly more pronounced in *C. bartocki*, resulting in a narrower neck (Figs. 2, 4). The female genitalia (Figs. 7, 8) of *C. wilsonarum* and *C. bartocki* cannot be distinguished with certainty.

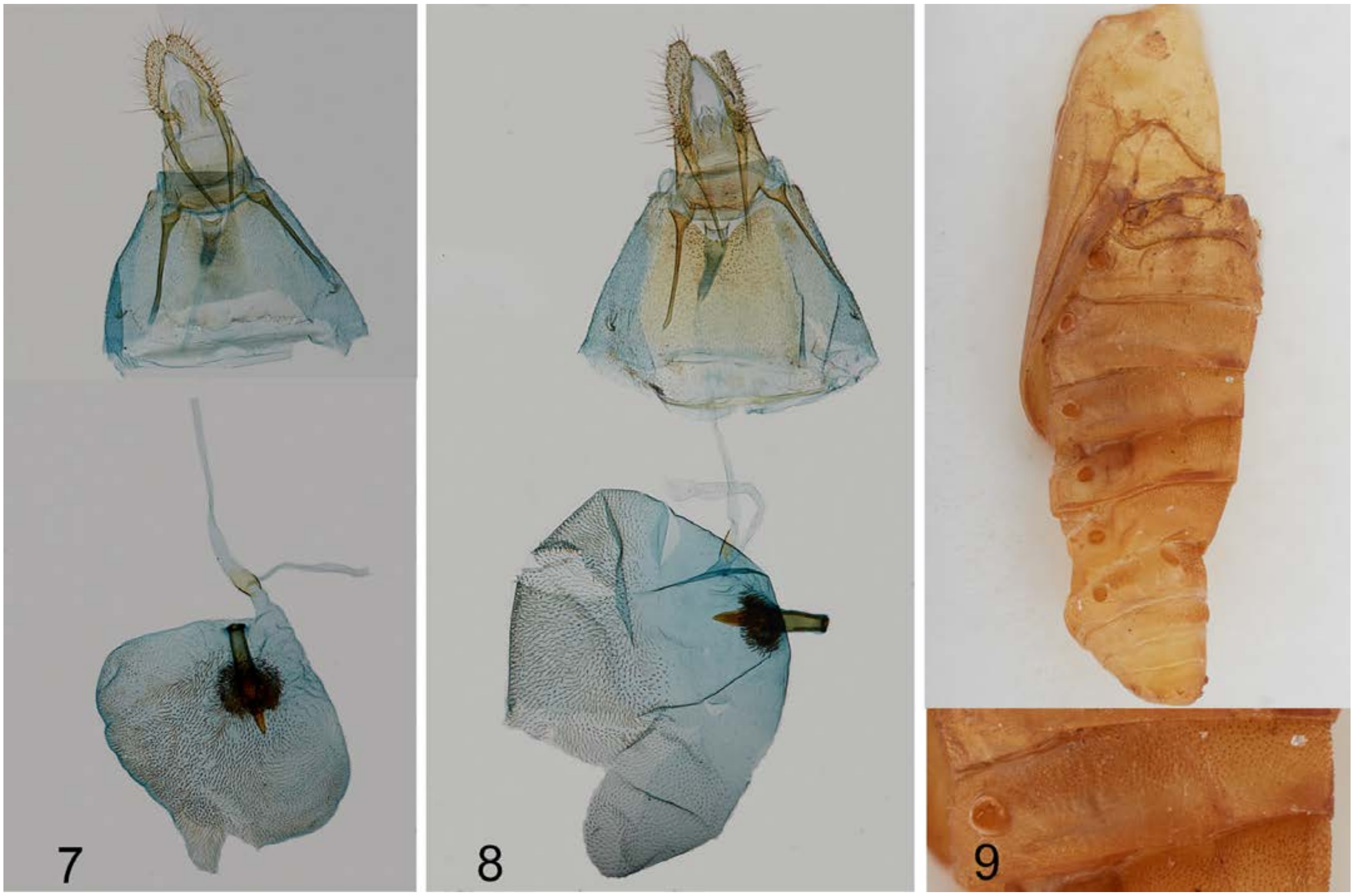
Description. **Head**: Vertex, frons, and labial palpus pale cream-orange; ocellus very large. **Thorax:** Dorsum orange. Forewing length 7.0-7.5 mm (mean = 7.3; n = 2) in males, 7.0-8.0 mm (mean = 7.5; n = 3) in females; forewing ground color orange, slightly browner in tornus and subterminal area; raised spots of opalescent orange scales roughly forming four diagonal lines, spots smallest along costa; some tiny blackish dots near termen; fringe cream, orange basally, brownish gray at tornus in females (Fig. 3). Hindwing nearly uniform dark brown; fringe whitish; frenulum with one spine in male, three in female. Abdomen: Male genitalia (Fig. 4) with pedunculi long, slender; vinculum well developed, broad; uncus short, broad, subrectangular, bearing a few long ventroterminal setae; socius pendant, broadly semicircular, with sparse hairs; valva slender, nearly parallel-sided, with large basal process, sacculus simple, cucullus ovoid with small ventral lobe, constriction near mid-venter of valva conspicuous, creating narrow neck, small warty process variably developed along ventral margin of valva near inner base of neck; juxta with small triangular basal plate and broad, subrectangular, hollow, shield-like plate covering phallobase; phallus simple, slender, vesica without cornuti. Female genitalia

Table 1. Sources of sequence data for phylogenetic analyses.

Species	Location	BOLD process ID / specimen code	BOLD BIN #	GenBank accession number	#bp
Concinocordis wilsonarum	Kenya, Western Province, Kakamega Forest	AFTOR145-12	ABW2379	KJ592132	654
Concinocordis wilsonarum	Kenya, Western Province, Kakamega Forest	AFTOR146-12	ABW2379	KJ592423	654
Concinocordis wilsonarum	Kenya, Western Province, Kakamega Forest	AFTOR149-12	ABW2379	KJ592163	654
Concinocordis wilsonarum	Kenya, Western Province, Kakamega Forest	AFTOR381-14	ABW2379	OR203620	654
Concinocordis wilsonarum	Kenya, Western Province, Kakamega Forest	AFTOR383-14	ABW2379	OR231695	307
Concinocordis bartocki	Kenya, Eastern Province, Mt. Kenya Forest	AFTOR147-12	ABW2377	KJ592273	654
Concinocordis bartocki	Kenya, Eastern Province, Mt. Kenya Forest	AFTOR148-12	ABW2377	KJ592223	654
Concinocordis bartocki	Kenya, Eastern Province, Mt. Kenya Forest	AFTOR382-14	ABW2377	OR203618	654
Concinocordis bartocki	Kenya, Eastern Province, Castle Forest Lodge, S slope of Mt. Kenya	TOR-DNA-0571	ABW2377	OQ933720	654
Concinocordis sp.	Kenya, Coast Province, Watamu	AFTOR384-14	ACM9378	OR203619	654
Enarmonia formosana	Finland, South Karelia	LEFIB495-10	AAC5227	HM871390	654
Eugnosta percnoptila	Kenya, Kiambu, Kereita Forest	AFTOR133-12	ABW2213	KJ592350	654
Paraccra mimesa	Kenya, Tharaka-Nithi, Mt. Kenya Forest	AFTOR014-12	ABV8114	KJ592208	654
Proteoteras obnigrana	Canada, Quebec, Aylmer	MEC417-04	AAB4843	GU096042	654
Auratonota auranticaDHJ02	Costa Rica, Alajuela	BLPDI015-09	AAA4018	GU697916	654
Hilarographa muluana	Thailand, Trang	KHCSP060-16	ADA7980	MN036538	666
Anthozela psychotriae	Kenya, Embu	AFTOR155-12	ABW2142	KJ592378	654
Ancylis diminutana	Finland, South Karelia	LEFIB511-10	AAB6876	HQ570265	654
Eucosmomorpha albersana	Norway, Ostfold	LON395-08	AAF2360	KX047726	654



Figures 1-6. *Concinocordis* species. 1. Female paratype of *C. wilsonarum*. 2. Male genitalia of paratype of *C. wilsonarum*, USNM slide 128,811. 3. Female paratype of *C. bartocki*. 4. Male genitalia of paratype of *C. bartocki*, USNM slide 145,613. 5. Male of *C.* sp. 6. Male genitalia of *C.* sp., USNM slide 144,874.



Figures 7-9. Female genitalia and pupal exuvium of *Concinocordis* species. 7. Paratype of *C. wilsonarum*, USNM slide 144,875. 8. Paratype of *C. bartocki*, USNM slide 145,614. 9. Pupal exuvium of *C. wilsonarum*, with close-up of abdominal segment four.

