Neotropical Biodiversity

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tneo20

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To cite this article: Carlos Federico Álvarez, Alejandra Clavijo-Giraldo, Sandra Inés Uribe, Tomasz Wilhelm Pyrcz, Cristiano Agra Iserhard, André Victor Lucci Freitas & Mario Alejandro Marín (2021) Sampling performance of bait traps in high Andean fruit-feeding butterflies, Neotropical Biodiversity, 7:1, 507-513, DOI: 10.1080/23766808.2021.2004802

To link to this article: https://doi.org/10.1080/23766808.2021.2004802

Published online: 11 Jan 2022.

NEOTROPICAL BIODIVERSITY
2021, VOL. 7, NO. 1, 507–513
https://doi.org/10.1080/23766808.2021.2004802

Sampling performance of bait traps in high Andean fruit-feeding butterflies

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ABSTRACT

Studies on model organisms such as butterflies are useful tools for conservation decision-making. However, in tropical ecosystems with an intrinsic high diversity a full understanding of biotic communities is difficult to obtain. Bait trap samplings have traditionally been used for community appraisals related to ecological and conservation issues. Nonetheless, in the Andes Mountains, there is little knowledge related to the effectiveness of bait traps for butterfly sampling. In this study, we tested the success of fermented fruits and rotten fish baits for butterfly sampling in four land-cover types (páramo, cloud forests, mixed, and pasture) in the upper Rio Chico basin of the northern Central Cordillera of the Colombian Andes. A butterfly survey was conducted between 2011 and 2014, in an elevation range of 2650 to 3300 masl, within a total of 132 field days. Three sampling units for each land cover were established with four standard Van Someren-Rydon traps (VSR) per sampling unit. Traps were baited alternatively with fermented fruits and carrion (rotten fish). All 57 recorded species were captured using rotten fish, while approximately 65% (37 species) were collected from fermented fruit. Moreover, species richness was higher in all sampled land covers using rotten fish bait, but the dominant species in the land covers differed between baits. The rotten fish bait proved to be highly effective for butterfly sampling in páramo and cloud forest, although the combination of traps baited with fermented banana and rotten fish, allowed the collection of data suitable for comparison among all studied land cover.

ARTICLE HISTORY
Received 1 December 2020
Accepted 8 November 2021

KEYWORDS
Biblidinae; cloud forest; páramo; rotten fish; Satyrinae

Introduction

In recent years, there has been further research in the butterfly fauna of high-altitude neotropical areas [1–7], and the search for standardized sampling protocols and methods to compare independent samples is a priority. The use of bait traps has proven to be one of the most reliable methods for comparing representative samples of fruit-feeding butterfly assemblages, although this method has had little success in montane habitats, where traps generally capture fewer butterflies than premontane and lowland tropical areas [8]. Fruit-feeding butterflies obtain most of their nutritional requirements from fermented fruits, feces, carcasses, decomposing organic matter, and fermented sap [9]. The group is non-monophyletic and is represented in the neotropical region by species of four Nymphalidae subfamilies: Satyrinae, Charaxinae, Biblidinae, and Nymphalinae (the latter to a lesser extent) (*sensu* Wahlberg et al. 2009) [8,10]. The tropical high Andean mountains are mainly represented by Satyrinae, particularly the subtribes Pronophilina (more than 600 species in 45 genera, Pyrcz in prep) and Euptychiina (mainly *Forsterinaria* sp.), followed by Biblidinae (*Epiphile*, *Perisama*, and *Orophila*), and some species of Charaxinae and Nymphalinae [11–14].

One of the most salient characteristics of fruit-feeding butterflies is that they can be sampled in a standardized manner to avoid human collector biases [15]. However, standardized butterfly sampling in neotropical montane habitats presents several difficulties when compared to lowland sampling. The vegetation of this region offers a reduced availability of fleshy fruits [16] and slower decomposition rates of organic material due to the low temperatures [17,18]. Consequently, fruit-feeding butterflies' assemblages at higher elevations are adapted to acquire nutrients from alternative sources, such as carrion and feces, or exchanged their food source completely to become nectar feeders (e. g. [19]). Accordingly, fermenting fruits are not a viable study base in montane habitats, requiring the search for novel sampling strategies to obtain a representative picture of the local assemblages. Previous studies in these habitats have successfully used traps baited

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Published online 11 Jan 2022

with the excrement of carnivorous mammals [5–7]. Although this bait was successful in sampling butterflies in high-altitude habitats, it is difficult to standardize feces to allow for comparative studies in different localities and in long-term projects. For biological assessment and monitoring projects it is necessary to use standardized methods that provide rigorous comparable data. This allows to combine results from various studies and facilitates the comparisons of species richness, composition and abundance within and among habitat types [15,20]. Therefore, the present study was designed to compare the performance of two different baits, fermented fruits and rotting fish, for the standardized sampling of fruit-feeding butterflies in a high mountain site.

