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Is the Existence of Two Lineages for *Hamadryas glauconome* (Lepidoptera: Nymphalidae) True? Molecular and Ecological Evidence

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Abstract: The genus *Hamadryas* has a neotropical distribution. In 1983, the subspecies *H. glauconome grisea* from Mexico was recognized with subtle and subjective differences in color, size and distribution and limited to the northwest. Since then, there has been a debate about whether it is a different lineage from *H. glauconome* because adult-stage morphology studies have not found significant differences. This study aims to delimitate *H. g. glauconome* and *H. g. grisea* lineages with two sources of evidence: ecological and molecular—the former through ecological niche modeling using the accessible area for the species and estimating the minimum volume ellipsoid overlapping as a fundamental niche using occurrences databases. The molecular evidence is found through the methods of phylogenetic inference and the generalized mixed yule coalescent approach, using sequences of cytochrome oxidase I. Ecological and molecular evidence suggest that *H. g. grisea* is a different lineage from *H. glauconome*. Also, molecular evidence of a third lineage from the south of Texas needs further study. This study suggests that different evidence should be provided when morphology is not enough for delimiting species, especially in recently diverged species. Furthermore, the *H. g. grisea* cytochrome oxidase I sequence (658 bp) is published for the first time.

Keywords: species delimitation; spatial distribution; ecological niche modeling; GMYC

Citation: Prado-Cuellar, B.R.; Lara-Pérez, L.A.; Trujano-Ortega, M.; Machkour-M'Rabet, S.; Pozo, C. Is the Existence of Two Lineages for *Hamadryas glauconome* (Lepidoptera: Nymphalidae) True? Molecular and Ecological Evidence. *Diversity* **2023**, *15*, 1196. https://doi.org/10.3390/d15121196

Academic Editors: Luc Legal and Shao-Ji Hu

Received: 31 October 2023
Revised: 1 December 2023
Accepted: 1 December 2023
Published: 6 December 2023

1. Introduction

Hamadryas is a Lepidoptera genus with a neotropical distribution [1]. This group was first studied by Fruhstorfer in 1916 [2], and later, Jenkins [1] made a comprehensive review of the genus. The genus comprises 20 species distributed in Latin America, the Caribbean, and Mexico [1]. In Mexico, there are nine of the twenty known species and eight subspecies, primarily distributed in the neotropical region. In the Yucatan Peninsula as a biotic province, including Campeche, Yucatan, Quintana Roo, northern Guatemala, and Belize [3,4], 10 species are known [5–8], including *Hamadryas julitta*, an endemic species restricted from northern Belize [6] to the northern Yucatan state.

In a previous study [7], genetic barcodes (a 658-base pair region of the cytochrome oxidase subunit I, COI gene) were used for the species identification of Lepidoptera in the Yucatan Peninsula. This study placed *H. julitta* and *H. glauconome* as sister species with a genetic divergence of 2%. This divergence is supported by further phylogenetic studies of the genus using morphology and molecular markers, which also place *H. julitta* and *H. glauconome* as sister species [9,10]. Morphologically, they are very similar, and in the past, *H. julitta* was considered a form of *H. glauconome* [2]. However, Jenkins [1]

provided a detailed description of both their external morphology and genitalia, along with a comparative table of their characteristics, and concluded that they are two separate species, changing the status of *H. julitta* to species. A recent study with *Hamadryas* eggs revealed clear differences in the exocorion between these two sister species, adding further distinguishing morphological characters [11].

The distribution of *H. glauconome* ranges from Mexico to Costa Rica [1,2]. In Mexico, subspecies *H. g. glauconome* and *H. g. grisea* are recognized [1,5]. The distribution of the former extends to the Pacific and Gulf of Mexico slopes, while the latter has a restricted distribution in northwestern Mexico [1,5]. However, the proposal of the subspecies *H. g. grisea* by Jenkins [1] is primarily based on distribution, coloration patterns, and subtle differences in genitalia. The study by Garzón-Orduña [9] highlights a group of *H. glauconome* that seems to be paraphyletic. A recent study [12] evaluated the taxonomic status of *H. julitta* and *H. glauconome* by morphology, color, and some climatic variables. It concluded that the variation between species is significant enough to consider them as different species. On the other hand, there was not a significant enough difference between *H. g. glauconome* and *H. g. grisea* to consider them different species and establish that the hindwing ocelli form is enough to differentiate among these subspecies. This affirmation states the need to study *H. glauconome* lineages under different approaches to provide a finer resolution and thus determine their status.

Studying the lineages of *H. glauconome* and *H. julitta* using different approaches will help us to understand how they are structured and the processes that led to their differentiation. If we consider species as lineages within a metapopulation that evolve independently and gradually diverge in various aspects such as morphology, genetics, behavior, and ecology, this provides evidence for the lineage delimitation of each species [13], especially when morphological characteristics are not discrete enough to discriminate between them.

Niche analysis has been used in previous studies for delineating lineages in vertebrates [14–16] and invertebrates like Lepidoptera [17–20]; it relies on the ecological species concept [21,22] and both Hutchinson's [23] and Maguire's [24] concept of niche as a species attribute. Species distribution is determined by abiotic favorable conditions and is equivalent to fundamental niche (A), biological interactions (B, biotic components), and accessible area (M). It is defined as the reachable area of a species, and it depends on dispersal capacity—the accessible areas of the world that have been available since the origins of the species [25]. Ecological niche modeling (ENM) uses species occurrences and environmental data to estimate the realized niche, defined as where A and B converge, which means where the species live [25]. Considering M while modeling instead of just A gives biological sense to the model as it depends on the dispersal capacity and evolutive history of the species [26]. Component B is very complex as it needs robust data in a broad temporal–space scale, and it is difficult to obtain because interactions change along the species distribution; thus, it is not included in ENM [26,27].

Another approach for species delimitation is the molecular one, and various criteria have been employed for this delimitation, ranging from divergence thresholds (such as genetic barcodes [28,29]) and phylogenetics through Bayesian inference [30] to methods that incorporate coalescence concepts [31,32]. Several methods include coalescence in their models, such as the GMYC method (generalized mixed yule coalescent), which uses the maximum likelihood model (ML). This is based on the prediction that lineages evolving independently lead to the emergence of distinct genetic clusters separated by long internal branches [31,32].

Considering this, our study proposes a multi-character approach, including ecological and molecular techniques to provide evidence for delimitating the lineages of *H. g. glauconome*, *H. g. grisea*, and *H. julitta*.

2. Materials and Methods

2.1. Ecological Analyses

2.1.1. Databases

Species and subspecies occurrence data in the distribution area were obtained from various Lepidoptera collections—Museo de Zoología de la Facultad de Ciencias from UNAM, El Colegio de la Frontera Sur in Chetumal, the McGuire Center for Lepidoptera and Biodiversity at the Florida University—and some were collected from recent fieldwork. Published data were included [1,33–36], a query from GBIF [37] was made only with preserved specimens, and doubtful data were dismissed. Missing coordinates were recovered with GEOLocate [38] and medium to low precision was validated with the QGIS version 3.20.3 [39] intersection tool using an America's countries shapefile (https://www.diva-gis.org, accessed on 20 October 2021). The remaining missing data were recovered with Google Earth [40]. Environmental variables were downloaded from WorldClim [41] with a 2.5′ resolution. The occurrences database was processed with RStudio [42] basic functions to eliminate duplicates, and the ecospat package [43] was used to avoid redundancy and bias by suppressing occurrences within 69 m, which was considered the dispersal capacity from the mean of the movement of some previously studied *Hamadryas* species [44].

2.1.2. Accessible Area

The accessible area (M) was estimated altogether, as sister lineages share an evolutive history [25]. The accessible area simulation was run using the R Grinnell package [45] with normal dispersal, spread = 1, maximum two dispersers, three replicates, and twenty dispersal events as simulation parameters. Environmental layers were cropped according to the simulated M.

