UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

PEDRO DE GUSMÃO RIBEIRO

POPULATION GENETICS OF THE WHITE SAND ECOSYSTEM SPECIALIST BUTTERFLY *HELICONIUS HERMATHENA* (LEPIDOPTERA:NYMPHALIDAE) ASSESSED BY MITOGENOME ANALYSIS

GENÉTICA DE POPULAÇÕES DA BORBOLETA ESPECIALISTA DE ECOSSISTEMAS DE AREIA BRANCA *HELICONIUS HERMATHENA* (LEPIDOPTERA:NYMPHALIDAE) OBTIDA POR ANÁLISE DE MITOGENOMA

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Os membros da Comissão Examinadora acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.

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“Todo começo é difícil em qualquer ciência.”

- Karl Marx

“[...] the empirical study of population genetics has always begun with and centered around the characterization of the genetic variation in populations”

- Richard C. Lewontin

Resumo

Os ecossistemas de areia branca da Amazônia (conhecidos como campinas e campinaranas) são manchas isoladas de vegetação escleromórfica que ocorrem sob solos de areia branca, exclusivamente na Amazônia. Esses ecossistemas apresentam baixa diversidade e alto endemismo floral e faunístico devido às características únicas do solo, alta luminosidade e temperatura. No entanto, esses ecossistemas são sub-representados em estudos geológicos e biológicos, especialmente no que diz respeito à estrutura genética e diversidade das espécies que os habitam. Neste trabalho, investigamos a variabilidade genética entre subpopulações de *Heliconius hermathena*, uma espécie de borboleta endêmica dos ecossistemas de areia branca da Amazônia. Utilizando todo o genoma mitocondrial como marcador molecular, inferimos alta diferenciação genética entre indivíduos de seis subespécies diferentes de *H. hermathena*, que ocorrem em oito localidades diferentes (subpopulações). Nossos dados confirmam a hipótese prévia sobre a diversificação da espécie, na qual o isolamento das áreas de areia branca pode ter desempenhado um papel importante na geração e manutenção da variabilidade genética e da estrutura populacional encontradas nas subpopulações de *H. hermathena*. Além disso, acreditamos que este estudo esteja de acordo com outros que estudaram espécies adaptadas a esses ambientes em relação a diferenciação genética, mostrando que é necessário avaliar o papel dos ecossistemas de áreas de areia branca de forma mais profunda, afim de entender melhor os padrões que levaram à grande biodiversidade amazônica.

Abstract

Amazonian white sand ecosystems (campinas and campinaranas) are strongly isolated patches of escleromorphic vegetation above white sandy soils that occur exclusively in the Amazon. These ecosystems present low diversity and high floral and faunal endemism due to unique soil characteristics, harsh luminosity and temperature conditions. Nevertheless, these ecosystems are underrepresented in geological and biological studies, especially regarding the genetic structure and diversity of the species inhabiting them. Here, we investigate the genetic differentiation among subpopulations of *Heliconius hermathena,* an endemic butterfly to the Amazonian white sand ecosystems. Using the whole mitochondrial genome as molecular marker, we inferred high values of genetic differentiation among individuals from six different subspecies of *H. hermathena* occuring in eight different localities (subpopulations). Our data confirms previous hypotheses regarding the species diversification in which the isolation of the patches of white sand areas might have played an important role in generating and maintaining the species structure across subpopulations. Furthermore, we believe that this work is in accordance with others on white sand areas genetic differentiation, showing that it is necessary to assess the role of white sand areas in generating and maintaining diversification patterns in the Amazon in order to better appreciate Amazonian biodiversity.

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Introdução geral

A história natural dos neotrópicos foi dramaticamente influenciada por variações climáticas ocorridas no passado, sobretudo no Pleistoceno e pós-Pleistoceno. Nesse período recente, sugere-se que as florestas neotropicais sofreram ciclos importantes de retração em momentos mais secos, permitindo, por exemplo, a expansão dos ecossistemas savânicos de modo geral, e de ampliação em momentos mais úmidos, quando os ecossistemas abertos persistiram como ilhas mais isoladas. Segundo a hipótese dos refúgios florestais (Haffer 1969), esse processo de expansão e retração da floresta amazônica foi fundamental para o isolamento e consequente diversificação de plantas e animais, como proposto por diversos trabalhos na literatura (e.g Haffer 1969; Vuilleumier 1971; Prance 1973; Brown 1976;). Novas evidências contrárias a essa hipótese (Knapp & Mallet 2003) e a favor (Pinheiro et al. 2013), mantém esse debate ainda atual. Mais que isso, a explicação desses processos a partir da biologia evolutiva e da genética de populações está ainda em curso, criando possibilidades para interessantes estudos nessa área de pesquisa.

Nesse contexto, investigamos a espécie de borboleta *Heliconius hermathena* Hewitson, 1854 (Lepidoptera: Nymphalidae), uma espécie endêmica da Amazônia e adaptada aos ecossistemas de areia branca conhecidos como campinas e campinaranas. Com sete subespécies descritas (*H. h. duckei, H. h. sheppardi, H. h hermathena, H. h. sabinae, H. h. curua* e *H. h. renatae* e *H. h. vereatta*), *H. hermathena* é considerada como uma das poucas espécies de *Heliconius* "não-miméticas", com exceção de *H. hermathena vereatta,* que apresenta um padrão de coloração das asas muito similar àqueles apresentados por *H. melpomene melpomene e H. erato hydara*, com as quais a subespécie é simpátrica. A distribuição das populações conhecidas de *H. hermathena* mostra que estas são bastante isoladas, seguindo a distribuição dos ecossistemas de areia branca, e acredita-se que sua diversificação esteja relacionada com a formação e fragmentação das campinas e campinaranas nas quais elas ocorrem, em um processo similar ao observado em espécies de *Heliconius* de floresta (Brown et al. 1974; Brown & Benson, 1977). Acreditamos que a variabilidade genética existente e sua distribuição nas diferentes subespécies e subpopulações de *H. hermathena* possa fornecer dados importantes para investigar a diversificação desta espécie e sua relação com esses importantes ecossistemas amazônicos.

Neste projeto, sequenciamos os genomas mitocondriais de 71 indivíduos de seis das sete subespécies de *H. hermathena* (*H. h. duckei, H. h. sheppardi, H. h hermathena, H. h. sabinae, H. h. curua* e *H. h. vereatta*) provenientes de oito localidades de coleta distribuídas nos seguintes municípios: Barcelos, Manaus, Maués, Presidente Figueiredo (todas no estado do Amazonas), Altamira, Faro e Santarém (todas no estado do Pará). Aplicamos análises de genética populacional e inferência bayesiana a fim de explicar como processos microevolutivos e a história do ambiente amazônico estão relacionados com a diversificação das populações de *H. hermathena*. Pretendemos obter, pela primeira vez, uma análise de genética de populações de um inseto especialista nos ecossistemas de areia branca, os quais, por sua vez, representam uma importante parcela da biodiversidade amazônica.

1.1 Ecossistemas de areia branca: campinas e campinaranas

Os ecossistemas de areia branca são formações únicas que ocorrem ao longo da bacia amazônica (Adeney et al. 2016). O fato de serem ambientes abertos, com vegetação predominantemente de estatura baixa a média, possuírem ciclos padronizados de queimadas, hidrografia particular e, principalmente, ocorrerem sobre solos arenosos, são características que os distinguem de outras formações savânicas como, por exemplo, o Cerrado (Eiten 1978, Adeney et al. 2016). Sua fisionomia é predominantemente esclerófila o que provavelmente se relaciona com sua composição edáfica, e isso pode ter sido importante do ponto de vista da formação de uma biota com baixa diversidade e rica em endemismo nesses ambientes (Anderson 1981). Os ecossistemas de areia branca ocorrem na região amazônica no Brasil, Peru, Venezuela, Colômbia, Suriname, Guiana e Guiana Francesa, alcançando uma área total de aproximadamente 334.879km^2 (Adeney et al. 2016; Eiten 1978). Todas essas características o tornam um ambiente único e importante da paisagem e amazônica. Sendo assim, o estudo de sua biota, tanto do ponto de vista taxonômico como dos pontos de vista ecológico e genético, se torna cientificamente relevante e imprescindível para se entender de forma mais profunda a biodiversidade amazônica.

Existem diversas nomenclaturas para os ambientes que são descritos como ecossistemas de areia branca amazônicos, mas para os objetivos deste trabalho consideramos apenas dois deles: as campinas e as campinaranas (Figura 1). As campinas existem de forma aberta (campina aberta), com vegetação arbustiva de tamanho médio, mas também podem

apresentar porções mais fechadas (campina fechada) conhecidas como '*scrubland*' (Adeney et al. 2016) (Figura 1). As campinaranas, por outro lado, são formações florestais sob solos arenosos (Adeney et al. 2016) (Figura 1).

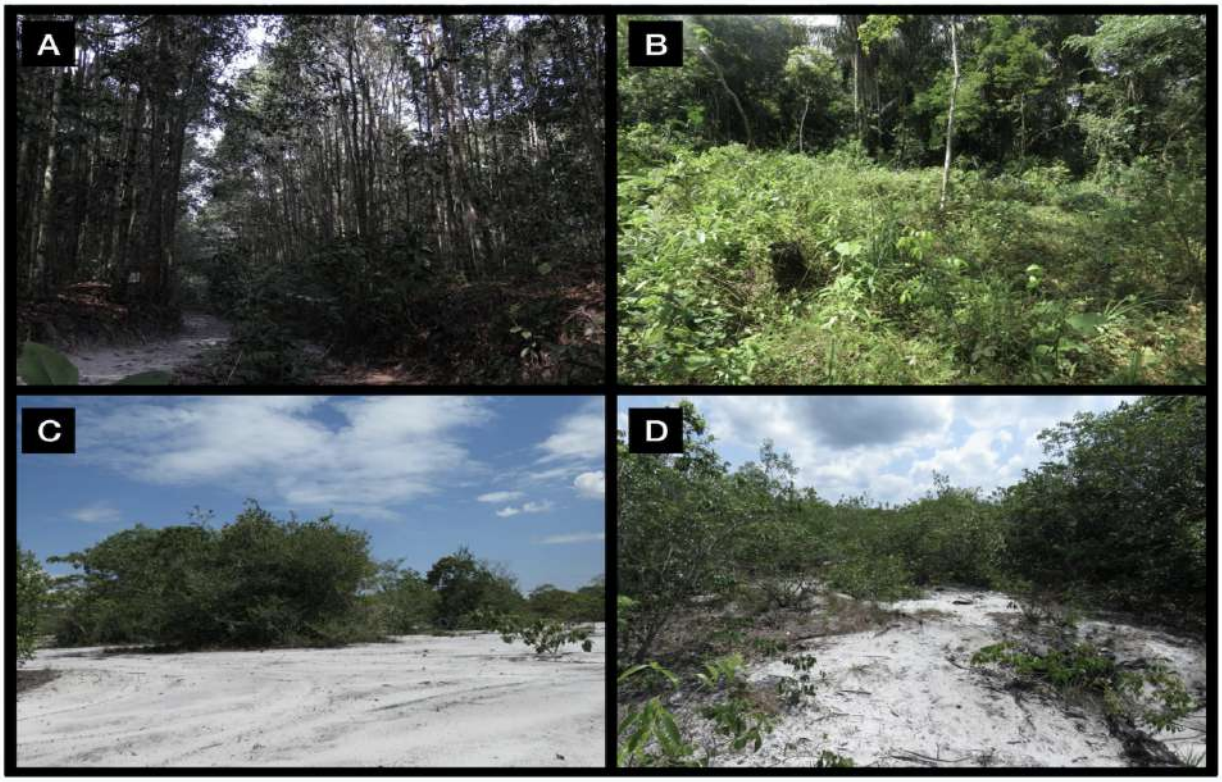

Figura 1 Fotos ilustrativas de ecossistemas de areia branca nos quais indivíduos de *H. hermathena* foram coletados. (A e B) Campinarana, ecossistema de areia branca tipicamente florestal; ainda que fechado e florestal, apresenta solos arenosos e "clarões" como em B. (C) Campina aberta com arbustos médios e pequenos. (D) Campina fechada ou *scrubland.* (Fotos: cortesia de R. R. Ramos)

Durante períodos de clima mais seco e de retração florestal no passado, supõe-se que os ecossistemas de areia branca apresentavam distribuição quase contínua na paisagem amazônica. De fato, alguns estudos demonstraram a possibilidade da existência de corredores de ambientes abertos como as campinas e campinaranas e, também, de sua expansão durante períodos de variação climática no passado (Quijada-Mascareñas et al. 2007; Vargas-Ramírez et al. 2010).

Com os períodos de expansão florestal, no entanto, esses ambientes se consolidaram como manchas isoladas, como ilhas de vegetação aberta cercadas por matriz florestal. Os processos que levaram à fragmentação desses ecossistemas podem então ter resultado no isolamento do conjunto gênico de *H. hermathena*, levando aos padrões atuais da distribuição de sua diversidade genética (Brown & Benson 1977). Sendo assim, esperaríamos observar

subpopulações da espécie com alta variabilidade interpopulacional e forte estrutura populacional. Nosso objetivo é entender se, de fato, tais padrões de distribuição da diversidade genética podem ser observados atualmente.

1.2 As borboletas do gênero *Heliconius* como modelos de estudos genéticos e evolutivos.

As borboletas do gênero *Heliconius* exibem uma grande diversidade de padrões de coloração que, por si só, são fortes atrativos para despertar a curiosidade sobre o gênero. Esses padrões, geralmente presentes em sistemas complexos de anéis miméticos distribuídos ao longo de grande parte dos Neotrópicos, fizeram com que as borboletas do gênero *Heliconius* se tornassem importantes modelos de estudo. Hoje, o conhecimento sobre essas borboletas se estende pelas nas mais diversas áreas da biologia como a ecologia, a sistemática, a biologia evolutiva e até a genética do desenvolvimento.

Os estudos acerca da herança e evolução dos padrões de coloração em borboletas do gênero *Heliconius* começam provavelmente em 1955 com um trabalho de William Beebe. Nesse trabalho, Beebe (1955) demonstra que alguns padrões de coloração que ocorrem naturalmente em áreas estreitas próximas à Trinidad, podem ser obtidos pelo retrocruzamento de padrões monomórficos que se distribuem mais amplamente na região. Essas áreas estreitas passaram, posteriormente, a serem chamadas de zonas híbridas. Em 1962, foi publicado o primeiro trabalho descrevendo a herança mendeliana presente nos caracteres que hoje se conhecem como *"dennis", "ray"* e a banda da asa anterior (*forewing 'FW' band*) em uma borboleta do gênero *Heliconius* (no caso, *H. melpomene*) (Turner & Crane 1962), envolvidos nos importantes processos do mimetismo presente nessas borboletas. Após esta publicação, diversos trabalhos se dedicaram a mapear os genes responsáveis por esses padrões e analisá-los do ponto de vista molecular (e. g Jiggins et al., 2005; Joron et al., 2006; Kronforst et al., 2006; Reed et al., 2011), o que possibilitou que se entendesse a dinâmica populacional desses genes em populações de *Heliconius* de ampla distribuição nos Neotrópicos. Hoje, sabe-se que processos como hibridização e introgressão são extremamente importantes na história evolutiva do gênero (Beltrán et al. 2002; Mallet et al. 2007; Pardo-Diaz et al. 2012 Wallbank et al. 2016; Jay et al. 2018; Kozak et al. 2018; Edelman et al. 2019; Jay et al. 2019). No entanto, apesar de menos presente na literatura atual, o papel

dos refúgios florestais na formação de raças, subespécies e espécies de *Heliconius* não pode ser ignorado.