Materials and methods

Study site and fieldwork

The study was conducted in the conservation area of the "Páramo de Belmira" (also known as "Páramo de Santa Inés"), located in the northernmost part of the Cordillera Central of the Colombian Andes (6°35′–6°51′ N and 75°47′–75°38′ W). The landscape of the region is dominated by four types of land cover: cloud forest, páramo (a native open vegetation typical of high altitude in the neotropics, dominated by grasses and other herbaceous plants; see [21,22]), cattle pastures, and mixed vegetation (a mosaic of regeneration vegetation in various successional stages, interspersed with cloud forest, and abandoned cattle pastures without a clear prevalence of any vegetation cover). The sampling was carried out essentially once a month from June 2011 to April 2014 in 12 localities at an elevation range of 2650 to 3300 m a.s.l. In each field trip, samplings were performed for 3 to 4 consecutive days, for a total of 132 field days. The design consisted of three sampling units for each habitat type, in which four standard Van Someren-Rydon traps (VSR) were used per sampling unit. Traps were located 1.5 to 2 m above the ground and separated by 100 m, following Hamer and Hill [23] and Benedick et al. [24]; those studies estimated the area of influence of a bait trap to be at a radius between 50 m and 100 m (Figure 1). The traps were baited alternately with 150 g of one of two feeds, fermented bananas or rotten fish (comercial avaliable trout). Baits were prepared in the butterfly house of the Laboratorio de Fisiología de Insectos of the Universidad Nacional de Colombia in Medellín, at an average temperature of 23.5 °C, a minimum of 21 °C and a maximum of 26.1 °C. The fermented bananas bait was a mixture of mashed mature banana with sugar and rum, fermented for four days, and the rotten fish bait consisted of pieces of trout, decomposed in a closed bag for five days. Bait replacement was done every 48 h during each sampling session, following Santos et al. [25]. For each field trip at the same site, the types of bait were rotated at all locations.

The collected specimens were identified based on the most recent updated faunal lists, revisions, and species accounts for the region [26–29]. The website of Butterflies of America [30] was also consulted, since it contains photographs of the type material for most

Figure 1. (a–c). Installation of bait traps; (d). fermented fruit bait; (e). Butterflies attracted to feces of local *Puma concolor* Linnaeus, 1771.

neotropical butterflies, and also reflects the current state of knowledge on the taxonomy of the group. Specimens were deposited in the collections of the Museo Entomológico Francisco Luis Gallego of the Universidad Nacional de Colombia Sede Medellín and in the collection of the Instituto Alexander von Humboldt in Villa de Leyva, Boyacá, Colombia.

Data analysis

A Venn diagram was used to compare the number of shared and exclusive species collected by each bait type. Diversity profiles based on Hill numbers through the q statistic [31,32] were calculated to measure the proportion of the diversity sampled for each method: q = 0 being the order based only on the species richness of each assemblage, q = 1 represents the exponential of Shannon entropy concerning equitability, and q = 2 represents the inverse of the Gini–Simpson dominance index. To evaluate differences in diversity, generalized linear models (GLMs) were developed according to the method proposed by Checa et al. [33]. GLMs were used to evaluate if the respose variables of abundance, observed species richness, or species diversity, differed with respect to sampling technique (predictor variables, fruit and rotten fish). The selection of possible models was based on the type of data: discrete (abundance, richness) or continuous (diversity), as well as Akaike Information Criterion (AIC) values and residual variance. The negative binomial distribution was the superior model over the Poisson or normal distribution for our data, in relation to abundance and species richness; the diversity of order q1 and q2 fit a Gaussian distribution and were tested with a standard linear model (LM). Analyses were performed in R version 3.6.3 [34] using the package vegan 2.5–6 [35].