(Fig. 8) with papillae anales slender; apophyses moderately long, slender, anteriores about as long as posteriores, distinctly curved medially; sterigma weakly sclerotized, minutely spined posteriorly; rim of ostium bursae slender; antrum weakly sclerotized, broader than ductus bursae; ductus bursae very slender, long, frail; ductus seminalis from ductus bursae immediately anterad of a small sclerite just posterad of junction of corpus bursae and ductus bursae; corpus bursae ovoid pear-shaped, with dense, extremely fine spines, except in posterior portion (longest spines ventrally and postmedially); signum a double-walled, tubular sclerite with extremely long, tubular capitulum with truncate end extending well outside corpus bursae and pointed end projecting inside corpus bursae, with a dense patch of inward projecting spines at junction of capitulum and corpus bursae.

Molecular characterization. The three DNA barcode sequences for *C. bartocki* have an average infraspecific variation of 1.53%, forming a BIN (BOLD:ABW2377) that is 9.31% different from its nearest neighbor.

Specimens examined. Holotype, ♂, Kenya, Coast Province, Ngangao Forest/Taita Hills, 1770 m, 3°22.33'S, 38°20.70'E, 2 May 2002, reared from unknown fruit, A&M 1981, USNM slide 124,421, R. S. Copeland (NMK).

Paratypes (1♂, 4♀). Kenya: Eastern Province: Mt. Kenya Forest: 2040 m, 0°14.256'S, 37°33.924'E, 6 Nov 2001 (1♀), reared from *Chrysophyllum gorungosanum*, A&M 1625, R. S. Copeland (NMK); 0°14.256'S, 37°33.924'E, 6 Nov 2001 (1♀), reared from *Chrysophyllum gorungosanum*, #1625, R. S. Copeland (USNM). 0°14.256'S, 37°33.924'E, 24 Jan 2002 (1♂, 1♀), reared from *Chrysophyllum gorungosanum*, #1705, R. S. Copeland (USNM). Castle Forest Lodge, S slope Mt. Kenya, 2080 m, 0°22'47''S, 37°18'39''E, 16 Dec 2010 (1♀), T. Gilligan & M. Epstein (Todd Gilligan Collection).

Distribution and biology. *Concinocordis bartocki* is recorded from Taita Hills of the Coast Province and Mt. Kenya Forest of the Eastern Province, Kenya, at elevations of 1770-2080 m, slightly higher than that recorded for *C. wilsonarum*. It has been reared only from *Chrysophyllum gorungosanum* (n = 5) (Sapotaceae). Although *C. wilsonarum* also utilizes the genus *Chrysophyllum*, it has been recorded from *C. albidum* (n = 2).

Etymology. This species is named for Craig Bartock, my long-time friend and former guitarist of the band Heart, for which the genus is named.

Remarks. The holotype and a female paratype of *Concinocordis bartocki* were originally included among the paratypes of *C. wilsonarum* in the description of the latter (Razowski & Brown, 2012).

Concinocordis sp.
(Figs. 5, 6)

Diagnosis. Based on the ML and Bayesian trees (Figs. 10a, b), a single specimen from the Coast Province of Kenya represents a third species in the genus. Because the specimen is in extremely poor condition (Fig. 5), no comparisons of maculation can be made. The male genitalia (Fig. 6) are most similar to those of *C. wilsonarum*, with a relatively broad neck of the valva that is

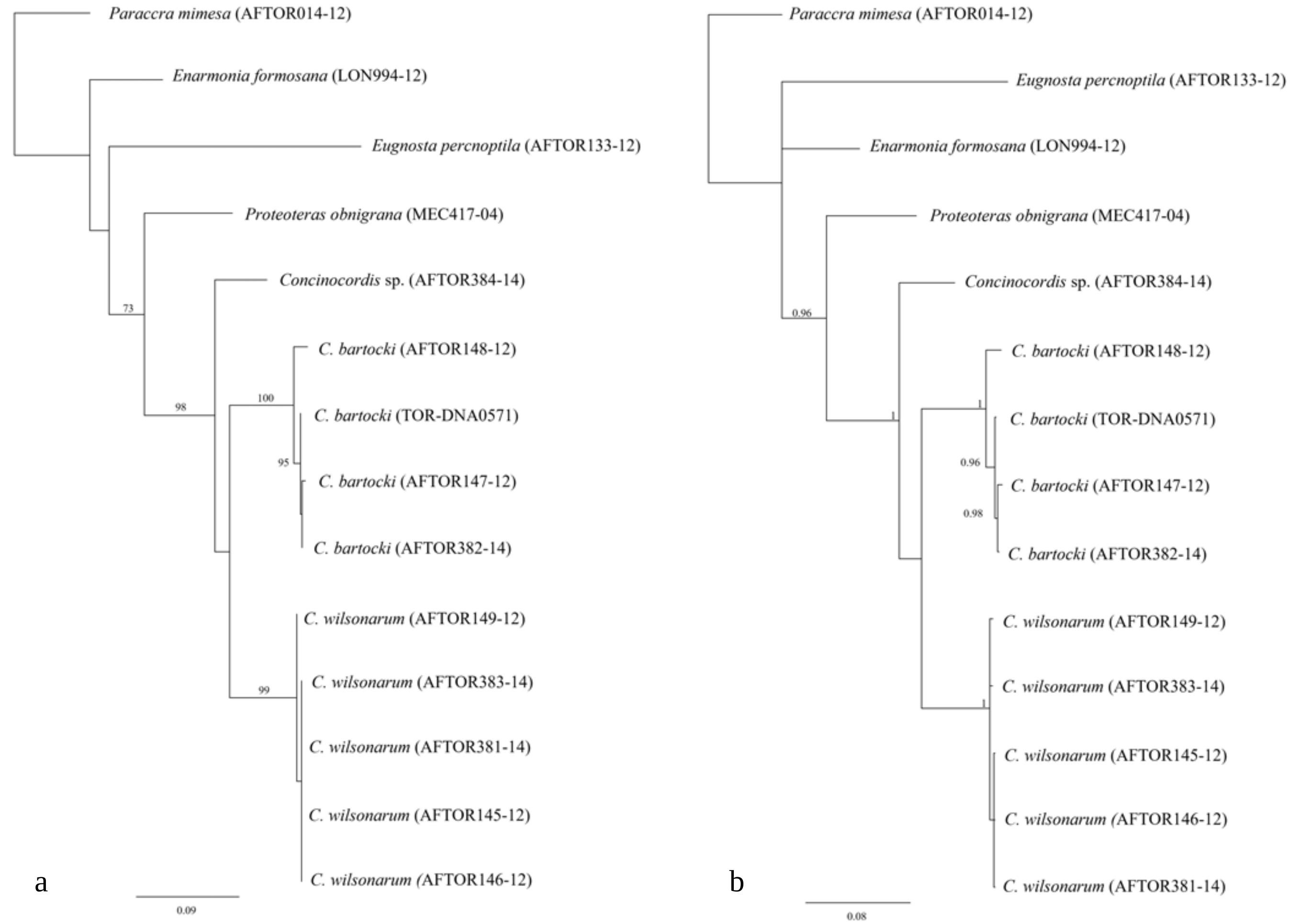


Figure 10. Maximum likelihood (a) and Bayesian (b) trees for all sequences of *Concinocordis* and four outgroup taxa using 654-bp of the cytochrome c oxidase subunit I gene. For the maximum likelihood algorithm, bootstrap values are based on 1000 replicates and only values >70% are shown above the nodes. Only Bayesian posterior probabilities greater than 0.70 are shown.

slightly more excavated than that of *C. wilsonarum*, but not as deep as that of *C. bartocki*.

Molecular characterization. The single barcode sequence for this species (BOLD:ACM9378) is 8.68% different from those of the nearest neighbor, *Concinocordis wilsonarum*.

Specimen examined. Kenya: Coast Province: Watamu, 3°21.75'S, 40°00.17'E, 20 ft [6 m elevation], 19 Feb 2000 (♂), reared from *Mimusopus obtusifolisa*, KIP-516, USNM slide 144,874, R. S. Copeland (NMK).

Distribution and biology. This species is known from a single male collected near Watamu at an elevation 6 m, a remarkably lower elevation locality than those its congeners. The specimen was reared from *Mimusopus obtusifolisa* (Sapotaceae), a plant that hosts no other congeners.

DISCUSSION

Brown *et al.* (2014) originally recognized that DNA barcodes of *C. wilsonarum* formed two distinct clusters separated by about 5% divergence; however, they were unable to distinguish the two groups based on morphology. Additional material and barcode sequences have revealed a third cluster (Figs. 10a, b), and these new data facilitated the recognition of subtle morphological characters and forewing pattern elements that distinguish the three detailed above.