Isso coloca essas borboletas no cerne de intensas discussões acerca de quais são os processos mais recorrentes que levaram à especiação nos Neotrópicos. Em diversos estudos, estas borboletas sustentaram tanto hipóteses de diversificação por isolamento (alopatria) em refúgios florestais (Brown et al. 1974; Brown 1976; Brown and Benson 1977, Brown 1979), como de diversificação em isolamento por distância (parapatria) e, também, em simpatria (Jiggins et al. 2001; Jiggins 2008). Por esses motivos, essas borboletas figuram em trabalhos que buscam desvendar desde os finos processos moleculares envolvidos na diversificação fenotípica do gênero, até os processos filogeográficos mais robustos que ajudam a explicar os padrões da biodiversidade neotropical.

1.3 *Heliconius hermathena*: taxonomia, ecologia e padrões de coloração.

A primeira descrição de um indivíduo de *Heliconius hermathena* foi feita por Hewitson em 1854, mas foi apenas em 1977 que um estudo mais amplo e detalhado foi realizado (Brown & Benson 1977). Nesse estudo, os autores descreveram pela primeira vez um paralelo que pode ser traçado entre a hipótese dos refúgios e a diferenciação de *H. hermathena.* Apesar de não terem se diferenciado nas áreas presumivelmente associadas aos refúgios florestais, a diferenciação de *H. hermathena* pode estar relacionada aos mesmos processos que causaram os refúgios: ciclos climáticos que ora expandiram e ora fragmentaram os ambientes onde a espécie está adaptada.

Heliconius hermathena possui sete subespécies descritas, diferenciadas por seus padrões de coloração e por históricos distintos de condições ecológicas (Brown & Benson 1977). Seis delas, *H. h. duckei, H. h. sheppardi, H. h hermathena, H. h. sabinae H. h. curua* e *H. h. renatae,* são consideradas como “não-miméticas” (não participam de anéis miméticos conhecidos) e são pouco diferenciadas entre si. Suas asas anteriores são pretas e apresentam uma banda vermelha e uma listra amarela tanto na parte ventral quanto na dorsal, enquanto as asas posteriores são pretas com uma banda transversal amarela e duas séries de pontos marginais amarelos. Por outro lado, *H. h. vereatta* apresenta a asa anterior preta com banda vermelha e a perda das bandas amarelas nas asas, sendo muito similar e voando junto com *H.*

melpomene melpomene e *H. erato hydara*, formando assim um anel mimético com elas, o que a faz ser considerada uma subespécie mimética de *H. hermathena*.

A maioria das subpopulações conhecidas de *H. hermathena* ocorre próximas ao Rio Negro, Rio Amazonas e Rio Madeira, exclusivamente em áreas de campinas e campinaranas. Suas populações podem ser bastante densas em algumas localidades (Seixas et al. 2017), mas em algumas localidades a densidade é bastante baixa, como é usual para espécies de *Heliconius*. Essas borboletas se alojam de forma gregária, debaixo de folhas vivas, geralmente bem próximas ao solo, diferenciando-se de espécies filogeneticamente próximas a elas como *H. erato,* as quais se alojam em galhos mortos e mais distantes do solo. Por outro lado, exibem um comportamento típico das *Heliconius,* conhecido como *"home range behavior"*, o qual limita sua capacidade de dispersão. Os adultos da espécie se alimentam de flores de plantas das famílias Rubiaceae, Apocynaceae, Fabaceae e Humiriaceae; já suas lagartas se alimentam exclusivamente de folhas de plantas do gênero *Passiflora* (*Passiflora faroana* e *P. hexagonocarpa*). A relação de *H. hermathena* com sua planta hospedeira parece ser mais especializada que a de espécies de floresta, pela disponibilidade e tipo de flores presentes nas campinas e campinaranas (Brown & Benson 1977).ß

Brown e Benson (1977) descreveram com bastante precisão os mais diversos aspectos da biologia de *H. hermathena*, inclusive aqueles relativos à sua diversificação, mesmo sem a utilização de dados moleculares. Hoje, podemos testar, se, de fato, a variabilidade genética presente na espécie reflete um processo de diversificação por isolamento ou, se, ao menos, ela indica que esse isolamento pode ser responsável por restringir ou inibir o fluxo gênico entre as subespécies no presente.

1.4 O genoma mitocondrial e seu uso nesse estudo.

Marcadores moleculares têm sido amplamente aplicados para inferir o fluxo gênico e o isolamento reprodutivo em populações de insetos (Sperling & Hickey 1994; Porretta et al. 2007). A subunidade I do Citocromo Oxidase (COI), por exemplo, foi um marcador pioneiro em estudos de biologia evolutiva e genética de populações; mais recentemente, sua aplicação foi proposta no sentido de integrar o conhecimento taxonômico com a variabilidade genética entre espécies num sistema universalizado, o *"DNA Barcode"* (Hebert et al. 2003a Rubinoff & Holland 2005). Por exemplo, nosso grupo de pesquisas utilizou à extremidade 5´ do COI para

auxiliar na identificação de uma nova subespécie de *H. hermathena*, a subespécie *H. h. curua* (Freitas et al. 2018). Nesse trabalho, algumas observações acerca da variabilidade haplotípica de subpopulações de *H. hermathena* foram feitas, sem o intuito de identificar como essa variabilidade se relaciona com a história evolutiva da espécie e com os ecossistemas de areia branca.

No entanto, existem críticas contundentes ao uso do COI e do mtDNA como marcadores moleculares em estudos filogenéticos e de genética de populações. Por vezes, tais críticas são feitas quando o mtDNA apresenta divergências em relação a marcadores nucleares e hipóteses taxonômicas pré-existentes (Shaw 2002); outras vezes, focam nas limitações estruturais e evolutivas de tais marcadores (Ballard & Whitlock 2004; Galtier, et al. 2009). Tais críticas são contestadas por autores que afirmam que, até o momento, as mesmas não são suficientes para contestar de forma ampla os estudos realizados ao longo de décadas com esses marcadores (Rubinoff & Holland 2005). Além disso, os mesmos afirmam que os argumentos que focam nas limitações estruturais e evolutivas tanto do COI como do mtDNA não são tão precisos para eliminar esses marcadores de estudos filogenéticos e de genética de populações, ressaltando suas vantagens e limitações (Rubinoff & Holland 2005; Silva-Brandão et al. 2009; DeSalle & Goldstein 2019). Atualmente, devido sobretudo ao avanço constante de tecnologias de sequenciamento de nova geração (NGS) e de métodos computacionais eficientes, tem-se utilizado vastamente o mtDNA completo (mitogenoma) em estudos evolutivos em insetos (e.g Haran et al. 2013; Simon & Hadrys 2013; Timmermans et al. 2014; Bourguignon et al. 2017; Condamine et al. 2018), humanos (e.g Zhang et al. 2013; Olivieri et al. 2017), peixes (e. g Carr & Marshall 2008; Teacher et al. 2012) e aves (e. g Ramos et al. 2018), por exemplo.

Neste trabalho, utilizamos o mitogenoma de 71 indivíduos de *H. hermathena,* afim de obter uma matriz completa e informativa de dados genéticos sobre a espécie. Devido à características como baixa taxa de recombinação, alta taxa de mutação, estrutura simples, herança materna e número efetivo populacional mais baixo (Avise et al. 1983; Moritz et al. 1987; Piganeau et al. 2004), acreditamos que essa matriz nos possibilitou acessar com precisão a variabilidade genética presente entre subespécies e subpopulações de *H. hermathena*, assim como fazer importantes inferências sobre a evolução da espécie.

Single chapter: Population genetics of the white sand butterfly *Heliconius hermathena* (Lepidoptera:Nymphalidae) assessed by mitogenome analysis

1. Introduction

White sand ecosystems (WSEs), usually known as *campinas* and *campinaranas,* are unique Amazonian environments characterized by their scleromorphic vegetation, white quartz sand soil, hydrology, fire regime and strongly patched distribution across the Amazon (Adeney et al. 2016). Varying from open grasslands to dense closed forests over white sandy soils, they reach Amazonian regions in Brazil, Peru, Venezuela, Colombia, Suriname, Guiana and French Guiana, totalizing around 334,879 km^2 (Eiten 1978; Adeney et al. 2016). Such broad distribution and specific features make white sand ecosystems a pivotal aspect of Amazonian landscape, notwithstanding, underrepresented in both geological and biological literature, especially those regarding the genetics of their inhabiting populations. Investigating such aspects of these unique environments, however, is crucial to understanding the processes by which Amazonian diversity has been generated.

Numerous hypotheses have been proposed to explain such processes, mostly focusing on allopatric speciation (e.g paleogeography hypothesis, river hypothesis, river-refuge hypothesis, refuge hypothesis, canopy-density hypothesis, museum hypothesis, disturbance-vicariance hypothesis and gradient hypothesis - revised by J. Haffer 2008). One of the most prominent, yet, criticized of these hypotheses is the refuge hypothesis. Originally proposed by Haffer (1969), it states that climatic fluctuations in the Pleistocene led to the retraction of the forest matrix, to the point where isolated areas of closed rainforest surrounded by open vegetation, such as currently present in savannas and WSEs, acted as centers of diversification for both plant and animal species, the so called “forest refugia” (Brown & Benson 1977; Haffer 1969). In effect, evidences of the formation of corridors and the expansion of open habitats such as savannas in the Amazon, suggest the retraction of the rainforest and the generation of the refugia areas (Quijada-Mascareñas et al. 2007; Vargas-Ramírez et al. 2010). Furthermore, many studies have demonstrated the possible role of forest refugia in generating patterns of differentiation in both the flora and fauna in the Amazon and in the Neotropics (Brown 1976; Brown et al. 1974; Fouquet et al. 2012; Haffer 1969; Pinheiro et al.

2013; Prance, 1973; Vuilleumier 1971). Nevertheless, there is strong debate on explaining Amazonian and, more broadly, Neotropical diversification solely based on the allopatry derived from the isolated forest refugia (Knapp & Mallet 2003). For instance, populations can become reproductively isolated by distance – parapatry – or even become adapted to specific niches in continuous ecosystems, such as the Amazon, because it is difficult to reach fully isolated habitats in such broad environments, which leads to sympatric diversification (Jiggins et al. 2001; Jiggins 2008). Hence, the importance of the refugia resides on explaining only part of the evolutionary history of the Neotropics, but not all of its complexity (Rull 2011).

Since *campinas* and *campinaras* currently occur in clear isolation, in the form of islands surrounded by forest matrix (Adeney et al. 2016), the present diversity in these areas may have arisen by allopatry and is maintained by the absence of gene flow. It is expected that when isolated in patches of white sand areas, populations inhabiting them might differentiate, making a strong case for allopatric diversification, such as proposed by the refuge hypothesis. Furthermore, the harsh conditions on these environments, such as high-light, high temperature and low humidity, might have acted as selective pressures that contributed to the formation of very specialized communities with high endemism in WSEs (Brown & Benson 1977; Anderson 1981; Adeney et al. 2016).

Even though the forest expansions that led to the isolation of WSEs are quite the opposite of the retractions that created presumed areas of forest refugia in the Pleistocene, there is of course a strong parallel between the existence of isolated WSEs and the isolated forest refugia. Nevertheless, only a small fraction of the literature investigated specifically the white sand areas, using ecological, morphological and distributional features of its species (e.g Brown & Benson 1977; Anderson 1981; Borges 2004; Poletto & Aleixo 2005; Ferreira 2009; Guilherme & Borges 2011). Even a smaller fraction explored the actual genetic diversity among populations inhabiting WSEs (Capurucho et al. 2013; Matos et al. 2016; Ferreira et al. 2018). Exploring this aspect is crucial to understand if WSEs provide an environment in which the genetic pools of its endemic populations become fully isolated, allowing the speciation processes to occur. It is not possible to fully understand Amazonian biodiversity without investigating the genetic diversity and the population differentiation within these unique environments, and the role of WSEs in the evolutionary processes that generated that outstanding diversity.

The white sand specialist butterfly *Heliconius hermathena* (Hewitson, 1854) (Lepidoptera: Nymphalidae) is a species that provides an excellent model to investigate the genetic diversity and population structure in WSEs. The seven described subspecies of *H. hermathena* are specialized to WSEs and occur in low-density populations across the isolated patches of open habitat vegetation (Brown & Benson 1977). It was proposed that the strong reduction in gene flow among subpopulations of *H. hermathena* led to the differentiated phenotypic patterns that can be currently appreciated (Brown & Benson 1977). While most of the subspecies (*H. h. curua, H. h. duckei, H. h. hermathena, H. h. renatae*, *H. h. sabinae,* and *H. h. sheppardi*) exhibit a non-mimetic color pattern with yellow streaks on the forewing, yellow stripes on the hindwing and two series of yellow spots on the hindwing, *H. h. vereatta* has no yellow markers at all, exhibiting only a postmedian red band on both ventral and dorsal sides of the forewing, as all the remaining subspecies (Brown & Benson 1977) (Fig 1). The unique pattern of *H. h. vereatta* within the species make it part of the mimetic ring composed by the sympatric *H. erato hydara* and *H. melpomene melpomene.* These color patterns are phenotypical features that allow the taxonomic identification of *H. hermathena* subspecies, but it is not yet known if these patterns correspond to current lineage differentiation among *H. hermathena* subpopulations. The thorough description of *H. hermathena* by Brown and Benson (1977) shows with much precision what can be seen in the field regarding distribution, systematics and ecology, although there was no further investigation on the genetic differentiation among its subpopulations. It is also unknown if the isolation of subpopulations provides an explanation for the genetic variability within the species, regardless of their phenotypically attributed taxonomy based on color patterns and geographical distribution.

Here we propose to investigate the genetic variability and structure within and among subpopulations of *H. hermathena* in its known areas of occurrence in Amazonian WSEs. Hence, we assembled a complete reference mitogenome for *H. hermathena* and used it to assemble individual mitogenomes for all sampled specimens. Mitogenomes have been vastly used in the last decade, especially in phylogenetics studies with insects (Haran et al. 2013; Simon & Hadrys 2013; Timmermans et al. 2014; Bourguignon et al. 2017; Condamine et al. 2018), fishes (Carr & Marshall 2008; Teacher et al. 2012) and birds (Ramos et al. 2018). In this work we apply the data from the mitogenome of *H. hermathena* in a population genetics framework to assess information related to the diversification of its lineages both in genetic and morphological characters, and to test if taxonomically relevant color patterns correspond

to current genetic differentiation. With the data gathered with this emblematic white sand specialist butterfly we expect to provide new evidence on the role of the Amazonian WSEs in the pattern of high diversity found in the whole Amazonian rainforest.

