

**Cracking the Code: Examination of Species Delimitations among *Hamadryas* Butterflies with DNA Barcodes Suggests Caribbean Cracker is *Hamadryas februa* Hübner (Nymphalidae: Biblidinae)**

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CRACKING THE CODE: EXAMINATION OF SPECIES DELIMITATIONS AMONG  
HAMADRYAS BUTTERFLIES WITH DNA BARCODES SUGGESTS CARIBBEAN CRACKER IS  
HAMADRYAS FEBRUA HÜBNER (NYMPHALIDAE: BIBLIDINAE)

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**ABSTRACT.** *Hamadryas februa* Hübner and close relatives *H. amphichloe* Boisduval, *H. glauconome* Bates and *H. julitta* Fruhstorfer are a homogeneous group of taxa loosely defined as different species mainly by a combination of wing color characters. Here we explored the utility of the mtDNA COI barcode region in delimiting these taxa and examined its overall performance to identify species of *Hamadryas*. We compiled all the barcode sequences available for 16 of the 20 species in the genus, and added new sequences from 34 samples for 8 species from Peru and Ecuador. The complete data set includes 260 individuals. After filtering out identical sequences, a parsimony analysis of 142 individuals with unique haplotypes found that almost all the samples grouped according to their traditional species identification. The exceptions were samples of *H. amphichloe diasia* Fruhstorfer from the Dominican Republic and *H. amphichloe ferox* Staudinger from Colombia, which grouped with samples of *H. februa*. The barcodes of widespread species or polytypic species such as *H. laodamia* Cramer, *H. arinome* Lucas and *H. februa* grouped according to the geographic limits of their subspecies, but this was not the case for *H. amphichloe*, *H. feronia* Linnaeus, *H. amphinome* Linnaeus and *H. guatemalena* Bates. Our results suggest that of the three subspecies of *H. amphichloe* included here, only *H. amphichloe amphichloe* Boisduval, originally described from Ecuador, should be considered a distinct species, and that *H. amphichloe diasia* and *H. amphichloe ferox* do not belong to that lineage, but instead are part of *H. februa*. Furthermore we found extensive intraspecific sequence variation that overlapped with interspecific genetic distances of closely related species. We offer some general comments on species delimitation by tree-based and distance-based approaches. In conclusion, our dataset shows that the majority of the species *Hamadryas* included here can be distinguished by their barcode sequence (11 of 16 or about half of the species in the genus). However, the geographical coverage of our dataset is somewhat limited. Broader sampling of widespread species and the comprehensive inclusion of samples from closely related species will determine the utility of the barcode with more confidence.

**Additional key words:** Neotropics, Biblidinae, taxonomy, barcoding, barcode gap, subspecies

A common theme in many butterfly genera is the presence of species that show extensive geographical variation in wing color, yet are largely homogenous in genitalic characters, one of the most important sources of diagnostic characters in Lepidoptera (Emsley 1965, Kristensen 2003). The butterflies in the genus *Hamadryas* Hübner conform to this pattern, and in times when the dominant idea was that every minor wing color variation deserved a formal title, this translated into a rapid proliferation of names (Fruhstorfer 1916, Jenkins 1983, 1984).

*Hamadryas* butterflies are medium sized nymphalids and conspicuous members of the neotropical fauna (DeVries 1987). Most species of *Hamadryas* are fairly abundant, and easy to recognize by their distinctive mosaic color pattern (also referred to as calico) on the dorsal surface of the forewing. Though not present in all species, the genus is best known for the ability of males to produce cracking sounds (Otero 1988, Yack et al. 2000, Marini-Filho & Benson 2010), thus leading to the common name of “Crackers”. Species of *Hamadryas* are distributed across exceptionally diverse habitats ranging from semi-desert to rain forest within Central America, the Caribbean and the three major Neotropical biomes

(i.e., the Amazon, the Andes and the Brazilian Atlantic forest). *Hamadryas* show a remarkable degree of intraspecific variability in wing color: prior to Jenkins' taxonomic revision (Jenkins, 1983), widespread species such as *H. februa* Hübner and *H. feronia* Linnaeus were split into 11 and 8 taxonomic entities, respectively. Jenkins reduced the number of valid taxa in *Hamadryas* by two-thirds, and since his revision only 20 species and 33 subspecies (including nominate subspecies) continue to be recognized in the genus (Lamas 2004).

Although the morphological and molecular divergences among species were sufficiently informative to provide a phylogenetic hypothesis for relationships among species in the genus (Garzón-Orduña 2012, Garzón-Orduña et al. 2013), these studies did not deal with the identity or circumscriptions of the species per se and, as in most phylogenetic studies, species determinations of specimens were exclusively based on morphology. One outcome of these studies hinted that a deeper look was needed to identify the limits of some species, namely, the lack of resolution among more closely related species. The mtDNA barcode is only a single, short gene region whose history might not reflect the true species phylogeny of *Hamadryas*, but it has

been used to reveal cryptic species and revise traditional taxonomies in the past (Brower 1996, Burns et al. 2007, Prudic et al. 2008, Burns et al. 2009, Janzen et al. 2009). We wanted to explore its efficacy in determining species boundaries in a morphologically diverse group like *Hamadryas* that also features a particularly wide geographical distribution. Given that erroneous species delimitations can have profound effects on the formulation of ecological and biogeographical hypothesis (Bickford et al. 2007), the aim of this study was to explore the utility of the mtDNA COI barcode region to delimit closely-related species of *Hamadryas* under tree-based and phenetic approaches, and concomitantly to test whether intraspecific variation at the molecular level also corresponds to distinctive recognized subspecies.

#### MATERIALS AND METHODS

We included samples from all the species of *Hamadryas* for which the mtDNA COI barcode was available. The COI dataset publicly available from BOLD was downloaded directly from <http://www.boldsystems.org> on August 2013. We found several samples of *H. feronia* misidentified as *H. guatemalena* Bates in BOLD, these were corrected prior to the analyses. One of these was specimen DHJ02 (Genbank # MHAAB470-05) whose match to one of their *H. feronia* samples led Prado et al. (2011) to suspect the presence of different (potentially new) species. Morphological and molecular data have shown that *H. feronia* and *H. guatemalena* are sister taxa (Garzón-Orduña 2012, Garzón-Orduña et al. 2013) and it is not uncommon to find exemplars of *H. feronia* misidentified as *H. guatemalena*. However, to be clear, adult *H. feronia* can be readily distinguished morphologically from *H. guatemalena* by the composition of its border ocelli on the dorsal surface of the hind wing and from characters of the male genitalia (Jenkins 1983). Sequences showing a high proportion of missing data were removed from the dataset: MHACG585-05, MHACG568-05, MHACG601-05, MHAAB471-05, MHACG752-05, MHACG633-05, MHMYC1217-09. Genbank COI sequence GU333980 (BOLD MHACG576-05) presents a spurious extra NNN at position 60. We checked the trace file present in BOLD (file 86512.ab1) and the triple N is not present and was thus removed from our dataset. Unpublished sequences for six species were also obtained from the Nymphalidae Systematics Group database (<http://www.nymphalidae.net/Vouchers.htm>).

#### New sequences

In addition to published data, we also included in our analysis new sequences for eight species from three

countries. Most of the new sequences came from adult specimens of *Hamadryas* collected from the San Martin department, Peru in September 2011, under research permit 0148-2011-AG-DGFFS-DGEFFS. Specimens were caught in the field using butterfly nets and preserved in glassine envelopes. Voucher specimens have been deposited in the collection of the "Evolution et Diversité Biologique" lab under accession numbers specified in Table 1 and corresponding information stored in the institution database (<http://edbase.ups-tlse.fr>). Also new were four specimens of *H. amphichloe* from Ecuador, obtained from Keith R. Willmott, and one specimen of *H. februa* and one of *H. glauconome* from Texas (USA), obtained from Berry and Laura Nall.