While the relationship among the three species is now clear, the phylogenetic position of *Concinocordis* within Tortricidae remains enigmatic. The presence of one row of scales per flagellomere on the antennae, the firm attachment of the phallus to the juxta, the eucosmine-like valvae with a distinct cucullus, and absence of a cremaster in the pupa all provide support for its assignment to Olethreutinae. Owing to its remarkable similarity to the Afrotropical *Enarmoniodes mirabilis* Ghesquière, 1940 (see Razowski *et al.* 2010: fig. 111) in forewing maculation and size, Razowski & Brown (2012) placed the genus in Enarmoniini. However, the highly unusual signum of the female genitalia is unlike that of any other member of Enarmoniini, or for that matter, any other tortricid of which we are aware. Furthermore, the absence of the characteristic paired rows of dorsal spines on the pupa of *Concinocordis* (Fig. 9) has been reported in only a few other tortricid taxa; e.g., the equally enigmatic *Tortrimosaica polypodivora* Brown & Baixeras, 2004 and at least two species of Hilarographini (Brown *et al.* 2004). This unusual character state may be linked to internal feeding behavior, although pupal spines are present in most fruit- and internal-feeding tortricids. The last two features (i.e., unusual female signum and absence of pupal spines) combine to make confident assignment of *Concinocordis* to a tribe impossible at present.

The ambiguity regarding the tribal assignment of *Concinocordis* likewise was reflected in our molecular results. In additional to our initial analysis (Figs. 10a, b), we constructed multiple trees based on various outgroup taxa, one example of which includes the following: *Paraccra mimesa* (Tortricini) (with similar raised forewing spots); *Eugnosta percnoptila* (Cochylini); *Ancylis dimunutana* (Haworth, 1811), *Eucosmomorpha albersana* (Hübner, 1811) and *Anthozela psychotriae* Brown & Razowski (all Enarmoniini) (putative tribal affinity); *Auratonota aurantica* (Chlidanotini); *Hilarographa mululana* Razowski (Hilarographini) (with similar pupal morphology); and *Proteoteras obnigrana* (Eucosmini) (with similar cucullus) (see Table 1). The results of this analysis are provided in Supplements S1 and S2, ML and Bayesian trees, respectively. None of the additional analyses provided meaningful insight into relationships between *Concinocordis* and the outgroup or among outgroup taxa in general. While outgroup relationships shifted, the monophyly of *Concinocordis* was maintained in every tree, with the North American eucosmine species *Proteoteras obnigrana* most frequently occupying the position of the closest relative. This lack of resolution is not surprising because COI typically has limited phylogenetic signal beyond the species level (Mallo and Posada, 2016; Trunz *et al.*, 2016). Although additional genomic data would undoubtedly provide greater resolution to the tribal assignment of *Concinocordis*, that analysis is beyond the scope of the present work.

ACKNOWLEDGMENTS

We thank Robert Copeland for the opportunity to examine specimens reared from native fruit in Kenya, and Scott Miller for facilitating the sequencing of the specimens. Barcoding was performed at the Institute for Biodiversity of Ontario at the University of Guelph, Canada and Colorado State University, Fort Collins, Colorado. Finally, we thank Leif Aarvik, Joaquin Baixeras, and Ryan St Laurent for helpful comments that increased the quality and clarity of the brief manuscript.

LITERATURE CITED

Brown, J. W., Baixeras, J., Solorzano-Filho, J. A., Kraus, J. E. 2004. Description and life history of an unusual fern-feeding tortricid moth (Lepidoptera: Tortricidae) from Brazil. *Annals of the Entomological Society of America* 97: 865-871.

Brown, J. W., Copeland, R. S., Aarvik, L., Luke, Q., Miller, S. E., Rosati, M. 2014. New host records for fruit-feeding Afrotropical Tortricidae (Lepidoptera). *African Journal of Entomology* 22: 343-376.

Brown, J. W., Powell, J. A. 1991. Systematics of the *Chrysoxena* group of genera (Lepidoptera: Tortricidae: Euliini). *University of California Publications in Entomology* 111. 87 pp. + figs.

Copeland, R. S., Wharton, R. A., Luke, Q., De Meyer, M. 2002. Indigenous hosts of *Ceratitis capitata* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America* 95: 672-694.

Craft, K. J., Pauls, S. U., Darrow, K., Miller, S. E., Hebert, P. D. N., Helgen, L. E., Novotny. V., Weiblen, G. D. 2010. Population genetics of ecological communities with DNA barcodes: An example from New Guinea Lepidoptera. *Proceedings of the National Academy of Sciences, U.S.A.* 107: 5041-5046.

Darriba, D., Taboada, G. L., Doallo, R., Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792-1797.

Ghesquière, J. 1940. Catalogues raisonnés de la faune entomologique du Congo Belge. Lépidoptères, Microlépidoptères (première partie). *Annales du Musee du Congo Belge. Zoologie. Serie III* (II)7(1): 1-120.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59(3): 307-321

Hebert, P. D. N., deWaard, J. R., Zakharov, E. V., Prosser, S. W. J., Sones, J. E., McKeown. J. T. A., Mantle, B., La Salle, J. 2013. A DNA 'Barcode Blitz': Rapid digitization and sequencing of a natural history collection. *PLoS One* 8 (7): e68535.

Huelsenbeck J.P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 754-755.

Mallo, D., Posada, D. 2016. Multilocus inference of species trees and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371(1702): 20150335.

Miller, M. A., Pfeiffer, W., Schwartz, T. 2010. *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*, pp. 1-8. 2010 Gateway Computing Environments Workshop (GCE).

Ratnasingham, S., Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife.org). *Molecular Ecology Notes* 7(3): 355-364.

Razowski, J. 1981. Nigerian Tortricini (Lepidoptera, Tortricidae). *Acta Zoologica Cracoviensia* 25: 319-340.

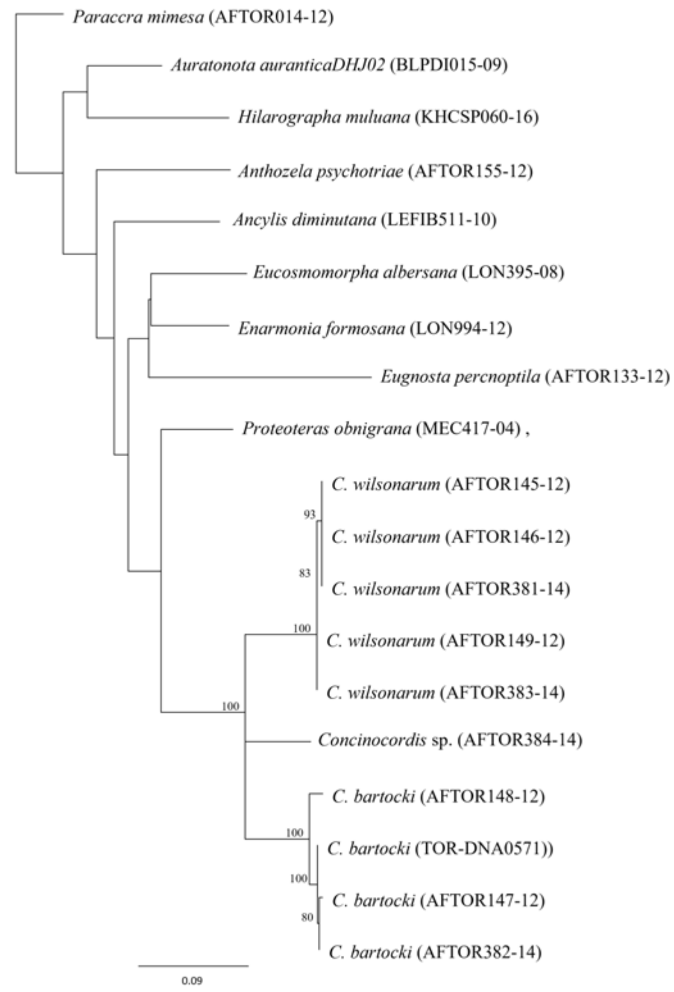
Razowski, J. 2002. *Tortricidae of Europe. Part I. Tortricinae and Chlidanotinae*. Bratislava, Frantisek Slamka. 247 pp.

Razowski, J., Aarvik, L., De Prins, J. 2010. An annotated catalogue of the types of Tortricidae (Lepidoptera) in the collection of the Royal Museum for Central Africa (Tervuren, Belgium) with descriptions of new genera and new species. *Zootaxa* 2469: 1-77.

