



A new subspecies in a *Heliconius* butterfly adaptive radiation (Lepidoptera: Nymphalidae)

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3 1 **A new subspecies in a *Heliconius* butterfly adaptive radiation**
4 2 **(Lepidoptera: Nymphalidae)**

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7 3 **Abstract**

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10 4 A fundamental goal in evolutionary biology is to understand how evolutionary
11 5 patterns and processes shape natural diversity. This, however, requires a
12 6 complete characterization of the phenotypic and genetic variation between
13 7 and within species. Here, we used molecular, morphological and behavioural
14 8 data to describe a new and stable subspecies of *Heliconius timareta*, named
15 9 *Heliconius timareta linaresi*. This race differs phenotypically from other red
16 10 coloured *H. timareta* and instead exhibits a black and yellow wing pattern
17 11 more similar to *H. cydno*. However, mtDNA, microsatellite and AFLP data
18 12 indicate a closer relationship with *H. timareta* than *H. cydno* and *H.*
19 13 *melpomene*. Larval morphology and host plant preferences are similar to
20 14 other *H. timareta* and *H. cydno* races. Thus, our combined data indicate that
21 15 this taxon is a novel subspecies of *H. timareta*, clearly differentiated from *H.*
22 16 *cydno* and *H. melpomene*.

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18 **Introduction**

19 The *Heliconius* butterflies have undergone an adaptive radiation in warning
20 coloration and are an excellent system in which to study how phenotypic
21 variation originates in nature (Emsley, 1965; Turner, Johnson & Eanes, 1979;
22 Turner, 1981; Brower, 1996b; Supple *et al.*, 2014; Merrill *et al.*, 2015).
23 Typically, *Heliconius* butterflies are involved in Müllerian mimicry rings where
24 distantly related species converge onto a few common colour patterns to
25 advertise their toxicity to predators (Müller, 1879). In contrast, closely related
26 taxa normally display divergent colour patterns and are part of different
27 mimicry rings (Mallet & Gilbert, 1995). This phenomenon has resulted in a
28 complex and colourful mosaic of patterns with more than 40 recognized
29 species and more than 400 colour pattern forms found in forest across tropical
30 Central and South America (Brown, 1979; Brown, 1981; Mallet & Gilbert,
31 1995; Mallet, McMillan & Jiggins, 1998; Lamas *et al.*, 2004). Despite
32 considerable taxonomic, field and molecular studies, we are still discovering
33 new taxa (Brower, 1996a; Giraldo *et al.*, 2008; Mallet, 2009; Moreira & Mielke,
34 2010; Mérot *et al.*, 2013; Nadeau *et al.*, 2014).

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36 The recently diverged species *H. melpomene* Linnaeus, *H. cydno* Doubleday
37 and *H. timareta* Hewitson form a closely related species complex with partially
38 overlapping distributions in the North Andes of South America and in Central
39 America (Brown, 1979; Brower, 1996a; Giraldo *et al.*, 2008; Mallet, 2009;
40 Mérot *et al.*, 2013). *Heliconius melpomene* is largely sympatric with both *H.*
41 *cydno* and *H. timareta*, while *H. cydno* and *H. timareta* are parapatric with
42 respect to each other. *Heliconius cydno* and *H. melpomene* are well-studied
43 species that show strong assortative mating, differ in habitat use, host plant
44 preference (Mallet *et al.*, 1998; Jiggins *et al.*, 2001; Naisbit, Jiggins & Mallet,
45 2001; Kronforst, Young & Gilbert, 2007; Merrill *et al.*, 2011; Merrill *et al.*, 2012;
46 Merrill *et al.*, 2013), and they mimic different and unrelated species of
47 *Heliconius*. *Heliconius cydno* has typically yellow or white elements and most
48 often mimics *H. sapho* Drury and *H. eleuchia* Hewitson, while *H. melpomene*
49 has red and yellow wing patterns and mimics *H. erato* Linnaeus (Flanagan *et*
50 *al.*, 2004). *Heliconius timareta*, the third member of this radiation, was

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3 51 previously thought to be limited to an isolated polymorphic population in
4 52 Ecuador. However, recent studies have revealed the existence of several
5 53 distinctive populations along the eastern slopes of the Andes from southern
6 54 Colombia to Peru (Brower, 1996a; Giraldo *et al.*, 2008; Mallet, 2009; Mérot *et*
7 55 *al.*, 2013; Nadeau *et al.*, 2014). These new forms generally exhibit red colour
8 56 pattern elements, which are acquired through adaptive introgression from *H.*
9 57 *melpomene* (Pardo-Díaz *et al.*, 2012; The *Heliconius* genome consortium,
10 58 2012; Wallbank *et al.*, 2016) (but see Brower, 2011; Brower, 2013) and are
11 59 almost indistinguishable from local *H. melpomene* races. Nonetheless, the
12 60 two are reproductively isolated both respect to mate choice and hybrid viability
13 61 (Sánchez *et al.*, 2015). In this case, assortative mating is almost certainly
14 62 related to differences in pheromones (Mérot *et al.*, 2015; Sánchez *et al.*,
15 63 2015). More extensive geographic sampling has revealed additional
16 64 populations of *H. timareta*, including a recent study that discovered a new
17 65 form in eastern Ecuador (Nadeau *et al.*, 2013; Nadeau *et al.*, 2014). With a
18 66 widespread sampling and new genetic data we are beginning to resolve the
19 67 evolutionary relationships among these three species and understand how
20 68 **these species** varies across the genome.

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35 70 Here, we used an integrative framework (similar to Braby, Eastwood &
36 71 Murray, 2012) that uses morphological (adult morphology, wing size, and
37 72 larval head capsule coloration), genetic (mtDNA, *Tpi*, microsatellites) and
38 73 behavioural data (host plant choice) to test the distinctiveness of a new race
39 74 of *H. timareta*, named here as *Heliconius timareta linaresi* Arias & Lamas.
40 75 This new race is endemic to the eastern cordillera of the Southern Colombian
41 76 Andes. Genetically it clusters within the *H. timareta* clade, but, unlike the most
42 77 of the more southern *H. timareta*, *linaresi* has a black-yellow *H. cydno* wing
43 78 colour pattern. Interestingly, *linaresi* does not seem to entirely mimic any other
44 79 taxa in its distribution, **although we cannot discard that other black-yellow**
45 80 ***Heliconius* occurring in the same general area (*H. congener* Weymer, *H. sara***
46 81 ***Fabricius* and *H. wallacei* Reakirt) could be comimetics. However, none of**
47 82 **them present a complete yellow forewing band. The only form that perfectly**
48 83 **match *H. t. linaresi* colour pattern is *H. c. cordula* Neustetter (Figure 1, Figure**
49 84 **2), a species found further north on the eastern slopes of the Andes. In**

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3 85 addition to describing morphological, ecological, and genetic attributes of the
4 86 *H. timareta linaresi*, we discuss the implications of this new form for our
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6 87 understanding of speciation in this group of butterflies.
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10 89 **Methods**

11 90 **Sampling and stock populations**

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13 91 Adult **individuals** of *Heliconius timareta linaresi* were collected between 2005-
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15 92 2009 in Las Morras (02°41'04" N, 79°53'17" W) and Puerto Rico (1°56'44" N,
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17 93 75°13'16" W) near the Pato River, in Caquetá (Colombia). A subset of these
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19 94 individuals was kept alive in separate insectaries (2 x 3 x 2 m³) in La Vega
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21 95 (Cundinamarca) and was used to establish **stock** populations. These were
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23 96 used to carry out host plant choice experiments and **larval** morphology
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25 97 description (see below). Adults were provided with ample nectar and pollen
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27 98 (*Lantana* and *Psiguria*), as well as artificial nectar solution (10% sugar
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29 99 solution). In addition, several *Passiflora spp.* host plants for immature stages
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31 100 were provided. Wings were removed from wild specimens and bodies
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33 101 preserved in DMSO for phenotypic and molecular analyses. DNA extractions
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35 102 were performed from one-third of the thorax of each individual by using a
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37 103 DNeasy tissue Kit (QIAGEN) following manufacturers' protocol.
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41 105 **DNA sequence analyses**

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43 106 We sequenced the mitochondrial region spanning the *Col* and *Coll* genes
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45 107 (1525 bp) for 12 *H. t. linaresi* individuals, and the nuclear Z (X)-linked gene
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47 108 *Tpi* (648 pb) for 11 *H. t. linaresi* specimens. Primer sequences and conditions
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49 109 were used as outlined by Beltrán *et al.* (2002). The fragments obtained were
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51 110 sequenced in an ABI Prism 3100 Sequencer (PE Applied Biosystems).
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53 111 Sequences for the *Col-Coll* and *Tpi* **gene regions** were downloaded from
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55 112 GenBank for *H. melpomene*, for other closely related species in the cydno
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57 113 complex (*H. cydno* races, *H. timareta* races, *H. heurippa* Hewitson, and *H.*
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59 114 *pachinus* Salvin) and for the outgroup species *H. numata* Cramer (GenBank
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115 accession numbers; Table 1SI). All sequences were aligned and checked by
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117 116 eye using MacClade 4.08a (Maddison & Maddison, 2001). The sequences
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119 117 generated in this study are available in GenBank (Accession numbers
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121 118 KU877714 - KU877725 [*Tpi*], KU877726 - KU877737[*Col-Coll*]; Table 1SI).