#### DNA extraction, amplification and sequencing

The DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) was used for tissue lysis and DNA purification following the manufacturer's protocol. The mitochondrial protein-encoding gene cytochrome c oxidase subunit I (COI) was amplified using the primer pairs LCO1490 – HCO (Folmer et al. 1994). Polymerase chain reactions (PCRs; 25 µL) included 1 µL of template DNA, 0.5 µL of each primer (20 µM), 0.5 µL of 10 µM dNTP's (Promega, Madison, WI, USA), 5 µL of 1x PCR buffer containing 1.5 mM MgCl<sub>2</sub> (Promega, Madison, WI, USA), 0.6 units of GoTaq DNA polymerase (Promega, Madison, WI, USA) and 1 µL of 10mg/mL BSA. PCRs were carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems), and involved an initial denaturation step (5 min at 93 degrees) followed by 35 cycles including denaturation at 93 degrees for 30 s, annealing at 47 degrees for 30 s, and extension at 68 degrees for 1 min, with a final extension step at 68 degrees for 10 min. The double-stranded PCR products were verified by agarose gel electrophoresis (1% agarose). PCR products were purified and sequenced directly with the same primer pairs as used for amplification at the national sequencing center of the Genopole. Chromatograms were edited and overlapping sequence fragments were assembled using Geneious 6.0.6 Pro (Biomatters, Auckland, New Zealand). Blast searches for each amplicon were conducted to check for putative contaminations. All new sequences have been deposited in GenBank under the accession numbers specified in Table 1.

#### Morphological examination

Concomitant to sequencing the mtDNA barcode, we also conducted morphological examinations of wing color and male genitalia on several specimens, including on some of the vouchers used for the DNA barcoding. Accordingly, male adult abdomens were soaked in a hot

solution of 10% KOH for 15–30 min and cleared of dissolved soft tissue with forceps and a fine paintbrush. The genitalia dissections were preserved on a solution of ethanol and glycerin and photographed under a light microscope. The images of the adults and genitalia dissections featured in this study have been deposited in Morphobank (<https://morphobank.org>) under project #P2688.

### Data Analysis

The DNA sequences were aligned using translator (Abascal et al. 2010) to ensure conservation of the reading frame. We evaluated the saturation of the full dataset, 1st and 2nd codon positions together and finally 3rd codon positions. We estimated saturation in the R package APE 3.0-8 (Paradis et al. 2004) by calculating the slope of the scatterplot of uncorrected versus K80 corrected distances (Kimura 1980) of all taxon pairs. We checked the compositional homogeneity of the sequences using a Chi square test (Jeremiin et al. 2004; Peters et al. 2011). Because 3rd positions were not saturated, and in fact provided most of the character support, we opted to analyze the data without differential character weighting. We used PAUP\* (Swofford, 2003) to estimate uncorrected p-distances among samples of the same species and between species.

A parsimony analysis with equal weights was conducted in TNT 1.5 (Goloboff & Catalano 2016). The tree search included 600 random addition sequences, holding three trees per replication, tree bisection and reconnection for branch swapping and 50 iterations of Ratchet. Branch support was calculated using absolute Bremer Support (holding suboptimal trees with up to 60 extra steps) and Jackknife (1000 replications).

### RESULTS

The final dataset comprises 260 mtDNA COI barcode sequences of 658 base pairs in length. However, only 142 were retained after removing redundant samples with identical sequences. The full locality data of the all specimens of *Hamadryas* used in this study can be found in Table 1, and samples with identical barcodes per species can be found in Supplemental material 1.

Comparison of cumulative intraspecific and interspecific uncorrected pairwise distances for all taxa showed overlap between maximal intraspecific and minimal interspecific divergences (Fig. 1a) and thus absence of a global barcode gap in *Hamadryas*. A more specific examination of variation within and between pairs of sister-taxa showed the presence of a gap in three of five species pairs compared (Fig. 1b). Generally the absence of a barcode gap appears to be caused by large amounts of intraspecific sequence divergence in

widespread species such as *H. amphinome*, *H. arinome* and *H. feronia*, that is greater than the distance to their sister species. Furthermore, five of the ten species in Fig. 1b feature intraspecific distances above 2%, violating the 2% rule (Hebert et al. 2003).

The equal-weights parsimony analysis of the dataset resulted in 643 most parsimonious trees (MPT) 805 steps long. A strict consensus of the MPTs is shown in Fig. 2. The samples of 11 of the 12 species for which we included more than one barcode sequence grouped according to their putative species identity. We found the barcode to be distinctive enough to separate closely related species that are otherwise very similar morphologically, such as *H. glauconome* from *H. julitta*, or *H. feronia* from *H. guatemalena*. The only instance in which samples of the same ostensible species failed to group together occurred among samples of *H. amphichloe ferox* and *H. amphichloe diasia*, which were grouped with samples of *H. februa februa* and not with samples of *H. amphichloe amphichloe* from Ecuador.

As expected, most of the widespread species for which subspecies have been described exhibited pronounced sequence variation, as indicated by the number of unique haplotypes (Fig. 2). These are, for example, *H. amphinome*, *H. arinome*, *H. feronia*, *H. laodamia* and *H. februa*. However only in the cases of *H. laodamia*, *H. arinome* and *H. februa* did this genetic variation correspond to traditionally recognized subspecies. Based on the barcode region, therefore, neither the two subspecies of *H. feronia* (the nominate *H. f. feronia* and *H. f. farinulenta*) nor the four subspecies in *H. amphinome* (nominate *amphinome*, *fumosa*, *mexicana* and *mazai*) can be readily distinguished. *Hamadryas julitta*, a species endemic to the peninsula of Yucatán (Jenkins 1983), exhibited an impressive amount of intraspecific sequence variation (Fig. 1a), in contrast to *H. guatemalena*, for which two subspecies have been described, but whose barcode sequences differed very little among individuals (Fig. 1b). For other polytypic species like *H. glauconome*, our sampling did not cover the full distributional range of the species and might not represent the entire mtDNA diversity. An example that could represent unsampled diversity could be *H. glauconome grisea*, which is confined to northwestern Mexico and to a diverse ecosystem currently threatened by urban development (Pfeiler et al. 2016).

### DISCUSSION

As mentioned previously, because of extensive wing color variation, name proliferation has been a prominent issue in the taxonomic history of *Hamadryas*, and one not exclusive to widespread species. Fruhstorfer (1916), for example, distinguished five different forms of *H.*