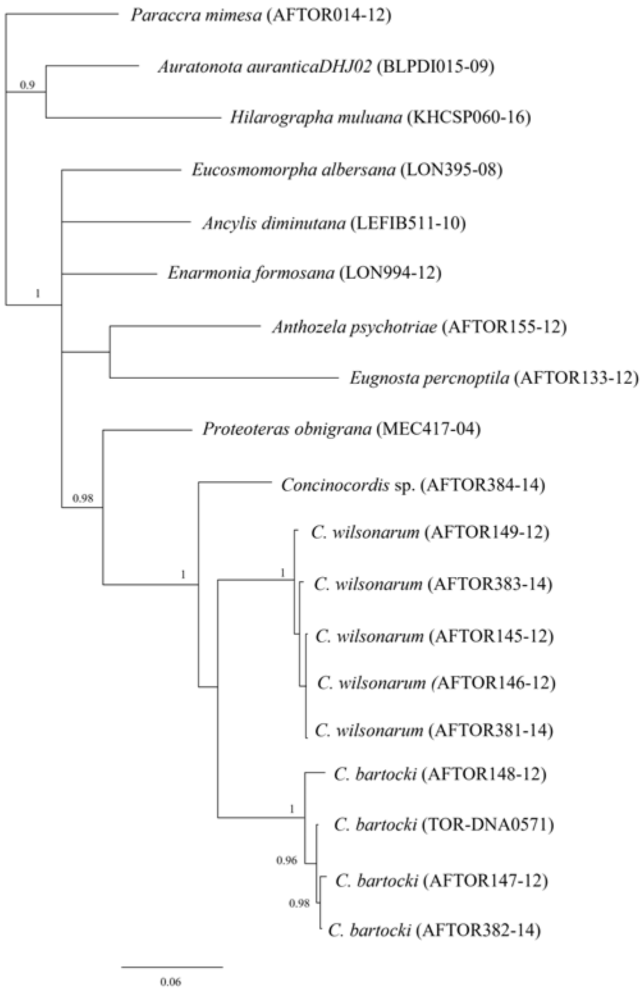
Razowski, J., Brown, J. W. 2012. Descriptions of new Tortricidae (Lepidoptera) reared from native fruit in Kenya. *Zootaxa* 3222: 1-27.

Trunz, V., Packer, L., Vieu, J., Arrigo, N., Praz, C. J. 2016. Comprehensive phylogeny, biogeography and new classification of the diverse bee tribe Megachilini: Can we use DNA barcodes in phylogenies of large genera? *Molecular Phylogenetics and Evolution* 103: 245-259.

Wilson, J. J. 2012. *DNA barcodes for insects*, pp. 17-46. *In*: Kress, W. J., Erickson, D. L. (Eds.), *DNA barcodes: Methods and Protocols*. New York, Springer.



S1. Supplemental tree. ML tree for three species of *Concinocordis* and nine outgroup taxa using 654-bp of the cytochrome c oxidase subunit I gene.



S2. Supplemental tree. Bayesian tree for *Concinocordis* and nine outgroup taxa using 654-bp of the cytochrome c oxidase subunit I gene.

The larval host plant and ant associate of *Nacaduba pavana georgi* (Lepidoptera: Lycaenidae) in Negros, Philippines

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Date of issue online: 27 October 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.10027012.

Abstract: The immature stages of the Small Four-Line Blue *Nacaduba pavana georgi* (Horsfield, [1828]) are described and illustrated from Mount Talinis, Negros Island, Philippines. Larvae fed on rambutan flowers (Sapindaceae: *Nephelium lappaceum* L.), a new host plant record, and associated with the ant *Philidris myrmecodiae* (Emery, 1887). We conclude by discussing the known host plant breadth and ant associates of other species of *Nacaduba* Moore, 1881.

Key words: host plant; life history; myrmecophily; *Nacaduba*; Negros; Philippines; rambutan.

INTRODUCTION

The Small Four-Line Blue, *Nacaduba pavana* (Horsfield, [1828]) (Lepidoptera: Lycaenidae), is widely distributed in Afghanistan, Pakistan, India, Nepal, Bangladesh, Myanmar, Thailand, Cambodia, Vietnam, Malaysia, Singapore, and the Philippines (Pinkert *et al.*, 2022), but is typically rare or locally distributed (Hardy & Lawrence, 2017; Corbet *et al.*, 2020). There are two recognized subspecies in the Philippines: ssp. *asaga* Fruhstorfer, 1916 in Palawan, and ssp. *georgi* Fruhstorfer, 1916, which occurs in Leyte, Luzon, Mindoro, Mindanao, Negros, Panay, and Sibuyan (Treadaway & Schroeder, 2012). Despite the wide distribution of the species, there are few records of host plants and none for its ant associates. In their field guide to Philippine butterflies, Hardy & Lawrence (2017) list three host plants for the species: *Andira inermis* (W.Wright) DC. and *Derris trifoliata* Lour. (both Fabaceae), and *Allophylus cobbe* (L.) Forsyth f. (Sapindaceae). No citation is provided for these records, and the first author (P. B. Hardy, pers. comm.) cannot confirm the source of these data, which are not from the authors' own rearing records. It seems likely that the records are from Singapore, as the same three host species are recorded by Tan *et al.* (2011). We are aware of no other host records and no ant associate records in the literature. This paper documents the life history of *Nacaduba pavana georgi* from the Philippine island of Negros, including a new host plant record and the first recorded ant associate, which is identified to the species level.

MATERIALS AND METHODS

An adult female butterfly (Figs. 1-2) was observed in May 2020 in a small, private rambutan plantation (Sapindaceae: *Nephelium lappaceum*) located along the Apolong Trail at the base of Mount Talinis. This area is a mosaic of secondary and primary forests with occasional or seasonal small-scale agriculture (600 m elevation; 9.281838 N, 123.206465 E). Inflorescences with eggs and three larvae were collected and reared.

The specimens were reared indoors in a space that was occasionally air conditioned. The flowers were periodically replaced, especially when showing signs of senescence. Two males and one female emerged from pupae. Voucher specimens of butterflies were deposited in the Rodolfo B. Gonzales Museum of Natural History, Biology Department, Silliman University, Dumaguete City, Negros Oriental, Philippines.

RESULTS

Nacaduba pavana georgi
(Figs. 1-26)

Immature stages:

Egg. We did not observe oviposition, but found eggs on flower buds of rambutan trees (Sapindaceae: *Nephelium lappaceum*) 5-10 m from the ground after observing a female fluttering in the treetops. The area had been modified into a lawn with remnant patches of forest trees, shrubs, and ferns. The sloping terrain of a small rambutan plantation made observations of the treetops easier (Fig. 27). The egg is white and cylindrical, with flat top and bottom (Figs. 3-4), and has a dorsal surface with dentations. The egg laid on the flower buds did not hatch when it was transferred to a container since the buds withered, so we were unable to document the first several instars.

Possibly 4th larval instar. We also found large, onisciform caterpillars in the inflorescences. They were pale green with 10-11 segments and whitish dorsal lines with conspicuous pale brown lines in the middle (Fig. 5). Midway between the dorsal

Figures 1-2 (1 above, 2 below). Adult female of *Nacaduba pavana georgi* preparing to oviposit.

Figures 3-4. Egg of *Nacaduba pavana georgi*.

Figure 5. Possible 3rd to 4th instar caterpillar of *Nacaduba pavana georgi*.

Figures 6-8. A *Philidris myrmecodiae* ant “piggy-back riding” on a *Nacaduba pavana georgi* caterpillar while the latter is searching for food.

Figures 9-12. *Nacaduba pavana georgi* caterpillar tended by five *Philidris myrmecodiae* ants.

Figure 13. Posterior region of *Nacaduba pavana georgi* caterpillar showing the dorsal nectary organ (white arrow) and the tentacle organ (red arrow).

Figures 14-15. Flower inflorescence of rambutan (Sapindaceae: *Nephelium lappaceum*).

and lateral part is a pale green wavy pattern. The body has setae especially anteriorly where they are longer and the body can be wider (appear flatter) or narrower (Fig. 6), likely assisting with moving along floral pedicels/peduncles and among tightly clustered flower buds. The anterior region is rounder while the posterior can be rounder or flatter. The larva was tended by ants.

Last larval instar. The last instar is greenish with tiny brown spots on mid-dorsum and a large brown, diamond-shaped marking on the posterior. The segments are not pronounced due to suture-like impressions. While feeding on flower buds, the larvae periodically secreted a droplet from their dorsal nectary organ (DNO) on the seventh abdominal segment. The ants did not appear to solicit these droplets, for example by antennating the area around the DNO, but eagerly consumed them (Fig. 9).