2.1.3. Model Calibration

For the calibration of the model, environmental values for each point were extracted with the raster [46] and dismo [47] R packages, and the correlation between variables was analyzed with the corr_var function in the lares package [48], where values ≥ 0.8 indicated a strong correlation. An analysis of variable contribution was conducted with a preliminary model using MaxEnt version 3.3.3 [49] with altogether lineages and the cropped environmental layers using the default basic parameters and running the jackknife test included in MaxEnt to measure variable importance. The permutation importance was used as a criterion to remove strongly correlated variables to avoid bias; if two variables had a strong correlation, the one with more permutation importance was kept. When two correlated variables are shuffled, one of them has little effect on the performance because the other one has very similar information, thus affecting the permutation importance in the final model.

2.1.4. Ecological Niche Modeling

Each lineage was modeled separately in MaxEnt with the 5 selected variables using 10 bootstrap replicates, random seed, a 20% random test, and a maximum number of background points and 500 iterations as a maximum, adding samples to the background and writing background predictions. A jackknife test was run from MaxEnt for variable importance. We used the area under the curve (AUC) generated in the program to assess the model performance. The plot of the mean of each species' replicates was used to visualize the suitability with Qgis version 3.26 [39].

2.1.5. Identity Test

An identity test was conducted with the identity.test function from ENMTools [50] R package using each species occurrences and selected bioclimatic layers (cropped as the accessible area). This calculates the empirical niche identity between lineages using Schoender's D estimate, which goes from 0 when there is no overlapping to 1 when there is complete overlapping. This function performs the test as in Warren [51] with a one-tailed

test. The parameters were 100 replicates, a MaxEnt model, and 1000 background points to construct a null distribution. Also, a critical value was calculated (p = 0.05), above of which 95% of values were higher, as well as compared results; if empirical D is above the critical value, the null hypothesis is accepted and no statistically significant difference exists between lineages niches.

2.1.6. Fundamental Niche Overlap

Each lineage data occurrence was used to visualize the niche in a multidimensional environmental space using NicheA version 3.0 [52]. The background was constructed using the cropped bioclimatic layers, and the minimum volume ellipsoid (MVE, fundamental niche) overlapping was calculated.

2.2. Molecular Analysis

2.2.1. Sequences

Sixteen *Hamadryas julitta*'s cytochrome oxidase I (COI) sequences (658 bp) from Barcode of Life Data Systems (BOLD, www.boldsystems.org, accessed on 28 September 2023) [53] under the project Nymphalidae of the Yucatan Peninsula (LNYM) were used. Additionally, 23 samples of *H. g. glauconome* and 5 from *H. g. grisea* were obtained from museum specimens' legs of the Lepidoptera Collection of Zoology Museum and the National Insects Collection, both from the Universidad Nacional Autónoma de México and the McGuire Center for Lepidoptera and Biodiversity at the Florida University. DNA extraction, amplification, and sequencing were performed at the Canadian Centre for DNA Barcoding under standard protocols [54]. A total of 44 (16 from *H. julitta*, 23 from *H. g. glauconome* and 5 from *H. g. grisea*) sequences were aligned with MUSCLE [55] and used to perform the molecular analyses. Interspecific and intraspecific distances were calculated with MEGA version 11.0.13 [56] using Kimura 2 parameters (K2P) [57] for a general overview.

2.2.2. Phylogenetic Inferences

The mtDNA tree was inferred employing both maximum likelihood (ML) in the IQ-Tree web server [58] and Bayesian inference (BI) in BEAST 2.4.6. [59] using all sequences whose GenBank accession numbers are in Table 1 (16 from *H. julitta*, 5 from *H. g. grisea* and 23 from *H. g. glauconome*) and sequences of *H. februa*, *H. feronia* and *H. amphicloe* as outgroups downloaded from GenBank (accession numbers GU659529, GU659523 and JN263324 respectively).

Sequence alignment was performed using the MUSCLE algorithm [55] included in the software MEGA X version 10.2.0 [60]. A maximum likelihood (ML) tree was run in the IQ-TREE web server [58] using the best fitting model, TIM2 + I, according to the Bayesian Information Criterion (BIC) calculated with jModelTest2 version 2.1.10 [61]. Analysis was run with standard bootstrap with 100 alignments to estimate the branch support analysis. The consensus tree was visualized using FigTree version 1.4.4 [62].

For BEAST analyses we specified the best fitting model TIM2 + I and the invariant proportion from jModelTest2 version 2.1.10 [61], an optimized relaxed clock and a log-normal constant-size coalescent tree prior. A run was performed with a length of 30 million generations and sampling every 5000 generations. We verified for convergence and evaluated the run performance with Tracer [63]. We used TreeAnnotator 2.4.6 [64] to generate a maximum credibility tree using mean heights for the nodes. The nodes with BI posterior probability (PP) values >0.95 and ML bootstrap (BS) values 70% or above were a priori regarded as strongly supported. In contrast, lower values were regarded as indicating no significant node support [65].

Table 1. Permutation and contribution analysis from the 19 environmental variables.

Variable	Permutation Importance	Percent Contribution
Precipitation of Driest Month (bio14) *	15	3.6
Mean Temperature of Wettest Quarter (bio08) *	13.8	9.2
Min Temperature of Coldest Month (bio06) *	10.8	12.2
Isothermality (bio03) *	9.3	1.2
Precipitation of Coldest Quarter (bio19) *	8.3	16.3
Temperature Seasonality (bio04)	7.7	5.9
Mean Diurnal Range (bio02)	7.3	3.5
Max Temperature of Warmest Month (bio05)	6.9	6.2
Mean Temperature of Warmest Quarter (bio10)	4	6
Precipitation of Warmest Quarter (bio18)	3.7	7.6
Precipitation of Wettest Month (bio13)	3.5	0.4
Mean Temperature of Driest Quarter (bio09)	3.3	3.3
Annual Precipitation (bio12)	2.4	11.1
Precipitation of Wettest Quarter (bio16)	2.4	3.3
Precipitation of Driest Quarter (bio17)	1	0.5
Annual Mean Temperature (bio01)	0.2	6.3
Mean Temperature of Coldest Quarter (bio11)	0.2	1.6
Precipitation Seasonality (bio15)	0.2	0.5
Temperature Annual Range (bio07)	0.1	1.4

* Variables selected to run the final model with each lineage.

2.2.3. Generalized Mixed Yule Coalescent (GMYC) Analysis

To delimit *H. glauconome* lineages, 28 sequences (including 23 from *H. g. glauconome* and 5 from *H. g. grisea*) were analyzed with a GMYC approach. For these sequences, the HKY + I substitution model was estimated with jModelTest2 version 2.1.10 [61] using Akaike's Information Criterion (AIC). An ultrametric tree was generated through Bayesian inference using BEAST version 2.7.5 [66] including a seed of 30,000,000 and substitution model of HKY + I with the invariant proportion from the jModelTest analysis. An optimized relaxed clock [67] was used to calculate the branch length under a coalescent constant population model with log-normal and a 30,000,000-length Monte Carlo Markov Chain. The tree was corroborated by Tracer version 1.7.2. [63] looking for an estimated sample size (ESS) of $\geq$200. The posterior maximum clade credibility tree was summarized by Treeannotator [64] with a 10% burn-in, and the final tree was visualized with FigTree version 1.4.4 [62]. Once the tree was generated, a GMYC was made with the single GMYC function of the splits package [68] in RStudio [42]. This function is an optimization of the method by Pons [32] and combines phylogenetic (Yule model) and phylogeographic (Coalescence model) approaches [68]. A second GMYC analysis was performed with a new ultrametric tree using the same parameters and adding 16 *H. julitta* sequences.