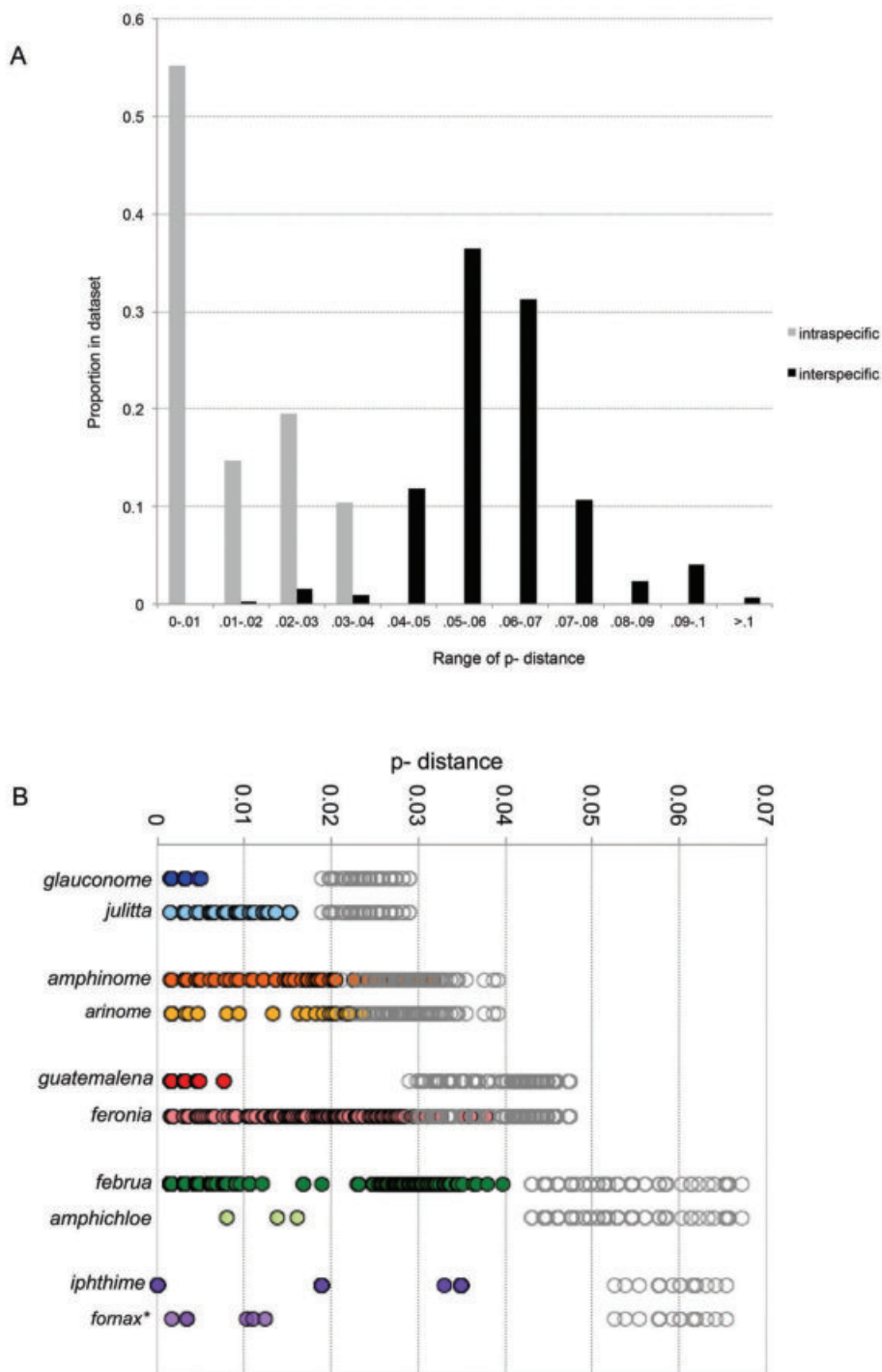


FIG. 1a. Frequency distribution of intraspecific and interspecific sequence divergence among all *Hamadryas* species sampled. Sequence divergence is measured as uncorrected p-distances. 1b. Intra and interspecific distances among pairs of sister taxa. Note that the intraspecific distances in *H. feronia*, *H. amphinome* and *H. arinome* overlap with the distance to their sister species. \*Note that according to the most recent phylogeny of *Hamadryas*, *H. epinome* is the sister species of *H. iphthime*, those together are sister to *H. formax*.



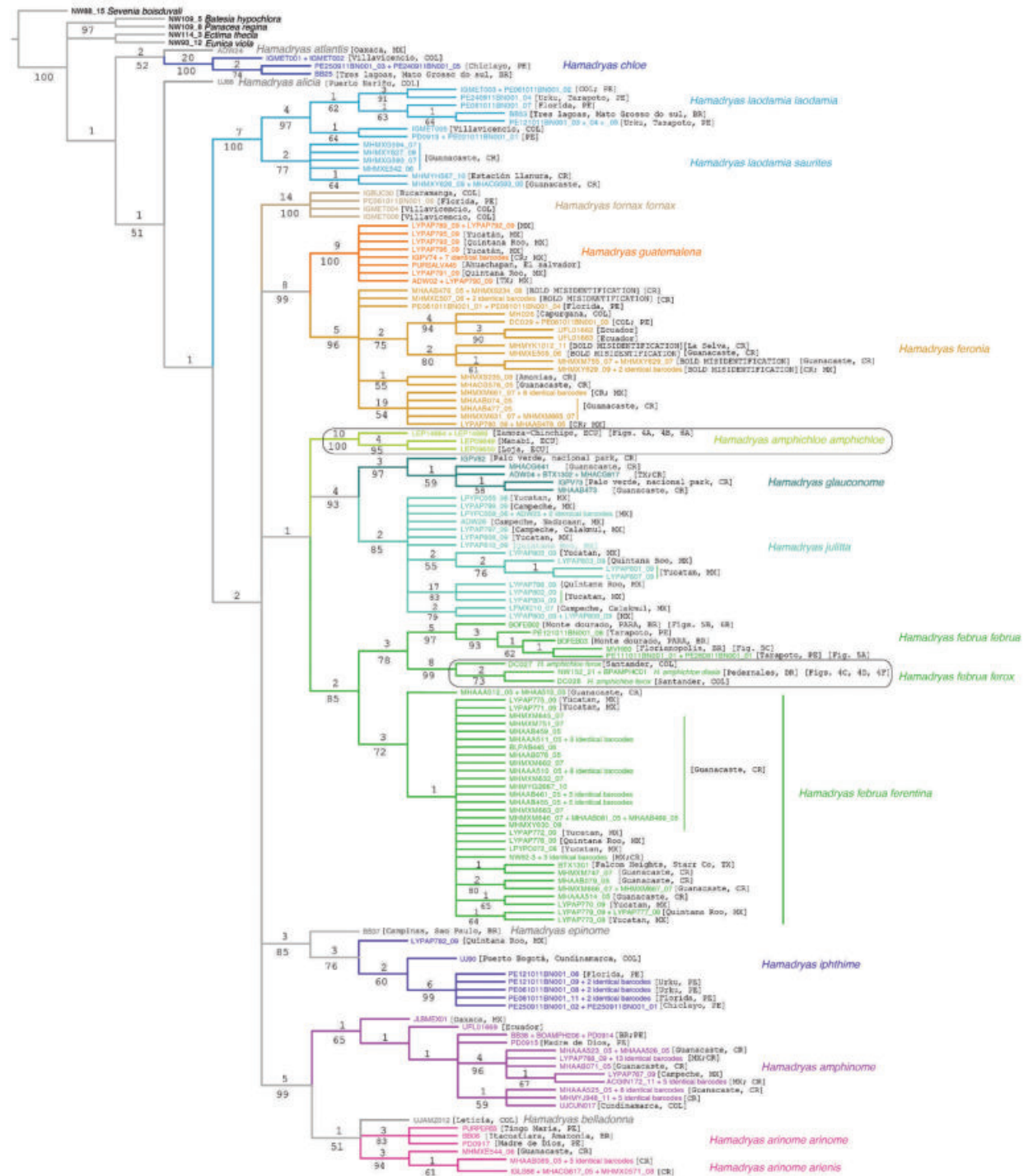


FIG. 2. Strict consensus of 643 MPTs (805 steps) from the parsimony analysis of 142 DNA barcodes. Samples with identical barcodes and/or BOLD misidentifications are labeled accordingly. Country codes are as follows: BR=Brazil, COL=Colombia, CR=Costa Rica, DR=Dominican Republic, ECU=Ecuador, MX=Mexico, PE=Peru, TX=Texas [USA]. For terminals with multiple identical barcodes only country codes are provided. The locality data for all the samples can be found in Table 1. Numbers above and below branches correspond to absolute Bremer support and to Jackknife values respectively; both calculated in TNT.



FIG. 3. Historical distributional records of *H. februa februa*, *H. f. ferentina*, *H. amphichloe amphichloe*, "*H. a. ferox*", "*H. a. diasia*" from Jenkins (1983). Note that these are historical distributional records and do not correspond to the current range of the species. For example, there are records of *H. februa ferentina* and "*H. amphichloe diasia*" from Texas (Jenkins 1984, Bordelon & Knudson 2009, Nall 2014).