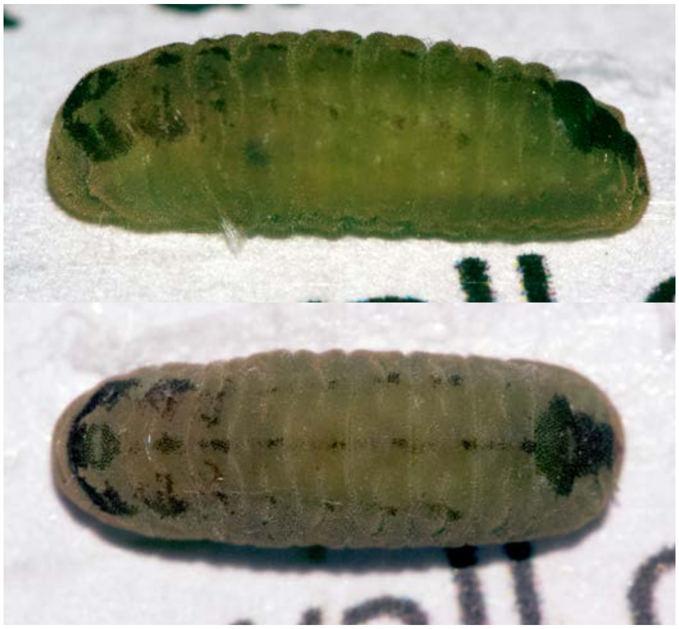
Prepupa. The prepupa is greenish with a slight purple composition, with brown spots and markings now more pronounced (Figs. 16-17). The larva is slightly shortened and the segments became visible as well as the suture-like impressions. The prepupa is held to the substrate by its cremaster and a silk girdle around the thorax.

Pupa. The late larval stage was documented in June 8, 2020 and pupated after 3 days (11 June). During the first few hours (Fig. 18) the pupa was pale brown, with greenish composition on the regions where wings develop, then turning brownish with dark brown spots (Figs. 19-20). Due to the impressions of the pupa, the future head, thorax, and abdomen are visible, even though the wings and the venation of the wings are visible (except during the later pupal stage). Adults emerged after 5-7 days.

Habitat: *Nacaduba pavana georgi* is found in primary and secondary forest at middle elevations of Mount Talinis, where rambutan trees are commonly planted. Females were seen flying around treetops, laying eggs on flower buds, and sipping rambutan nectar. Another female (Fig. 25) imbibed nectar from a nearby Tree-vine (*Leea manillensis* Walp. (Vitaceae)). We observed a male (Fig. 26) puddling at a nearby creek around 10:00 hrs and males were observed occasionally feeding on Tree-vine flowers from April to June (C. Chafer, pers. comm).

Hostplant: On Negros, the larvae were documented feeding on the inflorescences of rambutan (Sapindaceae: *Nephelium lappaceum*). This tree species is native to Southeast Asia and is widely cultivated for its fruit.

Ant associates. *Philidris myrmecodiae* (Emery, 1887) (Dolichoderinae) ants tended late larval instars; early larval instars were not observed. Identification of the ant was based on characters in Shattuck (1992) including the relative anterior position of the compound eye. During the middle instars, a single ant was observed “piggy-back riding” on the caterpillar (Figs. 6-8). It is possible that the ant, riding the caterpillar while it was foraging, was waiting for the caterpillar to find an inflorescence. Once the caterpillar starts feeding, the ant returns to its nest, leaving scent trails to recruit others from its colony. While feeding, the caterpillar was tended by approximately five ants. When threatened (as when the first author got close to take photographs), the ants seemed to assume defense positions in



Figures 16-17. Prepupa of *Nacaduba pavana georgi*.

Figures 18-20. Early and later stages of the pupa of *Nacaduba pavana georgi*.

Figures 21 (left) - **22** (right). Upperside and underside of male *Nacaduba pavana georgi*.

Figures 23 (left) - **24** (right). Upperside and underside of female *Nacaduba pavana georgi*.

which one ant stood on the head, another on the posterior, two on the dorsum, and another collected honeydew from the dorsal nectary organ (Figs. 9-13).

DISCUSSION

Nacaduba seems to be a genus of generalist herbivores that facultatively associate with ants. Of the 46 recognized *Nacaduba* species, only 14 have host plant records and 6 have records of ant associates, though the true number of ant-associated species in this genus is likely higher (Robinson *et al.*, 2020, 2021; Fiedler, 2021; Pierce & Dankowicz, 2022b). Eleven of the species with host plant records include Fabaceae in their host repertoire, which seems to be the most commonly eaten host plant family of the genus. Further, most *Nacaduba* species with known life histories appear to be polyphagous. For example, *N. kurava* (Moore, 1857) larvae feed on plants in 9 families from 6 orders, *N. berenice* (Herrich-Schäffer, 1869) larvae consume plants in 7 families from 7 orders, and *N. hermus* Felder, 1860 larvae feed on plants from 6 families in 4 orders (Robinson *et al.*, 2020).

Larval ant associations are recorded sporadically throughout the Lepidoptera, but are most common in the family Lycaenidae. In a typical association, a small number of ants "stand guard" over a butterfly larva as it feeds, attempting to protect it from predators and parasites, while the larva plies the ants with nutritious secretions from the dorsal nectary organ and apparently also from its cuticle (Pierce, 1984; Pierce *et al.*, 2002; Daniels *et al.*, 2005; Pierce & Dankowicz, 2022a). The DNO secretions of at least one lycaenid species alter the brain chemistry of their ant associates by decreasing dopamine, which reduces the ants' locomotory ability and causes them to remain near the larva (Hojo *et al.*, 2015). Some lycaenid-ant associations are obligate, meaning that larvae are always found with ants in the field, while others are facultative: larvae are occasionally observed without ants in nature. Pierce & Dankowitz (2022b) record 881 species of ant-associated lycaenid butterflies, and examination of their data and the tables of Fiedler (2021) suggest that this report is the first record of ant association in *Nacaduba pavana*.

Most other *Nacaduba* species that have been observed as larvae in the field seem to be facultatively myrmecophilous. In Sri Lanka, for example, *N. berenice*, *N. hermus*, *N. sinhala* (Ormiston, 1924) associate with *Technomyrmex* Mayr, 1872 spp. (Dolichoderinae), and *N. pactolus* (C. Felder, 1860) associates with *Camponotus* Mayr, 1861 sp. (Formicinae) (van der Poorten & van der Poorten, 2016). *Nacaduba kurava* and *N. calauria* (C. Felder, 1860) larvae have also been observed with unidentified ants. All of these interactions appear to be facultative, as larvae are sometimes found without ants (van der Poorten & van der Poorten, 2016). *Nacaduba berenice* is also reported to be facultatively myrmecophilous in Australia, where it has been recorded in association with *Camponotus* sp., *Oecophylla smaragdina* Fabricius, 1775, *Paratrechina* Motschulsky, 1863 sp., *Polyrhachis rufifemur* Forel, 1907, *P. vermiculosa* Mayr, 1876 (all Formicinae), and *Crematogaster* Lund, 1831 sp. (Myrmicinae) (Braby, 2000). In Papua New Guinea, *N. berenice* larvae are facultatively associated with

Figure 25. A female *Nacaduba pavana georgi* feeding on the flowers of a Tree-vine plant (*Leea manillensis*). Photograph by C. Chafer.

Figure 26. A male *Nacaduba pavana georgi* puddling on the side of a creek. Photograph by C. Chafer.

Figure 27. The first author documenting the larvae of *Nacaduba pavana georgi* on a rambutan tree. Photograph by J. H. Oracion.

an unidentified ant in the subfamily Dolichoderinae and *Anoplolepis gracilipes* F. Smith, 1857 (Formicinae) (Parsons, 1998).

Philidris Shattuck, 1992 ants frequently form mutualistic partnerships. *Philidris myrmecodiae* was originally described as a subspecies of *P. cordatus* (Emery, 1887), and this latter species associates with larvae of *Hypochrysops apollo* Miskin, 1891, *H. elgneri* (Waterhouse & Lyell, 1909), *H. narcissus* Fabricius, (1775), *H. theon* Felder & Felder, 1865, *Jamides cyta* (Boisduval, [1832]), and *Ogyris aenone* (Waterhouse, 1902) (all Lycaenidae) in Australia (Braby, 2000). The specific epithet *myrmecodiae* suggests this ant species also associates with *Myrmecodia* spp. (Rubiaceae), which are epiphytic ant-plants endemic to Southeast Asia and Australasia. Arboreal ants, including *Philidris cordatus* (Huxley, 1978), live in the modified rhizomes of *Myrmecodia* Jack, with mutual benefits for both parties (Janzen, 1974; Wallace, 1989).

Interactions between lycaenid larvae and ants are highly variable. Most are presumed to be mutualistic, including the association documented here. Other relationships can be parasitic, with butterfly larvae engaging in trophallaxis with the ants or consuming their larvae or Hemiptera symbionts (Pierce & Dankowitz, 2022b). The ability to make strong conclusions about evolutionary patterns of butterfly-ant associations, and to some extent butterfly-host associations, are constrained by the dearth of natural history observations recording larval biology (Kaliszewska *et al.*, 2015; Kawahara *et al.*, 2023). Observations like ours, which document novel interactions by identifying all organisms to the species or subspecies level and vouchering specimens in public museums, are necessary to fully understand the evolution of insects and their interactions with other organisms.