3. Results

3.1. Ecological Analyses

A database with 475 occurrences of the species *H. g. glauconome* (346), *H. g. grisea* (17) and *H. julitta* (112) was obtained through their distribution area. Variables correlation analysis resulted in 12 of them having a strong correlation with at least one other. Five variables were selected by applying the permutation importance criteria (Table 1).

The precipitation of the driest month (bio14) and the mean temperature of the wettest quarter (bio08) are the variables with the highest permutation importance. Butterflies are sensitive to precipitation and temperature, driving their phenology and distribution. Hence, they are excellent bioindicators [69,70]. The precipitation during the driest season is a key factor for these *Hamadryas* species. The modelling of each lineage is shown in Figure 1; all of them perform well, as AUC values show. From the five selected variables for the final model, the precipitation of the coldest quarter (bio19) had the highest permutation

importance for *H. g. glauconome*; meanwhile, the precipitation of the driest month (bio14) remained for the other two lineages (Table 2).

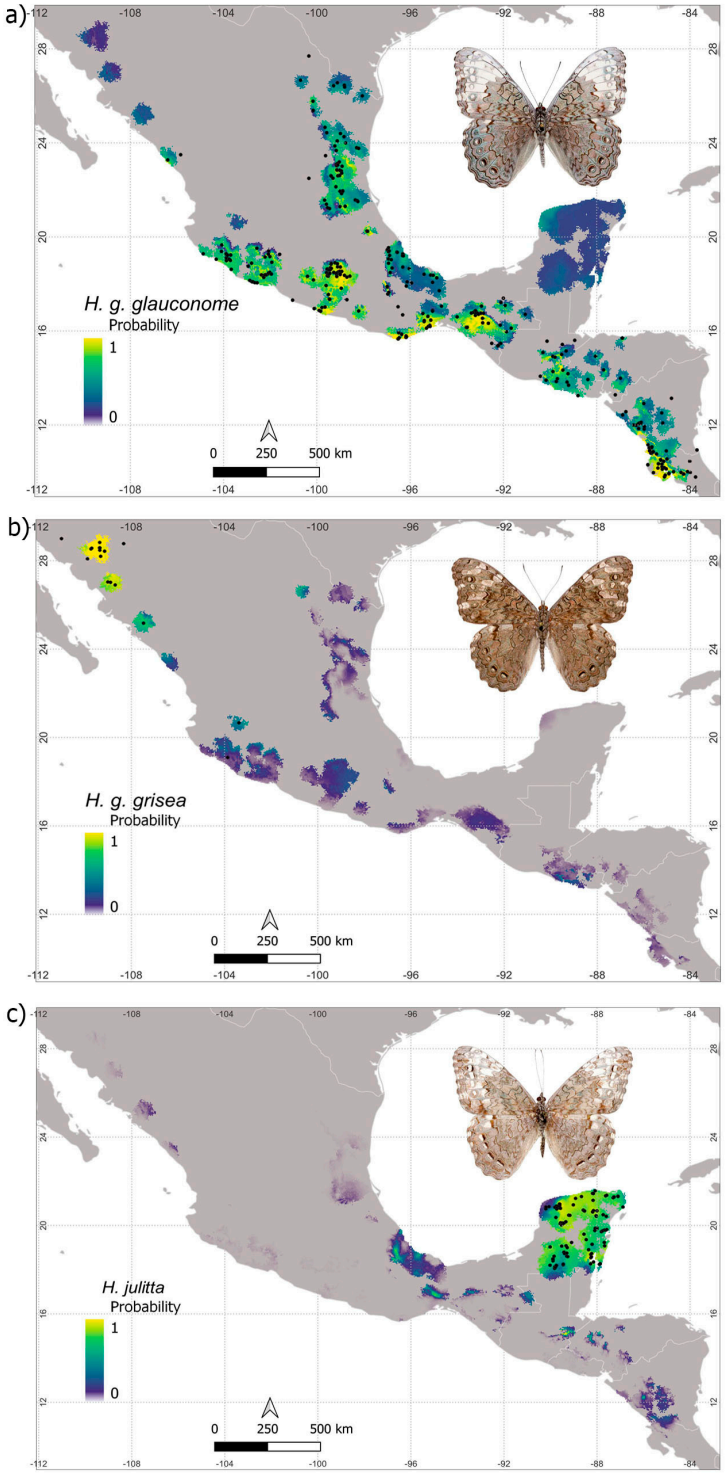

Figure 1. Ecological niche modeling for each lineage using the accessible area. Black dots are occurrences (**a**) *H. g. glauconome* AUC = 0.801 ± 0.007, (**b**) *H. g. grisea* AUC = 0.961 ± 0.014 and (**c**) *H. julitta* AUC = 0.919 ± 0.008. Images of *H. g. glauconome* and *H. julitta* from A. Warren; *H. g. grisea* from Kim Davis and Mike Strangeland, all images in Warren [71].

Table 2. Selected variables for final models, their percentage of contribution and permutation importance.

Variable	*H. g. glauconome*		*H. g. grisea*		*H. julitta*	
	Percent Contribution	Permutation Importance	Percent Contribution	Permutation Importance	Percent Contribution	Permutation Importance
bio03	12.9	10.8	0.1	2	18.9	25.4
bio06	19.5	27	56.1	41.3	13.4	7.3
bio08	8.3	9.5	9.8	3.3	14	16.7
bio14	19.4	16.5	28.2	48.4 *	43.6	30.3 *
bio19	39.9	36.2 *	5.8	5	10.1	20.3

* Variable with the highest permutation importance.

Schoender's D empirical estimation of niche identity shows that the *H. g. glauconome* niche overlaps more with *H. g. grisea* than with *H. julitta*, and the latter two are different (Table 3). The identity test leads to rejecting the null hypothesis and accepting that there is a significative difference between niches in all lineages.

Table 3. Identity test results of empirical values and critical values ($p = 0.05$). All empirical values are below critical values.

Species	Schoender's D Estimation	
	Empirical Value	Critical Value
H. g. glauconome-H. julitta	0.258	0.837
H. g. glauconome-H. g. grisea	0.613	0.724
H. g. grisea-H. julitta	0.279	0.646

Fundamental niche estimation with the MVE and its overlapping analysis shows more overlapping between *H. g. glauconome* and *H. g. grisea* than *H. g. glauconome* and *H. julitta*, and there is no overlapping between *H. g. grisea* and *H. julitta* (31.32 > 9.06 > 0, respectively (Figure 2))—a similar result to Shoender's D analysis.

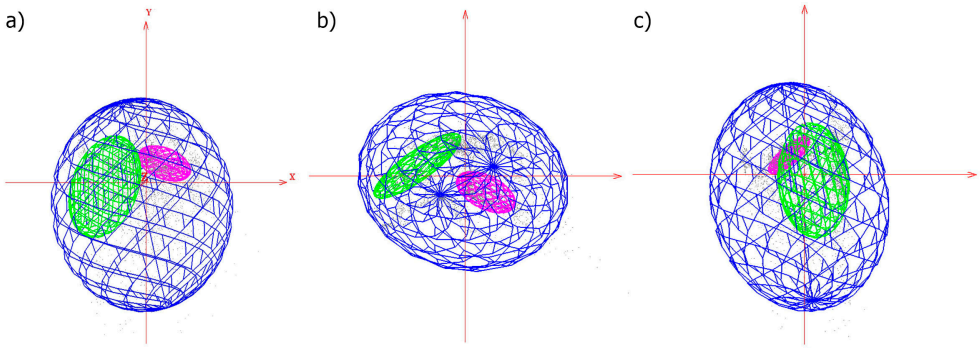

Figure 2. Minimum volume ellipsoid analysis, *H. g. glauconome* in color blue, *H. g. grisea* in green and *H. julitta* in pink. (**a**) X-Y view, (**b**) X-Z view and (**c**) Y-Z view.