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3 119 **Phylogenetic analyses** were conducted using Maximum likelihood (ML) with
4 120 RAxML Blackbox (Stamatakis, Hoover & Rougemont, 2008) and Bayesian
5 121 inference (BI) in Beast Xsede 1.7.5 (Drummond & Rambaut, 2007) from the
6 122 Cipres cluster web service (Miller, Pfeiffer & Schwartz, 2010). Both genes
7 123 were analysed using GTR+G nucleotide substitution model, which was
8 124 preferred by the Akaike Information Criterion (AIC) using JModel Test 1.1
9 125 (Darriba *et al.*, 2012). For ML analyses, branch stability was estimated after
10 126 5000 bootstrap replicates. BI analyses were modelled under a Yule speciation
11 127 process and branch lengths under the assumption of relaxed clock with an
12 128 uncorrelated lognormal distribution. The analysis was run for 40 million
13 129 generations and sampled every 4000 generations. Mixing properties and
14 130 convergence of the MCMC were evaluated by visual inspection of the
15 131 parameter trend plots and by examining that the effective sample size (ESS)
16 132 was higher than 200 after a burn in of 2500 samples in the Tracer program
17 133 (Drummond & Rambaut, 2007). Finally, 7500 trees from the posterior
18 134 distribution were evaluated and summarized with average branch length
19 135 values using the maximum credibility tree in TreeAnnotator 1.7.1 (Drummond &
20 136 Rambaut, 2007).

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35 36 138 **Multilocus microsatellite analyses**

37 139 Nuclear DNA variation was described at 7 microsatellite loci for 203
38 140 individuals [51 *H. cydno* individuals (18 *H. c. cydnides* Staudinger and 33 *H. c.*
39 141 *cydno* Doubleday), 83 *H. m. malleti* Lamas, 53 *H. t. florencía* Giraldo and 16
40 142 *H. t. linares*] using primers and conditions delineated in Arias *et al.* (2012).
41 143 Reaction fragments were run in an ABI Prism 3100 Sequencer (PE Applied
42 144 Biosystems). Allele sizes were established using ABI GeneMapper v4.0 (PE
43 145 Applied Biosystems) with Genescan Liz-500 (Applied Biosystems) as size
44 146 standard. Departure from Hardy–Weinberg equilibrium and linkage
45 147 disequilibrium were tested using Arlequin 3.5 (Schneider, Roessli & Excoffier,
46 148 2000). Levels of differentiation (F_{ST}) (Weir & Cockerham, 1984) among
47 149 populations were calculated with Arlequin 3.5 (Schneider *et al.*, 2000). We
48 150 used a Bayesian model–based clustering algorithm to assign individuals to
49 151 species and to detect admixed individuals using the software Structure 2.3.4
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3 152 (Pritchard, Stephens & Donnelly, 2000). The analysis was run under an
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5 153 admixture model with correlated allele frequencies between populations,
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7 154 which assumes that allele frequencies between populations are similar due to
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9 155 migration or ancestral polymorphism (Falush, Stephens & Pritchard, 2003).
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11 156 The number of ancestral clusters, K , were determined using an *ad hoc*
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13 157 statistic ΔK based on the rate of change in the log probability of data for K
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15 158 between 1 and 10 in 20 runs (Evanno, Regnaut & Goudet, 2005), where each
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17 159 run consisted of 10^6 iterations, after a burn in period of 10^4 iterations.
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161 **Adult morphology and larval coloration**

162 We assessed colour pattern qualitative diagnostic traits in **adult forewing and**
163 **hindwing** between wild caught individuals of *H. t. linaresi* (4 female, 13 males),
164 *H. c. cordula* (10 female, 10 males) and *H. t. timareta f. timareta* Hewitson (5
165 female, 5 males), which exhibit a similar phenotype (Figure 1 and Figure 2).
166 We further look at *H. t. linaresi* **head capsule larval** coloration. Giraldo *et al.*
167 (2008) found that *H. t. florenci*a displays similar head capsule coloration to *H.*
168 *cydno* races and different from *H. melpomene*. To test for differences between
169 *H. cydno*, *H. melpomene*, *H. t. florenci*a and *H. t. linaresi*, we raised six larvae
170 from six different wild caught females from Las Morras (Caqueta) and
171 compared them to 107 larvae head capsules from 54 *H. melpomene* (*H. m.*
172 *malleti*, *H. m. bellula* and *H. m. vulcanus*), 29 *H. cydno* (*H. c. cordula*, *H. c.*
173 *cydnides* and *H. c. zelinde*) and 24 *H. t. florenci*a individuals. Pictures were
174 taken with a Sony digital still camera DSC-S85 under similar conditions
175 with a colour standard, and processed with the software Scion Image (Scion
176 Corporation, Frederick, MD, USA). Four RGB indexes were calculated
177 following Giraldo *et al.* (2008) protocol. Finally, we tested for significant
178 differences between indexes with a one-way Analysis of Variance (ANOVA)
179 and Tukey's HSD *post hoc* test by using *R* statistical package (*R*, Core Team.
180 2014). *p*-Values less than 0.01 were taken to be statistically significant.

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182 **Host plant choice**

183 Individual insectaries were equipped with host plants of 6 *Passiflora* species
184 known to be used by races of the *H. cydno/H. melpomene* complex

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3 185 [*Passiflora edulis* Sims, *P. maliformis* Linnaeus, *P. ligularis* Juss, *P. arborea*
4 186 Spreng, *P. quadrangularis* Linnaeus, *P. oerstedii* Mast], and 2 host plant
5 187 controls frequently used by *H. erato* races [*P. suberosa* Linnaeus and *P. rubra*
6 188 Linnaeus] (Benson, Brown & Gilbert, 1975; Gilbert, 1982). All plants used
7 189 were fresh and of the same age. We tested plant preference of 7 females of
8 190 *H. t. linaresi*, 9 *H. t. florencia* and 10 *H. m. malleti* that were kept separated in
9 191 the individual insectaries. Eggs laid per plant by each female were collected
10 192 and counted twice a week. A multinomial laying probability $P_{j \times i}$, that represent
11 193 the probability of choice by a female type j to a plant type i , for each
12 194 combination of j -type female and i -type plant were obtained using Maximum
13 195 Likelihood (ML). The laying probability for each species/group is:
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$$P_{jxi} = \frac{P_i}{\sum_{i=1}^n P_i}$$

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28 198 where n is the total number of tested plants and P_i represents the laying
29 199 proportion of eggs for each plant. This probability was maximized by the
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31 200 expression:
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$$\sum_{j=1}^m \sum_{i=1}^n a \ln(P_{jxi})$$

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39 203 where m represent the total number of species/groups tested and a
40 204 correspond the total number of counted eggs per plant. Different parameter
41 205 models were compared using a reductionist strategy, starting with a model
42 206 that assumes three parameters corresponding to different laying proportions
43 207 across plants for the three groups (*H. m. malleti*, j_1 ; *H. t. florencia*, j_2 ; and *H. t.*
44 208 *linaresi*, j_3 ; [$j_1 \neq j_2 \neq j_3$]). These initial model was contrasted with a two (all
45 209 possible two species/groups arrangements [$j_1 = j_2 \neq j_3$; $j_1 \neq j_2 = j_3$; $j_1 = j_3 \neq j_2$]) and one
46 210 parameter model ($j_1 = j_2 = j_3$) using a $G = -2\Delta \text{LogeL}$ test, which asymptotically
47 211 follows a X^2 -distribution (Edwards, 1972).
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3 214 **Results**

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5 215 **Mitochondrial and *Tpi* data**

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8 216 We assessed variation at the mtDNA gene *Col-*Coll** (n= 150) and the Z(X)
9 217 linked nuclear gene *Tpi* (n=137) across the *H. cydno*, *H. melpomene* and *H.*
10 218 *timareta* radiations. MtDNA analyses with Maximum Likelihood (ML) and
11 219 Bayesian Inference (BI) generated a phylogeny with three clades: 1) a poorly
12 220 supported *H. melpomene* clade that appears paraphyletic with respect to *H.*
13 221 *cydno*; 2) a well supported clade containing *H. cydno* races, the 15 *H.*
14 222 *heurippa*, the 6 *H. t. florencía* individuals and one individual of *H. pachinus*;
15 223 and 3) a well supported clade that contained the remaining *H. pachinus*
16 224 specimens, all the *H. t. timareta* and *H. t. thelxinoe* individuals, a cluster
17 225 formed by 12 *H. t. linaresi* individuals and one *H. t. florencía* (Figure 3a,
18 226 Figure 1SI). In contrast, ML and BI analyses for the *Tpi* gene showed two well
19 227 supported clades: 1) a *H. melpomene* clade, mainly clustered by east and
20 228 west of the Andes; and 2) a clade containing *H. cydno* races, the closely
21 229 related species *H. heurippa* and *H. pachinus*, all *H. timareta* races and our 12
22 230 *H. t. linaresi* specimens (Figure 3b, Figure 2SI).