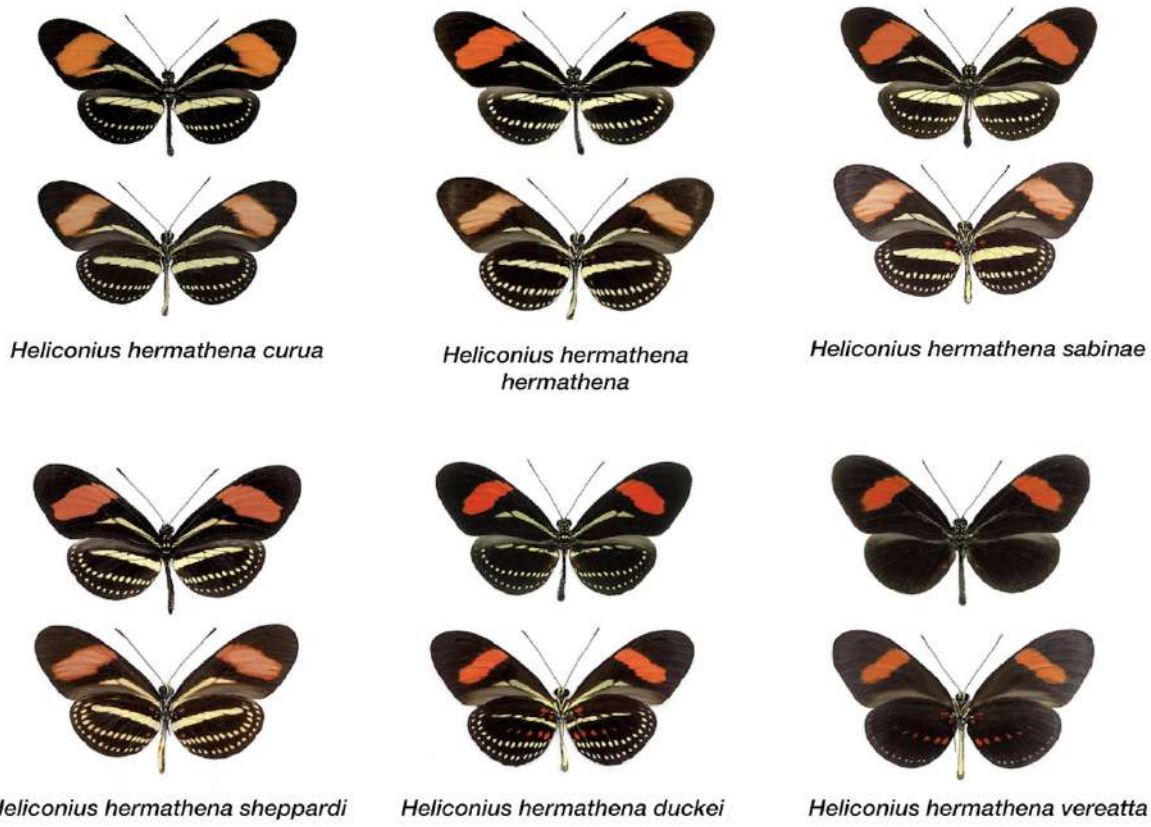

Figure 1. Subspecies of *Heliconius hermathena*. Upper picture of each individual represents dorsal view and lower picture represents ventral view.

2. Material and Methods

2.1. Data acquisition

We sampled 71 individuals from six subspecies of *Heliconius hermathena* in eight localities of white sand ecosystems (herein called subpopulations): Barcelos, Manaus, Maués and Presidente Figueiredo in Amazonas state, Curuá, Faro_Aibi, Faro_Tigre and Santarém in Pará state (Table 1, Figure 2). These areas include both patches of open *campina* vegetation and closed *campinaranas* surrounded by Amazonian rainforest.

Table 1 Sample localities (subpopulations) information. N = number of sampled individuals.

Subspecies	Localities	Locality Code	N	Coordinates
H. h. duckei	Faro, PA	Faro_Aibi	7	-1°58'48.60" S; 56° 42'32.46" W
H. h. vereatta	Faro, PA	Faro_Tigre	19	-2°11.23' S; -56°40.83' W
H. h. curua	Curuá, PA	Curuá	3	-8°43'52.46" S; -54°58'7.28" W
H. h. hermathena	Maués, AM	Maués	4	-3°22'36.55" S; -57°43'17.18" W
	Santarém,PA	Santarém	9	-2°27'38" S; -54°43'59" W
H. h. sabinae	Barcelos, AM	Barcelos	3	-0° 59'29.87" S; -62°55'31.28" W
H. h. sheppardi	Manaus, AM	Manaus	10	-2°35'29.23" S; -60°1'48.87" W
	Presidente Figueiredo, AM	P. Figueiredo	16	-1°59'7.5" S; -60°3'12.12" W

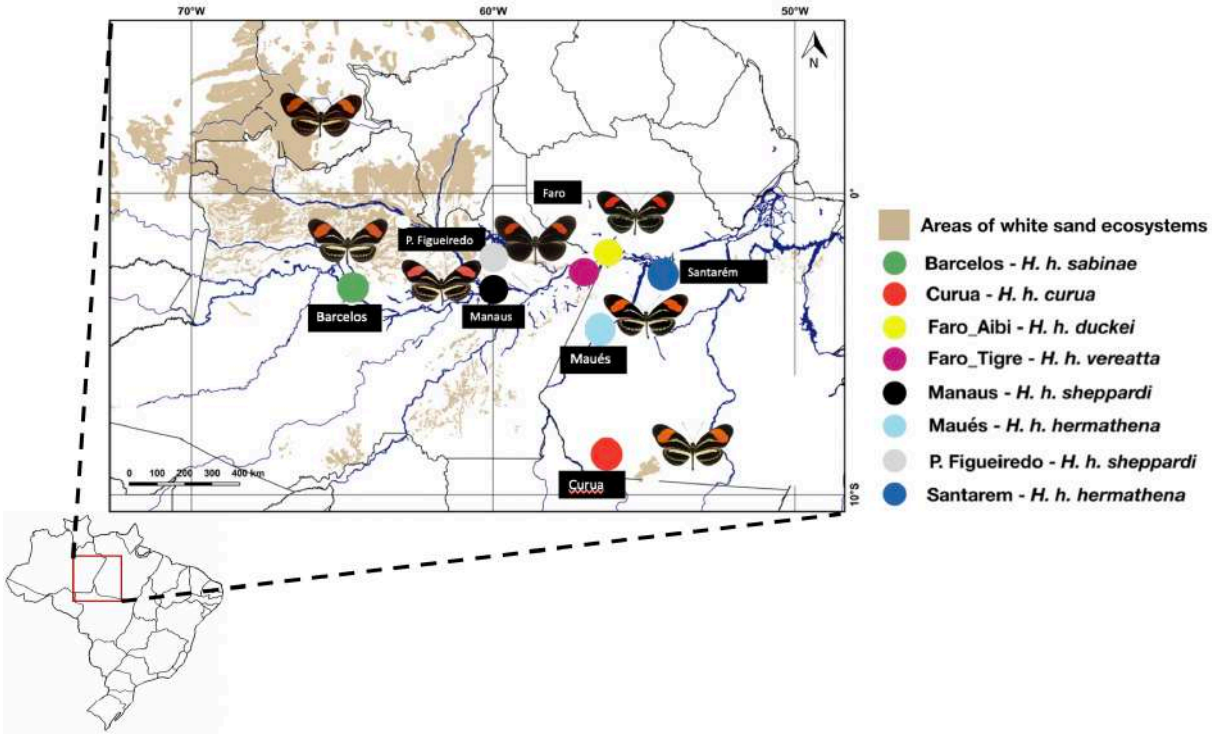

Figure 2 Map of the sampling localities of each subpopulation of *H. hermathena.*

Total genomic DNA was extracted from the thorax tissue of each specimen in a solution of 250 µl of a Tris HCL 1M, EDTA and 1% SDS solution, 1x PBS, 20 µl of proteinase-K, and 30 µl of RNAse A, incubated overnight at 56˚C. We then added 70 µl of KAC and incubated the material on ice for 30 minutes to posteriorly centrifuge it for 15 min at 13,000 rpm. The supernatant was transferred to new tubes for the addition of 500 µl of chloroform and centrifuged for 5 min at 13,000rpm. We repeated the same process one more time and then separated the supernatant in new tubes in which we added 900 µl of 100% ethanol and 30 µl

of 3 M NaAc. We mixed each tube by inversion and let them rest at -20˚C overnight. After this period, we centrifuged the tubes for 15 min at 13,000rpm and carefully discarded the supernatant. We centrifuged the precipitate with 1 ml of 80% ethanol for 5 min at 13,000rpm so we could later dry it and resuspend it with 55 μl of Tris HCl + 0,1mM EDTA.

Genomic DNA was fragmented with a Covaris sonicator (Covaris, Massachusets, USA) and individual genomic libraries were prepared following the procedure of the KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, USA). Twelve individual libraries were sequenced on an Illumina HiSeq 2500 lane in the Genomic Facility in the University of Chicago.

2.2. Mitogenome assembling, annotation and alignment

We visualized the quality control of paired-reads of the 71 individuals with FastQC (Andrews 2010) and MultiQC (Ewels et al. 2016), and removed adapters using Trim Galore. Since data was genomic, we filtered mitochondrial DNA (mtDNA) reads using the package MIRAbait of the MIRA assembler v. 4.0 (Chevreux et al. 1999) with the mitogenome of *Heliconius melpomene rosina* (NCBI Accession Number KP153600) as reference (*"bait"*), and k-mer = 15, since higher k-mer values resulted in poorer mitogenome assembly. We used default values for each program in the Galaxy platform (Cock et al. 2013), except for k-mer value in MIRAbait. We imported filtered reads for all paired-reads from the 71 individuals into Geneious v. 10 (Kearse et al. 2012) for the assembling of a *Heliconius hermathena* reference mitogenome, using the *Map to Reference* command, and *H. melpomene rosina's* mitogenome as reference.

We then separately assembled individual mitogenomes for each sampled specimen using the *Map to Reference* procedure, and the mitogenome assembled for *H. hermathena* as reference. We annotated the mitogenome sequences using the GeSeq tools (Tillich et al. 2017) on the Chlorobox website based on information for all available *Heliconius* mitogenomes on NCBI (selected from the Chlorobox website - https://chlorobox.mpimp-golm.mpg.de/). This produced the final sequences that were then aligned with the MAFFT (Katoh et al. 2002) plugin in Geneious v. 10 using the FFT-NS-i x1000 algorithm. We extracted protein coding genes (PCGs) from this alignment and concatenated them with the Geneious command "*Concatenate sequences or alignments",* which resulted in the final alignment composed by

all 71 individuals; only the PCGs were used in subsequent analyses. Final alignment was visually checked for possible errors with AliView (Larsson 2014).

2.3. Mitogenome architecture

We made a simple characterization of the *H. hermathena* reference mitogenome by visualizing the annotated mitogenome in Geneious v. 10 (Kearse et al. 2012) and identifying start and stop codons for PCGs, along with tRNAs and rRNAs. We downloaded annotated sequences of mitogenomes from other *Heliconius* species deposited in NCBI to structurally compare them. The downloaded sequences were: *H. clysonymus* (NC_027516.1), *H. cydno* (NC_024864.1), *H. ismenius* (NC_026463.1), *H. hecale* (NC_024744.1), *H. melpomene rosina* (KP100653.1), *H. pachinus* (NC_024741.1) and *H. sara* (NC_026564.1).

2.4. Sequence and haplotype diversity

We assessed nucleotide diversity within and between subpopulations using DnaSP v. 6 (Rozas et al. 2017). Individuals were assigned to their specific subpopulation, defined by its sampling locality (*Data > Define Sequence Set*), and the PCGs in the alignment were assigned as a domain by their lengths (*Data > Define Domain Set*). We used the command *DNA Polymorphism* to obtain summary statistics for both intrapopulational and interpopulational levels. In order to understand nucleotide diversity among mitochondrial genes for *H. hermathena* we used the *MultiDomain Analysis* (*Overview > MultiDomain Analysis*). We also performed Tajima's D (Tajima 1989) and Fu's F (Fu 1997) neutrality tests to infer variations in demographical patterns through time.

We proposed haplotype networks to infer the genetic relationships across subpopulations of *H. hermathena* based on mutational steps. We calculated a haplotype network for the whole mitogenome and for each individual PCG in order to understand how the information contained on each gene contributes to the haplotype segregation of *H. hermathena* subpopulations, using the median-joining network (epsilon = 0) algorithm on PopArt v 1.7 (Leigh & Bryant 2015).

2.5. Genetic differentiation and population structure

We used Arlequin v. 3.5 (Excoffier & Lischer 2010) in order to assess population differentiation by performing an analysis of molecular variance (AMOVA) and a pairwise F_{ST} analysis. AMOVA was performed for 1000 replicates and pairwise F_{ST} for 100 replicates calculating the distance matrix for pairwise differences. We also computed a pairwise distance matrix using Kimura-2-parameter (Kimura 1980) in MEGA X (Kumar et al. 2018). A Mantel test was performed with Arlequin in order to infer if there is any correlation between the differentiation estimated by pairwise F_{ST} and a pairwise linear geographic distance matrix across subpopulations.

2.6. Phylogenetic inference

Bayesian phylogenetic inference was performed to understand if individuals from a specific subpopulation are more closely related to individuals from the same subpopulation than with individuals from other subpopulations. We used two approaches to obtain phylogenetic trees: (1) a bayesian inference (BI) using MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003) in the CIPRES platform with a mixed prior of evolutionary models, unlinked state frequencies and shapes, and 100 million MCMC generations. Burn-in was set to 25% and the run was set for four chains; (2) a maximum likelihood tree (ML) obtained with IQ-TREE (Nguyen et al. 2015) setting the program to test for the best evolutionary model (-m TEST), and to run 1000 ultrafast bootstrap replicates (-bb 1000). Both trees were obtained with *Heliconius erato* as outgroup. We performed the partitioning of the data with PartitionFinder2 (Lanfear et al. 2016) in order to account for heterogeneity among the molecular evolution of the PCG's in our sequence matrix and to find the best fit model for different partition schemes, prior to running phylogenetic inferences.

2.7. Pearson correlation between morphological and genetic distance matrices

We used a Pearson correlation in order to analyze if morphological distances between subspecies and subpopulations are correlated with inferred genetic distances among them. In order to obtain the morphological distance matrix, we firstly obtained a taxonomical matrix

based on wing color pattern characters (Appendix Table 1). This taxonomical matrix was later converted into a pairwise morphological distance matrix using the package Claddis (Lloyd 2016) in R (R Core Team 2017) with RStudio (RStudio Team 2015). This matrix was obtained using the 'MORD' (Maximum Observable Rescaled Distance) (Lloyd 2016) argument, which computes every distance on a zero to one scale, comparable to that of the genetic distance matrix (obtained with MEGA X – section 2.5 of Materials and Methods). Both matrices were imported into Excel to compute the Pearson correlation.

3. Results

3.1. Mitogenome assembling and architecture

The assemblage of the reference mitogenome of *Heliconius hermathena* resulted in an overall confidence mean of Q39.1 (Phred Score) and at least 95% of the sequences have a Phred Score of Q30. The complete sequence is 15,352 bp in length (39.2% A, 40.9% T, 11.9% C, 7.9% G), composed of 13 protein coding genes (PCGs), 22 tRNA, two rRNAs and a A+T rich region, also called D-loop or control region (Fig. 3). Most of the PCGs (ND2, CO1, CO2, CO3, ATP8, ATP6, CO3, ND3, ND6 and CYTB) are present in forward position, coded on the majority strand, whilst the others (ND5, ND4, ND4L and ND1) are reversed and coded on the minority strand. The ND5 gene is the lengthiest and has 1736 base pairs while ATP8 is the shortest with 168 base pairs (Table 2). In accordance with other studies (e.g Lu et al. 2013), the start codon of the COI gene is CGA (Table 2). There is an overlap between the last seven bases of the ATP8 gene and the first seven bases of the ATP6, and this overlap was also documented in other works with Lepidoptera (Lu et al. 2013). There are no structural differences between *H. hermathena* mitogenome and mitogenomes from other *Heliconius* species.