3.2. Molecular Analyses

A total of 44 sequences were used for the molecular analysis (Table 4). Distances calculated with K2P in Mega show a mean interspecific distance of 1.53% between *H. g. grisea* and *H. g. glauconome*, 2.51% between *H. julitta* and *H. g. glauconome;* and 2.60% between *H. julitta* and *H. g. grisea* (Table 5). The mean intraspecific distance of *H. julitta* was 0.89%, *H. g. glauconome* was 0.20% and *H. g. grisea* was 0.34%.

Table 4. Sequences process ID from BOLD and GenBank Accession numbers.

	BOLD Process ID	GenBank Accession Number	Species		BOLD Process ID	GenBank Accession Number	Species
1	BRPC085-23	OR891501	*H. g. glauconome*	23	BRPC057-23	OR891504	*H. g. glauconome*
2	BRPC082-23	OR891505	*H. g. glauconome*	24	BRPC008-23	OR891516	*H. g. grisea*
3	BRPC081-23	OR891513	*H. g. glauconome*	25	BRPC007-23	OR891500	*H. g. grisea*
4	BRPC080-23	OR891499	*H. g. glauconome*	26	BRPC005-23	OR891510	*H. g. grisea*
5	BRPC079-23	OR891491	*H. g. glauconome*	27	BRPC003-23	OR891493	*H. g. grisea*
6	BRPC092-23	OR891506	*H. g. glauconome*	28	BRPC001-23	OR891509	*H. g. grisea*
7	BRPC073-23	OR891498	*H. g. glauconome*	29	LPMX210-07	JN201289	*H. julitta*
8	BRPC072-23	OR891503	*H. g. glauconome*	30	LYPAP807-09	GU659503	*H. julitta*
9	BRPC069-23	OR891511	*H. g. glauconome*	31	LYPAP769-09	GU659537	*H. julitta*
10	BRPC093-23	OR891490	*H. g. glauconome*	32	LPYPC059-08	JN201290	*H. julitta*
11	BRPC060-23	OR891508	*H. g. glauconome*	33	LYPAP810-09	GU659498	*H. julitta*
12	BRPC058-23	OR891494	*H. g. glauconome*	34	LYPAP809-09	GU659497	*H. julitta*
13	BRPC056-23	OR891507	*H. g. glauconome*	35	LYPAP808-09	GU659496	*H. julitta*
14	BRPC055-23	OR891495	*H. g. glauconome*	36	LYPAP805-09	GU659502	*H. julitta*
15	BRPC053-23	OR891502	*H. g. glauconome*	37	LYPAP804-09	GU659501	*H. julitta*
16	BRPC017-23	OR891512	*H. g. glauconome*	38	LYPAP803-09	GU659500	*H. julitta*
17	BRPC040-23	OR891514	*H. g. glauconome*	39	LYPAP802-09	GU659507	*H. julitta*
18	BRPC035-23	OR891496	*H. g. glauconome*	40	LYPAP801-09	GU659506	*H. julitta*
19	BRPC031-23	OR891497	*H. g. glauconome*	41	LYPAP800-09	GU659505	*H. julitta*
20	BRPC030-23	OR891492	*H. g. glauconome*	42	LYPAP799-09	GU659504	*H. julitta*
21	BRPC029-23	OR891515	*H. g. glauconome*	43	LYPAP798-09	GU659511	*H. julitta*
22	BRPC059-23	OR891489	*H. g. glauconome*	44	LYPAP797-09	GU659510	*H. julitta*

Table 5. Mean interspecific distance calculated in Mega with K2P.

Species	*H. g. grisea*	*H. julitta*
H. g. glauconome	0.0153	0.0251
H. g. grisea	-	0.0260

3.2.1. Phylogenetic Inferences

The BI and ML data set consisted of 47 sequences. Both analyses produced highly congruent topologies. In both trees, the three taxa are monophyletic. The trees show good resolution and branch support in the major clades; however, most basal nodes remained unresolved, but the nodes at the level of species groups were generally well-resolved and received strong support both in BI (Figure 3) and ML (Supplementary Figure S1) analyses.

3.2.2. Generalized Mixed Yule Coalescent (GMYC) Analysis

The single threshold GMYC (sGMYC) model using the ultrametric phylogenetic tree with *H. glauconome* lineages, created in BEAST, resulted in the identification of two putative species with high probabilities (confidence interval [CI] = 2-2, ML of null model = 197.35, ML of GMYC model = 207.03, p = 0.0000621). These are significative values that lead to rejecting the null hypothesis and accepting that there is more than one species in the analyzed data. A single *H. glauconome* sequence forms a separated entity ([CI] = 2-4) from a Texas specimen: the northern distribution limit for this species (Table 6). The GMYC model using the ultrametric tree including *H. julitta*, created in BEAST, resulted in the identification of three putative species with high probabilities (confidence interval [CI] = 3-3, ML of null model = 318.95, ML of GMYC model = 327.82, p = 0.00014). These are significative values that lead to rejecting the null hypothesis and accepting that there is more than one species in the analyzed data (Table 6).