Results

A total of 2939 fruit-feeding butterflies belonging to 57 species were collected. Among these, 430 individuals representing 37 species were captured in traps baited with fermented fruits, and 2509 butterflies included in all 57 species were found in the rotten fish traps (Table 1, Figure 2). Traps baited with rotten fish captured a higher number of species in all sampled habitats (Figure 3), with a different dominant species for each bait type: *Panyapedaliodes drymaea* (Hewitson, 1858) represented 25% of the individuals captured with fermented banana, while *Pedaliodes obstructa* Pyrcz and Viloria, 1999, represented 24% of those captured with rotting fish. Significant differences were observed in abundance (GLM Estimate = 1.7639, $p < 0.001$), richness (GLM estimate = 0.8315, $p = 0.002$), and q1 diversity (GLM Estimate = 3.766, $p = 0.041$). Abundance was higher in traps baited with rotten fish in the cloud forest, mixed vegetation, and páramo regions, while pastures showed a higher abundance in traps baited with fermented bananas (Table 2).

With a sample coverage higher than 91% in all sample sites, 47 species were captured in the cloud forest, comprising half of the collected specimens. In contrast, the pasture regions showed the lowest values for both parameters, with only 151 captured individuals of 21 species. All vegetation types except the pasture registered exclusive species: (1) *Eretris ocellifera* (C. Felder & R. Felder, 1867), *Lymanopoda obsoleta* (Westwood, 1851), *Manerebia germaniae* Pyrcz & J. Hall, 2006, *Mygona irmina* (E. Doubleday, [1849]), and *Proboscis propylea* (Hewitson, 1857) were exclusively captured in the cloud forest; (2) *Morpho sulkowskyi* Kollar, 1850, *Panyapedaliodes jephtha* (Thieme, 1905), and *Perisama yeba* (Hewitson, [1857]) were exclusively found in mixed vegetation; and (3) *Lymanopoda casneri* Pyrcz & Clavijo, 2016, *Pedaliodes griseola* Weymer, 1912, and *Pedaliodes negreti* Pyrcz, 1999 were collected solely in the páramo habitat.

Discussion

The present results clearly indicate that the two types of baits, resulted in different performances of sampling in fruit-feeding butterflies, with significant differences in abundance, richness, and diversity. Traps baited with rotting fish were more effective for obtaining a representative picture of the local assemblages. Considering all species of fruit-feeding butterflies in the region with 61 species [36], fermented bananas sampled 61% of the local assemblage, while rotting fish returned 93% of the total local species richness (including all species sampled with fermented bananas). The performance of the baits showed differences among the different land covers: a higher performance of the rotten fish was reported for páramo, cloud forest and mixed land covers, while the fermented bananas baits had a higher performance in pastures (with a 62% of the registered specimens), contributing significantly to the sampling coverage in this land cover. The difference in abundance performance of the baits in pastures is possibly related to a high abundance of one specific species, *Panyapedaliodes drymaea* (Hewitson, 1858), that apparently present a high preference by this kind of bait (Table 1). In short, the present results showed that the use of fermented fruit baits is not sufficient for collect a representative sample of the assemblage of fruit-feeding butterflies in high mountainous regions. Conversely, the use of

Table 1. Occurrence of fruit-feeding butterfly species in each type of bait and habitat.