ACKNOWLEDGMENTS

The senior author would like to thank Leandro S. Cabrera and Jean Henri Oracion for their time and assistance during their irregular survey and hiking trips along the Apolong Trail of Mount Talinis, Negros Island, Philippines. To Chris and Anna Chafer for their additional information regarding this butterfly in Mount Talinis, to the local people of Apolong for allowing us to hike and photograph birds and insects in the area (research was done at a private property thus obviating the need for a Gratuitous Permit), and to an anonymous reviewer of the manuscript. Jade Aster T. Badon was funded by the Tony Whitten Conservation Award.

LITERATURE CITED

Braby, M. F. 2000. *Butterflies of Australia: Their Identification, Biology, and Distribution*. Collingwood, CSIRO Publishing.

Corbet, A. S., Pendlebury, H. M., van der Poorten, G. M., van der Poorten, N. E. 2020. *The Butterflies of the Malay Peninsula. Fifth Edition*. Kuala Lumpur, Southdene Sdn Bhd. 634 pp + xiv.

Daniels, J. C., Pence, J. A., Emmel, T. C. 2005. A new hostplant record for *Strymon martialis* (Lycaenidae: Theclinae) in the Florida Keys. *Journal of the Lepidopterists' Society* 59(3): 174-175.

Emery, C. 1887 [1886]. Catalogo delle formiche esistenti nelle collezioni del Museo Civico di Genova. Parte terza. Formiche della regione Indo-Malese e dell'Australia. [part]. *Annali del Museo civico di storia naturale Giacomo Doria* 24[=(2)(4)]: 241-256.

Fiedler, K. 2001. Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. *Diversity and Distributions* 7: 45-60.

Fiedler, K. 2021. The ant associates of Lycaenidae butterfly caterpillar - revisited. *Nota Lepidopterologica* 44: 159-174.

Hardy, P. B., Lawrence, J. M. 2017. *Field Guide to Butterflies of the Philippines*. Manchester, Siri Scientific Press.

Hojo, M. K., Pierce, N.E., Tsuji, K. 2015. Lycaenid caterpillar secretions manipulate attendant ant behavior. *Current Biology* 25: 2260-2264.

Huxley, C.R. 1978. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* 80: 231-268.

Igarashi, S., Fukuda, H. 1997. *The Life Histories of Asian butterflies, Vol. 1*. Tokyo, Tokai University Press. 550 pp.

Janzen, D. H. 1974. Epiphytic myrmecophytes in Sarawak: Mutualism through the feeding of plants by ants. *Biotropica* 6:237-259.

Kaliszewska, Z. A., Lohman, D. J., Sommer, K., Adelson, G., Rand, D. B., Mathew, J., Talavera, G., Pierce, N. E. 2015. When caterpillars attack: Biogeography and life history evolution of the Miletinae (Lepidoptera: Lycaenidae). *Evolution* 69: 571-588.

Kawahara, A. Y., *et al***.** 2023. A global phylogeny of butterflies reveals their evolutionary history, ancestral hosts and biogeographic origins. *Nature Ecology & Evolution* 7: 903-913.

Parsons, M. 1998. *The Butterflies of Papua New Guinea*. San Diego, Academic Press.

Pierce, N. E. 1984. *Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants*, pp.197-200. *In*: Vane-Wright, R. I., Ackery, P. R. (Eds.), *The Biology of Butterflies*. London, Academic Press.

Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., Travassos, M. A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47: 733-771.

Pierce, N. E., Dankowicz, E. 2022a. Behavioral, ecological and evolutionary mechanisms underlying caterpillar-ant symbioses. *Current Opinion in Insect Science* 52(100898): 1-9.

Pierce, N. E., Dankowicz, E. 2022b. *The Natural History of Caterpillar-Ant Associations*, pp. 319-391. *In*: Marquis, R., Koptur, S. (Eds.), *Caterpillars in the Middle*. Chicago, Springer International Publishing.

Pinkert, S., Barve, V., Guralnick, R., Jetz, W. 2022. Global geographical and latitudinal variation in butterfly species richness captured through a comprehensive country-level occurrence database. *Global Ecology and Biogeography* 31: 830-839.

Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni, G. W., Hernández, L. M. 2020. HOSTS - a Database of the World's Lepidopteran Hostplants. http://www.nhm.ac.uk/our-science/data/hostplants/. Accessed 1 August 2020.

Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni, G. W., Hernández, L. M. 2001. *Hostplants of the Moth and Butterfly Caterpillars of the Oriental Region*. Kuala Lumpur, Southdene & Malaysian Nature Society. 744 pp.

Shattuck, S. O. 1992. Review of the dolichoderine ant genus *Iridomyrex* Mayr with descriptions of three new genera (Hymenoptera, Formicidae). *Journal of the Australian Entomological Society* 31: 13-18.

Tan, H., Ho, F., Chir, S., Khew, S. K. 2011. Life history of the Singapore Four-Line Blue (*Nacaduba pavana singapura*). https://butterflycircle.blogspot.com/2011/11/life-history-of-singapore-four-line.html. Accessed 30 October 2022.

Treadaway, C. G., Schroeder, H. G. 2012. Revised checklist of the butterflies of the Philippine islands (Lepidoptera: Rhopalocera). *Nachrichten des Entomologischen Vereins Apollo* S20: 1-64.

Van der Poorten, G. M., van der Poorten, N. 2016. *The Butterfly Fauna of Sri Lanka*. Colombo, Lepodon Books. 418 pp.

Wallace, B. J. 1989. *Vascular epiphytism in Australo-Asia*, pp. 261-282. *In*: Lieth, H., Werger, M. J. A. (Eds.), *Tropical Rain Forest Ecosystems*. Amsterdam, Elsevier.

A preliminary illustrated list of the subfamily Epipleminae (Geometroidea: Uraniidae) of Jamaica, West Indies

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Date of issue online: 27 October 2023
Electronic copies (ISSN 2575-9256) in PDF format at: https://journals.flvc.org/troplep; https://zenodo.org; archived by the Institutional Repository at the University of Florida (IR@UF), http://ufdc.ufl.edu/ufir; **DOI**: 10.5281/zenodo.10027053.

Abstract: The Jamaican species of the uraniid subfamily Epipleminae have been poorly studied. Only two species, a *Calizzia* and an *Erosia*, have been documented from Jamaica in recent species lists. Eight genera are now recognized from Jamaica from the present study. Records of Epipleminae from Florida and the Greater Antilles are tabulated.

Key words: Caribbean; Epipleminae; light traps; photography; Uraniidae.

INTRODUCTION

The Greater Antillean and Floridian representatives of the uraniid subfamily Epipleminae include thirteen genera. Seven genera each with a single species are recognized from Florida (FL) (Kimball, 1965; Heppner, 2003), seven genera with ten species from Cuba (CU) (Núñez & Barro Cañamero, 2012), and four genera with seven species in Puerto Rico (PR) (Moth Photographers Group, 2017). Hispaniola (HI) is expected to have more than the four species listed in Table 1. In Jamaica, an epiplemid identified as belonging to the genus *Callizzia* Packard, 1876, was collected and photographed by Barnes (2002). *Erosia incongua* Butler (1878), was also listed from Jamaica by Gowdey (1926), and next recorded during present studies in 2020. In these investigations eight genera have been collected or photographed in Jamaica between 2018 and 2023.

The preliminary information presented here is intended to form the basis for more intensive future studies. Many more collection sites across the island need to be explored and existing museum collections need to be examined. However, there have been no recent publications of Jamaican species in this subfamily.

MATERIALS AND METHODS

Adults were observed after being attracted to light sources at night using either a Honda Ex350 generator with 110 volt/350-amp output, ballast, and regulator, and 125W clear bulb; or 110/120V AC mains supply with clear 275-watt bulb and BioQuip #2819 Rain Shield and #2818S Poly Skirt, or mercury vapor (MV) 120-volt self-ballasted bulb, with or without use of a #2804 AC/DC black light. In December 2022, a new light source was added consisting of a LepiLED Maxi Switch powered by USB connection to a standard rechargeable Power Bank 26,000 mAh, QC 3.0. Each of these lights were placed in front of a large white collection sheet; the black light operated separately, away from the MV light source. Most trapping was conducted through the night from sunset until an hour before sunrise, usually around the time of a new moon.

Adults were photographed while at rest using a Nikon D800 camera with a Nikkor AF-S 18-200 mm lens or Nikon D850 camera with a Micro Nikkor AF-S 105 mm lens. These photographs were used to compare with published photographs of similar species. Points of similarities in wing markings between those specimens photographed and/or collected and those of specimens from neighboring territories were helpful in making tentative identifications. Differences in wing markings between those specimens collected in Jamaica and photographs found at online websites may be indicators of the presence of closely related taxa requiring further investigation, but such studies have not yet been conducted.