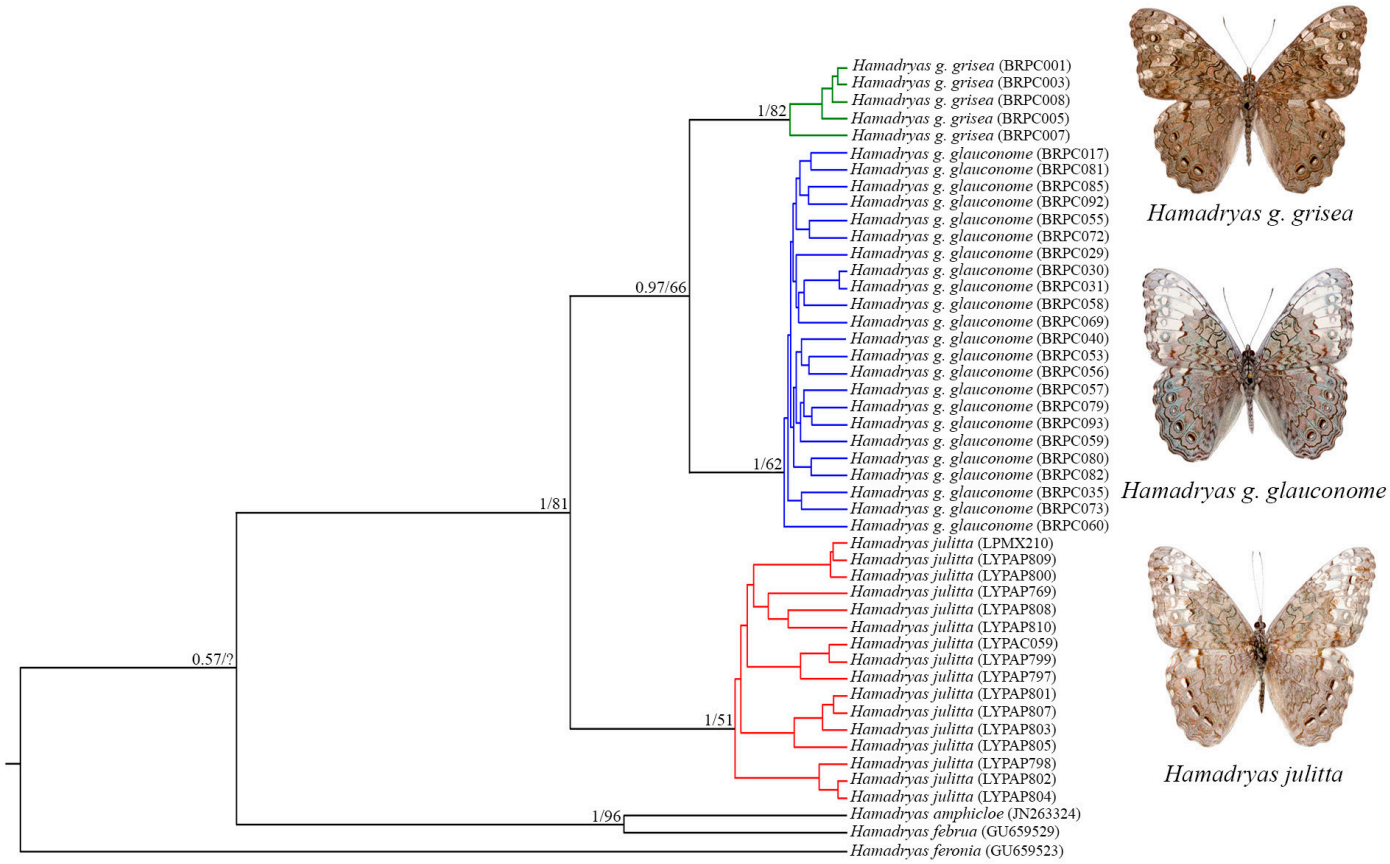

Figure 3. Ultrametric tree constructed using Bayesian inference and based on 47 sequences (658 bp) of the cytochrome oxidase c subunit I gene of specimens currently identified as *Hamadryas g. glauconome*; *H. g. grisea*, and *H. julitta*. This phylogenetic tree includes *H. februa, H. amphicloe* and is rooted using *H. februa* as an outgroup. The posterior probabilities of the major strongly supported clades are given in the nodes on the left side, and the bootstrap values from the maximum likelihood analysis are on the right side.

Table 6. Results of the GMYC entity assignments.

GMYC Entity Assignment of *H. glauconome* Sequences		
GMYC Species	**Number of Sequences**	**Species**
1	5	*H. g. grisea*
2	22	*H. g. glauconome*
3	1	*H. g. glauconome* *
GMYC entity assignment of sequences, including *H. julitta*		
1	5	*H. g. grisea*
2	23	*H. g. glauconome*
3	16	*H. julitta*

* Sequence of a specimen from South of Texas.

4. Discussion

Different traits evolve at diverse rates, challenging the species delimitation if only relayed in one of them, especially in recently diverged species [13]. In this study, ecological evidence suggests that *H. g. grisea* is a different lineage from *H. g. glauconome*, and even more, it reflects the relationship between them, as the fundamental niche overlap between MVE and Schoender's D identity is greater than with *H. julitta*. Nevertheless, identity tests among all niche lineages are significant enough to consider them different. It will be important to analyze if there is a hybridization zone in the distribution limits of *H. g. glauconome* and *H. g, grisea* where niches overlap, which could affect the species

designation of specimens from this area, as is seen in Lepidoptera species complexes [72]. The divergence niche is well known for other groups of sister species [16,73] and its importance in evolutive processes [74]. However, for some Lepidoptera specialist species, it has been proved that diversification processes are not associated with niche shift, and geographical distance is a preponderant factor [17]. Our study settles a baseline for further studies that should focus on biogeography and phylogeography to explain the processes that originated and shaped the distribution of these lineages.

The ENM for each species was very precise, as occurrences shown in the map and the AUC confirm. Suitability for each species is well differentiated along the distribution range, limiting *H. g. grisea* to the northwest of Mexico and *H. julitta* to the Yucatan Peninsula but also predicting its presence in some small parts of Veracruz, Guatemala, and Honduras. However, the model excludes biotic components and barriers that could restrict *H. julitta* distribution to the Yucatan Peninsula. *H. g. glauconome* is more suitable from Mexico to Costa Rica, but most on the Pacific slope. This distribution confirms the "Y" pattern described for Mexico, limited by the Sierra Madre Oriental, Sierra Madre Occidental, and Trans-Mexican volcanic belt reported for some Mexican butterfly species [5,75].

The distance between *H. g. grisea* and *H. g. glauconome* is 1.5%, which is below the threshold to consider a different species in most groups [7] but is above the mean intraspecific divergence of 0.20% in *H. g. glauconome* and 0.34% for *H. g. grisea*. Nevertheless, some Lepidoptera complex species groups have only 1.1% divergence, and different traits support their delimitation [76]. The genetic divergence threshold approaches, like barcoding, have limitations that often contain a degree of subjectivity and do not allow for hypothesis testing [77]. Bayesian inference and maximum likelihood are widely used to solve relationships among species; although our analysis recovers the three monophyletic groups, which means different lineages, it is not possible to establish relationships among the groups because of the low support. Additional markers, as more sequences, are needed to solve relationships among the lineages.

The GMYC approach, through the maximum likelihood optimization of the set of nodes defining the transition between intra and interspecific processes, is how clusters are delimited. This likelihood allows for statistical inferences and hypothesis testing throughout the tree [31]. The GMYC results in this study suggest that *H. g. grisea, H. g. glauconome and H. julitta* are different lineages. When excluding *H. julitta* from the GMYC analysis, one sequence from *H. g. glauconome* forms another entity, a specimen from the south of Texas; this is because of how clusters are defined in the GMYC analysis, as explained above. Because support for this node is low (0.24), further analysis that includes more samples and incorporates other molecular markers is needed. In a previous *Hamadryas* phylogenetic study [9], the glauconome group is considered paraphyletic as *H. julitta* is clustered within. Furthermore, certain divergence levels are seen among *H. glauconome* individuals: two from Costa Rica forming a cluster closer to *H. julitta* and one specimen from Texas that diverges from the others. It should be noted that in Garzón-Orduña et al. [9], no *H. glauconome* lineage from northwest Mexico was included, and the Texas specimen is different from the one in our study.

It is important to point out that morphology studies from these lineages have been performed with adult specimens [1,12], and the long-standing tradition of looking for genitalic differences responds to the morphological species concept, as a probe of reproductive isolation. However, in Lepidoptera, reproductive isolation is not only performed through genitalic morphology; behavior, chemical, and visual signaling act as a reproductive barrier between species [78–81]. In male *Hamadryas,* there is a structure called "rami" that is thought to be an organ that secretes pheromones to attract females [1]; in this way, it acts as a reproductive barrier. It proves the need to include different evidence aside from morphology when delimiting species.

In Lepidoptera complex species, sometimes the adults seem to be "cryptic"; nevertheless, when studying immature stages, morphological differences are evident [11,76]. A morphological approach that includes all lineages' immature stages will be necessary. Even

an exhaustive adult morphological search of distinctive characters in palpi, legs, or antenna is needed. The *H. g. glauconome* caterpillar is well known from Costa Rican specimens [82], and information about the *H. julitta* caterpillar has been published already [7]; meanwhile, the *H. g. grisea* caterpillar still needs to be described. The eggs of *H. julitta* and *H. g. glauconome* were described recently, with significant morphological differences between them [11].

Finally, knowledge of the diversity in complex groups is critical. Not recognizing the lineages that compose it poses a risk of losing them [83], especially the endemic lineages *H. julitta* and *H. g. grisea*, as the Yucatan Peninsula and the Gulf of California coastal region face significant threats to biodiversity [84–86]. Considering different lineages in our analyses provides a unique and precise view of diversity patterns in the taxonomic groups of interest [87].