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36 232 **Microsatellite analyses**

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38 233 We assessed variation at seven microsatellite loci for *H. t. linaresi*, *H. t.*
39 234 *florencia*, *H. m. malleti* and two allopatric populations of *H. cydno*. In general,
40 235 *H. t. linaresi* exhibited a mean observed heterozygosity of 0.45 ± 0.26 , which
41 236 was slightly lower than that of *H. t. florencía* and *H. cydno* (0.48 ± 0.21 and
42 237 0.48 ± 0.22 respectively). In contrast, *H. m. malleti* showed the highest
43 238 heterozygosity (0.50 ± 0.14). Some loci displayed significant deviations from
44 239 Hardy-Weinberg equilibrium within populations. These deviations were
45 240 caused by heterozygote deficiencies and were most likely due to presence of
46 241 null alleles, as is has been observed in prior studies (Flanagan *et al.*, 2002;
47 242 Mavarez & Gonzalez, 2006; Arias *et al.*, 2012). Genetic differentiation was
48 243 measured with and without null alleles correction with similar results. Overall,
49 244 genetic differentiation between *H. t. linaresi* and *H. m. malleti* was strong and

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3 245 significant ($F_{ST}=0.12$, $p<0.001$) and consistent with strong differentiation
4 246 between *H. timareta* and *H. melpomene* species (Giraldo *et al.*, 2008; Mérot
5 247 *et al.*, 2013). Congruently, *H. cydno* was also strongly and significantly
6 248 differentiated from *H. t. linaresi* ($F_{ST H. c. cydno}=0.14$, $F_{ST H. c. cydnides}=0.11$;
7 249 $p<0.001$). In contrast, genetic differentiation between *H. t. linaresi* and *H. t.*
8 250 *florencia* was lower but still significant ($F_{ST}=0.04$, $p<0.01$). The Bayesian
9 251 model-based clustering method implemented in Structure was consistent with
10 252 the observed genetic differentiation. The best estimate of the number distinct
11 253 clusters K was three, which corresponded to the three *Heliconius* species
12 254 (Figure 4a, Figure 3SI). These results are consistent with a similar analysis (a
13 255 population assignment test in structure) previously performed with a broader
14 256 scan of the genome with AFLP markers and a larger sample of *H. cydno*, *H.*
15 257 *timareta* and *H. melpomene* races (Figure 4b; Arias *et al.*, 2014).
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259 **Adult morphology, and larval head capsule coloration**

260 We investigated differences in colour pattern by comparing *H. t. linaresi*
261 individuals with *H. c. cordula* and *H. t. timareta f. timareta*, populations that
262 both display similar black-yellow wing pattern (Figure 2). We detected six
263 clear differences between *H. t. linaresi*, *H. c. cordula* and *H. t. timareta f.*
264 *timareta* specimens: a) in general, *H. t. linaresi* and *H. t. f. timareta* wings are
265 opaque black, whereas *H. c. cordula* wings are blackish-blue iridescent
266 (Figure 2a); b) the forewing (FW) yellow post-median band with a smooth
267 distal border in *H. t. linaresi* and *H. c. cordula* individuals, while *H. t. timareta f.*
268 *timareta* individuals present a narrower and more irregular FW post-median
269 band (Figure 2a,b); the yellow FW post-median spot ('oval' element), present
270 below vein Cu_2 that is shared between *H. t. linaresi* and *H. cordula*, but absent
271 in *H. t. timareta f. timareta*; d) a 'red line' at the base of the costal vein on the
272 ventral side of FW, present in *H. t. linaresi* and *H. t. timareta f. timareta*, but
273 rarely visible in *H. c. cordula* (less than 3% of the individuals present this line;
274 Figure 2c); e) the 'forceps' element on the ventral side of the hindwing (HW)
275 (Linares, 1989) that is present in all *H. c. cordula*, is absent in *H. t. timareta f.*
276 *timareta*; however, there is a small remnant of this element at the base of the
277 HW in *H. t. linaresi*; and f) the basal 'red spots' on the HW ventral side,

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3 278 present in *H. t. linaresi* and *H. t. timareta f. timareta* (Figure 2d), but absent in
4 279 *H. c. cordula* (a first dot is located between the A_{1+2} and the Cu_2 veins, a
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6 280 second dot at the base of the discal cell, and a third dot located between the
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8 281 $Sc+R_1$ and the Rs veins).
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13 283 We also found that *H. t. linaresi* has a dark yellow-orange larval head capsule
14 284 tone and light narrow bands on the dorsal view behind the head, similar to
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16 285 other *H. cydno/H. timareta* races. This was very different from the coloration
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18 286 of *H. melpomene* races where the larval head capsule is pale yellow and
19 287 there are two dark broad bands just behind the head. In fact, we found
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21 288 significant differences in larval head capsule coloration between
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23 289 species/groups (ANOVA, $df=4$, $p=2.2 \times 10^{-16}$). In particular, larval head capsule
24 290 coloration analyses showed that offspring of wild *H. t. linaresi* females share
25 291 similar colour indexes (b' and LM) with *H. t. florenci*a and other *H. cydno*
26 292 races (Tukey's HSD test $p>0.01$; Figure 4SI), but are significantly different
27 293 from *H. m. malleti* and other *H. melpomene* races (Tukey's HSD test $p<0.01$,
28 294 Figure 4SI).
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36 296 **Host plant choice**

37 297 Female laying behaviour was compared between 7 *H. t. linaresi* (353 eggs),
38 298 10 *H. m. malleti* (471 eggs) and 9 *H. t. florenci*a (729 eggs). *Heliconius t.*
39 299 *linaresi* females displayed a similar laying behaviour to *H. t. florenci*a,
40 300 ovipositing on several *Passiflora* species (*P. edulis* 63%, *P. ligularis* 19%, *P.*
41 301 *oerstedii* 17%, and *P. quadrangularis*, *P. arborea* and *P. maliformis* 1%;
42 302 Figure 5SI). In contrast, *H. melpomene* females laid eggs mainly on two
43 303 *Passiflora* species (*P. oerstedii* 70%, *P. ligularis* 29%, Figure 5SI). The model
44 304 that best explained the data was a two-parameter model, which distinguished
45 305 the laying behaviour of *H. timareta* races from that of *H. melpomene* ($\text{LnL}=-$
46 306 638.749 ; $p=0.00001$; $j_1 \neq j_2 = j_3$). Moreover, previous studies found that *H. c.*
47 307 *cordula* also oviposited on several *Passiflora* species, but in a different
48 308 proportion to *H. timareta* (*P. edulis* 16%, *P. ligularis* 38%, *P. maliformis* 26%
49 309 and *P. oerstedii* 20%) (Salazar unpublished data). These results suggest that
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3 310 *H. t. linaresi* shows different laying behaviour than *H. melpomene*, but
4 311 behaves more similar to *H. cydno* and *H. t. florencía*.

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8 313 **Discussion**

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10 314 A common practice is to define newly discovered local populations as
11 315 subspecies based on their different appearance from known populations.
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13 316 However, information about the complete phenotypic variation, geographic
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15 317 range, connectivity and natural history are not well understood in many cases.
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17 318 Furthermore, many such studies do not use a clear criterion to delimit species
18 319 and subspecies boundaries (Braby *et al.* 2012). In this study we used an
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20 320 integrative approach by gathering multiple sources of evidence (morphology,
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22 321 behaviour, genetics, ecology, etc) to diagnose distinctiveness in evolving
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24 322 populations. This approach has been used to delimit species and subspecies
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26 323 boundaries in Australian satyrine butterflies (Braby *et al.* 2012), *Philaethria*
27 324 butterflies (Barão *et al.* 2014), **European wood white butterflies (Dincă *et al.*,**
28 325 **2011)**, killer whales (Hoelzel *et al.*, 2007), Alaskan song sparrows (Pruett &
29
30 326 Winker, 2010) and other examples in Mallet (2008) and James (2010). All
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32 327 available evidence – molecular, morphological and ecological– are consistent
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34 328 with a new distinctive subspecies of *H. timareta* on the Southeastern slopes of
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36 329 the Colombian Andes, which we call *H. t. linaresi* (Appendix 1).

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38 331 **Molecular evidence**

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40 332 The molecular data strongly supported the hypothesis that *H. t. linaresi* is a
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42 333 new member of the *H. timareta* lineage. Mitochondrial DNA places *H. t.*
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44 334 *linaresi* as a discrete clade within the larger *H. timareta* radiation. This result is
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46 335 congruent with recent mtDNA analysis, which surveyed the ‘barcode’ region
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48 336 for a much larger number of races of *H. cydno*, *H. melpomene* and *H. timareta*
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50 337 (Arias *et al.*, 2014). In both studies, *H. timareta linaresi* clustered
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52 338 monophyletically with races of *H. timareta*, to the exclusion of both *H. cydno*
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54 339 and *H. melpomene*. Likewise, our microsatellite loci assigned all *H. t. linaresi*
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56 340 individuals to the *H. timareta* cluster, clearly differentiating it from both *H.*
57
58 341 *melpomene* and *H. cydno*. A similar pattern was observed in a previous AFLP
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60 342 analysis of this radiation, but using a larger sample of *H. cydno* races (Figure
343 343 4; Arias *et al.*, 2014). Congruently, F_{ST} estimates between *H. t. linaresi* and