	forest		mixed		paramo		pasture	
	fruit	fish	fruit	fish	fruit	fish	fruit	fish
Sample coverage	0.900	0.997	0.924	0.992	0.931	0.972	0.937	0.826
Species								
Biblidinae								
Epiphile chrysites (Latreille, [1809])	3	36		35		2	1	3
Orophila cardases (Hewitson, 1869)		4		8		1		
Perisama lebasii (Guérin-Méneville, [1844])				7				1
Perisama tryphena (Hewitson, [1857])		4		3				
Perisama yeba (Hewitson, [1857])				1				
Satyrinae								
Altopedaliodes belmira (Pyrcz and Rodríguez, 2004)	1	5		6	24	77		
Apexacuta orsedice (Hewitson, 1878)		2	1	5	1	4		
Corades chelonis Hewitson, 1863	5	69	3	26	1	2	1	2
Corades chirone Hewitson, 1863		9		7				
Corades cybele A. Butler, 1866	1	4		5	2	3		1
Corades dymantis Thieme, 1907	1	6		7				1
Corades medeba Hewitson, 1850	1	9	1	10				
Daedalma dinias Hewitson, 1858	2	11		3				
Eretris apuleja (C. Felder & R. Felder, 1867)	6	13	5	8		2		1
Eretris ocellifera (C. Felder & R. Felder, 1867)		2						
Forsterinaria difficilis (Forster, 1964)		31		1		9		
Forsterinaria rustica (A. Butler, 1868)	7	70	1	19	1	3		1
Junea doraete (Hewitson, 1858)			2	4	7	38		
Junea dorinda (C. Felder & R. Felder, 1862)		7		7		3		1
Lasiophila circe C. Felder & R. Felder, 1859	4	7	2	3	11	40		
Lasiophila prosymna (Hewitson, 1857)	1	10	2	14	1		1	
Lasiophila zapatoza (Westwood, 1851)	3	8		2		1		
Lymanopoda altis Weymer, 1890	1	30		2		2	1	
Lymanopoda casneri Pyrcz & Clavijo, 2016						1		
Lymanopoda ionius Westwood, 1851		2			1	7		
Lymanopoda labda Hewitson, 1861		5		7				
Lymanopoda obsoleta (Westwood, 1851)		3						
Manerebia germaniae Pyrcz & J. Hall, 2006	2	1						
Manerebia inderena (Adams, 1986)	1		1	5				
Morpho sulkowskyi Kollar, 1850			1	1				
Mygona irmina (E. Doubleday, [1849])		3						
Panyapedaliodes drymaea (Hewitson, 1858)	4	6	15	8	4		84	25
Panyapedaliodes jephtha (Thieme, 1905)				1				
Panyapedaliodes phila (Hewitson, 1862)	1	25		1	1	3		
Panyapedaliodes rojasi Pyrcz & Álvarez, 2016					2	8	2	
Panyapedaliodes silpa (Thieme, 1905)	1	3				1		
Pedaliodes baccara Thieme, 1905	37	96	6	17		1		3
Pedaliodes griseola Weymer, 1912						6		
Pedaliodes hebena Pyrcz & Viloria, 1999	2	10		3		1		
Pedaliodes negreti Pyrcz, 1999						1		
Pedaliodes nutabe Pyrcz & Álvarez, 2016		1			1	40		
Pedaliodes obstructa Pyrcz & Viloria, 1999	22	393	21	124	20	65	1	12
Pedaliodes peucestas (Hewitson, 1862)	5	4	2	3				1
Pedaliodes phaedra (Hewitson, 1870)	1	20	7	12	6	12	1	
Pedaliodes pimienta Adams, 1986		1						1
Pedaliodes pollonia Adams, 1986	4	44	7	22		1		1
Pedaliodes polusca (Hewitson, 1862)	3	35	5	25	28	64		
Pedaliodes porcia (Hewitson, 1869)	1	67		18	4	59		
Pedaliodes praemontagna Pyrcz & Viloria, 2007		93	1	27	1	2		2
Pedaliodes praxithea (Hewitson, 1870)	1	1				1		
Pedaliodes rodriguezi Pyrcz & Andrade, 2013	3	67	2	11		1		
Proboscis propylea (Hewitson, 1857)		2						
Pronophila epidipnis Thieme, 1907	4	97	5	129		3	2	
Pseudomaniola loxo (Dogin, 1891)		3		6		1		
Steremnia monachella (Weymer, 1911)				12		2		
Steremnia selva Adams, 1986		3		22		1		1
Steroma bega Westwood, [1850]	1	21	1	3		1		
Total	129	1343	91	640	116	469	94	57

rotting fish or a combination of both bait types was more effective for obtain a great sample coverage of comparable data for high mountain butterfly assemblages among different land covers.