*glaucanome*, a species distributed from Mexico to Costa Rica. Jenkins' (1983) comprehensive revision of the genus winnowed out much of this nomenclatural redundancy, improving significantly the taxonomy of the genus and facilitating the study of the phylogenetic relationships among species (Garzón-Orduña 2012, Garzón-Orduña et al. 2013). Our DNA barcode results suggest that work is still needed, and that the study of intraspecific variation in some species of *Hamadryas* could benefit from exploring an alternative source of characters to corroborate the status of subspecies, some of which might not correspond to distinctive lineages, as Jenkins (1983: p. 20) suspected.

### Resolution of *Hamadryas* species with DNA barcodes

Our tree-based result shows that all the included species of *Hamadryas* can be readily distinguished by their mtDNA barcode sequences (Fig. 2). This turned out to be particularly revealing in the case of *H. februa* and its close relative *H. amphichloe*. These two species are sympatric in northern South America (Fig. 3), are

mainly distinguished by wing color characters, and lack differentiating diagnostic features in the male genitalia. The traditional taxonomy of *H. februa* has been difficult due to its extensive phenotypic variation and wide geographical range (Fig. 3): Fruhstorfer (1916) identified more than 10 forms of the species (and described six), most of which were synonymized by Jenkins (1983, 1984). One taxon though, *H. februa diasia* Fruhstorfer (with type locality Puerto Rico and Haiti) was maintained by Jenkins (1983) as a subspecies of *H. amphichloe*. After examining more than a thousand specimens of *H. februa*, Jenkins maintained (albeit reluctantly) only two subspecies (*H. f. februa* and *H. f. ferentina*), which our study shows are further discernable based on their mtDNA barcodes (Fig. 2). The taxonomic history of *H. amphichloe* as a species is newer: Although described (as a subspecies of *H. februa*) in 1870, it was not formally recognized as a species until Jenkins' revision, mainly due to Boisduval's (1870) vague original description, which led to major confusion of *H. amphichloe* with *H. februa*. In contrast to his views on the subspecies of *H. februa*, Jenkins





FIG. 4. **A** and **B**, male and Female vouchers of *Hamadryas amphichloe amphichloe* from Ecuador respectively; **C** and **D**, male and female vouchers of *H. amphichloe diasia* from the Dominican republic; **E**, male of "*Hamadryas amphichloe ferox*" from Venezuela (not included in barcode analysis); **F**, male voucher "*H. amphichloe diasia*" from the Dominican republic.





FIG. 5. Adults *Hamadryas februa februa* included in the barcode analysis. **A.** voucher from Peru; **B.** voucher from Monte dourado, Brazil; **C.** voucher from Florianopolis, Brazil.

confidently distinguished the three subspecies within *H. amphichloe* (Jenkins 1983), further arguing that in fact they could be called different species. In this study we included specimens identified as '*H. amphichloe*' from three localities that corresponded to the distributional ranges of three of its subspecies (Jenkins 1983) (Figs. 3, 4): *H. amphichloe diasia* from the Dominican Republic, *H. amphichloe ferox* from northeastern Colombia, and *H. amphichloe amphichloe* from west and east Ecuador. The barcodes of these subspecies did not group together (Fig. 2). Rather, "*amphichloe*" individuals from Colombia and from the Dominican Republic grouped

with individuals of *H. februa februa* from Peru and Brazil (Fig. 5). Samples of *H. amphichloe amphichloe* from eastern and western Ecuador formed a distinct clade; Ecuador (Guayaquil) is the type locality of *H. amphichloe*, allowing us to conclude that these individuals correspond to the nominate *H. amphichloe*. The samples initially considered *H. amphichloe diasia* and *H. amphichloe ferox*, from the Dominican Republic and Colombia, respectively, correspond instead to *H. februa*. Furthermore, we consider the recognition of these taxa as *H. februa ferox* to be justified since they possess a distinctive patch of white (chalky) scales in

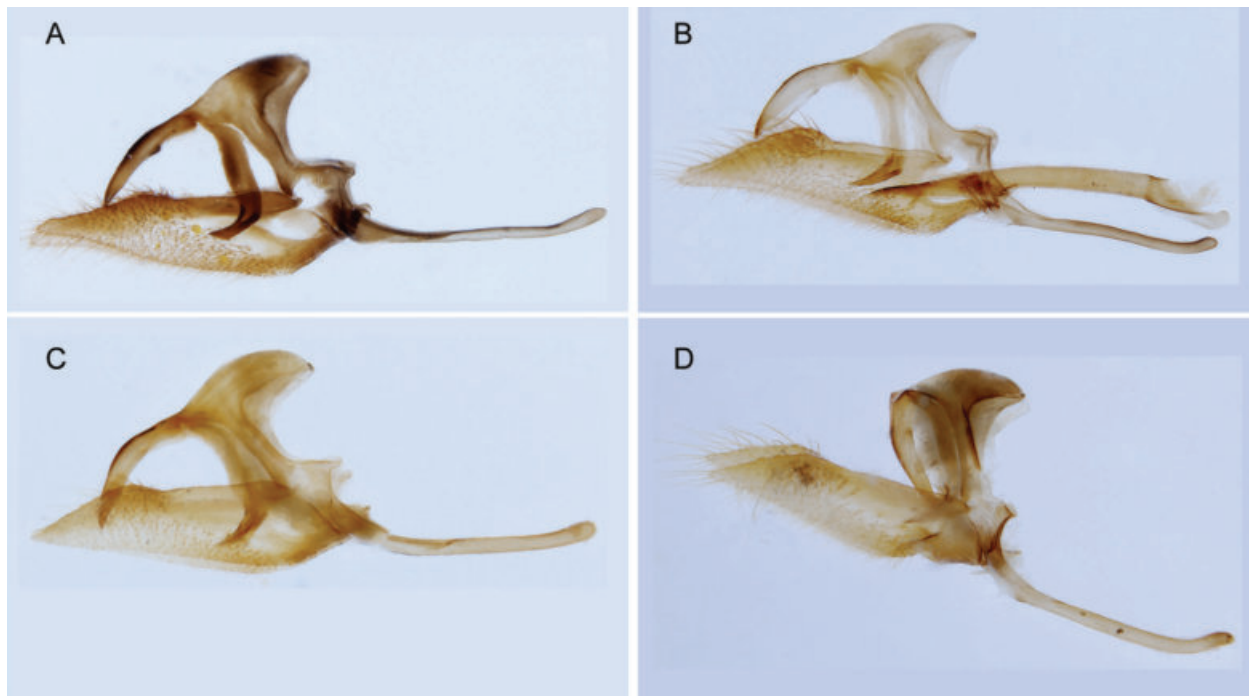


FIG. 6. Male genitalia in lateral view. **A.** *Hamadryas amphichloe amphichloe* from Ecuador (voucher Lep14666, genitalia dissection 15-32); **B.** *H. februa februa* from Brazil (voucher BOFEB02, genitalia dissection 15-33); **C.** "*H. amphichloe ferox*" from Colombia (specimen not included in barcode analysis); **D.** *H. februa februa* from Peru (genitalia dissection 14-14, specimen not included in barcode analysis).

their forewing (FW), not present in the two other subspecies of *H. februa* (Fig.3). Finally, although Jenkins (1983) claimed that *H. februa* and *H. amphichloe* are sympatric in Ecuador, efforts at the FLMNH to retrieve the Ecuadorean *februa* specimens used by Jenkins have failed (K.R. Willmott, pers. comm.). As mentioned above, the two species are rather similar, however the presence of a dark submarginal ring with a white center at the VFW in Cu1–Cu2 remains diagnostic of *H. amphichloe*. In any case, whether or not Ecuadorian *H. februa* and *H. amphichloe* are the same entity remains to be determined.