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3 344 both *H. m. malleti* and *H. cydno* races where high and similar to comparable
4 345 estimates from earlier studies of genetic differentiation among the *H.*
5 346 *melpomene*, *H. cydno* and *H. timareta* radiations (Giraldo *et al.*, 2008; Martin
6 347 *et al.*, 2013; Mérot *et al.*, 2013; Nadeau *et al.*, 2013; Arias *et al.*, 2014).
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10 348
11 349 The Z(X) linked gene *Tpi* similarly differentiated all *H. cydno* and *H. timareta*
12 350 individuals from all *H. melpomene* individuals. As expected, *H. t. linaresi* fell
13 351 within a distinctive lineage containing *H. cydno*/*H. timareta*. Within this
14 352 lineage, there were two clear clusters of *Tpi* alleles, both containing multiple
15 353 races of *H. cydno* and *H. timareta*. However, within both lineages, there are
16 354 no *Tpi* alleles shared between the two species. In contrast, the *H. t. linaresi*
17 355 individuals we analysed shared nearly identical alleles with both *H. t. timareta*
18 356 and *H. t. florencia*. Perhaps, the strong differentiation observed between *H.*
19 357 *melpomene* and *H. cydno*/*H. timareta* clades could be the result of rapid
20 358 coalescent of the species alleles due to lower effective population size of loci
21 359 on the Z chromosome and/or the accumulation of Z linked factors that
22 360 contributes to postzygotic isolation. In fact, previous studies have found a
23 361 statistical association between *Tpi* or linked loci with sterility in F₁ females
24 362 between *H. cydno* females and *H. melpomene* males (Naisbit *et al.*, 2002;
25 363 Salazar *et al.*, 2005). There was a similar pattern between *H. t. linaresi* and *H.*
26 364 *melpomene*, where F₁ female hybrids between *H. t. linaresi* female and *H. m.*
27 365 *malleti* male were sterile (Sánchez *et al.*, 2015). The pattern observed in
28 366 these studies suggest that *H. timareta* and *H. cydno* show a similar degree of
29 367 Z effect in their postzygotic isolation with *H. melpomene*. Additionally, *H. t.*
30 368 *linaresi* and *H. m. malleti* showed strong prezygotic reproductive isolation
31 369 (Sánchez *et al.*, 2015). In contrast, experimental crosses between *H. t. linaresi*
32 370 and *H. c. cordula* are completely interfertile (Sánchez *et al.*, 2015).
33 371 Nonetheless, there is evidence for some premating isolation where females of
34 372 *H. t. linaresi* mate at a low frequency with *H. c. cordula* males, while *H. t.*
35 373 *linaresi* males easily mate with *H. c. cordula* females (Sánchez *et al.*,
36 374 2015). This asymmetric mating preference, with an almost identical colour
37 375 pattern form (e.g. *H. c. cordula*, see below), suggests that mechanisms other
38 376 than colour pattern, such as pheromone signals and/or courtship behaviour,
39 377 are likely to be involved.
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5 379 Notably, genetic differentiation as measured by F_{ST} analysis of microsatellite
6 380 loci between *H. t. linaresi* and other *H. timareta* subspecies was
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8 381 approximately 5 times lower than that observed between *H. timareta*
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10 382 subspecies and either *H. cydno* and *H. melpomene*. Nonetheless, these
11 383 differences were significant suggesting restricted gene flow among
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13 384 parapatrically distributed *H. timareta* races. As an example, genetic
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15 385 differences between *Heliconius t. linaresi* and *H. t. florenci*a were slight, but
16 386 significant. The two subspecies have a parapatric distribution and hybrids
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18 387 between them have been collected in areas of overlap. However, the strong
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20 388 differences in colour pattern between *H. t. linaresi* and *H. t. florenci*a (black-
21 389 yellow vs. dennis-ray respectively, Figure 1) likely play a role in some degree
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23 390 of pre-mating reproductive isolation between the two (Sánchez *et al.*, 2015).
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26 392 ***Morphological and natural history evidence***

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28 393 In the eastern foothills of the Andes there are two other taxa that are
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30 394 phenotypically similar to *H. t. linaresi*: to the north *H. c. cordula* and further
31 395 south, one of the forms of the polymorphic race of *H. t. timareta* from eastern
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33 396 Ecuador. When we compared FW and HW patterns, we found five main traits
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35 397 that differ between these taxa. Three of these, the lack of wing iridescence,
36 398 'red line' and 'red spots', were shared between *H. t. linaresi* and *H. t. timareta*;
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38 399 one trait (FW yellow band size and form) was shared between *H. t. linaresi*
39
40 400 and *H. c. cordula* and one trait was different between the three taxa ('forceps';
41 401 Figure 2). We further compared head capsule larval coloration between *H. t.*
42
43 402 *linaresi* and several *H. melpomene*, *H. cydno* and *H. timareta* races (Figure
44
45 403 4SI). These results indicate that *H. t. linaresi* is more closely related to the *H.*
46 404 *timareta*/*H. cydno* clade and is clearly divergent from *H. melpomene* (except
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48 405 for *H. m. malleti*; Figure 4SI). Similar results were found previously in
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50 406 comparisons between *H. t. florenci*a/*H. cydno* and *H. melpomene* races
51 407 (Giraldo *et al.*, 2008).
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55 409 Ecological differentiation between *H. melpomene* and *H. cydno* has been
56 410 documented in habitat preference and host-plant use (see Jiggins, 2008;
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58 411 Merrill *et al.*, 2013). Typically, *H. timareta*/*H. cydno* races are associated with
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3 412 high altitudinal ranges (between 500 to 2000 m) in the understory forest of the
4 413 northeastern Andes, while *H. melpomene* races are usually correlated with
5 414 tropical lowland habitats (0 to 1000 m) across Central and South America.
6 415 *Heliconius t. linaresi* has been collected during the last 10 years (~120
7 416 individuals) at two different locations on the understory forest of the
8 417 northeastern Andes slopes: 1) at Puerto Rico (Caquetá, Colombia) within an
9 418 altitude of ~1100 *masl* and 2) at Las Morras (Caquetá, Colombia) within an
10 419 altitude of ~1300 *masl*. Interestingly, *H. timareta* and *H. cydno* races,
11 420 including *H. t. linaresi*, are geographic replacements of each other along the
12 421 northeastern slopes of the Andes (Figure 1), which suggest some kind of
13 422 competitive exclusion between these two species. Additionally, *H. cydno*/*H.*
14 423 *timareta* and *H. melpomene* show contrasting host-plant use. On the one
15 424 hand, *Heliconius timareta*/*H. cydno* races are usually not host-specific,
16 425 whereas *H. melpomene* races, in general, are more host-specific (Smiley,
17 426 1978; Giraldo *et al.*, 2008; Jiggins, 2008; Merrill *et al.*, 2013). The host-plant
18 427 choice experiments carried out here imply that *H. t. linaresi* is not host-
19 428 specific, similar to *H. t. florencía* and other *H. cydno* races (Figure 5SI).
20 429 Therefore, habitat preference and host-plant use again imply some degree of
21 430 ecological isolation between *H. t. linaresi* and *H. melpomene*. Overall,
22 431 morphological and natural history data support the existence of a well-
23 432 established population clearly differentiated from *H. melpomene* races and
24 433 more closely related to the *H. timareta*/*cydno* clade. Moreover, despite the
25 434 phenotypic similarity of the black-yellow pattern between *H. t. linaresi* and *H.*
26 435 *c. cordula*, distinctive colour pattern traits suggest that this novel entity is more
27 436 closely related to the *H. timareta* lineage.
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438 ***Implications for the Heliconius butterfly radiation***

439 The rapid radiation of the Neotropical *Heliconius* butterflies has long
440 interested biologists. In particular, the diversification of the closely related
441 species *H. melpomene*, *H. cydno* and *H. timareta* has received great attention
442 in the last few years (Jiggins, 2008; Kronforst & Papa, 2015; Merrill *et al.*,
443 2015). Recent phylogenetic and phylogeographic studies of the radiation,
444 support the separation of *H. melpomene*, *H. cydno* and *H. timareta* as three
445 distinct species (Nadeau *et al.*, 2013; Arias *et al.*, 2014; Kozak *et al.*, 2015).

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3 446 However, the history of *H. cydno/H.timareta* diversification is still not well
4 447 understood. It has been suggested that a proto *H. cydno/H. timareta* evolved
5 448 in Central America and spread down the northeastern slopes of the Andes
6 449 and into the Magdalena and Cauca valleys, across to the western slopes of
7 450 the Andes (Arias *et al.*, 2014). At some point the *H. timareta* lineage diverged
8 451 and acquired colour pattern alleles through introgression from *H. melpomene*
9 452 and started mimicking races of this species in the northeastern slopes of the
10 453 Andes (Pardo-Diaz *et al.*, 2012; The *Heliconius* genome consortium, 2012)
11 454 (but see Brower, 2011; Brower, 2013) whereas the *H. cydno* lineage largely
12 455 tracked the phenotypic variation of the distantly related *H. sapho* and *H.*
13 456 *eleuchia*. In general *H. cydno* races have a complete yellow or white FW band
14 457 (Figure 1), suggesting that the proto *H. timareta/H.cydno* form likely had a
15 458 similar pattern. Perhaps, *H. t. linaresi* represents a remnant population of the
16 459 proto *H. cydno/H. timareta* that spread into the slopes of the northeastern
17 460 Andes and that later has acquired red elements by introgression from *H.*
18 461 *melpomene* races in different locations. Notably, *H. t. linaresi* is not obviously
19 462 mimetic with other taxa in its distribution, which contrasts with other *H.*
20 463 *timareta* races, which fall in the same mimicry ring as *H. melpomene* (Lamas,
21 464 1997; Giraldo *et al.*, 2008; Nadeau *et al.*, 2014).

22 465
23 466 In general, the genetic pattern observed together with morphological and
24 467 natural history data support the existence of a well-established race of *H.*
25 468 *timareta*, clearly differentiated from *H. cydno* and *H. melpomene* species. This
26 469 study highlights the importance of the use of a comprehensive approach to
27 470 understand the phenotypic and genetic diversity within species, which is
28 471 crucial to completely comprehend the mechanisms that promotes adaptation
29 472 and speciation in recent radiations.