Unlike previous studies, where different baits were used to inventory all butterfly communities [33], the present study aimed to provide a standardized protocol for sampling and monitoring butterflies in montane habitats, similar to those proposed for other forest types (see [8,10,37–39]). The present proposal of the use of trout represented a clear methodological advantage for the following reasons: (1) trout is an introduced freshwater species now present in many neotropical montane sites, including the Andean region, the mountains from Southeastern Brazil and Central America; (2) trout are not as expensive as squid and shrimp and are

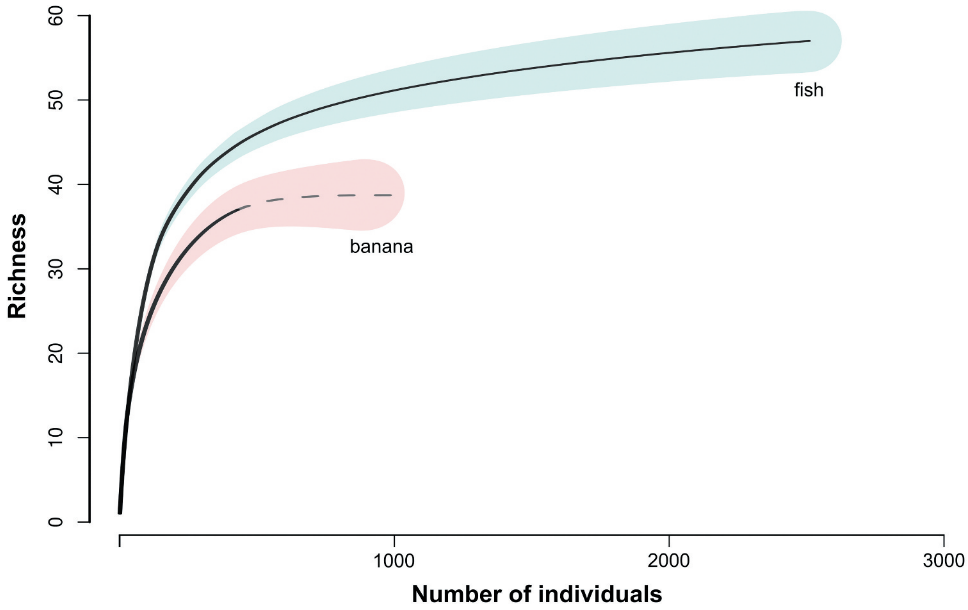

Figure 2. Rarefaction comparing baits, fermented banana (banana) and rotten fish (fish).

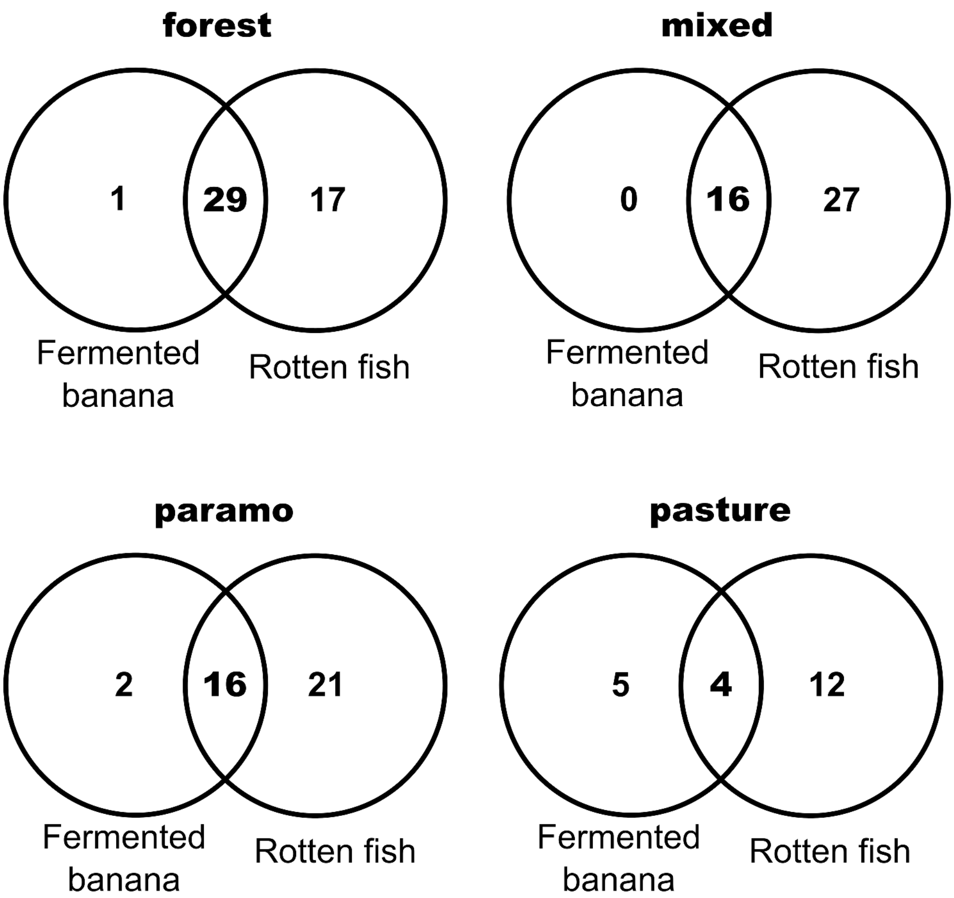

Figure 3. Venn diagrams, showing the exclusive and shared species registered in each bait in the different habitats.