5. Conclusions

Hamadryas g. grisea, *H. g. glauconome* and *H. julitta* are different lineages, as ENM, Bayesian inference, maximum likelihood and GMYC approaches suggest. We recommend reviewing the status of the subspecies to elevate it to species. Also, it is important to conduct a morphological analysis including immature stages, as discriminating characters among lineages could be detected. An analysis is also important in looking for hybridization zones at both lineages' limit distributions. Relationships among the lineages should be solved by including different markers and more samples. When delimiting species, we suggest providing different sources of evidence when morphology is not enough. This is the first time that the *H. g. grisea* COI sequence (barcode) has been published.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15121196/s1, Figure S1. The ML tree was generated with IQ-TREE web server. The analysis includes 47 COI (658bp) sequences (16 from *H. julitta*, 5 from *H. g. grisea*, 23 from *H. g. glauconome*, and sequences of *H. februal*, *H. amphicloe*, and *H. feronia* as outgroup). The nodes are shown with bootstrap support.

Author Contributions: Conceptualization, B.R.P.-C., C.P., L.A.L.-P., M.T.-O. and S.M.-M.; methodology, B.R.P.-C. and M.T.-O.; writing—review and editing, B.R.P.-C., C.P., L.A.L.-P., M.T.-O. and S.M.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All sequences are available on GenBank with de Accession numbers provided in the article. Also, sequences datasets are available on www.boldsystems.org in the dataset "Delimiting species with GMYC, Bayesian inference and Ecological Niche Modeling" (DS-GLAUCO).

Acknowledgments: We are grateful to Armando Luis Martínez from Facultad de Ciencias, UNAM, to Ivonne Garzón-Orduña from Instituto de Biología, UNAM, and Andy Warren and Keith Willmott from the McGuire Center for Lepidoptera and Biodiversity at the Florida University for all the facilities in the Lepidoptera collection from each institute. Also, Noemí Salas-Suárez and Estela Domínguez Mukul for their help in processing samples. M.T.-O was supported by a posdoctoral grant from CONAHCYT (Estancias Posdoctorales por México para la Formación y Consolidación de las y los Investigadores por México; CVU131802). B.R.P.-C. thanks CONAHCYT for the granted scholarship during her graduate program (CVU215222).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jenkins, D.W. Neotropical Nymphalidae I. Revision of Hamadryas. *Bull. Allyn. Mus.* **1983**, *81*, 1–146.
2. Fruhstorfer, H. Ageronia. In *Die Gross-Schmetterlinge der Erde: Eine Systematische Bearbeitung der bis Jetzt Bekannten Gross-Schmetterlinge*; Seitz, A., Ed.; Alfred Kernen: Stuttgart, Germany, 1916; Volume Bd.5, pp. 537–542.
3. Rzedowski, J. Capítulo 6. Provincias Florísticas de México. In *Vegetación de México*; Limusa: Mexico City, Mexico, 1978.
4. Morrone, J.J. Hacia una síntesis biogeográfica de México. *Rev. Mex. Biodivers.* **2005**, *76*, 207–252. [CrossRef]

5. Luis Martínez, A.; Llorente-Bosuquets, J.; Vargas-Fernández, I. *Nymphalidae de México I (Danainae, Apaturinae, Biblidinae y Heliconiinae): Distribución Geográfica e Ilustración*; Prensas de Ciencias: Mexico City, Mexico, 2003; ISBN 9789703206933.
6. Meerman, J.; Boomsma, T. Occasional Papers of the Belize Natural History Society. *J. Belizean Na. Hist.* **1993**, *2*, 37–46.
7. Prado, B.R.; Pozo, C.; Valdez-Moreno, M.; Hebert, P.D.N. Beyond the Colours: Discovering Hidden Diversity in the Nymphalidae of the Yucatan Peninsula in Mexico through DNA Barcoding. *PLoS ONE* **2011**, *6*, e27776. [CrossRef] [PubMed]
8. Salinas-Gutiérrez, J.L.; Méndez, C.; Barrios, M.; Pozo, C.; Llorente-Bousquets, J. Hacia Una Síntesis de Los Papilionoidea (Insecta: Lepidoptera) de Guatemala Con Una Reseña Histórica. *Caldasia* **2009**, *31*, 407–440.
9. Garzón-Orduña, I.J.; Marini-Filho, O.; Johnson, S.G.; Penz, C.M. Phylogenetic Relationships of Hamadryas (Nymphalidae: Biblidinae) Based on the Combined Analysis of Morphological and Molecular Data. *Cladistics* **2013**, *29*, 629–642. [CrossRef]
10. Garzón-Orduña, I.J.; Brower, A.V.Z.; Kamilari, M.; Iribar, A.; Murienne, J. Cracking the Code: Examination of Species Delimitations among Hamadryas Butterflies with DNA Barcodes Suggests Caribbean Cracker Is Hamadryas Februa Hübner (Nymphalidae: Biblidinae). *J. Lepid. Soc.* **2018**, *72*, 53–73. [CrossRef]
11. Nieves-Uribe, S.; Flores-Gallardo, A.; Llorente-Bousquets, J.; Luis-Martínez, A.; Pozo, C. Use of Exochorion Characters for the Systematics of Hamadryas Hübner and Ectima Doubleday (Nymphalidae: Biblidinae: Ageroniini). *Zootaxa* **2019**, *4619*, 77–108. [CrossRef]
12. Esquivel-Ruíz, E.S. *Reevaluación Del Status Taxonómico de Hamadryas Julitta Respecto a Hamadryas Glauconome Por Medio de Análisis Cuantitativos de Caracteres Morfológicos*; Maestría, UNAM: Ciudad de México, Mexico, 2022.
13. De Queiroz, K. Species Concepts and Species Delimitation. *Syst. Biol.* **2007**, *56*, 879–886. [CrossRef]
14. Raxworthy, C.J.; Ingram, C.M.; Rabibisoa, N.; Pearson, R.G. Applications of Ecological Niche Modeling for Species Delimitation: A Review and Empirical Evaluation Using Day Geckos (Phelsuma) from Madagascar. *Syst. Biol.* **2007**, *56*, 907–923. [CrossRef]
15. Rissler, L.J.; Apodaca, J.J. Adding More Ecology into Species Delimitation: Ecological Niche Models and Phylogeography Help Define Cryptic Species in the Black Salamander (Aneides Flavipunctatus). *Syst. Biol.* **2007**, *56*, 924–942. [CrossRef] [PubMed]
16. Vaissi, S.; Rezaei, S. Niche Divergence at Intraspecific Level in the Hyrcanian Wood Frog, Rana Pseudodalmatina: A Phylogenetic, Climatic, and Environmental Survey. *Front. Ecol. Evol.* **2022**, *10*, 774481. [CrossRef]
17. Imada, Y.; Kawakita, A.; Kato, M. Allopatric Distribution and Diversification without Niche Shift in a Bryophyte-Feeding Basal Moth Lineage (Lepidoptera: Micropterigidae). *Proc. R. Soc. B Biol. Sci.* **2011**, *278*, 3026–3033. [CrossRef] [PubMed]
18. Talavera, G.; Kaliszewska, Z.A.; Heath, A.; Pierce, N.E. Recent Diversification of Chrysoritis Butterflies in the South African Cape (Lepidoptera: Lycaenidae). *Mol. Phylogenet. Evol.* **2020**, *148*, 106817. [CrossRef]
19. Moraes, S.D.E.S.; Gueratto, P.E.; Dos Santos, J.P.; Santos, M.H.; Freitas, A.V.L.; Duarte, M. Niche Modelling and Comparative Morphology Untangle Taxonomy of the Dysschema Eurocilia Clade (Lepidoptera: Erebidae) and Reveal a Relictual Pleistocene Arc Distribution. *Syst. Biodivers.* **2022**, *20*, 1–39. [CrossRef]
20. Campbell, E.O.; MacDonald, Z.G.; Gage, E.V.; Gage, R.V.; Sperling, F.A.H. Genomics and Ecological Modelling Clarify Species Integrity in a Confusing Group of Butterflies. *Mol. Ecol.* **2022**, *31*, 2400–2417. [CrossRef] [PubMed]
21. Andersson, L. The Driving Force: Species Concepts and Ecology. *Taxon* **1990**, *39*, 375–382. [CrossRef]
22. Van Valen, L. Ecological species, multispecies, and oaks. *Taxon* **1976**, *25*, 233–239. [CrossRef]
23. Hutchinson, G.E. Concluding Remarks. *Cold Spring Harb. Symp. Quant. Biol.* **1957**, *22*, 415–427. [CrossRef]
24. Maguire, B. Niche Response Structure and the Analytical Potentials of Its Relationship to the Habitat. *Am. Nat.* **1973**, *107*, 213–246. [CrossRef]
25. Soberón, J.; Peterson, A. Interpretation of Models of Fundamental Ecological Niches and Species' Distributional Areas. *Biodivers. Inform.* **2005**, *2*, 1–10. [CrossRef]
26. Barve, N.; Barve, V.; Jiménez-Valverde, A.; Lira-Noriega, A.; Maher, S.P.; Peterson, A.T.; Soberón, J.; Villalobos, F. The Crucial Role of the Accessible Area in Ecological Niche Modeling and Species Distribution Modeling. *Ecol. Model.* **2011**, *222*, 1810–1819. [CrossRef]
27. Soberón, J.M. Niche and Area of Distribution Modeling: A Population Ecology Perspective. *Ecography* **2010**, *33*, 159–167. [CrossRef]
28. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological Identifications through DNA Barcodes. *Proc. R. Soc. Lond. B Biol. Sci.* **2003**, *270*, 313–321. [CrossRef] [PubMed]
29. Hebert, P.D.N.; Ratnasingham, S.; de Waard, J.R. Barcoding Animal Life: Cytochrome c Oxidase Subunit 1 Divergences among Closely Related Species. *Proc. R. Soc. Lond. B Biol. Sci.* **2003**, *270*, S96–S99. [CrossRef]
30. Dumas, P.; Barbut, J.; Le Ru, B.; Silvain, J.-F.; Clamens, A.-L.; d'Alençon, E.; Kergoat, G.J. Phylogenetic Molecular Species Delimitations Unravel Potential New Species in the Pest Genus Spodoptera Guenée, 1852 (Lepidoptera, Noctuidae). *PLoS ONE* **2015**, *10*, e0122407. [CrossRef] [PubMed]
31. Fujisawa, T.; Barraclough, T.G. Delimiting Species Using Single-Locus Data and the Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on Simulated Data Sets. *Syst. Biol.* **2013**, *62*, 707–724. [CrossRef]
32. Pons, J.; Barraclough, T.G.; Gomez-Zurita, J.; Cardoso, A.; Duran, D.P.; Hazell, S.; Kamoun, S.; Sumlin, W.D.; Vogler, A.P. Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. *Syst. Biol.* **2006**, *55*, 595–609. [CrossRef]
33. Miller, J.; Matthews, D.; Warren, A.; Solis, M.; Harvey, D.; Gentili-Poole, P.; Lehman, R.; Emmel, T.; Covell, C. An Annotated List of the Lepidoptera of Honduras. *Insecta Mundi.* **2012**, *205*, 1–72. Available online: https://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1724&context=insectamundi (accessed on 31 October 2021).