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Appendix: Description of the New Subspecies

476 ***Heliconius timareta linaresi* Arias & Lamas, new subspecies (Figure 5SI)**

477 **Diagnosis**

478 As other recently described subspecies in the *Heliconius timareta* group
 479 (Giraldo *et al.*, 2008; Mérot *et al.*, 2013) this new taxon belongs to the
 480 *Heliconius cydno* clade [part of the ‘*numata* group’ of Holzinger & Holzinger
 481 (1994) and of the ‘*melpomene–cydno* lineage’ of Brower & Egan (1997)] (Arias
 482 *et al.*, 2014). *Heliconius timareta linaresi* is phenotypically similar to *H.*
 483 *timareta timareta* f. *timareta* Hewitson, 1867 and *H. cydno cordula* Neustetter
 484 (1913) (Figure 2 and 6SI). However, it is clearly distinguishable by the
 485 following six traits: a) the opaque black background coloration typical of *H.*
 486 *timareta* subspecies that contrasts with the blackish-blue iridescent coloration
 487 of *H. c. cordula*; b) the form of the forewing (FW) yellow post-median band
 488 with a smooth distal border in *H. t. linaresi* and *H. c. cordula*, but irregular in
 489 *H. t. timareta* f. *timareta* (Figure 2); c) the yellow FW post-median spot present
 490 below vein Cu₂ present in *H. t. linaresi* and *H. cordula* but absent in *H. t.*
 491 *timareta* f. *timareta*; d) a ‘red line’ at the base of the costal vein on the ventral
 492 side of FW, present in *H. t. linaresi* and *H. t. timareta* f. *timareta*, but absent in
 493 *H. c. cordula* (Figure 2); e) the ‘forceps’ element on the ventral side of the
 494 hindwing (HW), absent in *H. t. timareta* f. *timareta*, present in *H. c. cordula*
 495 and a small remnant in *H. t. linaresi*; and f) the basal ‘red spots’ on the HW
 496 ventral side ventral side, present in *H. t. linaresi* and *H. t. timareta* f. *timareta*,
 497 but absent in *H. c. cordula*.(Figure 2 and 6SI).

498

499 **Male**

500 FW length 38–44mm (mean 41.8mm; N=9). *Dorsal* wing colour opaque black,
 501 FW traversed by a yellow post-median band that extends from the distal end
 502 of the discal cell to the base of vein R₃, curving smoothly from subcosta to
 503 vein Cu₂, a yellow trapezoidal spot in the distal third of the discal cell, and
 504 another yellow, oval spot in the distal fourth of cell Cu₂-2A; HW completely
 505 opaque black, except for the area above subcosta, which has a shiny light
 506 buff. *Ventral* wing colour paler brown, FW with a ‘red line’ element (2.43–5.10

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3 507 mm in length; N=12) located at the base of the costal cell and crossed by a
4 508 pale yellow post-median band mirroring that present on the dorsal side; HW
5 509 with a series of three (>2mm in length) basal 'red spot' elements from cell
6 510 Sc+R1-Rs to cell Cu₂-2A, a conspicuous yellow costal streak, extending from
7 511 the base to one half to two-thirds the length of the costa, and a brown
8 512 remnant of the 'forceps' element behind vein 2A to the anal margin.

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15 514 **Female**

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17 515 FW length 37-44mm (mean 40.5mm; N=4). Phenotypically similar to the male,
18 516 but distinguishable by the dull dark brown costal area of the dorsal HW, the
19 517 five-segmented prothoracic tarsus (fused together in males) and the clearly
20 518 different external genitalia.

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25 520 **Type material:**

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27 521 Holotype ♂, Colombia, Caquetá, San Vicente del Caguán, Vereda Las
28 522 Morras, close to the Pato river, 2°06'49.61" N 74°47'09.40"W, 1300 m,
29 523 12.ii.2006 (M. Linares)(IAvH-E-163671), in the Colección de Mariposas del
30 524 Instituto de Investigación de Recursos Biológicos Alexander von Humboldt,
31 525 Villa de Leyva, Colombia (Figura 5SI). Paratype ♂, same data as the
32 526 holotype, but 5.ix.2009 (M. Linares) (ANDES-E16852), deposited in the
33 527 Museo de Historia Natural de la Universidad de los Andes, Bogotá, Colombia
34 528 (Figura 5SI); Paratype ♀, same data as holotype, 1300 m, but 14.i.2007 (M.
35 529 Linares) (IAvH-E-163672), in the Colección de Mariposas del Instituto de
36 530 Investigación de Recursos Biológicos Alexander von Humboldt, Villa de
37 531 Leyva, Colombia.

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47 533 **Etymology**

48 534 Here we propose to name this new *H. timareta* subspecies as *H. timareta*
49 535 *linaresi* after Mauricio Linares who has dedicated his scientific career to
50 536 studying evolution and speciation of Colombian *Heliconius* butterflies. A noun
51 537 in the genitive case.

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3 540 **Taxonomy and variation**

4 541 All available evidence (morphological, genetic, biogeographic and
5 542 behavioural) supports the existence of a well-established race of *H. timareta*,
6 543 a member of the *H. cydno* clade (Arias *et al.*, 2014 and this study). Individual
7 544 variation is not pronounced, however, there is some variation on the size of
8 545 the 'forceps' element.
9 546

10 547 Known (described and undescribed) *Heliconius timareta* subspecies are as
11 548 follows (in a latitudinal distribution, north to south): a) *linaresi* (this study); b)
12 549 *florencia* Giraldo *et al.* (2008), from Caquetá, eastern Colombia; c) *tristero*
13 550 *stat. nov.* Brower (1996a) [new status *sensu* Mérot *et al.* (2013)], from
14 551 Putumayo, southeastern Colombia; d) *timareta* Hewitson 1867, a polymorphic
15 552 subspecies from eastern Ecuador; e) an as yet undescribed subspecies from
16 553 eastern Ecuador Nadeau *et al.* (2014); f) an undescribed subspecies from
17 554 southeastern Ecuador Holzinger and Holzinger (1994); g) *timoratus* Lamas
18 555 (1997), from northern Amazonas, Peru, close to the border with Ecuador and
19 556 h) *thelxinoe* Mérot *et al.* (2013), from northeastern Peru (Figure 1).
20 557

21 558 **Distribution**

22 559 Currently known near the vicinity of San Vicente del Caguán, (Caquetá) along
23 560 the eastern slopes of the Colombian Andes, at elevations between 1100 and
24 561 1300 m.
25 562

26 563 **Habitat and behaviour**

27 564 *Heliconius timareta linaresi* is found in the forest understory, usually foraging
28 565 as adults on orange Cucurbitaceae flowers, such as *Psiguria* or *Gurania*, in
29 566 small sunny gaps or at forest edges. Males are more frequently seen than
30 567 females, flying fast in sunny patches and chasing females or other males.
31 568 Females lay solitary eggs, usually on young stems of the host plant. Host-
32 569 plant choice experiments suggest that *H. t. linaresi* is not host-specific. The
33 570 larva has a dark yellow-orange head capsule tone and light narrow bands on
34 571 the dorsal region behind the head similar to *H. t. florencia*.
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802 **Figures**

803 **Figure 1. Distribution of *Heliconius timareta* subspecies.** In general, *H.*
804 *timareta* subspecies form mimetic rings with *H. melpomene*. However, there
805 are a number of exceptions. Notably, *H. heurippa* and *H. t. linaresi* are not
806 obviously mimetic with other taxa in their distribution. In addition, there is a
807 polymorphic population in Ecuador that are not obviously involved in mimicry
808 with the local butterfly community. Interestingly, *H. t. linaresi* resembles
809 almost perfectly *H. c. cordula* and *H. t. timareta f. timareta*, species found on
810 the eastern slopes of the Andes.

811
812 **Figure 2. Colour pattern similarities and differences among *H. c. cordula*, *H. t.***
813 ***linaresi* and *H. t. timareta f. timareta*.** From left to right *H. c. cordula*, *H. t. linaresi*
814 and *H. t. timareta f. timareta* are presented: a) Complete dorsal view of the three
815 taxa. Note the black-blue iridescent coloration of *H. c. cordula*, but the opaque black
816 coloration of *H. t. linaresi* and *H. t. timareta f. timareta*; b) Dorsal view of the
817 Forewing (FW)– the FW has three principal pattern elements in a black background
818 consisting by an irregular yellow postmedial band extending from distal end of discal
819 cell to R₁-R₃ fork and laterally from subcostal to CU_{1b}, a yellow bowtie element in the
820 discal cell and an oval element below CU_{1b}. Both *H. t. linaresi* and *H. c. cordula* show
821 a similar FW yellow band, but irregular in *H. t. timareta f. timareta*; c) Ventral view of
822 FW– the ventral FW has two principal pattern elements in a black background
823 consisting by a yellow band similar between the three taxa and a ‘red line’ present in
824 *H. t. linaresi* and *H. t. timareta f. timareta*, but absent in *H. c. cordula*. d) Ventral view
825 of Hindwing (HW)– the ventral HW has two color pattern elements in a black
826 background, the ‘forceps’ that is present in all *H. c. cordula*, absent in *H. t. timareta f.*
827 *timareta*, but with a small remnant of this element in *H. t. linaresi*; the ‘red spots’
828 element which is present in *H. t. linaresi* and *H. t. timareta f. timareta*, but absent in
829 *H. c. cordula*. Arrows point to important diagnostic traits between the three forms.

830
831 **Figure 3. Col-Coll and Tpi Phylogenetic trees.** Phylogenetic relationships of
832 *Heliconius timareta linaresi* (indicated with a star) and closely related
833 cognates (*H. cydno*, *H. melpomene* and *H. timareta* subspecies). Posterior
834 probability values (on the numerator) were estimated using Bayesian analysis
835 and bootstrap support (on the denominator) derived from a Maximum
836 Likelihood analysis are displayed over the branches.

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4 838 **Figure 4. Population assignment test.** a) Multilocus microsatellite data for
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6 839 *H. timareta linaresi* (highlighted in grey), another *H. timareta* subspecies
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8 840 (*florenzia*), *H. cydno* and *H. melpomene*. For comparison in panel b) AFLP
9
10 841 data modified from Arias *et al.*, 2014 is presented. Bar plots showing
11
12 842 Bayesian assignment probabilities from the software Structure 2.3.4. Each
13
14 843 horizontal bar corresponds to one individual. The proportion of colour on the
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16 844 bar represents an individual's assignment probability to the different clusters.
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18 845 Black-vertical bars on the left and right of each panel show phenotypic and
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20 846 species classification respectively.