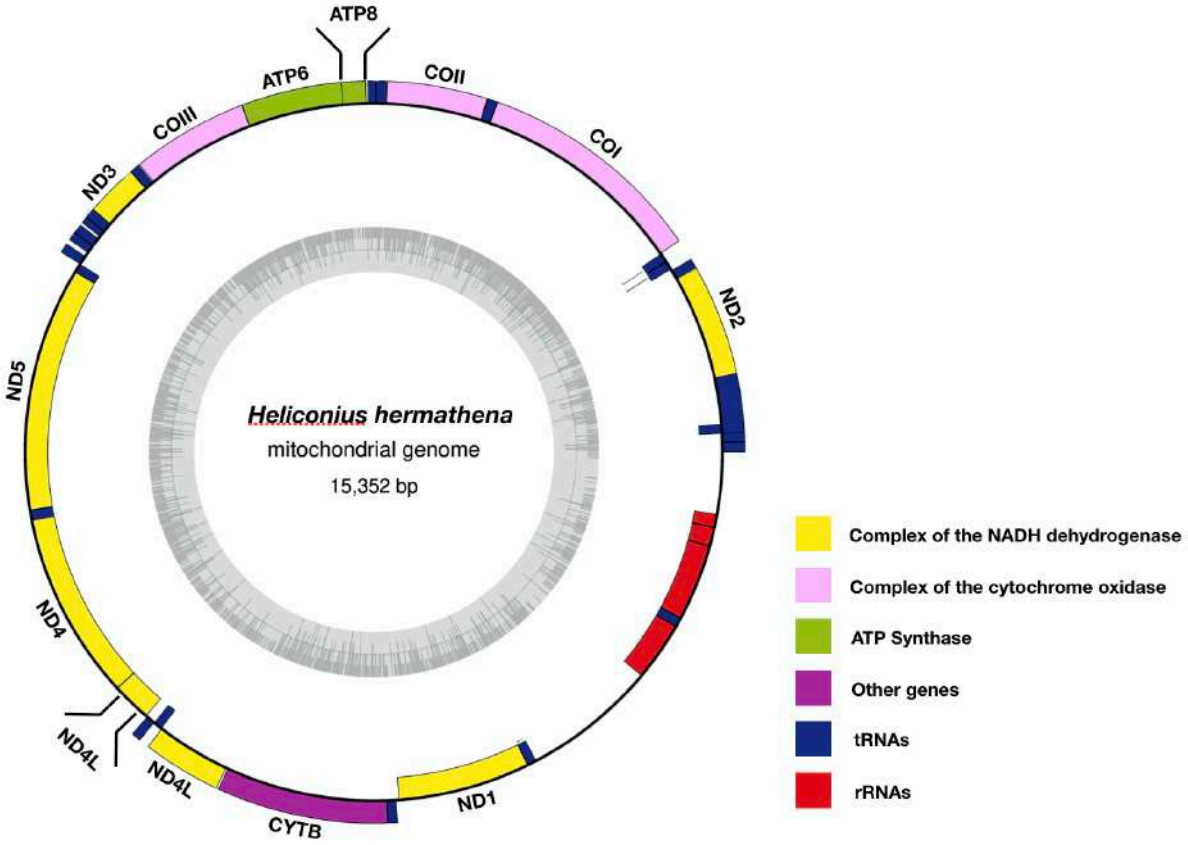

Figure 3 Graphic representation of the mitogenome of *Heliconius hermathena.*

Table 2 Summary of mitogenome architecture. * indicates reverse position of the gene

PCGs and tRNAs	Length (Position)	Start Codon	Stop Codon	Anticodon
ND2	1015 (255-1269)	ATT	TAA	-
COI	1531 (1480-3010)	CGA	ATT	-
COII	674 (3078-3751)	ATG	TTA	-
ATP8	165 (3891-4055)	ATT	TAA	-
ATP6	678 (4049-4276)	ATG	TAA	-
COIII	786 (4726-5511)	ATG	TAA	-
ND3	354 (5582-5935)	ATT	TAA	-
ND5 *	1736 (6350-8085)	ATT	TAA	-
ND4 *	1536 (8158-9043)	ATG	GAT	-
ND4-L *	282 (9493-9774)	ATG	TAA	-
ND6	531 (9915-10445)	ATT	TAA	-
CYTB	1149 (10458-11606)	ATG	TAA	-
ND1 *	939 (11690-12628)	TTG	TAA	-
trnM1	57 (1-57)	-	-	GTA
trnI	64 (69-132)	-	-	CTA
trnQ	69 (130-198)	-	-	AAC
trnM2	11 (514-524)	-	-	GTA
trnW	65 (1273-1337)	-	-	AGT
trnC	65 (1348-1412)	-	-	CGT

trnA1	67 (1348-1414)	-	-	ACG
trnY *	65 (1413-1477)	-	-	CAT
trnL1	67 (3011-3077)	-	-	ATT
trnK	71 (3754-3824)	-	-	GAA
trnD	52 (3826-3877)	-	-	CAG
trnG	65 (5527-5581)	-	-	AGG
trnA2	69 (5934-6002)	-	-	ACG
trnR	47 (6002-6048)	-	-	AGC
trnN	65 (6066-6130)	-	-	CAA
trnS1	60 (6129-6188)	-	-	CGA
trnE	67 (6220-6286)	-	-	AAG
trnF *	66 (6350-6285)	-	-	CTT
trnH *	69 (8086-8154)	-	-	CAC
trnT	65 (9783-9847)	-	-	ACA
trnP *	65 (9848-9912)	-	-	ACC
TrnS2	68 (11605-11672)	-	-	ACT
trnL2 *	67 (12699-12695)	-	-	ATC
trnV *	66 (14047-14139)	-	-	ATG

3.2. Sequence and haplotypic diversity across subpopulations

The dataset used for all of the analyses is a 11,173 bp matrix composed of all of the 71 sequenced individuals and 13 concatenated PCGs. Subpopulations presented low intrapopulational nucleotide diversity (π) and high interpopulational nucleotide diversity, indicating that genetic diversity is mostly retained among subpopulations, rather than within them (Table 3). Presidente Figueiredo presented the highest value of nucleotide diversity and Manaus has the lowest one, even though both localities have specimens identified as *H. h. sheppardi* and are only 66,89 km apart. Barcelos presented an even lower nucleotide diversity than Manaus, but since only three individuals were sampled in this locality, we assume that the whole diversity could not be appreciated in this dataset. Tajima's D and Fu's F neutrality tests were insignificant both for each subpopulation and overall. Nucleotide diversity present in each PCG showed that the cytochrome oxidase subunit 1 (COI) has the highest number of segregating sites and the highest value of nucleotide diversity (Table 4).

Table 3 Overall (interpopulational) and within (intrapopulational) genetic diversity among subpopulations of *H. hermathena*. N = number of individuals; S = segregating sites; H = number of haplotypes; Hd = haplotypic diversity; π = nucleotide diversity; Fs = Fu's F; D = Tajima's D; SD = standard deviation; numbers in **bold** p < 0.05

Subpopulation	N	S	H	Hd (SD)	π (SD)	Fs	D
Barcelos	3	0	1	0.000 (0.000)	0.00000 (0.00000)	--	--
Curua	3	42	3	1.000 (0.272)	0.00251 (0.00100)	--	--
Faro_Aibi	7	16	4	0.714 (0.181)	0.00043 (0.00019)	**1.621**	**-1.503**
Faro_Tigre	19	23	6	0.725 (0.083)	0.00086 (0.00006)	**5.755**	**1.815**
Manaus	10	2	3	0.511 (0.164)	0.00006 (0.00002)	**-0.272**	**-0.184**
Maués	4	2	2	0.500 (0.265)	0.00009 (0.00005)	**1.099**	**-0.701**
P. Figueiredo	16	131	9	0.892 (0.054)	0.00286 (0.00122)	**6.092**	**-0.85**
Santarém	9	6	5	0.722 (0.159)	0.00000 (0.00005)	**-1.113**	**-0.849**
Overall	71	293	33	0.958 (0.010)	0.00669 (0.00031)	**13.299**	**0.686**

Table 4 MultiDomain Analysis performed on DnaSP v 6.0 on each of the 13 PCG's considering all *H. hermathena's* individuals (N = 71). S = segregating sites; Hap = number of haplotypes; Hd = haplotype diversity; VarHd = variance of haplotype diversity; π = nucleotide diversity

PCG	Number of Sites	S	Hap	Hd	Var Hd	π
ATP8	165	1	2	0.369	0.00284	0.00224
ATP6	678	14	13	0.829	0.00074	0.00369
COI	1531	51	16	0.894	0.00021	0.00928
COII	674	17	8	0.778	0.00061	0.00486
COIII	786	22	17	0.907	0.00017	0.00555
CYTB	1149	32	13	0.879	0.00030	0.00778
ND1	939	35	15	0.910	0.00013	0.00878
ND2	1015	21	9	0.793	0.00073	0.00576
ND3	354	9	8	0.783	0.00065	0.00562
ND4	1536	42	12	0.866	0.00033	0.00840
ND4L	282	6	6	0.756	0.00058	0.00632
ND5	1736	33	13	0.870	0.00029	0.00546
ND6	531	10	13	0.874	0.00026	0.00444
Overall	11173	293	33	0.959	0.00010	0.00667

The haplotype network (Fig. 4) resulted in a total of 33 haplotypes, in agreement with the total number of haplotypes (Overall HD – Table 3) inferred by DnaSP (Overall HD – Table 3). Individuals from Pres. Figueiredo presented the highest number of haplotypes, nine, while all three individuals sampled in Barcelos presented the same haplotype; with the same sampling size, Curuá showed three different haplotypes. Each subpopulation is genetically isolated from the others, although this is not true for the two subpopulations from Faro (Faro_Aibi and Faro_Tigre): despite inhabited by individuals of two highly morphologically different subspecies, they do not form discrete haplogroups, even though some haplotypes are exclusive for each subpopulation. Individuals from Maués and Santarém, identified as *H.*

h. hermathena, are genetically close, although individuals from Maués are clearly closer to each other than with individuals from Santarém. Manaus and P. Figueiredo, both with individuals identified as *H. h. sheppardi*, present the highest distance between two groups (Fig. 4)

Overall, most of the PCGs recover a haplotype network which resembles the complete mitogenome's network: at least six discrete haplogroups genetically isolated by different amounts of mutational steps (Fig. 5). These haplogroups are generally formed by: (1) Barcelos, (2) Curuá, (3) both of the two subpopulations from Faro subpopulations, (4) Manaus, (5) Maués and Santarém, and (6) Presidente Figueiredo. The degree of isolation of the pair of subpopulations in Maués and Santarém varies according to the gene that is under analysis, as does the isolation of Barcelos and Presidente Figueiredo. The genes COI, COIII and ND1 genes recover almost exactly the same network pattern recovered with the complete mitogenome (except by the number of mutational steps separating subpopulations), and they present the highest number of diverse haplotypes (17 COIII, 16 COI and 15 ND1). The least genetic information is present at the ATP8 gene, which only recovers two haplotypes, one of them presented in individuals from Barcelos and Presidente Figueiredo and the other in individuals of all other subpopulations. The network recovered with the gene COII, shows one individual from Curuá sharing its haplotype with individuals from Maués and Santarém (Fig 5).

3.3. Genetic differentiation and population structure

The lowest pairwise genetic distance was found between the two subpopulations from Faro (Table 5). Presidente Figueiredo and Manaus do not present the highest pairwise genetic distance, although they are clearly distant from each other. Barcelos has the highest genetic distances in relation to all other subpopulations, which is probably due to the small sampling at this locality, resulting in the lowest nucleotide diversity and lowest haplotypic diversity (Table 3). The genetic distance between Barcelos and P. Figueiredo is clearly lower than the distances between Barcelos and the other subpopulations, indicating a genetic proximity between them, a result obtained across all analyses.

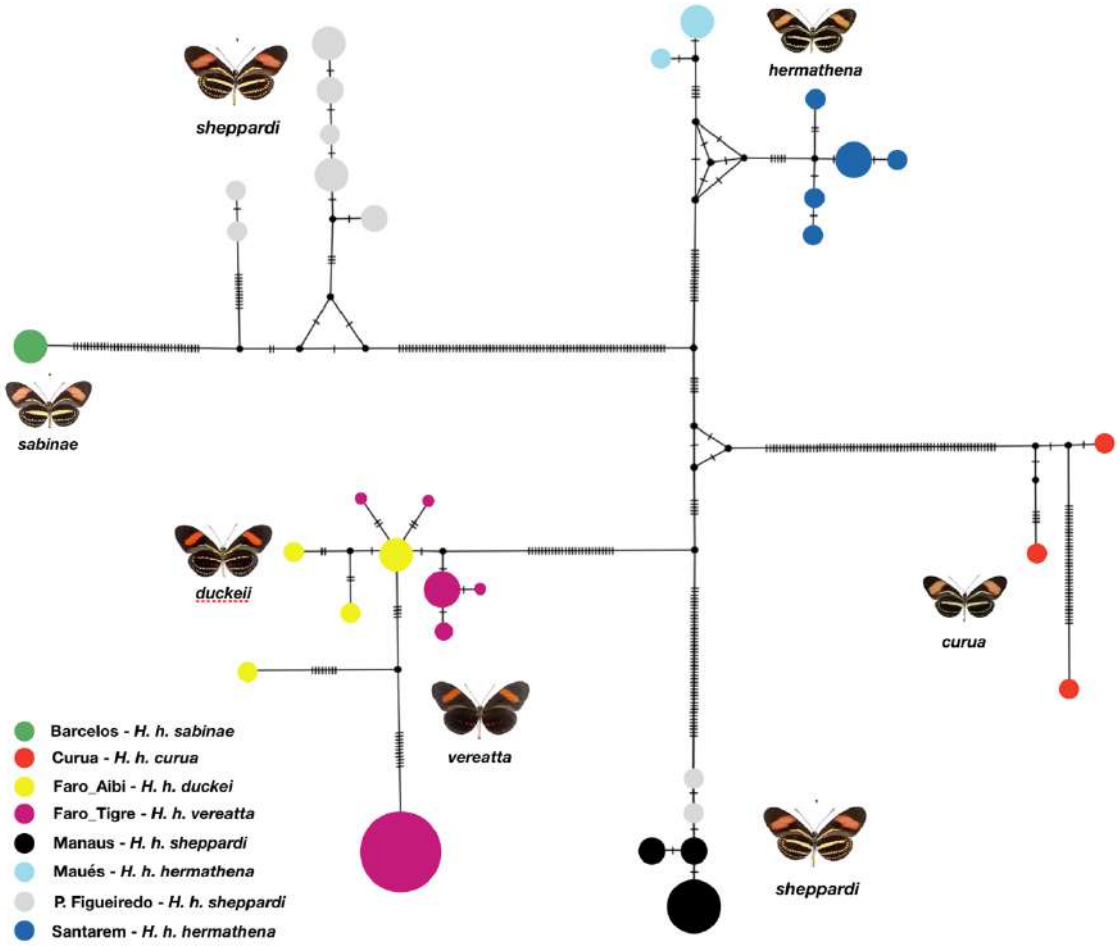

Figure 4 Haplotype network (median joining network) for *H. hermathena* subpopulations. Mutational steps are shown as small bars crossing the main lines. The size of circles indicates the number of individuals sharing the same haplotype, and smallest circles represent one individual. Colors of circles indicate sampling localities.

Table 5 Intrapopulational genetic distances within each of the subpopulations (first diagonal, **bold**), and pairwise mean genetic distances among subpopulations. Distances were obtained using Kimura-2-Paramaters as substitution model.