Over the years, regional inventories have identified the Caribbean cracker in multiple ways, including as *H. ferentina* (Barcant 1970), as *H. amphichloe diasia* (Schwartz 1989, Smith et al. 1994) or *H. februa diasia* (Brown & Heineman 1972; Riley 1975); our study suggests that none of these trinomina accurately reflects the affinities of this lineage. We herein therefore reclassify *H. amphichloe ferox* as *H. februa ferox* and synonymize *H. amphichloe diasia* with it.

*Hamadryas februa ferox* (Staudinger, 1886)

(*Ageronia*) **stat. nov.**

=*Ageronia ferox diasia* Fruhstorfer, 1916

**syn. nov.**

=*Ageronia februa antillana* Hall, 1925

=*Ageronia ferentina diasia* Bates, 1936

=*Hamadryas februa diasia* Brown & Heinemann, 1972

=*Hamadryas amphichloe diasia* Jenkins, 1983; Lamas, 2004

### **Forewing patch of white (chalky) scales is a plesiomorphy.**

The difficulties in identifying *H. amphichloe* specimens from the Dominican Republic and north-eastern Colombia rest on two factors: one is the absence of diagnostic features in the male genitalia of *H. februa* or *H. amphichloe* (Fig. 5). We dissected our four specimens of the nominal *H. amphichloe* from Ecuador, and found no distinctive characters in the female or male genitalia that would readily distinguish them from *H. februa februa* or from *H. februa ferentina*. The other factor is the presence of a patch of matte-white (chalky) scales on the forewing of males of both species (Fig. 3). One of the implications of our results is that the characteristic FW patch of *H. glauconome*, *H. julitta*, and *H. amphichloe* should now be considered present also in *H. februa* and thus is not an evolutionary novelty for these three species, as previously hypothesized (Garzón-Orduña 2012). An additional implication of our results is that the total evidence study

of Garzón-Orduña et al. (2013) did not include representatives of *H. amphichloe*, but rather sampled multiple populations of *H. februa*; the fact that the FW patch is polymorphic in *H. februa* makes the case for the chalky patch trait to be homologous among the four species, regardless of relationships among them.

### **The success of DNA barcoding and the failure of the barcoding gap**

While the success of the DNA barcode in other genera of butterflies has yielded mixed results (Silva-Brandañ et al. 2009), its efficacy in the case of *Hamadryas* was hinted by the study of Prado et al. (2011), where the presence of unusually high intraspecific sequence divergence (>7 %) among samples thought to be individuals of *H. feronia* “flagged” the presence of *H. ipthime* Bates. We note that *H. feronia* and *H. ipthime* can be differentiated by morphology by the composition of the dorsal border ocelli on the HW, as well as by genitalic characters (Jenkins 1983). Our results support the overall observation made by Prado’s study over a more extensive taxonomical and geographical coverage. However while the DNA barcode is able to effectively discriminate samples according to recognized species, this is not to say that future identification of specimens relying solely on estimated sequence divergences from DNA barcodes will lead to correct identifications (or that “discovering” new species of *Hamadryas* might not result in over splitting) given the absence of a barcode gap (Figs. 1a, 1b).

From the dawn of DNA barcoding (Hebert et al. 2003), the idea of a barcode gap—a break in the pattern of within- and between-species pairwise sequence divergence levels (often about 2%)—has been suggested as a diagnostic shibboleth to tell putative species from one another. Almost as soon as DNA barcodes became popular, questions about the existence of the barcode gap began to arise (Meyer & Paulay 2005, Wiemers & Fiedler 2007), and it has been clear for some time that it is not a general pattern (Goldstein & DeSalle 2010). There are two reasons why the barcode gap, especially if restricted to some arbitrary percentage, often may not correspond to species boundaries. First, if sampling is done only from a single locality (e. g., Guanacaste, Costa Rica), then the diversity of intraspecific sequences may be underestimated, resulting in inferred gaps when they do not actually exist in more widespread populations (Brower 2006). Second, if some species exhibit a lot of variability across their ranges, then the intraspecific variation may overlap interspecific variation. This is especially likely to be true if all pairwise distances from all taxa in a study are amalgamated into a single

distribution, as in our Fig. 1a. In butterflies specifically, the absence of the barcode gap seems to be the rule rather than the exception (Whinnett et al. 2005, Wiemers and Fieldler 2007, Elias et al. 2007, Verovnik & Wiemers 2016, this study).

Fortunately, the success of mtDNA in diagnosing species does not require the existence of the barcode gap. Further evidence of this is the fact that the absence of a barcode gap in plants has not impeded progress of botanical taxonomy (e.g., Molina et al. 2015). Unlike distance-based approaches, autapomorphies do not affect cladistic methods, which identify groups based on synapomorphies. Thus species identity can be maintained even in the presence of large amounts of intraspecific sequence variation. Likewise, two clusters of haplotypes are recognized as reciprocally distinct, even by only a single fixed substitutional change (Brower 1999). Note that we do not consider the presence of a clade of haplotypes to indicate “species monophyly,” since monophyly is a quality of interspecific relationships (Nixon and Wheeler 1990).

However challenging it might be to delimit closely related species, we acknowledge that species delimitation should be based ultimately on a wide array of evidence (e.g. multiple genes, morphology, biology, ecology, geography, karyotype) (Rubinoff & Holland 2005, Will et al. 2005, Brower 2006, Knowles & Carstens 2007). We hope our study contributes towards a clearer taxonomy of *Hamadryas* and towards a better understanding of the number (as well as limits) of lineages that make part of the *februa* species complex. Furthermore, it remains to be seen whether the DNA barcode is equally effective at distinguishing among other closely-related species, such as *H. iphthime* and *H. epinome* Felder & Felder, for which there are not yet many existing barcode sequences but which could potentially harbor significant sequence variation (Fig. 1b shows that *H. iphthime* does). As more barcodes become available, we hope to add more observations to this dataset and thus cover many of its geographical limitations.

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#### ADDENDUM

While this paper was in press, a new study by Lavinia et al., 2017 published 24 barcodes for five species of *Hamadryas* (*H. amphinome*, *H. epinome*, *H. feronia*, *H. formax* and *H. februa*). We added the 16 new haplotypes from their study to our matrix for a last minute analysis, and confirmed that all of their samples fell within our circumscriptions of those species.

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TABLE 1. Voucher specimen information for DNA sequence data. The genbank accession numbers for the new sequences will be provided upon acceptance.