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H. c. cordula



H. heurippa



H. t. linaresi



H. t. florenzia



H. t. tristero stat. nov.



H. t. timareta f. timareta



H. t. timareta f. configua



H. t. timoratus



H. t. thelxinoe



H. m. mallei



H. m. bellula

H. m. ecuadorensis



H. m. amaryllis



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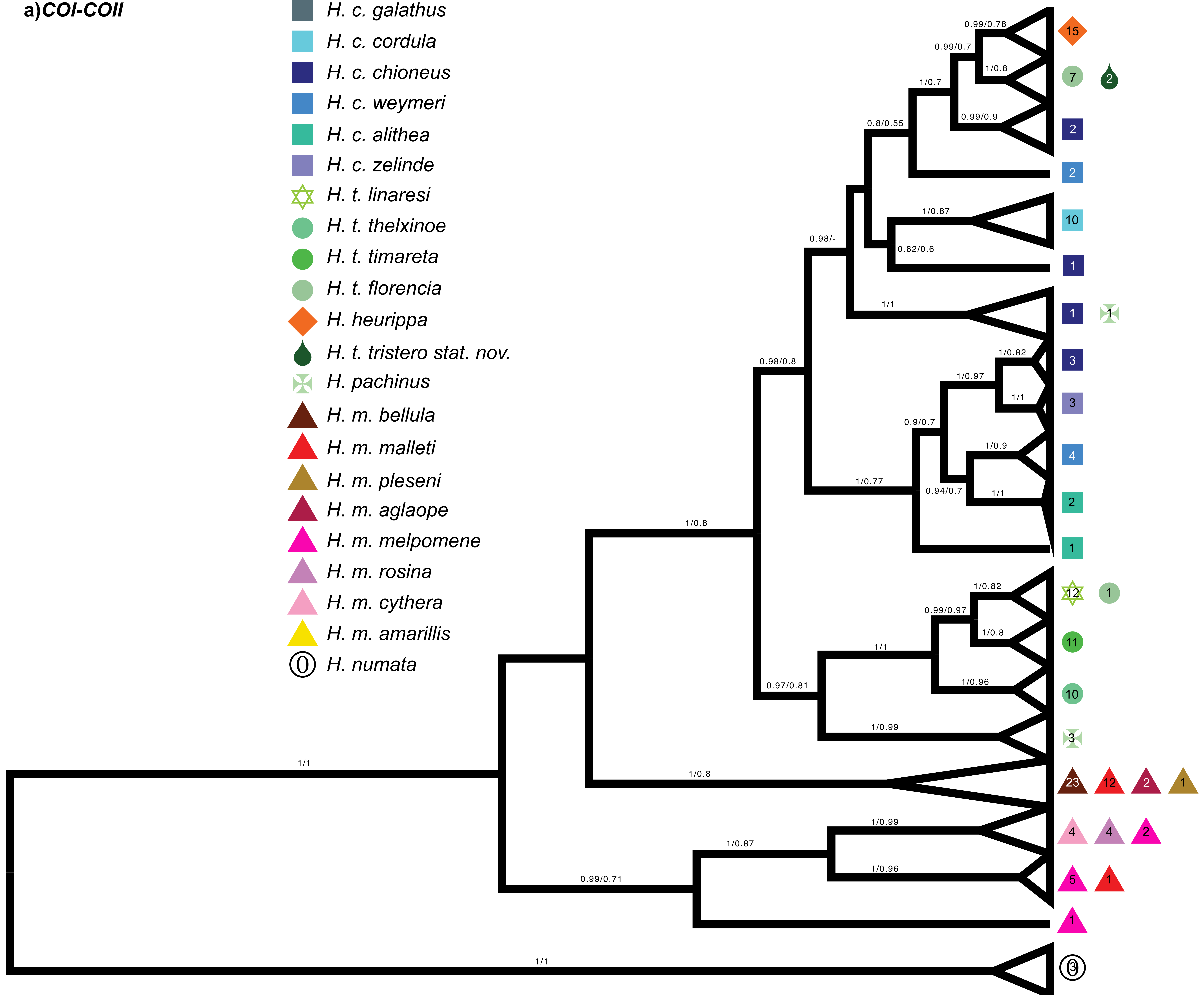
H. c. cordula

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H. t. timareta

a) COI-COII

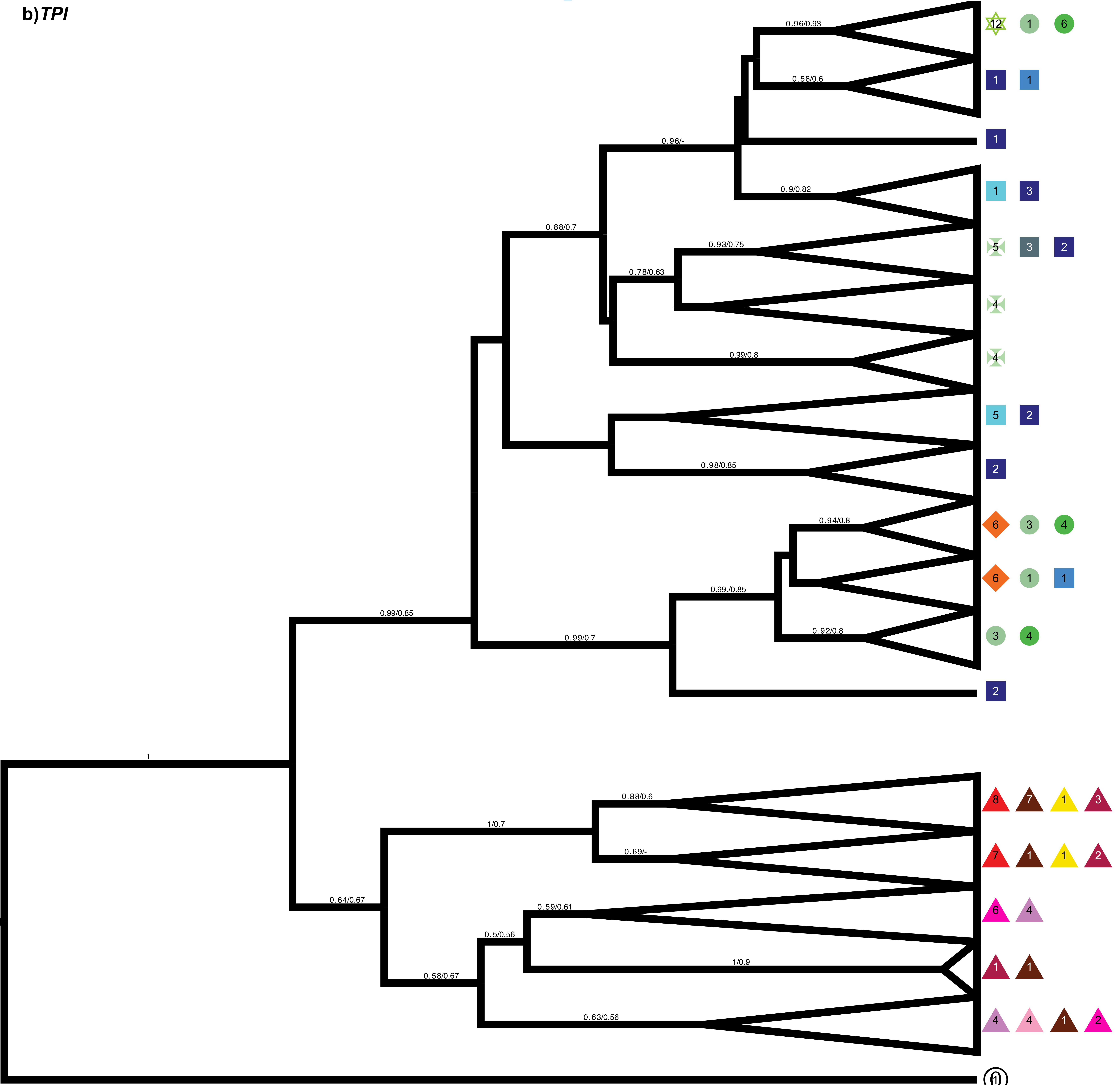
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- H. t. linaresi*
- H. t. thelxinoe*
- H. t. timareta*
- H. t. florencia*
- H. heurippa*
- H. t. tristero stat. nov.*
- H. pachinus*
- H. m. bellula*
- H. m. malleti*
- H. m. pleseni*
- H. m. aglaope*
- H. m. melpomene*
- H. m. rosina*
- H. m. cythera*
- H. m. amarillis*
- H. numata*