34. Calderón-Vásquez, M.A. *Evaluación de Cuatro Años de Monitoreo de Mariposas En La Finca Agroecológica de Zamorano, Honduras*; Escuela Agrícola Panamericana: Zamorano, Honduras, 2020.
35. Medina, M.; Orozco, J.; Hernández, J. Diversidad de Especies de Lepidoptera: Nymphalidae, Papilionidae y Pieridae, En "El Cacao", Telica, León, Nicaragua, En 2018. *Revista Nicaragüense de Entomología.* **2020**, *211*, 3–64.
36. Gallardo, R.J.; Diaz, O. *Guide to the Butterflies of Honduras*; Robert Gallardo (privately published): Santa Bárbara, Honduras, 2022.
37. GBIF Occurrence Download. Available online: https://www.gbif.org/occurrence/download/0008894-231002084531237 (accessed on 9 October 2023).
38. Rios, N.E.; Bart, H.L. GEOLocate 2010 (Version 3.22). Tulane University Museum of Natural History: Belle Chasse, LA, USA. Available online: https://www.geo-locate.org/standalone/default.html (accessed on 31 October 2021).
39. QGIS.org QGIS Geographic Information System. Available online: https://www.qgis.org/ (accessed on 31 October 2021).
40. Google Earth. Available online: https://earth.google.com/ (accessed on 4 February 2022).
41. Hijmans, R.J.; Cameron, S.E.; Parra, J.L.; Jones, P.G.; Jarvis, A. Very High Resolution Interpolated Climate Surfaces for Global Land Areas. *Int. J. Climatol.* **2005**, *25*, 1965–1978. [CrossRef]
42. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2023.
43. Di Cola, V.; Broennimann, O.; Petitpierre, B.; Breiner, F.T.; D'Amen, M.; Randin, C.; Engler, R.; Pottier, J.; Pio, D.; Dubuis, A.; et al. Ecospat: An R Package to Support Spatial Analyses and Modeling of Species Niches and Distributions. *Ecography* **2017**, *40*, 774–787. [CrossRef]
44. Lourenço, G.M.; Dáttilo, W.; Ribeiro, S.P.; Freitas, A.V.L. Biological Aspects and Movements of Neotropical Fruit-Feeding Butterflies. *Neotrop. Entomol.* **2022**, *51*, 43–53. [CrossRef] [PubMed]
45. Machado-Stredel, F.; Cobos, M.E.; Peterson, A.T. A Simulation-Based Method for Selecting Calibration Areas for Ecological Niche Models and Species Distribution Models. *Front. Biogeogr.* **2021**, *13*, e48814. [CrossRef]
46. Hijmans, R.J.; van Etten, J. Raster: Geographic Analysis and Modeling with Raster Data. R Package Version 2.0-12 2012. Available online: https://rspatial.org/raster (accessed on 31 October 2021).
47. Hijmans, R.J.; Phillips, S.; Leathwick, J.; Elith, J. Dismo: Species Distribution Modeling. R Package Version 1.0-12 2015. Available online: https://rspatial.org/raster/sdm/ (accessed on 31 October 2021).
48. Lares, B. Lares: Analytics & Machine Learning Sidekick. R Package Version 5.2.4.9000 2023. Available online: https://github.com/laresbernardo/lares (accessed on 31 October 2021).
49. Phillips, S.J.; Dudík, M. Modeling of Species Distributions with Maxent: New Extensions and a Comprehensive Evaluation. *Ecography* **2008**, *31*, 161–175. [CrossRef]
50. Warren, D.L.; Beaumont, L.J.; Dinnage, R.; Baumgartner, J.B. New Methods for Measuring ENM Breadth and Overlap in Environmental Space. *Ecography* **2019**, *42*, 444–446. [CrossRef]
51. Warren, D.L.; Glor, R.E.; Turelli, M. Environmental Niche Equivalency Versus Conservatism: Quantitative Approaches to Niche Evolution. *Evolution* **2008**, *62*, 2868–2883. [CrossRef]
52. Qiao, H.; Peterson, A.T.; Campbell, L.P.; Soberón, J.; Ji, L.; Escobar, L.E. NicheA: Creating Virtual Species and Ecological Niches in Multivariate Environmental Scenarios. *Ecography* **2016**, *39*, 805–813. [CrossRef]
53. Ratnasingham, S.; Hebert, P.D.N. Bold: The Barcode of Life Data System (Http://Www.Barcodinglife.Org). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef]
54. Ivanova, N.V.; DeWaard, J.R.; Hebert, P.D.N. An Inexpensive, Automation-Friendly Protocol for Recovering High-Quality DNA. *Mol. Ecol. Notes* **2006**, *6*, 998–1002. [CrossRef]
55. Edgar, R.C. MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [CrossRef] [PubMed]
56. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]
57. Kimura, M. A Simple Method for Estimating Evolutionary Rates of Base Substitutions through Comparative Studies of Nucleotide Sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef] [PubMed]
58. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
59. Bouckaert, R.; Heled, J.; Kühnert, D.; Vaughan, T.; Wu, C.-H.; Xie, D.; Suchard, M.A.; Rambaut, A.; Drummond, A.J. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput. Biol.* **2014**, *10*, e1003537. [CrossRef] [PubMed]
60. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
61. Sullivan, J.; Joyce, P.; Posada, D.; Crandall, K.A. JModelTest 2: More Models, New Heuristics and Parallel Computing. *Nat. Methods* **2012**, *36*, 716–723. [CrossRef]
62. Rambaut, A. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. 2010. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 31 October 2021).
63. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [CrossRef]
64. Drummond, A.J.; Rambaut, A. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. *BMC Evol. Biol.* **2007**, *7*, 214. [CrossRef]