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
UJ88	<i>H. alicia</i>	–	Colombia	San Martin de Amacayacu, Puerto Nariño	KC541634	Garzón-Orduña et al. 2013
LEP-09650	<i>H. amphichloe</i>	<i>amphichloe</i>	Ecuador	Loja, Santuario San Vicente	MF098653	This study K.R. Willmot
LEP-09649	<i>H. amphichloe</i>	<i>amphichloe</i>	Ecuador	Manabí, Reserva Lalo Loor	MF098652	This study K.R. Willmot
LEP-14664	<i>H. amphichloe</i>	<i>amphichloe</i>	Ecuador	Zamora-Chinchipe	MF098654	This study K.R. Willmot
LEP-14666	<i>H. amphichloe</i>	<i>amphichloe</i>	Ecuador	Zamora-Chinchipe	MF098655	This study K.R. Willmot
BB38	<i>H. amphinome</i>		Brazil	Mata de Santa Genebra, Campinas, Sao Paulo	MF108842	Karina Silva-Brandão
BO-AMPH206	<i>H. amphinome</i>	<i>amphinome</i>	Brazil	Melgaco, caxiuana, Para El Azulillo, Candelaria	MF098656	Bill Overall Garzón-Orduña et al. 2013
JLBMEX1	<i>H. amphinome</i>	<i>mexicana</i>	Mexico	Loxicha; Oaxaca	KC541620	Garzón-Orduña et al. 2013
UFL01669	<i>H. amphinome</i>	<i>fumosa</i>	Ecuador		KC541632	Garzón-Orduña et al. 2013
PD0915	<i>H. amphinome</i>	<i>amphinome</i>	Peru	Madre de Dios, los amigos	KC541626	Garzón-Orduña
PD0914	<i>H. amphinome</i>	<i>amphinome</i>	Peru	Madre de Dios, los amigos research center	KC541625	Garzón-Orduña et al. 2013
UJCUN017	<i>H. amphinome</i>	<i>amphinome</i>	Colombia	Cundinamarca, Puerto Bogota	KC541638	Garzón-Orduña et al. 2013
MHMYJ948–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Camino Pozo Tres	JQ529751	BOLD
MHMYJ1154–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Tajo Angeles	JQ539182	BOLD
MHMYJ1155–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Camino Pozo Tres	JQ539183	BOLD
MHMYJ1156–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Camino Pozo Tres	JQ539184	BOLD
MHMYJ1157–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Tajo Angeles	JQ539185	BOLD
MHMYJ1158–11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Camino Pozo Tres	JQ539186	BOLD
MHMXO582–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536265	BOLD
MHMXO583–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536266	BOLD
MHMXO574–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536257	BOLD
MHMXO576-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536259	BOLD
MHAAA525–05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU333987	BOLD
MHMXO584–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536267	BOLD
MHMXO578–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536261	BOLD
MHMXO575–08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536258	BOLD
MHAAA527–05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU333986	BOLD
LYPAP768–09	<i>H. amphinome</i>	<i>mexicana</i>	Mexico	Campeche, Calakmul	GU659536	BOLD

TABLE 1. (continued) Table 1 page 2

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
MHAAA523-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU333988	BOLD
MHAAA526-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU333985	BOLD
LYPAP767-09	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU659535	BOLD
LYPAP766-09	<i>H. amphinome</i>	<i>mexicana</i>	Mexico	Campeche	GU659542	BOLD
ACGIN172-11	<i>H. amphinome</i>	<i>mexicana</i>	Mexico	Quebrada Lajosa	JQ574512	BOLD
ACGIN173-11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Bosque Aguirre	JQ574513	BOLD
ACGIN174-11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Quebrada Lajosa	JQ574514	BOLD
ACGIN175-11	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Quebrada Lajosa	JQ574515	BOLD
MHAAA524-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU333989	BOLD
MHAAB071-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU157214	BOLD
MHMXO573-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536256	BOLD
MHMXO577-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536260	BOLD
MHMXO572-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536255	BOLD
LYPAP764-09	<i>H. amphinome</i>	<i>mexicana</i>	Mexico	Campeche	GU659541	BOLD
MHAAB072-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU157213	BOLD
MHMXO586-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536269	BOLD
MHAAB070-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU157215	BOLD
MHMXO581-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536264	BOLD
MHMXO580-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536263	BOLD
MHMXO585-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536268	BOLD
MHAAB475-05	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	GU157212	BOLD
MHMXO579-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536262	BOLD
MHMXO587-08	<i>H. amphinome</i>	<i>mexicana</i>	Costa Rica	Area de Conservación Guanacaste	JQ536270	BOLD
BB06	<i>H. arinome</i>	<i>arinome</i>	Brazil	Itacoatiara, Amazonia	MF108839	Karina Silva-Brandão
PURPER55	<i>H. arinome</i>	<i>arinome</i>	Peru	Tingo Maria	KC541628	Garzón-Orduña et al. 2013
PD0917	<i>H. arinome</i>	<i>arinome</i>	Peru	Madre de Dios, los amigos research center	KC541638	Garzón-Orduña et al. 2013
IGLS66	<i>H. arinome</i>	<i>arinome</i>	Costa Rica	La Selva	KC541610	Garzón-Orduña et al. 2013
PE121011BN001-01	<i>H. arinome</i>	<i>ariensis</i>	Peru	Urku, Tarapoto	MF098643	This study

Table 1. Continued Table 1 page 3

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
MHMXE544-06	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	JQ543434	BOLD
MHMXO571-08	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	JQ536254	BOLD
MHACG617-05	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	GU333971	BOLD
MHAAB069-05	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	GU157183	BOLD
MHMYA1408-09	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Estación Quica	GU647389	BOLD
MHMXE543-06	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	JQ543433	BOLD
MHACG751-05	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	GU333970	BOLD
MHACG625-05	<i>H. arinome</i>	<i>ariensis</i>	Costa Rica	Area de Conservación Guanacaste	GU333972	BOLD
ADW24	<i>H. atlantis</i>		Mexico	Oaxaca	KC541602	Garzón-Orduña et al. 2013
UJAMZ012	<i>H. belladonna</i>	–	Colombia	Leticia	KC541637	Garzón-Orduña et al. 2013
BB25	<i>H. chloe</i>	<i>chloe</i>	Brazil	Tres lagoas, Mato Grosso do Sul	MF108840	Karina Silva-Brandão
IGMET001	<i>H. chloe</i>	<i>chloe</i>	Colombia	Villavicencio, Meta	KC541611	Garzón-Orduña et al. 2013
IGMET002	<i>H. chloe</i>	<i>chloe</i>	Colombia	Villavicencio, Meta	KC541612	Garzón-Orduña et al. 2013
PE240911BN001-05	<i>H. chloe</i>	<i>chloe</i>	Peru	Urku, Tarapoto	MF098617	This study
PE250911BN001-03	<i>H. chloe</i>	<i>chloe</i>	Peru	Shiclayo	MF098618	This study
BB37	<i>H. epinome</i>	–	Brazil	Campinas, Sao Paulo	MF108841	Karina Silva-Brandão
DC027	<i>H. februa</i>	<i>ferox</i>	Colombia	Chicamocha, Santander	KC541606	Garzón-Orduña et al. 2013
DC028	<i>H. februa</i>	<i>ferox</i>	Colombia	Rio Suarez, Santander	KC541607	Garzón-Orduña et al. 2013
BPAMPHCO1	<i>H. februa</i>	<i>ferox</i>	Dominican Republic	Perdenales	KC541605	Garzón-Orduña et al. 2013
NW152-21	<i>H. februa</i>	<i>ferox</i>	Dominican Republic	Pedernales, Parque Nacional Jaragua	MF098657	Niklas Wahlberg (NSG)
MVH60	<i>H. februa</i>	<i>februa</i>	Brasil	Florianópolis, Mata Atlantica	KC541622	Garzón-Orduña et al. 2013
BO-FEB-02	<i>H. februa</i>	<i>februa</i>	Brasil	Monte dourado, Para	MF098648	Bill Overall
BO-FEB-03	<i>H. februa</i>	<i>februa</i>	Brasil	Monte dourado, Para	MF098649	Bill Overall
PE260911BN001-01	<i>H. februa</i>	<i>februa</i>	Peru	Urahasha	MF098647	This study
PE111011BN001-01	<i>H. februa</i>	<i>februa</i>	Peru	Hotel La Patarasca, Tarapoto	MF098646	This study
PE121011BN001-08	<i>H. februa</i>	<i>februa</i>	Peru	Urku, Tarapoto	MF098645	This study
NW62_3	<i>H. februa</i>	<i>ferentina</i>		Butterfly House supplier	AY090216	Niklas Wahlberg (NSG)