H. cydno / *H. heurippa* / *H. t. florencia* / *H. t. tristero* / *H. pachinus* *H. timareta* *H. melpomene*

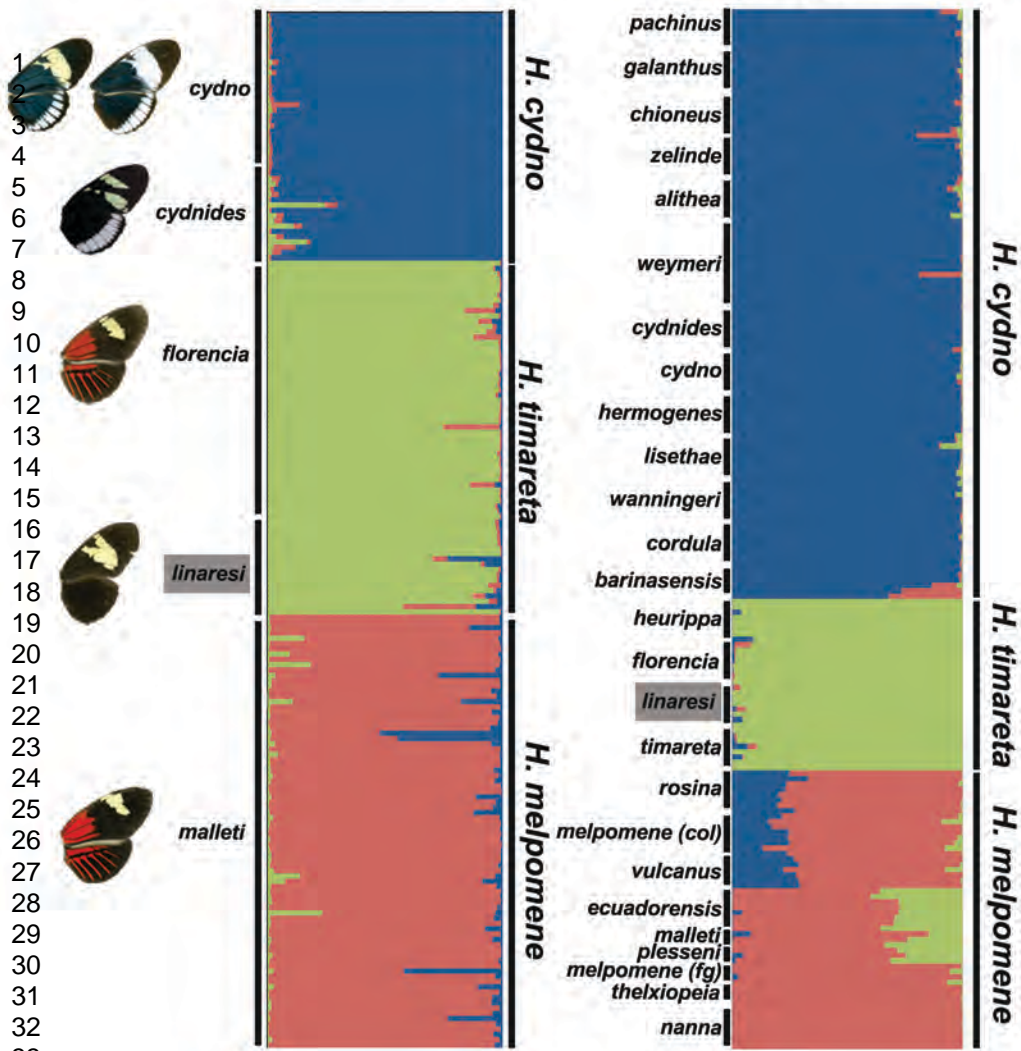
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b) TPI



H. cydno / *H. timareta* / *H. heurippa* / *H. pachinus* *H. melpomene*

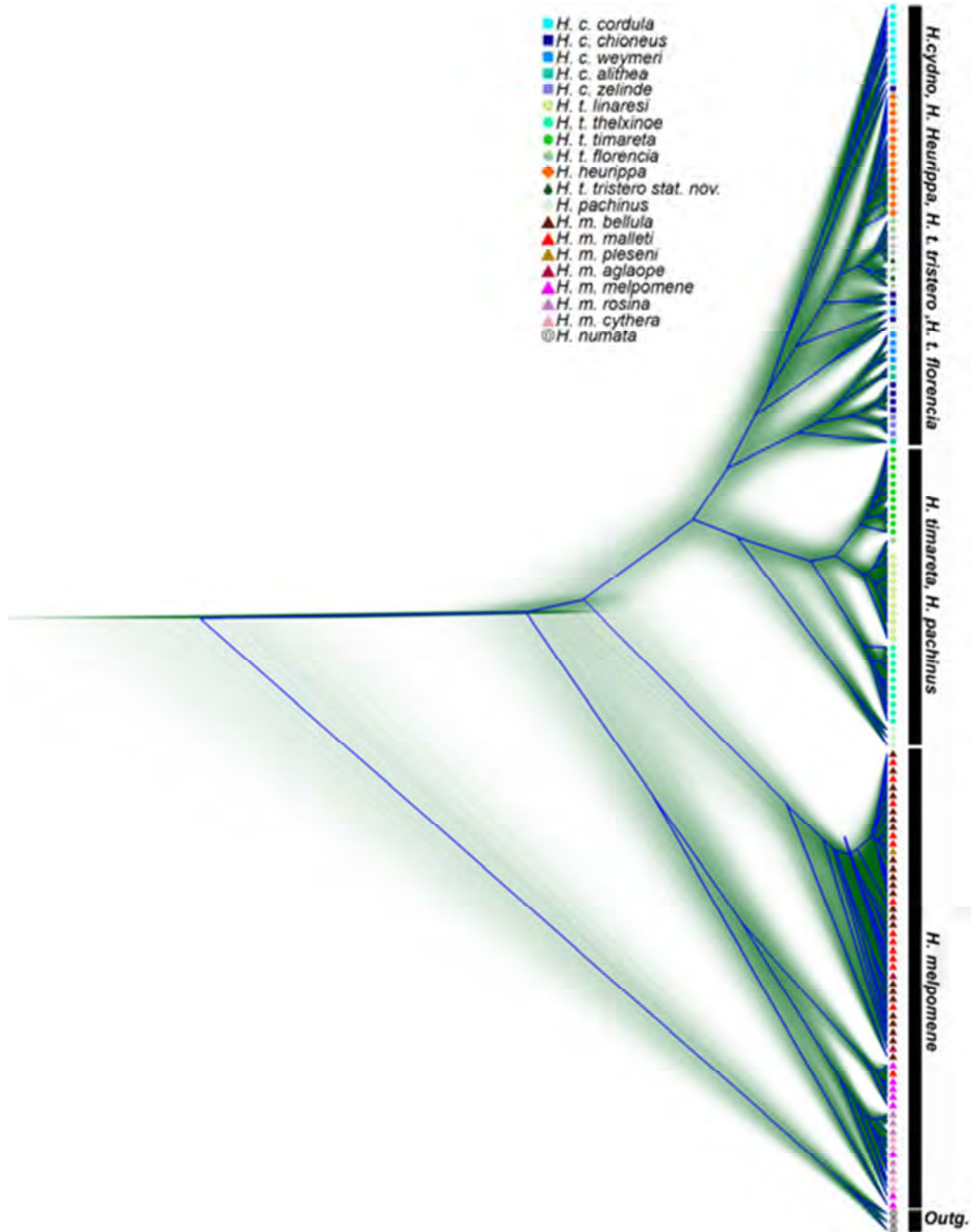
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1 **Supporting information**