65. Huelsenbeck, J.P.; Hillis, D.M. Success of Phylogenetic Methods in the Four-Taxon Case. *Syst. Biol.* **1993**, *42*, 247–264. [CrossRef]
66. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kühnert, D.; De Maio, N.; et al. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef] [PubMed]
67. Zhang, R.; Drummond, A. Improving the Performance of Bayesian Phylogenetic Inference under Relaxed Clock Models. *BMC Evol. Biol.* **2020**, *20*, 54. [CrossRef] [PubMed]
68. Ezard, T.; Fujisawa, T.; Barraclough, T.G. Splits: Species' Limits by Threshold Statistics. R Package Version 2009. Available online: https://r-forge.r-project.org/projects/splits/ (accessed on 31 October 2021).
69. Pozo, C.; Luis-Martínez, A.; Llorente-Bousquets, J.; Salas-Suárez, N.; Maya-Martínez, A.; Vargas-Fernández, I.; Warren, A.D. Seasonality and Phenology of the Butterflies (Lepidoptera: Papilionoidea and Hesperioidea) of Mexico's Calakmul Region. *Fla. Entomol.* **2008**, *91*, 407–422. [CrossRef]
70. Scoble, M.J. *The Lepidoptera: Form, Function and Diversity*; Natural History Museum Publications; Natural History Museum: New York, NY, USA, 1995; ISBN 9780198549529.
71. Warren, A.D.; Davis, K.J.; Stangeland, E.M.; Pelham, J.P.; Willmott, K.; Grishin, N.V. Illustrated Lists of American Butterflies. Available online: https://www.butterfliesofamerica.com (accessed on 27 November 2023).
72. Jasso-Martínez, J.M.; Machkour-M'Rabet, S.; Vila, R.; Rodríguez-Arnaiz, R.; Castañeda-Sortibrán, A.N. Molecular Evidence of Hybridization in Sympatric Populations of the Enantia Jethys Complex (Lepidoptera: Pieridae). *PLoS ONE* **2018**, *13*, e0197116. [CrossRef] [PubMed]
73. Graham, C.H.; Ron, S.R.; Santos, J.C.; Schneider, C.J.; Moritz, C. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution* **2004**, *58*, 1781–1793. [CrossRef] [PubMed]
74. Pitteloud, C.; Arrigo, N.; Suchan, T.; Mastretta-Yanes, A.; Vila, R.; Dincă, V.; Hernández-Roldán, J.; Brockmann, E.; Chittaro, Y.; Kleckova, I.; et al. Climatic Niche Evolution Is Faster in Sympatric than Allopatric Lineages of the Butterfly Genus Pyrgus. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, 20170208. [CrossRef] [PubMed]
75. Maya-Martínez, A.; Schmitter-Soto, J.J.; Pozo, C. Panbiogeography of the Yucatan Peninsula Based on Charaxinae (Lepidoptera: Nymphalidae). *Fla. Entomol.* **2011**, *94*, 527–533. [CrossRef]
76. Hebert, P.D.N.; Penton, E.H.; Burns, J.M.; Janzen, D.H.; Hallwachs, W. Ten Species in One: DNA Barcoding Reveals Cryptic Species in the Neotropical Skipper Butterfly Astraptes Fulgerator. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14812–14817. [CrossRef]
77. DeSalle, R.; Goldstein, P. Review and Interpretation of Trends in DNA Barcoding. *Front. Ecol. Evol.* **2019**, *7*, 302. [CrossRef]
78. Lassance, J.-M.; Svensson, G.P.; Kozlov, M.V.; Francke, W.; Löfstedt, C. Pheromones and Barcoding Delimit Boundaries between Cryptic Species in the Primitive Moth Genus Eriocrania (Lepidoptera: Eriocraniidae). *J. Chem. Ecol.* **2019**, *45*, 429–439. [CrossRef]
79. Smadja, C.; Butlin, R.K. On the Scent of Speciation: The Chemosensory System and Its Role in Premating Isolation. *Heredity (Edinb)* **2009**, *102*, 77–97. [CrossRef] [PubMed]
80. González-Rojas, M.F.; Darragh, K.; Robles, J.; Linares, M.; Schulz, S.; McMillan, W.O.; Jiggins, C.D.; Pardo-Diaz, C.; Salazar, C. Chemical Signals Act as the Main Reproductive Barrier between Sister and Mimetic Heliconius Butterflies. *Proc. R. Soc. B Biol. Sci.* **2020**, *287*, 20200587. [CrossRef] [PubMed]
81. Vane-Wright, R.I.; Boppré, M.; Butlin, R.K.; Guilford, T.; Krebs, J.R. Visual and Chemical Signalling in Butterflies: Functional and Phylogenetic Perspectives. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **1997**, *340*, 197–205. [CrossRef]
82. Janzen, D.H.; Hallwachs, W. Dynamic Database for an Inventory of the Macrocaterpillar Fauna, and Its Food Plants and Parasitoids, of Area de Conservacion Guanacaste (ACG), Northwestern Costa Rica (Nn-SRNP-Nnnnn Voucher Codes). Available online: http://janzen.sas.upenn.edu (accessed on 30 September 2020).
83. D'Amen, M.; Zimmermann, N.E.; Pearman, P.B. Conservation of Phylogeographic Lineages under Climate Change. *Glob. Ecol. Biogeogr* **2013**, *22*, 93–104. [CrossRef]
84. Islebe, G.A.; Schmook, B.; Calmé, S.; León-Cortés, J.L. Introduction: Biodiversity and Conservation of the Yucatán Peninsula, Mexico. In *Biodiversity and Conservation of the Yucatán Peninsula*; Islebe, G., Calmé, S., León-Cortés, J., Schmook, B., Eds.; Springer: Cham, Switzerland, 2015.
85. Pfeiler, E. Butterfly Biodiversity in a Threatened Coastal Desert Ecosystem of Northwestern Mexico, with a Focus on the Life History and Ecology of Potentially Endangered Species. *J. Lepid. Soc.* **2016**, *70*, 47–60. [CrossRef]
86. Garza-García, J. Environmental NGOs and the Political Ecology of Biodiversity Conservation in Mexican Forests. Ph.D. Thesis, Macquarie University, Sidney, Australia, 2023.
87. Rissler, L.J.; Hijmans, R.J.; Graham, C.H.; Moritz, C.; Wake, D.B. Phylogeographic Lineages and Species Comparisons in Conservation Analyses: A Case Study of California Herpetofauna. *Am. Nat.* **2006**, *167*, 655–666. [CrossRef]