Table 1. Continued Table 1 page 4

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
BTX-1301	<i>H. februa</i>	<i>ferentina</i>	USA	Falcon Heights, Starr Co,	MF098650	This study
MHAAA512-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU333978	BOLD
MHAAA513-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU333975	BOLD
MHAAB474-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157186	BOLD
MHAAB461-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157190	BOLD
MHAAB468-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157188	BOLD
MHAAB457-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157185	BOLD
MHAAA510-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU333976	BOLD
MHAAB456-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157187	BOLD
MHAAB078-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157201	BOLD
MHAAB080-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157202	BOLD
MHAAB465-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157194	BOLD
MHAAA514-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU333977	BOLD
MHAAB079-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157199	BOLD
MHAAB469-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157196	BOLD
MHAAB081-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157200	BOLD
MHAAB463-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157197	BOLD
MHAAB459-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157193	BOLD
MHAAB460-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157195	BOLD
MHAAA511-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU333974	BOLD
MHAAB462-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157192	BOLD
MHAAC223-06	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ548262	BOLD
MHAAB466-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157189	BOLD
MHAAB455-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157184	BOLD
MHAAB467-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157198	BOLD
MHAAB458-05	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU157191	BOLD
MHMXM660-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535621	BOLD



Table 1. Continued Table 1 page 5

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
MHMXM632-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535595	BOLD
MHMXM645-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535608	BOLD
MHMXM644-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535607	BOLD
MHMXM659-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535620	BOLD
MHMXM662-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535623	BOLD
MHMXM630-09	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU666790	BOLD
MHMXM667-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535628	BOLD
MHMXM666-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535627	BOLD
MHMXM663-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535624	BOLD
MHMXM646-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535609	BOLD
MHMXM751-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535702	BOLD
MHMXM747-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535701	BOLD
MHMXM664-07	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ535625	BOLD
MHMYG2667-10	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Estación Llanura	HM886090	BOLD
MHMYC1218-09	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU651433	BOLD
MHMYC1219-09	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	GU651434	BOLD
MHMYJ1153-11	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ539181	BOLD
BLPAB445-06	<i>H. februa</i>	<i>ferentina</i>	Costa Rica	Area de Conservación Guanacaste	JQ578180	BOLD
LYPAP771-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Reserva Cuxtal, Tahdzibichen, Yucatan	GU659531	Prado et al. 2011
LYPAP776-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Quintana Roo	GU659528	Prado et al. 2011
LYPAP777-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Quintana Roo	GU659529	Prado et al. 2011
LYPAP773-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Celestun, camino a Chunchucmil, Yucatan	GU659533	Prado et al. 2011
LYPAP779-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Quintana Roo	GU659524	Prado et al. 2011
LYPAP770-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Uman, X tepen, Yucatan	GU659538	Prado et al. 2011
LPYPC072-08	<i>H. februa</i>	<i>ferentina</i>	Mexico	Yucatan	JN201288	Prado et al. 2011
LYPAP778-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Sabidos, Quintana Roo	GU659530	Prado et al. 2011
LYPAP774-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Yucatan	GU659534	Prado et al. 2011
LYPAP772-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Yucatan	GU659532	Prado et al. 2011

Table 1. Continued Table 1 page 6

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
LYPAP775-09	<i>H. februa</i>	<i>ferentina</i>	Mexico	Yucatan	GU659527	Prado et al. 2011
UFL01662	<i>H. feronia</i>	<i>feronia</i>	Ecuador		KC541630	Garzón-Orduña et al. 2013
UFL01663	<i>H. feronia</i>	<i>feronia</i>	Ecuador		KC541631	Garzón-Orduña et al. 2013
MH026	<i>H. feronia</i>	<i>feronia</i>	Colombia	Capurgana, Choco.	KC541621	Garzón-Orduña et al. 2013
PE061011BN001-03	<i>H. feronia</i>	<i>feronia</i>	Peru	Florida	MF098627	This study
PE061011BN001-01	<i>H. feronia</i>	<i>feronia</i>	Peru	Florida	MF098628	This study
PE061011BN001-04	<i>H. feronia</i>	<i>feronia</i>	Peru	Florida	MF098629	This study
DC029	<i>H. feronia</i>	<i>feronia</i>	Colombia	Suarez River, Santander	KC541608	Garzón-Orduña et al. 2013
MHMYK1012-11	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	La selva	JQ529755	BOLD MissID as <i>H. guatemalena</i>
MHMYJ1152-11	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Estación Llanura	JQ539180	BOLD MissID as <i>H. guatemalena</i>
MHMYG2665-10	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Estación Llanura	HM886089	BOLD
MHMXE509-06	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ543405	BOLD MissID as <i>H. guatemalena</i>
MHMXM755-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535703	BOLD MissID as <i>H. guatemalena</i>
MHMXM629-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535593	BOLD MissID as <i>H. guatemalena</i>
MHMXY629-09	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU666789	BOLD MissID as <i>H. guatemalena</i>
MHMXS234-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Amonias	JQ537091	BOLD MissID as <i>H. guatemalena</i>
MHMXE510-06	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ543406	BOLD MissID as <i>H. guatemalena</i>
MHMXE507-06	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ543404	BOLD MissID as <i>H. guatemalena</i>
MHMXS235-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Amonias	JQ537092	BOLD
MHMXM661-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535622	BOLD
MHMXY628-09	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU666788	BOLD
MHMXM631-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535594	BOLD

Table 1. Continued Table 1 page 7

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
MHMXM665-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535626	BOLD
MHMXS233-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Estación Pitilla	JQ537090	BOLD
MHMXS236-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Piedrona	JQ537093	BOLD
MHMXE510-06	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ543406	BOLD MissID as <i>H. guatemalena</i>
MHMXE507-06	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ543404	BOLD MissID as <i>H. guatemalena</i>
MHMXS235-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Amonias	JQ537092	BOLD
MHMXM661-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535622	BOLD
MHMXY628-09	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU666788	BOLD
MHMXM631-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535594	BOLD
MHMXM665-07	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	JQ535626	BOLD
MHMXS233-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Estación Pitilla	JQ537090	BOLD
MHMXS236-08	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Piedrona	JQ537093	BOLD
MHAAB470-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157210	BOLD MissID as <i>H. guatemalena</i>
MHAAB476-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157211	BOLD MissID as <i>H. guatemalena</i>
MHAAB472-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157203	BOLD
MHAAB477-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157206	BOLD
MHAAB074-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157207	BOLD
MHAAB478-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU157204	BOLD
MHACG576-05	<i>H. feronia</i>	<i>farinulenta</i>	Costa Rica	Area de Conservación Guanacaste	GU333980	BOLD
LYPAP781-09	<i>H. feronia</i>	<i>farinulenta</i>	Mexico	Ejido Buenavista, Isla de Luzbel, Quintana Roo	GU659526	BOLD
LYPAP786-09	<i>H. feronia</i>	<i>farinulenta</i>	Mexico	Ejido Nuevo Becal, camino al Chorro, Campeche	GU659523	BOLD
LYPAP780-09	<i>H. feronia</i>	<i>farinulenta</i>	Mexico	Quintana Roo	GU659525	BOLD
PE061011BN001-06	<i>H. fornax</i>	<i>fornax</i>	Peru	Florida	MF098630	This study
IG-MET004	<i>H. fornax</i>	<i>fornax</i>	Colombia	Villavicencio, Meta	KC541614	Garzón-Orduña et al. 2013