The focus so far has been on enumerating species not recently or previously identified from Jamaica. Species are arranged in alphabetical order by genus. Representatives of species collected have been deposited at the Natural History Museum Jamaica, at the Institute of Jamaica (NHMJ, IJ), Kingston in accordance with the terms of the permit issued by the National Environmental Protection Agency of Jamaica.

With reference to Fig. 1, trapping was conducted at the following sites: **Guava Ridge [1], St. Andrew Parish:** 18°1'40.9"N, 76°40'36.6"W. Elevation 975 m. Secondary forest on shales and conglomerate soils facing the southwestern slopes of the Blue Mountain range. **Mount Diablo [2], St. Ann Parish:** 18°12'0"N, 77°7'0"W. Elevations from 332 m to 858 m. Unpaved road south from Moneague to Hollymount through disturbed primary wet limestone forest southwest of Mount Diablo. **Marshall's Pen [3], Mandeville, Manchester Parish:** 18°3'0"N, 77°31'60"W. Elevation 655 m. Undisturbed primary wet limestone forested hilltops with cattle pastures between. **Silver Hill Gap [4], St. Andrew Parish:** 18°5'23"N, 76°41'21"W. Elevation 1,035 m. Disturbed land with coffee plantations overlooking undisturbed montane riverine forest in valleys below. **Windsor Great House [5], Trelawny Parish:** 18°22'0"N, 77°37'0"W. Elevation 180 m. Edge of primary minimally disturbed wet limestone forest. **Portland Ridge [6], Clarendon Parish:** 17°44'22"N, 77°9'27"W. Elevation 157 m. Dry limestone forest.

NOTES ON JAMAICAN EPIPLEMINAE

[1] Genus *Antiplecta* Warren, 1900

Antiplecta triangularis (Warren, 1906). Fig. 2, Table 1. Average wingspan 12.5 mm; forewing length 5.4 mm (n=5).

Two *Antiplecta* examined in this study were photographed at the Marshall's Pen trapping site on 12 March 2021, and 4 June 2022. Seventy-five specimens were observed at Portland Ridge, Clarendon Parish in dry limestone forest on 22 May 2023. The specimens at this site were very small with wingspans of only 11 mm. We have observed these much smaller than normal sizes in Cossidae specimens, also in this dry habitat. However, the forewing shape and markings and antennal color indicate that this species is *Antiplecta triangularis* when compared with photographs of this species documented by the Moth Photographers Group (2017), and it conforms to Moths of North America ID # 7652.

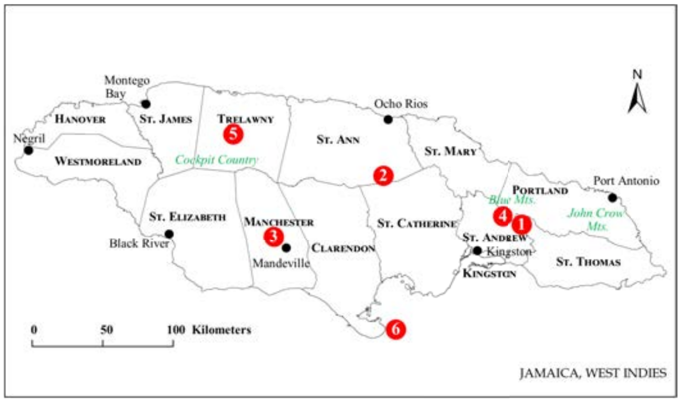

Figure 1. Map of Jamaica showing principal moth trapping locations for this study: 1. Guava Ridge; 2. Mount Diablo; 3. Marshall's Pen; 4. Silver Hill Gap; 5. Windsor Great House; 6. Portland Ridge.

[2] Genus *Calledapteryx* Grote, 1868

Calledapteryx dryopterata Grote, 1868. Fig. 3, Table 1. Average wingspan: 25.5 mm; forewing length 12.5 mm (n=4).

Not previously listed for Jamaica. The first specimen of this moth was photographed at Marshall's Pen on 5 August 2018. Two specimens were photographed at the same site in April 2020 and another in June 2022. The well-defined indentation in the outer wing margin below the apex is only minimally expressed

Figures 2-10. 2. *Antiplecta triangularis* (Warren, 1906). Portland Ridge, Clarendon Parish, 22 May 2023. **3**. *Calledapteryx dryopterata* Grote, 1868. Marshall's Pen, Manchester Parish, 29 April 2023. **4**. *Callizzia* nr. *armorata* Packard, 1876. Guava Ridge, St. Andrew Parish, 1989. Photo courtesy of Matthew Barnes. **5**. *Epiplema incolorata* (Guenée, [1858]). Marshall's Pen, Manchester Parish, 6 October 2018. **6**. *Erosia incongua* Butler, 1878. Marshall's Pen, Manchester Parish, 3 February 2022. **7**. *Nedusia fimbriata* Herrich-Schäffer, 1870. Marshall's Pen, Manchester Parish, 20 June 2023. **8**. *Philagraula slossoniae* Hulst, 1896. Marshall's Pen, Manchester Parish, 5 August 2018. **9**. *Schidax squammaria* Hübner, 1818. Marshall's Pen, Manchester Parish, 8 September 2021. **10**. *Schidax coronaria* Strecker, 1899? Ocho Rios, St. Ann Parish, 13 January 2023. Photo courtesy of Sheldon Logan.

Table 1. Epipleminae of Florida and the Greater Antilles (References to dates of descriptions are provided in the Cited Literature).

Species	FL	CU	JA	HI	PR
Antiplecta triangularis (Warren, 1906)	●	●	■		
Calledapteryx dryopterata Grote, 1868	●		■	●	●
Callizzia armorata Packard, 1876	●				
Callizzia nr *armorata* Packard, 1876			■		
Epiplema ecludaria Möschler, 1890					●
Epiplema incolorata (Guenée, [1858])	●	●	■	●	
Epiplema ineptaria Möschler, 1890					●
Epiplema obvallataria Möschler, 1890					●
Erosia incongua (Butler, 1878)			■		
Nedusia fimbriata Herrich-Schäffer, 1870		●	■		
Phazaca infans (Warren, 1900)	●				
Philagraula slossoniae Hulst, 1896	●	●	■	●	
Schidax anosectaria (Guenée, [1858])		●			
Schidax coronaria Strecker, 1899	●	●	?		
Schidax squammaria Hübner, 1818		●	■		
Syngria reticularia Möschler, 1890		●		?	●
Syngria ramosaria (Möschler, 1890)		●			●
Trotorhombia metachromata (Walker, 1861)		●		●	●

in Jamaican specimens but all other forewing markings and color patterns conform to *Calledapteryx dryopterata* Grote. Source of identification: Moth Photographers Group (2017) Moths of North America ID # 7653.

[3] Genus *Callizzia* Packard, 1876

Callizzia nr. *armorata* Packard, 1876. Fig. 4, Table 1.
Wingspan: 18.5 mm; forewing length 10.5 mm; (n=1).

This specimen was collected by Matthew Barnes on Guava Ridge near the town of Mavis Bank, in 1989. The wing markings are more lightly expressed than specimens of *Callizzia armorata* Packard from Florida but the outer postdiscal band and evenly rounded forewing-tip, along with the characteristic shapes and patterns typical of shape of the hindwing margin display the characteristic shapes and pattern typical of *Callizzia*. The subdiscal transverse forewing band in this specimen is not well-defined, and the dark marginal lunule on the outer forewing found on *Callizzia armorata* is absent, replaced by a row of marginal spots as found in *C. certiorata* Pearsall. While this does appear to be a species of *Callizzia* the differences noted suggest this is possibly an undescribed species.

[4] Genus *Epiplema* Herrich-Schäffer, [1855]

Epiplema incolorata (Guenée, [1858]). Fig. 5, Table 1.
Syn. *Gymnoplocia mamillata* Felder & Rogenhöfer, 1875
Syn. *Powondrella cingillaria* Geyer, 1850
Average wingspan 15 mm; forewing length 8 mm; (n=7).

This species is found across the island and is not uncommon, with eight specimens photographed during this study. These were observed in the months of May at Windsor in the north of the primary forest Cockpit Country in Trelawny Parish, and in August and October in Mandeville and on Mt. Diablo. There are also recent records from Ocho Rios in March 2023, and from Port Antonio in July 2023. On two occasions, specimens were observed at rest on low vegetation in the daytime. Ground color varies from off-white to pale brown with darker brown markings arranged in a pattern consistent with specimens of *E. incolorata*. The costal vein of the forewing protrudes just beyond the remainder of the forewing margin creating a minute apical point. Source of identification: Moth Photographers Group (2017) Moths of North America ID# 7649.