	Santarém	Manaus	Faro_Aibi	Faro_Tigre	P. Figueiredo	Curuá	Barcelos	Maués
Santarém	***0,00015***							
Manaus	0,00701	***0,00006***						
Faro_Aibi	0,00574	0,00673	***0,00042***					
Faro_Tigre	0,00586	0,00712	0,00091	***0,00086***				
P. Figueiredo	0,00924	0,00920	0,00964	0,00996	***0,002891***			
Curuá	0,00927	0,00984	0,00928	0,00951	0,010518	***0,002515***		
Barcelos	0,01117	0,01154	0,01130	0,01156	0,005108	0,010589	***0,000000***	
Maués	0,00119	0,00693	0,00553	0,00564	0,009346	0,009082	0,010956	***0,000090***

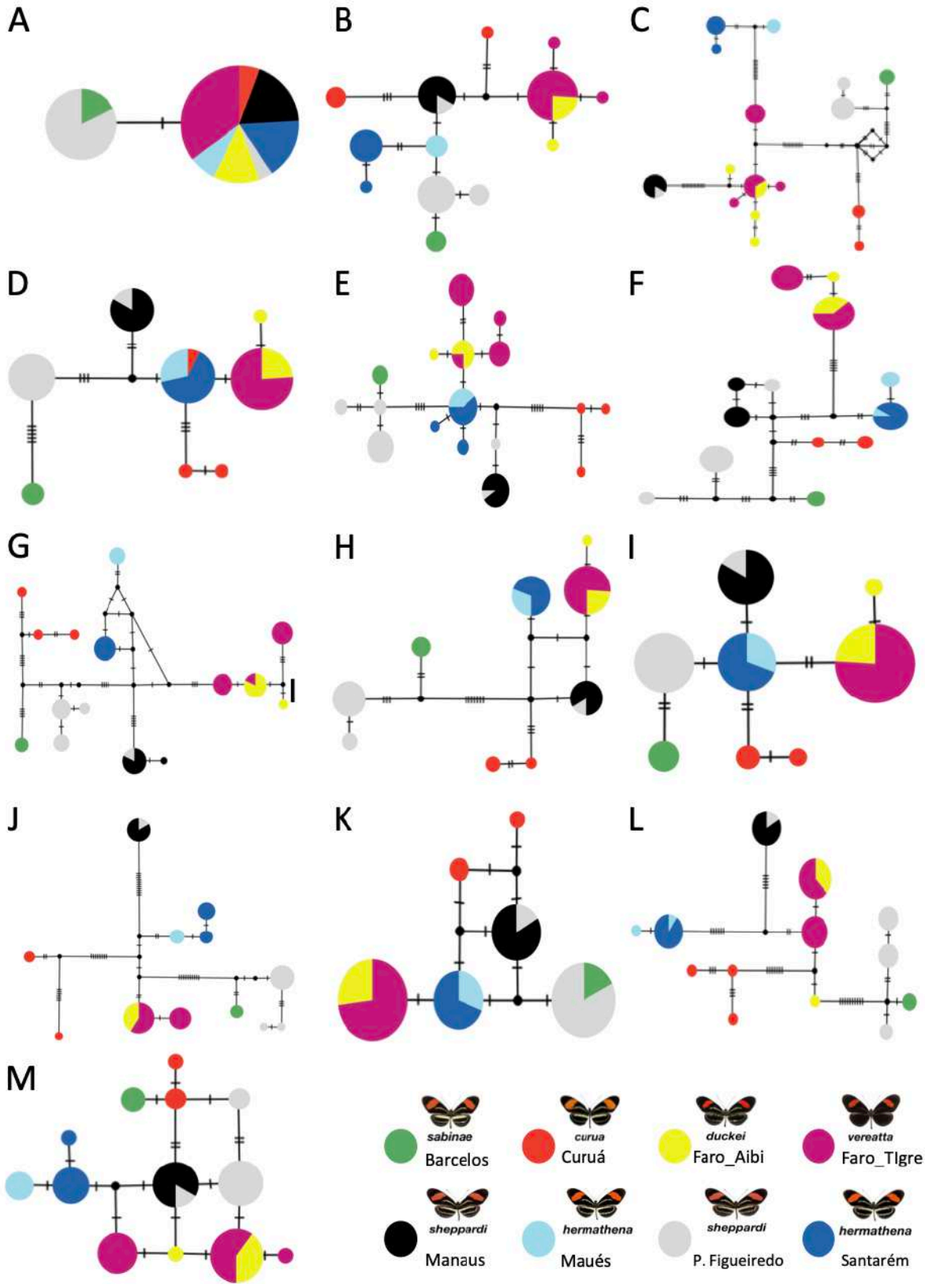

Figure 5 Haplotype networks for each of the PCGs in the *H. hermathena's* mitogeome. A) ATP8; B) ATP6; C) COI; D) COII; E) COIII; F) CYTB; G) ND1; H) ND2; I) ND3; J) ND4; K) ND4-L; L) ND5; M) ND6.

The AMOVA indicated strong genetic differentiation among subpopulations (Table 6). Most of the molecular variance (86.11%) is retained between among subpopulations whilst only 13.92% is present within them, which is an evidence of the importance of their isolation in retaining the present genetic differentiation. Pairwise F_{ST} is high for most of the pairwise comparisons. The lowest value is found between Faro_Aibi and Faro_Tigre (0.247). Presidente Figueiredo and Barcelos also have a lower F_{ST} (0.561) in comparison with all other values.

Table 6 Overall and pairwise F_{ST} among subpopulations of *H. hermathena* obtained in Arlequin v 3.5 with 100 permutations and computing the distance matrix for pairwise differences. * $p < 0.05$; ** $p < 0.001$; **number in bold** $p > 0.05$. AMOVA results obtained with 1000 permutations are shown below overall F_{ST}. Inside parenthesis are the degrees of freedom for the AMOVA analysis.

	Santarém	Manaus	Faro_Aibi	Faro_Tigre	P. Figueiredo	Curuá	Barcelos	Maués
Santarém	--							
Manaus	0.984**	--						
Faro_Aibi	0.951**	0.969**	--					
Faro_Tigre	0.891**	0.917**	0.247*	--				
P. Figueiredo	0.797**	0.807**	0.782**	0.818**	--			
Curuá	0.928*	0.945*	0.893*	0.885*	0.731*	--		
Barcelos	0.988*	0.995*	0.971*	0.933**	0.561*	**0.880**	--	
Maués	0.883*	0.990**	0.943*	0.870**	0.757*	0.880*	0.995*	--
Overall F_{ST}	0,861**							
Within subpopulation molecular variation	13.92% (7) **							
Among supopulation molecular variation	86.11% (63) **							

We also performed a Mantel test, for which a weak p-value for isolation-by-distance was found ($p = 0.056$). Regardless of subpopulations clear isolated distribution, this result does not comprehensively support a hypothesis of isolation-by-distance.

3.4. Phylogenetic relationships among subpopulations

We found no differences in our phylogenetic topology when using partitioned or non-partitioned data. Both Bayesian phylogenetic inference and Maximum Likelihood tree both resulted in the same topology with high support values, although only the Bayesian inference is shown (Fig. 6). There are five main monophyletic clades: (1) Barcelos and Presidente Figueiredo, (2) Curua, (3) Manaus, (4) Santarém and Maués, (5) Faro_Aibi and Faro_Tigre.

Within these clades, there is clear distinction between groups formed by individuals from each of the isolated subpopulations, which resemble the haplogroups presented in the complete mitochondrial haplotype network (Fig. 4). This result indicates that the genetic differentiation maintained by the isolation of *H. hermathena* subpopulations in WSEs formed distinct mitochondrial lineages. In accordance with the low pairwise F_{ST}, close genetic distances and the haplotype network, Faro subpopulations (Faro_Aibi and Faro_Tigre) show a clear paraphyletic pattern. These two subpopulations, therefore, cannot be genetically distinguished by any of our analyses.

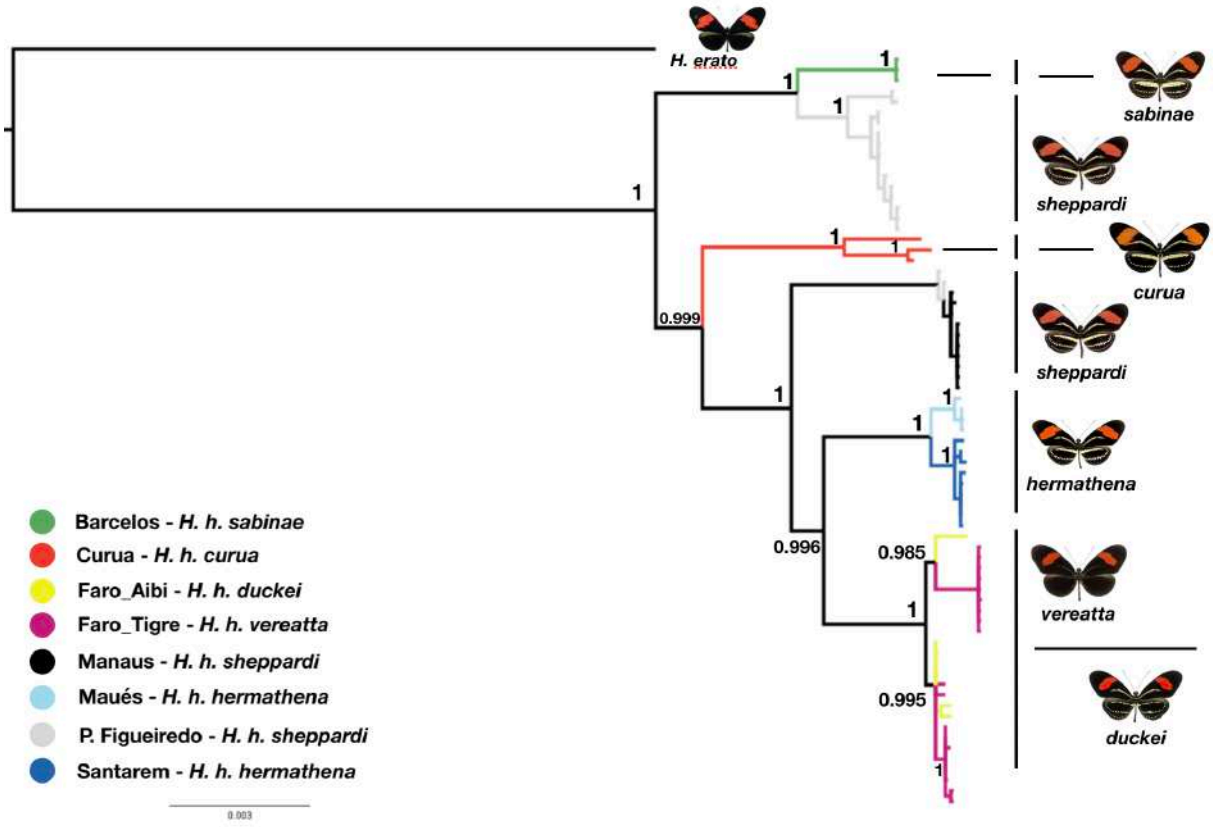

Figure 6 Bayesian inference phylogeny for subpopulations of *H. hermathena* based on complete mitogenome. Numbers on nodes indicate posterior probability.

3.5. Morphological and genetic correlation

The Pearson correlation resulted in a Pearson coefficient of 0.1538 and $R^2 = 0.0237$ indicating an insignificant, low correlation between morphological and genetic distances. This result shows that, for the analyzed matrices, color pattern characters cannot explain the observed genetic distances among subpopulations, corroborating the idea that, for *H.*

hermathena, at least the demographical history and genetic differentiation patterns are not maintained by color patterns. Nevertheless, we highlight four particular patterns of the relationship between genetic and morphological distances: (A) a pair of subpopulations showing high genetic distance and low morphological distance (Fig. 7A); (B) a pair of subpopulations showing low genetic distance and low morphological distance (Fig. 7B); (C) a pair of subpopulations showing low genetic distance and intermediate to high morphological distance (Fig. 7C); and (D) a pair of subpopulations showing high genetic distance and high morphological distance (Fig. 7D). Regardless of low genetic and morphological distances in case (B), involving subpopulations from Maués and Santarém, (identified as *H. h. hermathena* (Fig. 7B) subpopulations, it is clear by other analyses that both of these subpopulations are structured and represent two distinct haplogroups. These patterns highlight the complexity of *H. hermathena's* diversification and point to cases where it would be difficult to clearly establish species delimitation (according to Barley et al. 2013).

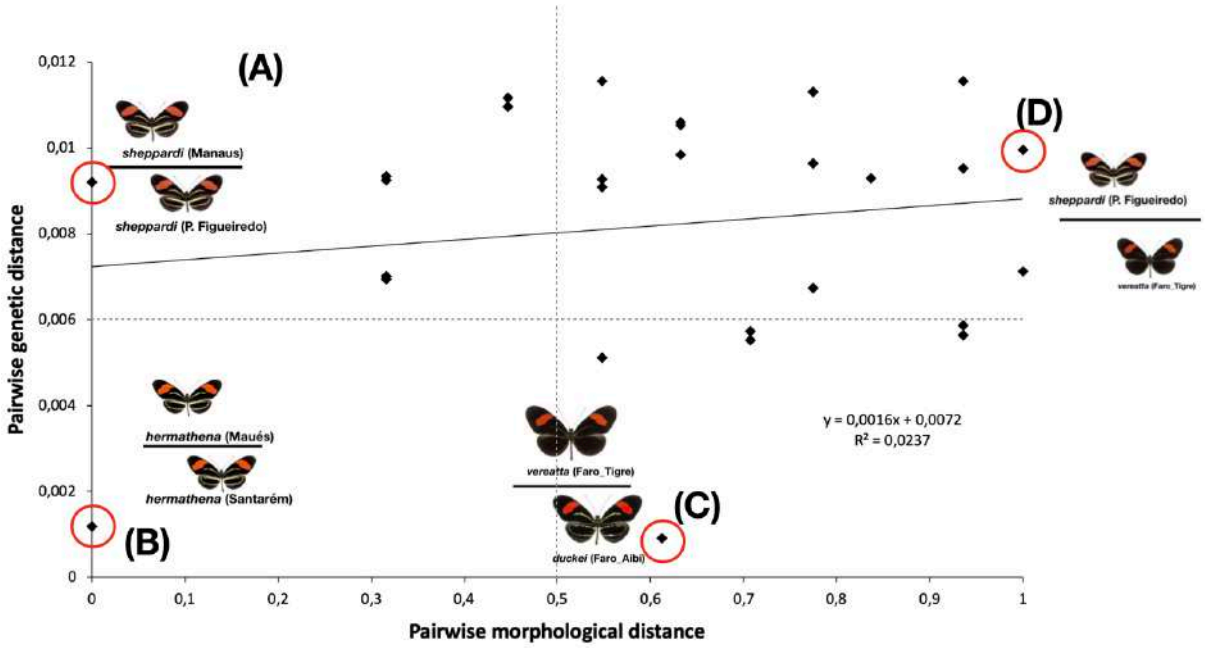

Figure 7 Pearson correlation between pairwise genetic distances and morphological genetic distances. Highlighted patterns of relationship between genetic and morphological distances: (A) high genetic distance and low morphological distance; (B) low genetic distance and low morphological distance; low genetic distance and intermediate to high morphological distance; (D) high genetic distance and high morphological distance. Each of these specific points are highlighted with red circles.

4. Discussion

The high genetic structure and differentiation of *H. hermathena* subpopulations is perhaps what first stands out in our results in Amazonian WSEs. Four main lines of evidence corroborate this pattern: (1) nucleotide diversity and molecular variance are retained among subpopulations rather than within them, as seen by the lower levels of intrapopulation nucleotide diversity in comparison with interpopulation nucleotide diversity (Table 3); (2) haplotype network clearly shows the genetic isolation of subpopulations (Fig. 4); (3) subpopulations are strongly structured, as indicated by overall F_{ST}, pairwise F_{ST} and AMOVA (Table 6); (4) phylogenetic inference shows that each subpopulation can be represented by an isolated mitochondrial lineage (Fig. 6).