Table 1. Continued Table 1 page 8

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
IG-MET008	<i>H. fornax</i>	<i>fornax</i>	Colombia	Villavicencio, Meta	KC541616	Garzón-Orduña et al. 2013
IG-BUC30	<i>H. fornax</i>	<i>fornax</i>	Colombia	Bucaramanga, Santander	KC541609	Garzón-Orduña et al. 2013
ADW-04	<i>H. glauconome</i>	<i>glauconome</i>	USA	Starrco. Rio Grande City, Fort Ringold, Texas	KC541601	Garzón-Orduña et al. 2013
BTX-1302	<i>H. glauconome</i>	<i>glauconome</i>	USA	Falcon Heights, Starr Co, TX	MF098651	This study
IG-PV73	<i>H. glauconome</i>	<i>glauconome</i>	Costa Rica	Palo Verde Biological Station, Guanacaste	KC541617	Garzón-Orduña et al. 2013
IG-PV82	<i>H. glauconome</i>	<i>glauconome</i>	Costa Rica	Palo Verde Biological Station, Guanacaste	KC541619	Garzón-Orduña et al. 2013
MHAAB473	<i>H. glauconome</i>	<i>glauconome</i>	Costa Rica	Area de Conservación Guanacaste	GU157208	BOLD
MHACG817	<i>H. glauconome</i>	<i>glauconome</i>	Costa Rica	Area de Conservación Guanacaste	GU333984	BOLD
MHACG641	<i>H. glauconome</i>	<i>glauconome</i>	Costa Rica	Area de Conservación Guanacaste	GU333983	BOLD
ADW-02	<i>H. guatemalena</i>	<i>marmorice</i>	USA	Texas: Starrco. Rio Grande City, Fort Ringold	KC541600	Garzón-Orduña et al. 2013
IG-PV74	<i>H. guatemalena</i>	<i>guatemalena</i>	Costa Rica	Palo Verde Biological Station, Guanacaste	KC541618	Garzón-Orduña et al. 2013
MHAAB073-05	<i>H. guatemalena</i>	<i>guatemalena</i>		Area de Conservación Guanacaste	GU157209	BOLD
PURSALVA45	<i>H. guatemalena</i>	<i>guatemalena</i>	El Salvador	Ahuachapan	KC541629	Garzón-Orduña et al. 2013
LYPAP783-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659520	BOLD
LYPAP784-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659521	BOLD
LYPAP785-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659522	BOLD
LYPAP787-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Uman, X tepen, Yucatan	GU659516	BOLD
LYPAP788-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Yucatan	GU659517	BOLD
LYPAP789-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Campeche	GU659518	BOLD
LYPAP790-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Campeche	GU659519	BOLD
LYPAP791-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659512	BOLD
LYPAP792-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659513	BOLD
LYPAP793-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Quintana Roo	GU659514	BOLD
LYPAP794-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Reserva de la Biosfera de Calakmul, Campeche	GU659515	BOLD
LYPAP795-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Yucatan	GU659508	BOLD
LYPAP796-09	<i>H. guatemalena</i>	<i>guatemalena</i>	Mexico	Yucatan	GU659509	BOLD
PE121011BN001-06	<i>H. iphthime</i>	–	Peru	Urku, Tarapoto	MF098642	This study



Table 1. Continued Table 1 page 9

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
PE250911BN001-01	<i>H. iphthime</i>	–	Peru	Shiclayo	MF098640	This study
PE121011BN001-10	<i>H. iphthime</i>	–	Peru	Urku, Tarapoto	MF098639	This study
PE121011BN001-07	<i>H. iphthime</i>	–	Peru	Urku, Tarapoto	MF098637	This study
PE061011BN001-08	<i>H. iphthime</i>	–	Peru	Florida	MF098632	This study
PE061011BN001-12	<i>H. iphthime</i>	–	Peru	Florida	MF098636	This study
PE121011BN001-09	<i>H. iphthime</i>	–	Peru	Urku, Tarapoto	MF098638	This study
PE061011BN001-09	<i>H. iphthime</i>	–	Peru	Florida	MF098633	This study
PE250911BN001-02	<i>H. iphthime</i>	–	Peru	Shiclayo	MF098641	This study
PE061011BN001-10	<i>H. iphthime</i>	–	Peru	Florida	MF098634	This study
PE061011BN001-11	<i>H. iphthime</i>	–	Peru	Florida	MF098635	This study
PE061011BN001-05	<i>H. iphthime</i>	–	Peru	Florida	MF098631	This study
LYPAP782-09	<i>H. iphthime</i>	–	Mexico	Quintana Roo	HM431617	BOLD
UJ90	<i>H. iphthime</i>	–	Colombia	Puerto Bogota, Cundinamarca	KC541635	Garzón-Orduña et al. 2013
ADW25	<i>H. julitta</i>	–	Mexico	Campeche, Nadzcaan	KC541603	Garzón-Orduña et al. 2013
ADW26	<i>H. julitta</i>	–	Mexico	Campeche, Nadzcaan	KC541604	Garzón-Orduña et al. 2013
LPYPC055-08	<i>H. julitta</i>	–	Mexico	Yucatan	JN201291	BOLD
LPYPC059-08	<i>H. julitta</i>	–	Mexico	Yucatan	JN201290	BOLD
LYPAP769-09	<i>H. julitta</i>	–	Mexico	Campeche	GU659537	BOLD
LYPAP797-09	<i>H. julitta</i>	–	Mexico	Campeche, Calakmul	GU659510	BOLD
LYPAP798-09	<i>H. julitta</i>	–	Mexico	Quintana Roo	GU659511	BOLD
LYPAP799-09	<i>H. julitta</i>	–	Mexico	Campeche	GU659504	BOLD
LYPAP800-09	<i>H. julitta</i>	–	Mexico	Quintana Roo	GU659505	BOLD
LYPAP801-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659506	BOLD
LYPAP802-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659507	BOLD
LYPAP803-09	<i>H. julitta</i>	–	Mexico	Quintana Roo	GU659500	BOLD
LYPAP804-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659501	BOLD
LYPAP805-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659502	BOLD

Table 1. Continued Table 1 page 10

Voucher code	Species	Subspecies	Country	Locality	Accession number	Source
LYPAP807-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659503	BOLD
LYPAP808-09	<i>H. julitta</i>	–	Mexico	Yucatan	GU659496	BOLD
LYPAP809-09	<i>H. julitta</i>	–	Mexico	Campeche, Calakmul	GU659497	BOLD
LYPAP810-09	<i>H. julitta</i>	–	Mexico	Quintana Roo	GU659498	BOLD
LYPAP811-09	<i>H. julitta</i>	–	Mexico	Quintana Roo	GU659499	BOLD
LPMX210-07	<i>H. julitta</i>	–	Mexico	Campeche, Calakmul	JN201289	BOLD
MHMXG594-07	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	JQ544124	BOLD
MHMXG627-09	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	GU666787	BOLD
MHMXG593-07	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	JQ544123	BOLD
MHMXE542-06	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	JQ543432	BOLD
MHMXG626-09	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	GU666794	BOLD
MHACG593-05	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Area de Conservación Guanacaste	GU333991	BOLD
MHMYH567-10	<i>H. laodamia</i>	<i>saurites</i>	Costa Rica	Estación Llanura	HQ574588	BOLD
BB53	<i>H. laodamia</i>	<i>laodamia</i>	Brazil	Tres lagoas, Mato Grosso do Sul	MF108843	Karina Silva-Brandão
IGMET005	<i>H. laodamia</i>	<i>laodamia</i>	Colombia	Villavicencio, Meta	KC541615	Garzón-Orduña et al. 2013
PD0913	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Los Amigos Research Center	KC541624	Garzón-Orduña et al. 2013
IGMET003	<i>H. laodamia</i>	<i>laodamia</i>	Colombia	Villavicencio, Meta	KC541613	Garzón-Orduña et al. 2013
PE121011BN001-02	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Urku, Tarapoto	MF098619	This study
PE021011BN001-01	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Boca Toma, Pongo	MF098620	This study
PE240911BN001-04	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Urku, Tarapoto	MF098622	This study
PE061011BN001-02	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Florida	MF098621	This study
PE061011BN001-07	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Florida	MF098626	This study
PE121011BN001-04	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Urku, Tarapoto	MF098624	This study
PE121011BN001-03	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Urku, Tarapoto	MF098623	This study
PE121011BN001-05	<i>H. laodamia</i>	<i>laodamia</i>	Peru	Urku, Tarapoto	MF098625	This study