[5] Genus *Erosia* Guenée, 1857

Erosia incongua (Butler, 1878). TL Jamaica. Fig. 6, Table 1.
Syn. *Erosia praeflorata* Möschler, 1886.
Average wingspan 26 mm; forewing length 12 mm; (n= 5).

Apart from in the website http://focusonnature.com (Hill, 2022) there have been no recent published records documenting the presence of this endemic species in Jamaica since it was described by Butler in 1878 and listed by Gowdey in 1926. Five specimens were photographed in this study in the months of February, August, November, and December in Mandeville. Two specimens were collected at the Windsor Great House. Identification was determined by the series of fine notches at the forewing apex and arrangement of the middle series of dorsal markings that cross the forewing, and by the shape and patterns of the dorsal hindwing.

[6] Genus *Nedusia* Hübner, 1818

Nedusia fimbriata Herrich-Schäffer, 1870. Fig. 7, Table 1.
Wingspan: 30-34 mm; forewing length 13.5-15.5 mm; (n=2).

This moth was photographed on 9 July 2021 at Marshall's Pen and identified as *N. fimbriata* after comparison with photographs of the Cuban species. A second female specimen was photographed on 20 June 2023. This species is not in the Gowdey collection at the NHMJ, IJ and does not appear on any recent moth lists for the island though occurring in several localities.

[7] Genus *Philagraula* Hulst, 1896

Philagraula slossoniae Hulst, 1896. Fig. 8, Table 1.
Wingspan: 10.5 mm; forewing length 5 mm; (n=1).

This small specimen was photographed on 12 March 2021 at Marshall's Pen. The postdiscal and submarginal dark bands of the forewing do not cross the entire wing in a single arc but display the more triangular markings of *P. slossoniae.* The lack of pale buff scales on the head and the shape of the hindwings also conforms best with *P. slossoniae.* Source of identification: Moth Photographers Group (2017) Moths of North America ID# 7654. Not listed from Jamaica in any recent lists.

[8] Genus *Schidax* Hübner, 1818

Schidax squammaria Hübner, 1818. Fig. 9, Table 1.
Wingspan: 33 mm; forewing length 18.0 mm; (n=1).

A single specimen of this distinctive species was photographed at Marshall's Pen on 8 September 2021. A photograph of a specimen from Jamaica in the Avinoff collection at the Carnegie Museum of Natural History, Pittsburgh, USA, confirms this identification. This species is present in Cuba.

After submission of this article for review, a photograph of another *Schidax* was posted on www.inaturalist.org. The wings are shaped differently to those of *Schidax squammaria,* appearing to be similar to those of *Schidax coronaria* Strecker, 1899. This specimen (Fig. 10) was photographed in a residential area near Ocho Rios by Sheldon Logan on 13 January 2023. There are no specimens available for examination at this time. Neither species previously listed for Jamaica.

ACKNOWLEDGMENTS

These investigations were made possible by the issuance of permit 18/27 by the National Environment Protection Agency, Kingston. Matthew Barnes kindly provided permission to reproduce his photograph of *Callizzia*. Our thanks to Dr. Kevin Keegan and Vanessa Verdecia at the Carnegie Museum of Natural History for photographic identification of *Schidax squammaria,* to Sheldon Logan for the photograph of a *Schidax* species, to Dr. Susan Koenig, Windsor, Trelawny Parish, and Dr. Ann Haynes-Sutton, Mandeville, Manchester, for permission to operate moth traps at their locations. Special thanks to the reviewers of this paper for their appropriate queries and comments.

LITERATURE CITED

Barnes, M. J. C. 2002. *A Preliminary Illustrated Catalogue of the Larger Moths of Jamaica*. http://www.mbarnes.force9.co.uk/jamaicamoths/jamaicahome.htm. Accessed 31 December 2022.

Blanchard, É. 1845. *Histoires des Insectes Traitant de Leurs Mouers et de leurs Métamorphoses en Général, et Comprenant une Nouvelle Classification Fondée sur leurs Rapparts Naturels. 2*. Paris, Didot, IV + 524 pp.

Butler, A. G. 1878. On a small collection of Lepidoptera from Jamaica. *Proceedings of the Zoological Society of London* 1878: 480-495.

Felder, C., Rogenhofer, A. F. 1875. *Reise der osterreichischen Fregatte Novara um die Erde (zoologischer Theil).* Vienna. Band 2 (5): 1-20. Pls. 108-140.

Gowdey, C. C. 1926. Catalogue of Jamaican Insects. *Jamaica Department of Agriculture Entomological Bulletin* 4(3).

Grote, A. R. 1868. On the synonomy, etc. of certain species of American Lepidoptera. *Transactions of the American Entomological Society* 2: 115-122.

Guenée, A. 1857 [1858]. Uranides et Phalénites Tome 1. *In*: Boisduval, J. A., Guenée A. *Histoire naturelle des insectes; spécies général des lépidoptères Tome IX*. Paris, Roret. 514 pp.

Geyer, C. 1850. *In*: Hübner, J., *Zuträge zur Sammlung exotischer Schmetterlinge, bestehend in Bekundigung einzelner Fliegmuster neuro der rarer nichteuropäischer Gattungen*. Augsburg. (5): 52 pp., figs. 401-500.

Hampson, G. F. 1892. *Moths (Vol. 1). In*: Blanford, W. T. (Ed.), *The Fauna of British India, including Ceylon and Burma (Moths)*. London, Taylor and Francis. 1: xxiii, 527 pp.

Hill, A. 2022. *Focus on Nature Tours*. http://focusonnature.com. Accessed 31 December 2022.

Heppner, J. B. 2003. *Lepidoptera of Florida. Part 1. Introduction and catalog*. Gainesville, Florida Department of Agriculture & Consumer Services. (*Arthropods of Florida and Neighbouring Land Areas*. Volume 17). x + 670 pp.

Herrich-Schäffer, G. A. W. [1855]. *Sammlung neuer oder wenig bekannter aussereuropäische Schemtterlinge. Heterocera (Nachtfalter)*. Regensburg, G. J. Manz. (1) (13-17), pl. 49-58, figs. 259-390.

Herrich-Schäffer, G. A. W. 1870. Die Schmetterlinge fauna der Insel Kuba. *Korresplondenzblatt des Zoologisch-Mineralogischen* 24: 180-190.

Hübner, J. 1818-[1823], 1816. *Verzeichniss bekannter Schmettlinge*. Augsburg, Jacob Hübner. (1): 27, pl. 28, figs. 161, 162; (I): 30, 291, 315.

Hulst, G. D. 1896. A classification of the Geometrina of North America with descriptions of new genera and species. *Transactions of the American Entomological Society* 23: 245-386.

Kimball, C. P. 1965. *The Lepidoptera of Florida. An Annotated Checklist*. Gainesville, State of Florida Department of Agriculture, Division of Plant Industry. v + 363 pp., 26 pls.

Leach, W. E. 1815. *Entomology*, pp. 57-172. *In*: Brewster, D. (Ed.), *The Edinburgh Encyclopedia*. Volume 9. Edinburgh, W. Blackwood.

Möschler, H. B. 1886. Beitrage zur schmetterlings-fauna von Jamaica. *Ablandlungen von der senckerbergischen Naturforschender Gesellschaft* 14(3): 25-84.

Möschler, H. B. 1890. Die Lepidopteren-Fauna der Insel Portorico. *Abhandlungen der Senkenbergischen Naturforschenden Gesellschaft* 16: 70-360, pl. 1

Moth Photographers Group. 2017. *Digital Guide to Moth Identification: Greater Antilles Species List; Checklist of the Lepidoptera of the Antilles*. https://mothphotographersgroup.msstate.edu/Antilles/AntillesChecklist.shtml. Accessed December 2022.

Núñez, R., Barro Cañemaro, A. 2012. A list of Cuban Lepidoptera (Arthropoda: Insecta). *Zootaxa* 3384(1): 1-59.

Packard, A. S. 1876. *A Monograph of the Geometrid Moths or Phalaenidae of the United States*. Washington, Report of the U. S. Geological Survey. X, 314.

Strecker, H. 1899. *Lepidoptera, Rhopaloceres and Heteroceres: Indigenous and Exotic*. Reading, the author. Supplement no. 2: 8.

Walker, F. 1861. *List of specimens of Lepidopterous insects in the collection of the British Museum*. London, British Museum.

Warren, W. 1900. New genera and species of American Drepanidae, Thryridae, Epiplemidae and Geometridae. *Novitates Zoologicae* 7(2): 117-225.

Warren, W. 1906. Descriptions of new genera and species of South American geometrid moths. *Proceedings of the United States National Museum* 30(1456): 399-557.

TROPICAL LEPIDOPTERA
Research

VOLUME 33, NUMBER 2 December 2023

TABLE OF CONTENTS