Both Bayesian inference and haplotype network indicate the strong structure among *H. hermathena* subpopulations. There is clear distinction between clades that are strictly related to each of the subpopulations, and the same pattern is observed within haplogroups in the haplotype network. All phylogenetic lineages are strongly supported by high values of posterior probability and indicate that *H. hermathena* mitochondrial genome have been evolving in isolation. Individuals from the same subpopulation are more closely related among them regardless of their subspecies (see the strong differentiation between the subpopulations from Manaus and P. Figueiredo which are both identified as *H. h. sheppardi*), indicating that evolution of different lineages is mostly explained by their geographical locality and not by their named subspecies. This is also corroborated by the fact that there is no correlation between morphological and genetic distances. In this scenario, it is clear that allopatry is in fact preventing gene-flow, at least at present, even between individuals from the same subspecies.

Allopatry, for instance, has been the most held hypothesis to explain why speciation occurs (Mayr 1963; Coyne & Orr, 2004). It is quite straightforward to understand that when populations are kept geographically isolated for long enough, they will no longer present gene flow and, therefore, undergo speciation. This seems to be the case for *H. hermathena.* The uniqueness of WSEs and its strongly patched distribution may have driven *H. hermathena* to have genetically isolated subpopulations, as our results indicate. Allopatry may not be the sole reason why *H. hermathena* diversified, but rather indicate that, for this species, rainforest

surrounding WSEs act as a barrier for gene flow in present days, maintaining a pattern of high genetic differentiation among subpopulations.

Population genetics studies with other white sand specialist insects are absent, but patterns of low genetic structure were described for white sand specialist birds, such as *Xenopipus atronitens, Polytmus theresiae* and *Tachyphonus phoenicius* (Capurucho et al. 2013; Matos et al. 2016). Mainly because of behavioral and flying capability, and because of the ability of these birds in inhabiting neighboring *igapó* flooded forests, their dispersal is greater, allowing for a broader gene flow among populations. Conversely, *H. hermathena* butterflies seems to present a very specialized relationship with WSEs, and other amazonian open habitats, even neighboring ones, are not suitable for them (Brown & Benson 1977). Furthermore, *H. hermathenas*, as many other *Heliconius*, exhibit home-range behavior, which along with a very limited choice of host plant (only *P. hexagonocarpa* and *P. faroana*), greatly restricts their dispersal capability. Such behavior has been demonstrated to not necessarily prevent gene-flow (Mallet 1986), but its combination with the strongly patched WSEs might have isolated *H. hermathena* subpopulations in such a way that contact and gene-flow with other subpopulations is close to non-existent. Altogether, this features of *H. hermathena's* biology approximates its patterns of genetic variability and population structure to that of the birds of the *Galbula leucogastra/chalcothorax* species complex, for which the isolation of WSEs also act as a barrier for gene flow among populations (Ferreira et al. 2018).

4.1 Genetic variability and haplotypic patterns

Since our study was developed with all of the PCGs in the mitogenome of *H. hermathena*, we assessed the different haplotypic patterns generated by each gene (Fig. 5), which was insightful in exploring possible past relationships among subpopulations. Furthermore, this type of analysis allowed us to understand the contribution of each PCG to the information present in the whole mitogenome. The gene COI alone was able to retrieve a pattern very similar to the one recovered with the whole mitogenome, in which all of the same subpopulations can be distinguished, by different number of mutational steps. This highlights the importance of the contribution of the COI sequences in studies where variability between closely related species (in this case, subspecies), needs to be inferred. We confirm that the COI sequence is a reliable tool to characterize patterns of genetic differentiation within and

among populations, in accordance with the ever increasing use of this tool (DeSalle & Goldstein 2019). On the other hand, ATP8, the gene with the least segregating sites and nucleotide diversity, generated an interesting pattern whereby Barcelos and Presidente Figueiredo share a common haplotype for all individuals that is different from a second haplotype, shared by all other individuals from every other subpopulation. A star haplotype pattern was found for these two subpopulations based on COI barcode region sequences (Freitas et al., 2018). Furthermore, individuals from Barcelos and Presidente Figueiredo are genetically closer across our analyses and have the second lowest value of pairwise F_{ST}. Taken together, these results suggest that individuals from Barcelos and Presidente Figueiredo individuals share a recent evolutionary history and that Barcelos subpopulation might have originated from migrants from Presidente Figueiredo subpopulation.

Individuals from Maués, Santarém and Curúa subpopulations share a common haplotype for the COII gene, which does not happen for any other PCG (Fig. 5). It is not trivial to conclude that the subpopulation from Curuá originated from *H. h. hermathena* (Maués and Santarém) subpopulations, or vice-versa. Nevertheless, there are at least three important rivers surrounding these subpopulations – Tapajós, Xingu and Teles Pires – and as suggested, one possible reason for the expansion of white sand areas is their transportation by rivers, resulting from erosion and displacement of river channels (Latrubesse 2002). Although they do not share the same color pattern and are from different subspecies, a connection between Curuá, Maués and Santarém in the past seems reasonable in the light of the Latrubesse's hypothesis for the expansion of WSEs areas in the Amazon. This scenario could have led to gene-flow among them and the generation of the current mitogenomic variability patterns.

The whole mitogenome haplotype network shows how subpopulations are genetically isolated, because many mutational steps clearly separate those subpopulations in distinguished haplogroups. Nevertheless, it would not be possible to see patterns of shared haplotypes solely based on this analysis, and, therefore, to make assumptions on closest relationships among subpopulations that could lead to a better understanding of the evolutionary history of the species.

4.2. Phylogeographical structure and phenotypic variation

When analyzing the phylogeographic pattern of pairs of subpopulations and combining them with the phenotypic variation of each subspecies, it becomes clear that *H. hermathena* diversification is much more complex than expected. This pattern indicates that other mechanisms besides allopatry acted to generate the current pattern of diversification within this species.

Zamudio et al. (2016) describe several possible phylogeographic scenarios to explain different patterns of genetic and geographical divergence among populations, and we are able to find more than one of these scenarios explaining present patterns within *H. hermathena*. The overall pattern indicates that subspecies are phylogeographically clustered while phenotypic patterns are also geographically grouped and isolated in each subpopulation. This pattern suggests that neutral divergence, local adaptation or divergent sexual selection might be active mechanisms driving diversification (Warwick et al. 2015; Winger & Bates 2015; Waldrop et al. 2016; Zamudio et al. 2016). The two cases that, otherwise, point to the possible role of other mechanisms related to different patterns of phylogeographical and phenotypic structure are *H. h. vereatta* and *H. h. duckei,* both from Faro, and *H. h. sheppardi*, the subspecies that occurs both in Manaus and P. Figueiredo. For the first pair (*H. h. vereatta* and *H. h. duckei*), phylogeographic structure is absent and phenotypic structure is geographically clustered, a pattern that might have resulted from rapid diversification, phenotypic plasticity or gene-flow between them (Rice & Pfennig 2010; Faulks et al. 2015; Mason & Taylor 2015; Zamudio et al. 2016). We believe that a mechanism of rapid diversification best explains the current pattern found for this pair of subspecies since, other than *H. h. duckei*, *H. h. vereatta* is part of the mimetic ring of the sympatric *H. erato hydara* and *H. melpomene melpomene* (Brown and Benson 1977), and, therefore, introgression might have strongly acted in the formation of this mimetic form. This mimicry switch of *H. h. vereatta* is associated with the *cortex* gene (Van Kuren et al. submitted) which was proposed to generate adaptive differences associated with wing color pattern in *Heliconius* butterflies (Nadeau et al. 2016). Since male mating preference is associated with mating cues (i.e the *optix* gene – associated with the formation of the red band in *Heliconius's* forewings, also present in *H. h. vereatta*) in *Heliconius* butterflies (Merrill et al. 2019), assortative mating can be facilitated, acting as an ecological barrier for gene-flow and, therefore, driving speciation. In this scenario, in the

presence of predation pressure in the mimetic ring of *H. h. vereatta, H. m. melpomene* and *H. e. hydara,* selection against non-mimetic forms (e.g *H. h. duckei*) in the system might have acted to rapidly fix the mimetic form of *H. h. vereatta* in some areas in Faro.

For the pair of subpopulations from Manaus and Presidente Figueiredo, both identified as *H. h. sheppardi*, possible mechanisms to explain the pattern of high genetic differentiation, presence of phylogeographical structure but undetectable phenotypic variation, include stabilizing selection or cryptic diversification (Zamudio et al. (2016); Paupério et al. 2012; Singhal & Moritz 2013; Barley et al. 2015; Reilly & Wake 2015). Both our phylogenetic hypothesis and haplotype network indicate that two individuals sampled in P. Figueiredo have a mitogenome more similar to the one found in the Manaus lineage, although they are slightly different from the mitogenomes of Manaus (Fig. 6 and Fig 5). When these individuals are assigned to the Manaus subpopulation in a nucleotide diversity analysis, intrapopulational π of P. Figueiredo decreases (0.00254 to 0.00064, supplementary Table 1), slightly increasing Manaus's nucleotide diversity. Both individuals represent a haplogroup that is more similar to the Manaus haplogroup, but not exactly the same, which indicates that this is not an ongoing process of migration. On the other hand, due to mitochondrial features such as lower effective population size, high evolutionary rates and lack of recombination (Avise et al. 1983; Moritz et al. 1987; Piganeau et al. 2004), it would be expected that if contact between Manaus and P. Figueiredo happened in a more distant past, the differentiation of these two individual's haplogroup with the haplogroup from Manaus would be even greater. Evidence that contact across non-forest habitats such as savannas and WSEs occurred in a more recent past (Capurucho et al. 2013; Matos et al. 2016), might indicate that Manaus and P. Figueiredo could have been recently in contact. Therefore, there was not enough time for a greater differentiation of a previous Manaus haplogroup, that remained in the vicinity of P. Figueiredo until present days and is represented by two individuals in this study.

4.3. *H. hermathena* and WSEs: a myriad of investigation possibilities

Heliconius literature has become extremely competitive since the genus has been proven to be a model that can elucidate evolutionary processes in natural populations in a fine scale. Many works with the genus have demonstrated the roles of sympatry (Jiggins et al. 2001) and parapatry (Jiggins et al. 1996; Mallet et al. 1998) in speciation processes, which is

an important shift in the way biologists think about speciation. Gene flow and hybridization are common phenomena across the evolution of the genus (Beltrán et al. 2002; Mallet et al. 2007) and introgression has been shown to occur as an important genetic mechanism driving the evolution of these butterflies (Pardo-Diaz et al. 2012 Wallbank et al. 2016; Jay et al. 2018; Kozak et al. 2018; Edelman et al. 2019; Jay et al. 2019). There is even strong rejection of allopatric diversification in a specific 'suture zone' in Peru based in coalescent modeling (Dasmahapatra et al. 2010) thanks to the study of *Heliconius.* All of this was achieved through the deep genetic investigation of forest *Heliconius* species, where contact between species is expected when environments such as WSEs are isolated. These types of studies are currently lacking for species, such as *H. hermathena*, where allopatry seems to be an important factor in maintaining the genetic differentiation among populations. This scenario makes it scientifically relevant to assess the genetic mechanisms underlying *H. hermathena's* diversification in the context of allopatric occurrence.

The intimate relationship between *H. hermathena* and WSEs also provides an opportunity to explore the roles of ecological speciation. For instance, exploring the genetic basis of the dependence of *H. hermathena's* on its host plant could help to elucidate the roles of insect-plant interactions in the maintenance of isolated and structured populations of insects (Oliver 2006; Dover & Settele 2009). In fact, in *Heliconius* literature, the genetic basis of the relationship between the butterflies and their host plant in the *Passiflora* genus is still vastly unexplored (de Castro et al. 2018), and *H. hermathena* provides an opportunity for studies in this area. Furthermore, since edaphic composition seems to be a major factor in maintaining high endemism in WSEs (Anderson 1981; Adeney et al. 2016), we could explore if it could also be a part of the restriction of *H. hermathena* to those habitats. For instance, it would be necessary to explore loci under selection that can be putatively associated with adaptations to WSEs, such as resistance for high luminosity and low humidity, or even for the preference of host plant. In this context, those loci could lead to adaptive divergence through ecological isolation (Nosil et al. 2008; Nosil et al. 2009a). In general, the reasons for which *H. hermathena* is the only *Heliconius* species adapted to WSEs are still unexplored and, obviously, provide many research opportunities.

Advancing the study of WSE's and *H. hermathena* also means to establish a solid comparative framework with forest species of *Heliconius*, which is relevant since comparing species with different biogeographical backgrounds can lead to more sophisticated

evolutionary assumptions. This type of comparison has led to a better understanding of the evolution of birds in the Amazon. Works involving the population genetics of WSEs specialist birds have benefited from the amount of population genetics works with forest bird species (Ribas et al. 2012; Sousa-Neves et al. 2013; Fernandes et al. 2014; Thom & Aleixo 2015). For example, Capurucho et al. (2013) and Matos et al. (2016) have demonstrated that *X. atronitens, Polytmus theresiae* and *Tachyphonus phoenicius* have shallow genetic diversity and low population structure which is the opposite of what is seen for *terra firme* birds (Ribas et al. 2012; Sousa-Neves et al. 2013; Fernandes et al. 2014; Thom & Aleixo 2015). Understanding these differences has certainly allowed for a more robust proposition of the biogeographical origins of those populations. For them, it is possible to assess if such differences are related to the history of the formation of *terra firme* and WSEs environments. Our work along with another (Van Kuren et al. submitted), perhaps provide the first steps in creating a scenario favorable for these comparisons, which can certainly lead to a broader understanding of evolution in the Amazon.

It is often common when studying *Heliconius* to use updated, sophisticated methods, to end up confirming hypotheses that have so elegantly been proposed in the past by authors such as Keith Brown Jr. and Woodruff W. Benson. The methods applied in this work and the results obtained here are no different, since, ultimately, we show for the first time with genetic data, that, in fact, local subpopulations of *H. hermathena* have diversified in isolation due to the patchy distribution of WSEs, as proposed by Brown and Benson (1977). It is outstanding that one species, inhabiting one unique environment such as WSEs, can provide such a myriad of possibilities for ecological and evolutionary assumptions. This reinforces the importance of understanding not only general aspects within Amazonian evolution, but also the detailed processes within specific and unique environments of the Amazonian landscape. As stated by Keith Brown and Woodruff Benson in ther *Heliconius hermathena* 1977 article, non-forest habitats such as WSEs may provide “the most significant observations of the contemporary action of eco-evolutionary forces in the Neotropics” and it is therefore mandatory to more thoroughly investigating both *H. hermathena* and WSEs in general.

5. References

Adeney, J. M., Christensen, N. L., Vicentini, A., & Cohn-haft, M. (2016). White-sand Ecosystems in Amazonia. *Biotropica*, *48*(1), 7–23.

Adrian Quijada-Mascareñas, J., Ferguson, J. E., Pook, C. E., Salomão, M. D. G., Thorpe, R. S., & Wüster, W. (2007). Phylogeographic patterns of trans-Amazonian vicariants and Amazonian biogeography: The Neotropical rattlesnake (Crotalus durissus complex) as an example. *Journal of Biogeography*, *34*(8), 1296–1312. https://doi.org/10.1111/j.1365-2699.2007.01707.x

Andrews, S. (2010) FastQC: a quality control tool for high throughput sequence data. Retrieved from http://bioinformatics.babraham.ac.uk/projects/fastqc

Anderson, J. R. (1981). White sand vegetation of brazilian amazonia. *Biotropica*, 7, 326–343.