available in inland regions; (3) the more common species of trout are available throughout the neotropical region, which allows for a relatively standardized bait (which is extremely difficult in the case of feces). Rotten fish and feces have shown to be particularly useful for short-term inventories in high Andean environments [4,6,7]. However, for environmental community comparison and long-term diversity monitoring studies, is necessary to use standardized and replicable sampling methods. The present study shows that comparative standardized studies in Neotropical montane habitats can be made by the combined use of traps baited with fermented banana and rotten fish, allowing the collection of robust ecological data suitable for

Table 2. Butterfly diversity: abundance, observed species richness, and diversity (q1 and q2) recorded, comparing traps baited with fermented banana and rotten fish in the different studied habitats. Result of the GLMs test, significant <0.01 **, <0.05 *, determining whether butterfly diversity differed with respect to sampling technique.

		Abundance **			Species richness **			q1 *			q2		
	Cov.	banana	fish	total	banana	fish	total	banana	fish	total	banana	fish	total
forest	0.998	129	1343	1472	30	46	47	14.54	16.50	17.18	7.88	8.62	9.02
mixed	0.994	91	640	731	21	43	43	13.02	19.54	20.22	9.09	10.62	11.21
paramo	0.976	116	469	585	18	37	39	9.19	13.42	13.19	6.68	9.63	9.27
pasture	0.914	94	57	151	9	16	21	1.74	6.99	3.68	1.25	4.04	1.88
all		430	2509	2939	37	57	57	15.20	22.42	23.06	8.94	11.84	12.71

statistical analysis, concerning general patterns of diversity and structuration of fruit-feeding butterflies assemblages and monitoring programs.

Acknowledgments

We would like to thank the local community of Belmira, in particular Hector Rojas (Corantioquia-DMI Belmira) for field support, GSM members, volunteers, and all the people who helped in field work. Corporación Universitaria Lasallista and the Universidad Nacional de Colombia sede Medellín for logistic support. Specimen collection was done under license number 4 of 7 May 2011 and a resolution 503 on 24 May 2013 by ANLA (Agencia Nacional de Licencias Ambientales, Colombia).

Disclosure statement

No potential conflict of interest was reported by the author (s).

Funding

This work was supported by the [Ministerio de Ciencia Tecnología e Innovación - Colciencias (Colombia)] under Grant [Ministerio de Ciecnia Tecnología e Innovación (CO) 528/2011]; [Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (Brazil)] under Grant [Fundação de Amparo à Pesquisa do Estado de São Paulo (BR) 2011/50225–3] and [2018/11910–1]; [Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Brazil)] under Grant [563332/2010–7] and [303834/2015–3]; [Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Brazil)] under Grant [Finance code 001]; [National Science Foundation - NSF (USA)] under Grant [Directorate for Biological Sciences DEB-1256742]; and [narodowe centrum nauki – NCN (Poland)] under Grant [narodowe centrum nauki Harmonia-10 2018/30/M/NZ8/00293] “Evolutionary biogeography and diversification of the predominantly Andean butterfly subtribe Pronophilina (Nymphalidae, Satyrinae) based on phylogenetic data generated using modern molecular methods”.

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Author contributions

CFA, CAI, AVLF, and MAM conceived and designed the study. CFA, ACG, and MAM performed the field study. CFA and MAM wrote the first draft of the manuscript. CFA, ACG, and TWP perforemd, sample processing, taxonomy support, and specimen identification. CFA and MAM analyzed the data. SIU, TWP, CAI, and AVLF reviewed and improved the manuscript.

Geolocation information

Páramo de Belmira, Antioquia, Colombia. Elevation range of 2650 to 3,300 meters of elevation. Coordinates (N 6°35′ to 6° 51′) (W 75°47′ to 75°38′).

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