Avise, J. C., Shapiva, J. F., Lansman, R. A., & Daniel, W. (1983). Mitochondrial DNA differentiation during the speciation process in Peromyscus. *Molecular Biology and Evolution*, 1(1), 38–56. https://doi.org/10.1093/oxfordjournals.molbev.a040301

Ballard, J. W. O., & Whitlock, M. C. (2004). The incomplete natural history of mitochondria. *Molecular Ecology*, 13(4), 729–744.

Barley, A. J., White, J., Diesmos, A. C., & Brown, R. M. (2013). The challenge of species delimitation at the extremes: Diversification without morphological change in philippine sun skinks. *Evolution*, *67*(12), 3556–3572.

Barley, A. J., Monnahan, P. J., Thomson, R. C., Grismer, L. L., & Brown, R. M. (2015). Sun skink landscape genomics: Assessing the roles of micro-evolutionary processes in shaping genetic and phenotypic diversity across a heterogeneous and fragmented landscape. *Molecular Ecology*, *24*(8), 1696–1712. https://doi.org/10.1111/mec.13151

Beebe, W. (1955). Polymorphism in reared broods of Heliconius butterflies from Surinam and Trinidad. *Zoologica: Scientific Contributions of the New York Zoological Society*, 40, 139–143.

Beltrán, M., Jiggins, C. D., Bull, V., Linares, M., Mallet, J., McMillan, W. O., & Bermingham, E. (2002). Phylogenetic discordance at the species boundary: Comparative gene genealogies among rapidly radiating Heliconius butterflies. *Molecular Biology and Evolution*, *19*(12), 2176–2190. https://doi.org/10.1093/oxfordjournals.molbev.a004042

Benson, W. W. (1972). Natural Selection for Miillerian Mimicry in. *Science*, *176*(4037), 936–

939. https://doi.org/10.1126/science.176.4037.936

Borges, S. H. (2004). Species poor but distinct: Bird assemblages in white sand vegetation in Jaú National Park, Brazilian Amazon. *Ibis*, *146*(1), 114–124. https://doi.org/10.1111/j.1474-919X.2004.00230.x

Borges, S. H., Cornelius, C., Moreira, M., Ribas, C. C., Conh-Haft, M., Capurucho, J. M et al. and Almeida, R. (2016). Bird Communities in Amazonian White-Sand Vegetation Patches: Effects of Landscape Configuration and Biogeographic Context. *Biotropica*, *48*(1), 121–131. https://doi.org/10.1111/btp.12296

Bourguignon, T., Lo, N., Sobotnik, J., Ho, S. Y. W., Iqbal, N., Coissac, E., et al. and Evans, T. A. (2017). Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. *Molecular Biology and Evolution*, *34*(3), 589–597. https://doi.org/10.1093/molbev/msw253

Brown, K. S. (1972). The heliconians of Brazil (Lepidoptera: Nymphalidae). Part III. Ecology and biology of Heliconius nattereri, a key primitive species near extinction, and comments on the evolutionary development of Heliconius and Eueide. *Zoologica*, *57*(41), 59.

Brown, K. S., Sheppard, P. M., & Turner, J. R. G. (1974). Quaternary refugia in tropical America: evidence from race formation in Heliconius butterflies. *Proceedings of the Royal Society of London - Biological Sciences*, *187*(1088), 369–378. https://doi.org/10.1098/rspb.1974.0082

Brown, K. S. (1976). Geographical patterns of evolution in Neotropical Lepidoptera . Systematics and derivation of known and new Heliconiini (Nymphalidae: Nymphalinae). *Journal of Entomology Series B, Taxonomy*, *44*(3), 201–242.

Brown, K. S., & Benson, W. W. (1977). Heliconians of Brazil (Lepidoptera, Nymphalidae). Part VII. Evolution in Modern Amazonian Non-Forest Islands : Heliconius hermathena. *Biotropica*, *9*(2), 95–117.

Brown, K. S. (1979). Ecologia geográfica e evolução nas florestas neotropicais. Universidade Estadual de Campinas, Campinas.

Capurucho, J. M. G., Cornelius, C., Borges, S. H., Cohn-Haft, M., Aleixo, A., Metzger, J. P., & Ribas, C. C. (2013). Combining phylogeography and landscape genetics of Xenopipo atronitens (Aves: Pipridae), a white sand campina specialist, to understand Pleistocene landscape evolution in Amazonia. *Biological Journal of the Linnean Society*, *110*(1), 60–76. https://doi.org/10.1111/bij.12102

Carr, S. M., & Dawn Marshall, H. (2008). Phylogeographic analysis of complete mtDNA genomes from walleye pollock (Gadus chalcogrammus Pallas, 1811) shows an ancient origin of genetic biodiversity. *Mitochondrial DNA*, *19*(6), 490–496. https://doi.org/10.1080/19401730802570942

Chevreux, B., Wetter, T., & Suhai, S. (1999). Genome Sequence Assembly Using Trace Signals and Additional Sequence Information. *Computer Science and Biology: Proceedings of the German Conference on Bioinformatics (GCB)*, 99, 45-56.

Cock, P. J. A., Grunnig, B. A., Paszkiewicz, K., Pritchard, L. (2013). Galaxy tools and workflows sequence analysis with applications in molecular plant pathology. *Peer J*. Retrieved from https://doi.org/10.7717/peerj.167

Condamine, F. L., Nabholz, B., Clamens, A.-L., Dupuis, J. R., & Sperling, F. A. H. (2018). Mitochondrial phylogenomics, the origin of swallowtail butterflies, and the impact of the number of clocks in Bayesian molecular dating. *Systematic Entomology*, 43(3), 460-480. https://doi.org/10.1111/syen.12284

Coyne, J. A., & Orr, H. A. (2004). *Speciation* (1st ed.). Sunderland: Sinauer Associates.

Dasmahapatra, K. K., Lamas, G., Simpson, F., & Mallet, J. (2010). The anatomy of a "suture zone" in Amazonian butterflies: A coalescent-based test for vicariant geographic divergence and speciation. *Molecular Ecology*, *19*(19), 4283–4301. https://doi.org/10.1111/j.1365-294X.2010.04802.x

de Castro, É. C. P., Zagrobelny, M., Cardoso, M. Z., & Bak, S. (2018). The arms race between heliconiine butterflies and Passiflora plants – new insights on an ancient subject. *Biological Reviews*, *93*(1), 555–573. https://doi.org/10.1111/brv.12357

DeSalle, R., & Goldstein, P. (2019). Review and Interpretation of Trends in DNA Barcoding. *Frontiers in Ecology and Evolution*, *7*(September), 1–11. https://doi.org/10.3389/fevo.2019.00302

Dover, J., & Settele, J. (2009). The influences of landscape structure on butterfly distribution and movement: A review. *Journal of Insect Conservation*, *13*(1), 3–27. https://doi.org/10.1007/s10841-008-9135-8

Edelman, N. B., Frandsen, P. B., Miyagi, M., Clavijo, B., Davey, J., Dikow, R. B., et al. and Patterson, N. (2019). Genomic architecture and introgression shape a butterfly radiation. *Science, 599*(November), 594–599.

Eiten, G. (1978). Delimitation of the Cerrado concept. *Vegetatio*, *36*(3), 169–178.

Erlich, P. R., & Gilbert, L. E. (1973). Population Structure and Dynamics of the Tropical Butterfly Heliconius ethilla. *Biotropica*, *5*(2), 69–82.

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Faulks, L., Svanbäck, R., Eklöv, P., & Östman, Ö. (2015). Genetic and morphological divergence along the littoral-pelagic axis in two common and sympatric fishes: Perch, Perca fluviatilis (Percidae) and roach, Rutilus rutilus (Cyprinidae). *Biological Journal of the Linnean Society*, *114*(4), 929–940. https://doi.org/10.1111/bij.12452

Fernandes, A. M., Wink, M., Sardelli, C. H., & Aleixo, A. (2014). Multiple speciation across the Andes and throughout Amazonia: The case of the spot-backed antbird species complex (Hylophylax naevius/Hylophylax naevioides). *Journal of Biogeography*, *41*(6), 1094–1104. https://doi.org/10.1111/jbi.12277

Ferreira, C. A. C. (2009). Análise comparativa de vegetação lenhosa do ecossistema campina na Amazônia brasileira. 277.

Ferreira, M., Fernandes, A. M., Aleixo, A., Antonelli, A., Olsson, U., Bates, J. M., et al and Ribas, C. C. (2018). Evidence for mtDNA capture in the jacamar Galbula leucogastra/chalcothorax species-complex and insights on the evolution of white-sand ecosystems in the Amazon basin. *Molecular Phylogenetics and Evolution*, *129*(June), 149–157. https://doi.org/10.1016/j.ympev.2018.07.007

Fouquet, A., Noonan, B. P., Rodrigues, M. T., Pech, N., Gilles, A., & Gemmell, N. J. (2012). Multiple quaternary refugia in the eastern guiana shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology*, *61*(3), 461–489. https://doi.org/10.1093/sysbio/syr130

Edelman, N. B., Frandsen, P. B., Miyagi, M., Clavijo, B., Davey, J., Dikow, R. B., et al and Patterson, N. (2019). Genomic architecture and introgression shape a butterfly radiation. *Science*, *599*(November), 594–599.

Freitas, A. V. L., Ramos, R. R., Silva-Brandão, K. L., Coutouné, N., Magaldi, L. M., Pablos, J. L., et al, and Brown, K. S. (2018). A New Subspecies of Heliconius hermathena (Nymphalidae:

Heliconiinae) from Southern Amazonia. *Neotropical Entomology*. https://doi.org/10.1007/s13744-018-0658-8

Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*(2), 915–925.

Galtier, N., Nabholz, B., GlÉmin, S., & Hurst, G. D. D. (2009). Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecology*, 18(22), 4541–4550.

Guilherme, E., & Borges, S. H. (2011). Ornithological Records from a Campina / Campinarana Enclave. *The Wilson Journal of Ornithology. 123*(1), 24–32. https://doi.org/10.2307/23033480

Haffer, J. (2008). Hypotheses to explain the origin of species in Amazonia. *Brazilian Journal of Biology*, *68*(4 SUPPL.), 917–947. https://doi.org/10.1590/S1519-69842008000500003

Haffer, J. (1969). Speciation in Amazonian Forest Birds. *Science*. *165*(3889), 131–138.

Haran, J., Timmermans, M. J. T. N., & Vogler, A. P. (2013). Mitogenome sequences stabilize the phylogenetics of weevils (Curculionoidea) and establish the monophyly of larval ectophagy. *Molecular Phylogenetics and Evolution*, *67*(1), 156–166. https://doi.org/10.1016/j.ympev.2012.12.022

Jay, P., Chouteau, M., Whibley, A., Bastide, H., Llaurens, V., Parrinello, H., & Joron, M. (2019). Mutation accumulation in chromosomal inversions maintains wing pattern polymorphism in a butterfly. *BioRxiv*, 1–12. https://doi.org/10.1101/736504

Jay, P., Whibley, A., Frézal, L., Rodríguez de Cara, M. Á., Nowell, R. W., Mallet, J., … Joron, M. (2018). Supergene Evolution Triggered by the Introgression of a Chromosomal Inversion. *Current Biology*, *28*(11), 1839-1845.e3. https://doi.org/10.1016/j.cub.2018.04.072

Jiggins, C. D., McMillan, W. O., Neukirchen, W., & Mallet, J. (1996). What can hybrid zones tell us about speciation? The case of Heliconius erato and H. himera (Lepidoptera: Nymphalidae) . *Biological Journal of the Linnean Society*, *59*(3), 221–242. https://doi.org/10.1111/j.1095-8312.1996.tb01464.x

Jiggins, C. D., Naisbit, R. E., Coe, R. L., & Mallet, J. (2001). *Reproductive isolation caused by colour pattern mimicry*. *411*(MAY), 302–305.

Jiggins, C. D., Mavarez, J., Beltrán, M., McMillan, W. O., Johnston, J. S., & Bermingham, E. (2005). A genetic linkage map of the mimetic butterfly Heliconius melpomene. *Genetics*, *171*(2), 557–570. https://doi.org/10.1534/genetics.104.034686

Jiggins, C. D. (2008). Ecological Speciation in Mimetic Butterflies. *BioScience*, *58*(6), 541–548.

https://doi.org/10.1641/b580610

Joron, M., Papa, R., Beltrán, M., Chamberlain, N., Mavárez, J., Baxter, S., … Jiggins, C. D. (2006). A conserved supergene locus controls colour pattern diversity in Heliconius butterflies. *PLoS Biology*, *4*(10), 1831–1840. https://doi.org/10.1371/journal.pbio.0040303

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12136088%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC135756

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. and Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*, *16*(1330), 111–120. https://doi.org/10.1007/BF01731581

Knapp, S., & Mallet, J. (2003). Refuting Refugia? A Glimpse into tmRNA-Mediated. *Science*, *300*(April), 71–72.

Kozak, K. M., McMillan, W. O., Joron, M., & Jiggins, C. D. (2018). Genome-wide admixture is common across the Heliconius radiation. *BioRxiv*, 1–18. https://doi.org/10.1101/414201

Kronforst, M. R., Young, L. G., Kapan, D. D., McNeely, C., O'Neill, R. J., & Gilbert, L. E. (2006). Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(17), 6575–6580. https://doi.org/10.1073/pnas.0509685103

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). *PartitionFinder 2 : New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses*. *34*(3), 772–773. https://doi.org/10.1093/molbev/msw260

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Latrubesse, E. M. (2002). Evidence of quaternary palaeohydrological changes in middle Amazonia: The Aripuanã-Roosevelt and Jiparaná "fans." *Zeitschrift Fur Geomorphologie, Supplementband*, *129*(January), 61–72.

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lloyd, G. T. (2016). Estimating morphological diversity and tempo with discrete character-taxon matrices: Implementation, challenges, progress, and future directions. Biological Journal of the Linnean Society, 118(1), 131–151. https://doi.org/10.1111/bij.12746

Lu, H. F., Su, T. J., Luo, A. R., Zhu, C. D., & Wu, C. S. (2013). Characterization of the Complete Mitochondrion Genome of Diurnal Moth Amata emma (Butler) (Lepidoptera: Erebidae) and Its Phylogenetic Implications. *PLoS ONE*, *8*(9). https://doi.org/10.1371/journal.pone.0072410

Mallet, J. L. B., & Jackson, D. A. (1980). The ecology and social behaviour of the Neotropical butterfly Heliconius xanthocles Bates in Colombia. *Zoological Journal of the Linnean Society*, *70*(1), 1–13. https://doi.org/10.1111/j.1096-3642.1980.tb00845.x

Mallet, J. (1986). Dispersal and gene flow in a butterfly with home range behavior: Heliconius erato (Lepidoptera : Nymphalidae). *Oecologia*, *68*, 210–217.

Mallet, J., Mcmillan, W. O., & Jiggins, C. D. (1998). Mimicry and warning color at the boundary between races and species. In *Endless Forms: Species and Speciation* (pp. 309–403). New York: Press, Oxford University.

Mallet, J., Beltrán, M., Neukirchen, W., & Linares, M. (2007). Natural hybridization in heliconiine butterflies: The species boundary as a continuum. *BMC Evolutionary Biology*, *7*, 1–16. https://doi.org/10.1186/1471-2148-7-28

Mason, N. A., & Taylor, S. A. (2015). Differentially expressed genes match bill morphology and plumage despite largely undifferentiated genomes in a Holarctic songbird. *Molecular Ecology*, *24*(12), 3009–3025. https://doi.org/10.1111/mec.13140

Matos, M. V., Borges, S. H., d'Horta, F. M., Cornelius, C., Latrubesse, E., Cohn-Haft, M., & Ribas, C. C. (2016). Comparative Phylogeography of Two Bird Species, Tachyphonus phoenicius

(Thraupidae) and Polytmus theresiae (Trochilidae), Specialized in Amazonian White-sand Vegetation. *Biotropica*, *48*(1), 110–120. https://doi.org/10.1111/btp.12292

Mayr, E. (1963). *Animal species and evolution*. Cambridge, Massachussets: Harvard University Press.

Merrill, R. M., Rastas, P., Martin, S. H., Melo, M. C., Barker, S., Davey, J., … Jiggins, C. D. (2019). Genetic dissection of assortative mating behavior. *PLoS Biology*, *17*(2), 1–21. https://doi.org/10.1371/journal.pbio.2005902

Moritz, C., Dowling, T. E., & Brown, W. M. (1987). Evolution of Animal Mitochondrial DNA: Relevance for Population Biology and Systematics. *Annual Review of Ecology and Systematics*, *18*(1), 269–292. https://doi.org/10.1146/annurev.es.18.110187.001413

Nadeau, N. J., Pardo-Diaz, C., Whibley, A., Supple, M. A., Saenko, S. V., Wallbank, R. W. R., et al. and Jiggins, C. D. (2016). The gene cortex controls mimicry and crypsis in butterflies and moths. *Nature*, *534*(7605), 106–110. https://doi.org/10.1038/nature17961

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nosil, P., Egan, S. P., & Funk, D. J. (2008). Heterogeneous genomic differentiation between walking-stick ecotypes: “Isolation by adaptation” and multiple roles for divergent selection. *Evolution*, *62*(2), 316–336. https://doi.org/10.1111/j.1558-5646.2007.00299.x

Nosil, P., Funk, D. J., & Ortiz-Barrientos, D. (2009). Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, *18*(3), 375–402. https://doi.org/10.1111/j.1365-294X.2008.03946.x

Oliver, J. C. (2006). *Population genetic effects of human-mediated plant range expansions on native phytophagous insects. 112*(2), 456–463.

Pardo-Diaz, C., Salazar, C., Baxter, S. W., Merot, C., Figueiredo-Ready, W., Joron, M., et al. and Jiggins, C. D. (2012). Adaptive introgression across species boundaries in Heliconius butterflies. *PLoS Genetics*, *8*(6). https://doi.org/10.1371/journal.pgen.1002752

Paupério, J., Herman, J. S., Melo-Ferreira, J., Jaarola, M., Alves, P. C., & Searle, J. B. (2012). Cryptic speciation in the field vole: A multilocus approach confirms three highly divergent lineages in Eurasia. *Molecular Ecology*, *21*(24), 6015–6032. https://doi.org/10.1111/mec.12024

Piganeau, G., Gardner, M., & Eyre-Walker, A. (2004). A broad survey of recombination in

animal mitochondria. *Molecular Biology and Evolution*, *21*(12), 2319–2325. https://doi.org/10.1093/molbev/msh244

Pinheiro, F., Cozzolino, S., de Barros, F., Gouveia, T. M. Z. M., Suzuki, R. M., Fay, M. F., & Palma-Silva, C. (2013). Phylogeographic Structure And Outbreeding Depression Reveal Early Stages Of Reproductive Isolation In The Neotropical Orchid Epidendrum Denticulatum. *Evolution*, *67*(7), 2024–2039. https://doi.org/10.1111/evo.12085

Poletto, F., & Aleixo, A. (2005). Implicações biogeográficas de novos registros ornitológicos em um enclave de vegetação de campina no sudoeste da Amazônia brasileira. *Revista Brasileira de Zoologia*, *22*(4), 1196–1200. https://doi.org/10.1590/s0101-81752005000400055

Porretta, D., Canestrelli, D., Bellini, R., Celli, G., & Urbanelli, S. (2007). Improving insect pest management through population genetic data: A case study of the mosquito Ochlerotatus caspius (Pallas). *Journal of Applied Ecology*, 44(3), 682–691.

Prance, G. T. (1973). Phytogeographic support for the theory of Pleistocene forest refuges in the Amazon Basin , based on evidence from distributioh patterns in Caryocaraceae , Chrysobalanaceae , Dichapetalaceae and Lecythidaceae. *Acta Amazonica*, *3*(3), 5–28.

Ramos, B., González-Acuña, D., Loyola, D. E., Johnson, W. E., Parker, P. G., Massaro, M., et al. and Vianna, J. A. (2018). Landscape genomics: Natural selection drives the evolution of mitogenome in penguins. *BMC Genomics*, *19*(1), 1–17. https://doi.org/10.1186/s12864-017-4424-9

Reed, R. D., Papa, R., Martin, A., Hines, H. M., Kronforst, M. R., Chen, R., … Mcmillan, W. O. (2011). optix Drives the Repeated Convergent Evolution of Butterfly Wing Pattern Mimicry. *Science*, *333*(August), 1137–1142.

Reilly, S. B., & Wake, D. B. (2015). Cryptic diversity and biogeographical patterns within the black salamander (Aneides flavipunctatus) complex. *Journal of Biogeography*, *42*(2), 280–291. https://doi.org/10.1111/jbi.12413

Ribas, C. C., Aleixo, A., Nogueira, A. C. R., Miyaki, C. Y., & Cracraft, J. (2012). A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1729), 681–689. https://doi.org/10.1098/rspb.2011.1120

Rice, A. M., & Pfennig, D. W. (2010). Does character displacement initiate speciation? Evidence of reduced gene flow between populations experiencing divergent selection. *Journal of*

Evolutionary Biology, *23*(4), 854–865. https://doi.org/10.1111/j.1420-9101.2010.01955.x

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rubinoff, D., & Holland, B. S. (2005). Between Two Extremes: Mitochondrial DNA is neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference. *Systematic Biology*, 54(6), 952–961.

Rull, V. (2011). Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology and Evolution*, *26*(10), 508–513. https://doi.org/10.1016/j.tree.2011.05.011

Seixas, R. R., Santos, S. E., Okada, Y., & Freitas, A. V. L. (2017). Population Biology of the Sand Forest Specialist Butterfly Heliconius hermathena hermathena (Hewitson) (Nymphalidae: Heliconiinae) in Central Amazonia). *Journal of the Lepidopterists' Society*, 71(3), 133–140. https://doi.org/10.18473/lepi.71i3.a2

Shaw, K. (2002). Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences*, 99(25), 16122-16127

Silva-Brandão, K. L., Lyra, M. L., & Freitas, A. V. L. (2009). Barcoding lepidoptera: current situation and perspectives on the usefulness of a contentious technique. Neotropical Entomology, 38(4), 441–451. https://doi.org/10.1590/s1519-566x2009000400001

Simon, S., & Hadrys, H. (2013). A comparative analysis of complete mitochondrial genomes among Hexapoda. *Molecular Phylogenetics and Evolution*, *69*(2), 393–403. https://doi.org/10.1016/j.ympev.2013.03.033

Singhal, S., & Moritz, C. (2013). Reproductive isolation between phylogeographic lineages scales with divergence. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1772). https://doi.org/10.1098/rspb.2013.2246

Sousa-Neves, T., Aleixo, A., & Sequeira, F. (2013). Cryptic patterns of diversification of a widespread Amazonian Woodcreeper species complex (Aves: Dendrocolaptidae) inferred from multilocus phylogenetic analysis: Implications for historical biogeography and taxonomy. *Molecular Phylogenetics and Evolution*, *68*(3), 410–424. https://doi.org/10.1016/j.ympev.2013.04.018

Sperling, F. A. H., & Hickey, D. A. (1994). Mitochondrial DNA sequence variation in the spruce budworm species complex (Choristoneura: Lepidoptera). *Molecular Biology and Evolution*, 11, 656–665.

Tajima, F. (1989). Statistical Method fot Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics*, *123* (November), 585–595.

Teacher, A. G., André, C., Merilä, J., & Wheat, C. W. (2012). Whole mitochondrial genome scan for population structure and selection in the Atlantic herring. *BMC Evolutionary Biology*, *12*(1). https://doi.org/10.1186/1471-2148-12-248

Thom, G., & Aleixo, A. (2015). Cryptic speciation in the white-shouldered antshrike (Thamnophilus aethiops, Aves - Thamnophilidae): The tale of a transcontinental radiation across rivers in lowland Amazonia and the northeastern Atlantic Forest. *Molecular Phylogenetics and Evolution*, *82*(PA), 95–110. https://doi.org/10.1016/j.ympev.2014.09.023

Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E. S., Fischer, A., Bock, R., & Greiner, S. (2017). GeSeq - Versatile and accurate annotation of organelle genomes. *Nucleic Acids Research*, *45*(W1), W6–W11. https://doi.org/10.1093/nar/gkx391

Timmermans, M. J. T. N., Lees, D. C., & Simonsen, T. J. (2014). Towards a mitogenomic phylogeny of Lepidoptera. *Molecular Phylogenetics and Evolution*, *79*(1), 169–178. https://doi.org/10.1016/j.ympev.2014.05.031

Turner, J., & Crane, J. (1962). The genetics of some polymorphic forms of the butterflies Heliconius melpomene Linnaeus and H. erato Linnaeus. I. Major genes. *Zoologica : Scientific Contributions of the New York Zoological Society.*, *47*(13), 141–152.

Turner, J. R. G. (1971). Experiments on the Demography of Tropical Butterflies. II. Longevity and Home-Range Behaviour in Heliconius erato. *Biotropica*, *3*(1), 21. https://doi.org/10.2307/2989703

Vargas-Ramírez, M., Maran, J., & Fritz, U. (2010). Red- And yellow-footed tortoises, Chelonoidis carbonaria and C. denticulam (Reptilia: Testadines: Testudinidae), in South American savannahs and forests: Do their phylogeographies reflect distinct habitats? *Organisms Diversity and Evolution*, *10*(2), 161–172. https://doi.org/10.1007/s13127-010-0016-0

Vuilleumier, B. (1971). Pleistocene changes in the fauna and flora of South America. *Science*, *173*(4), 771–780.

Waldrop, E., Hobbs, J. P. A., Randall, J. E., Dibattista, J. D., Rocha, L. A., Kosaki, R. K., et al. and Bowen, B. W. (2016). Phylogeography, population structure and evolution of coral-eating butterflyfishes (Family Chaetodontidae, genus Chaetodon, subgenus Corallochaetodon). *Journal of Biogeography*, *43*(6), 1116–1129. https://doi.org/10.1111/jbi.12680

Wallbank, R. W. R., Baxter, S. W., Pardo-Diaz, C., Hanly, J. J., Martin, S. H., Mallet, J., et al. and Jiggins, C. D. (2016). Evolutionary Novelty in a Butterfly Wing Pattern through Enhancer Shuffling. *PLoS Biology*, *14*(1), 1–16. https://doi.org/10.1371/journal.pbio.1002353

Warwick, A. R., Travis, J., & Lemmon, E. M. (2015). Geographic variation in the Pine Barrens Treefrog (Hyla andersonii): Concordance of genetic, morphometric and acoustic signal data. *Molecular Ecology*, *24*(13), 3281–3298. https://doi.org/10.1111/mec.13242

Winger, B. M., & Bates, J. M. (2015). The tempo of trait divergence in geographic isolation: Avian speciation across the Marañon Valley of Peru. *Evolution*, *69*(3), 772–787. https://doi.org/10.1111/evo.12607

Zamudio, K. R., Bell, R. C., & Mason, N. A. (2016). Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(29), 8041–8048. https://doi.org/10.1073/pnas.1602237113

Appendix

Table 7 Matrix of characters used to obtain the pairwise morphological distance matrix based on wing color patterns of individuals from each of the subpopulations.

Characters	Barcelos	Curuá	Faro Aibi	Faro Tigre	Manaus	Maués	P. Figueiredo	Santarém
1- yellow cubital stripes on dorsal hindwings	3	2	0	4	1	1	1	1
2- two series of doubled intervenal submarginal yellow spots, the internal series with spots larger than the external series	1	1	1	0	1	1	1	1
3- first line of the doubled intervenal submarginal yellow spots on dorsal hindwings	0	0	0	0	1	0	1	0
4- ventral hindwing with red cubital spots	0	0	1	1	0	0	0	0
5- yellow streak over the forewing cubitus	1	2	1	0	1	1	1	1
6- tip of the yellow streak over the hindwing cubitus	0	1	0	-	0	0	0	0
7- postmedian red band on dorsal forewing	0	0	1	1	0	0	0	0
8- proximity of postmedian red band on dorsal forewing to the margin	0	0	1	1	0	0	0	0
9-yellow cubital stripes on dorsal hindwing mixed with numerous black scales,	1	0	0	-	0	0	0	0

resulting in a grayish tone								
10-Tone of the transverse red postmedian band	0	0	1	1	0	0	0	0

Table 8 Characters and character states used to generate the matrix of morphological characters based on *H. hermathena's* subspecies wing color patterns.

Characters	Character states				
	0	1	2	3	4
1- yellow cubital stripes on dorsal hindwings, when present	Very narrow	Narrow	Media	Broad	Absent
2- two series of doubled intervenal submarginal yellow spots, the internal series with spots larger than the external series	Absent	Present	-	-	-
3- first line of the doubled intervenal submarginal yellow spots on dorsal hindwings	Weak through the end	Strong through the end	-	-	-
4- ventral hindwing with red cubital spots	Absent	Present	-	-	-
5- yellow streak over the forewing cubitus	Absent	Present	Usually Mergin	-	-
6- tip of the yellow streak over the hindwing cubitus	Thin	Rounded	-	-	-
7- postmedian red band on dorsal forewing	Broad	Narrow	-	-	-
8- proximity of postmedian red band on dorsal forewing to the margin	Near	Distant	-	-	-
9-yellow cubital stripes on dorsal hindwing mixed with numerous black scales, resulting in a grayish tone	Present	Absent	-	-	-
10-Tone of the transverse red postmedian band	Orangish	Red	-	-	-

Table 9 Pairwise morphological distances between each of the subpopulations obtained using the Maximum Observable Rescaled Distance method.

	Santarém	Manaus	Faro Aibi	Faro Tigre	P. Figueiredo	Curuá	Maués	Barcelos
Santarém	0							
Manaus	0.31622	0						

Faro_Aibi	0.70710	0.77459	0					
Faro_Tigre	0.93541	1	0.61237	0				
P. Figueiredo	0.31622	0	0.77459	1	0			
Curuá	0.54772	0.63245	0.83666	0.93541	0.63245	0		
Barcelos	0.44721	0.54772	0.77459	0.93541	0.54772	0.63245	0	
Maués	0	0.31622	0.70710	0.93541	0.31622	0.54772	0.44721	0

Attachment I

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As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese, de Mestrado/Doutorado, intitulada **GENÉTICA DE POPULAÇÕES DA BORBOLETA DE AREIA BRANCA *HELICONIUS HERMATHENA* (LEPIDOPTERA:NYMPHALIDAE) AVALIADA POR ANÁLISE DO MITOGENOMA,** não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

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Attachment II

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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Dissertação de Mestrado, intitulada "***GENÉTICA DE POPULAÇÕES DA BORBOLETA DE AREIA BRANCA HELICONIUS HERMATHENA (LEPIDOPTERA:NYMPHALIDAE) AVALIADA POR ANÁLISE DO MITOGENOMA***", desenvolvida no Programa de Pós-Graduação em Genética e Biologia Molecular do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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