2 **Figure 1SI. Coalescent reconstruction in BEAST for the *COI-COII* locus.**

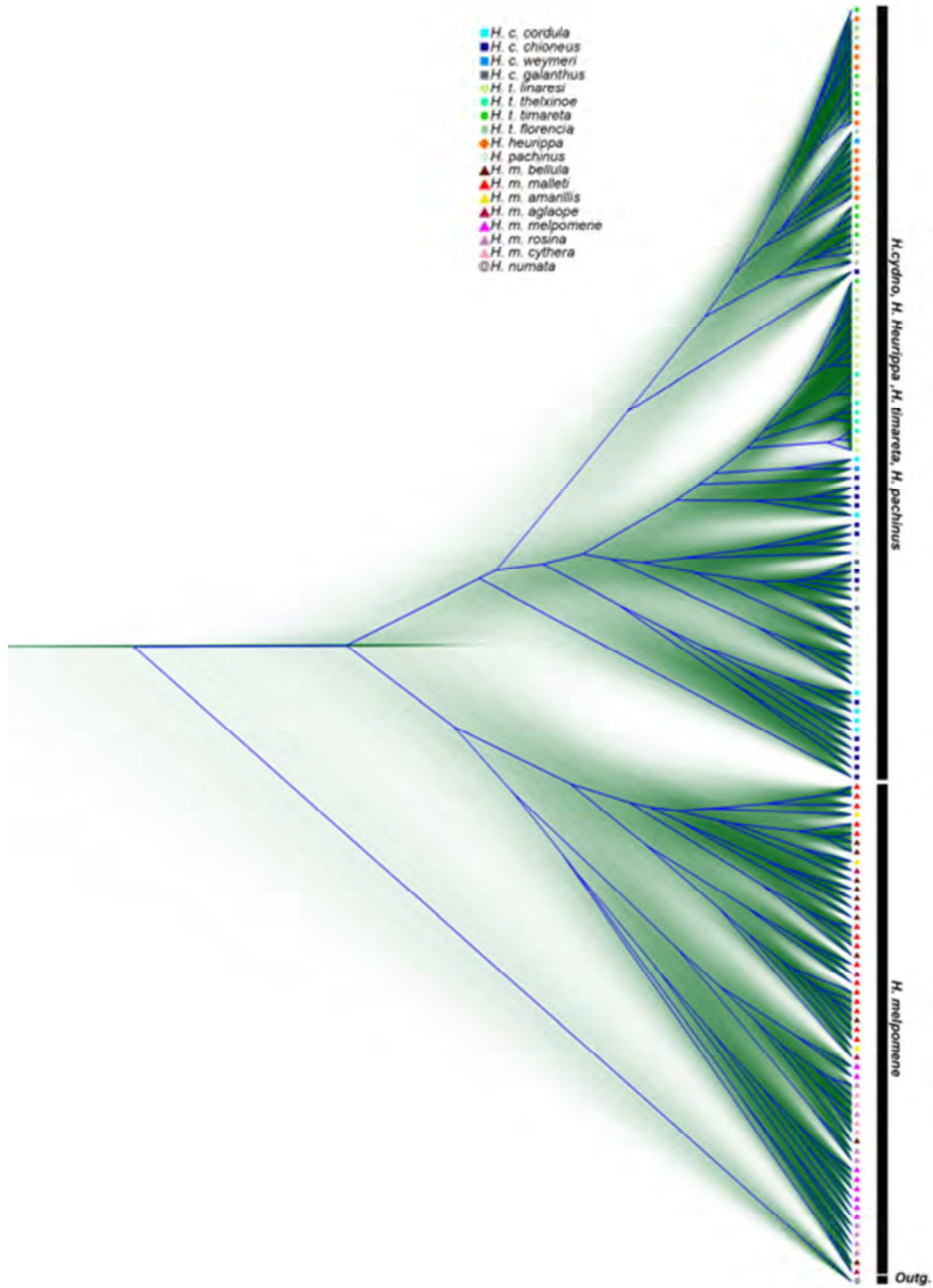
3 Trees are visualised with DensiTree (Bouckaert & Heled, 2014) displaying all
 4 trees of the Markov chain Monte Carlo chain with a burn-in of 7500 trees.
 5 Higher levels of uncertainty are represented by lower densities in green. A
 6 root-canal tree is presented in blue to guide the eye.
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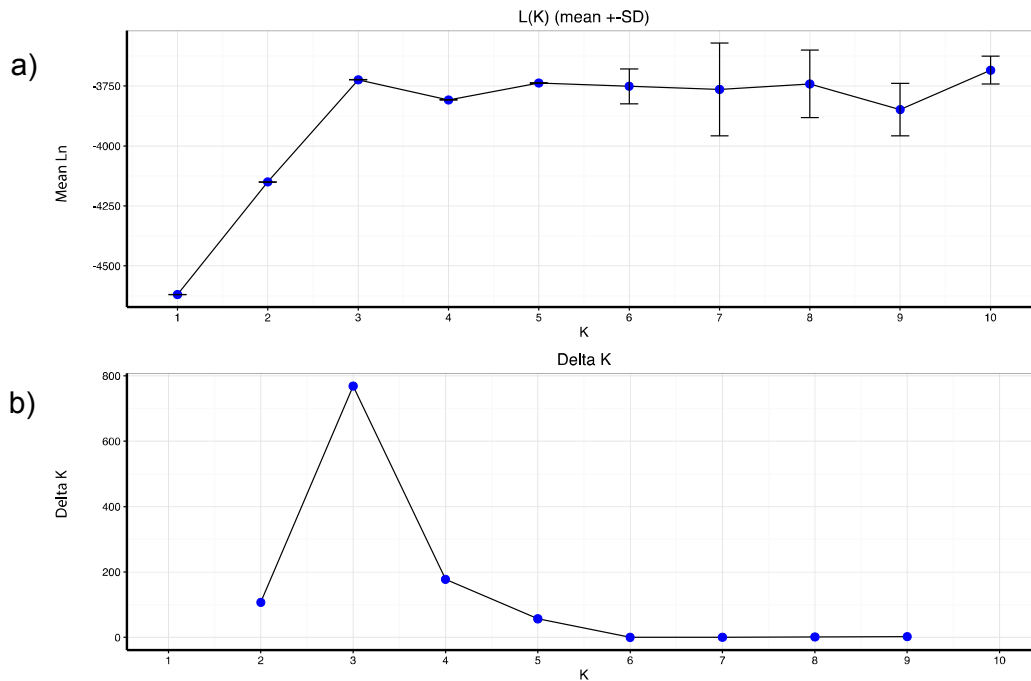
10 **Figure 2SI. Figure 1SI. Coalescent reconstruction in BEAST for the *TPI***
 11 **locus.** Trees are visualised with DensiTree (Bouckaert & Heled, 2014)
 12 displaying all trees of the Markov chain Monte Carlo chain with a burn-in of
 13 7500 trees. Higher levels of uncertainty are represented by lower densities in
 14 green. A root-canal tree is presented in blue to guide the eye.



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17 **Figure 3SI. Estimation of the number of *Heliconius* clusters (K).** In a) is
 18 showed the mean Ln probability of the data (Ln P(K)) (Pritchard, Stephens &
 19 Donnelly, 2000) and in b) is displayed the second-order rate of change (ΔK)
 20 (Evanno, Regnaut & Goudet, 2005). The highest point in b) suggest that the
 21 best estimate is K=3.



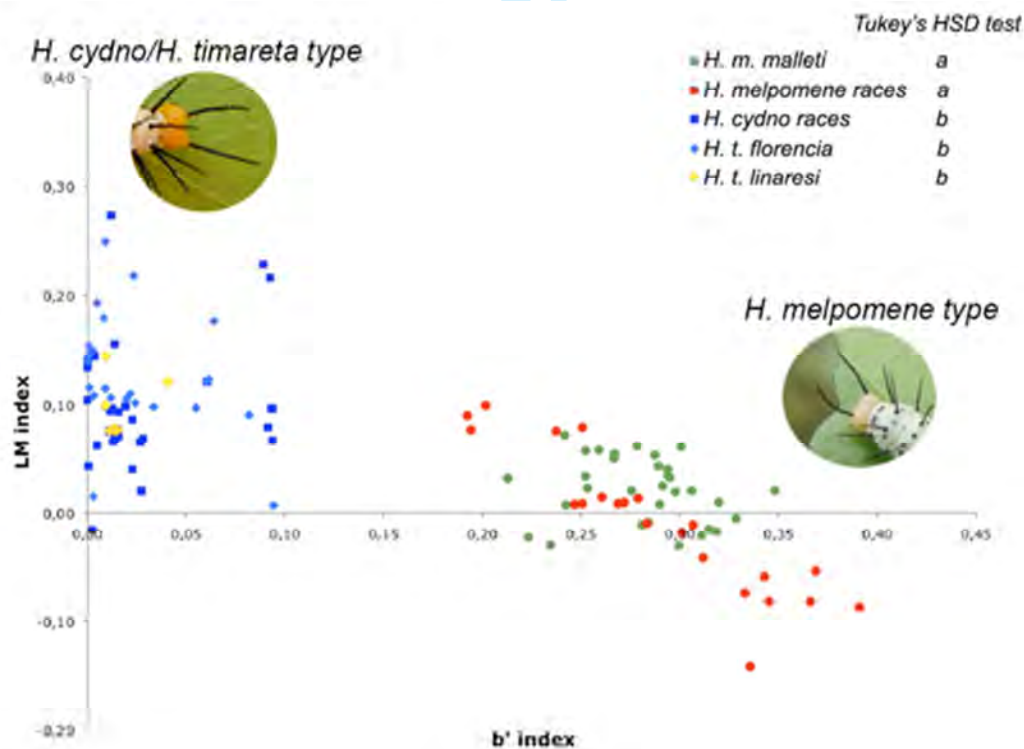
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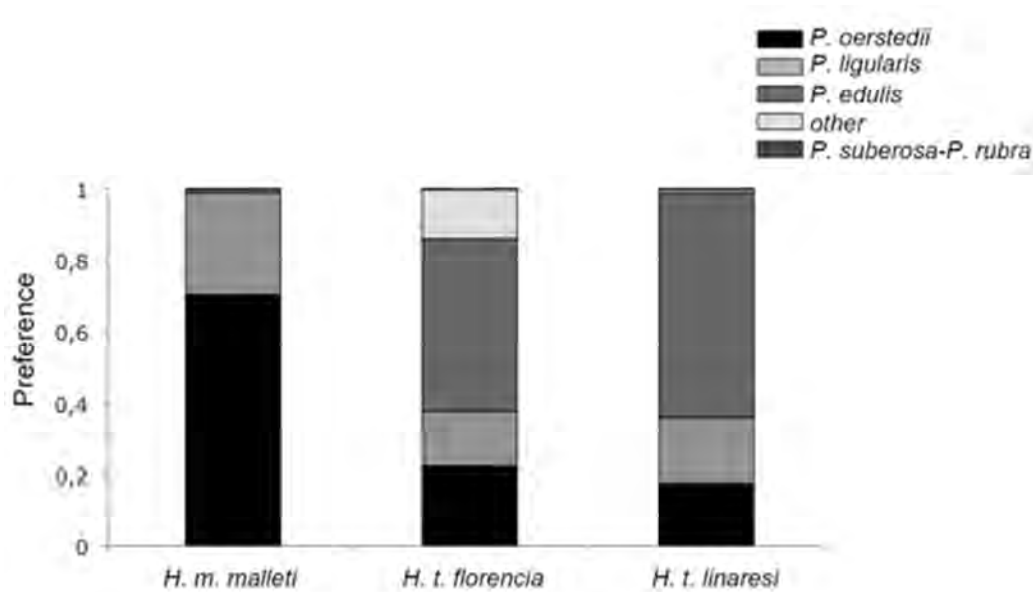
24 **Figure 4SI. Larvae colour dispersion index b' and index LM, based on**
 25 **intensity and brightness of the head capsule.** Larvae of *H. melpomene*
 26 and *H. cydno/H. timareta* are distinguishable by cephalic colour, which is pale
 27 yellow in *H. melpomene* and bright orange in *H. cydno/H. timareta*. Moreover,
 28 *H. cydno/H. timareta* larvae has two light narrow bands on the dorsal view
 29 behind the head, while *H. melpomene* larvae present two dark broad bands
 30 just behind the head. *Heliconius melpomene* races are: *H. m. malleti*, *H. m.*
 31 *bellula* and *H. m. vulcanus*; and *H. cydno* races are: *H. c. cordula*, *H. c.*
 32 *cydnides* and *H. c. zelinde*. In fact, we found significant differences in head
 33 capsule coloration between species/groups (ANOVA, $df=4$, $p=2.2 \times 10^{-16}$). In
 34 particular, head capsule coloration analyses showed that offspring of wild *H. t.*
 35 *linaresi* females share a similar colour index with *H. t. florencia* and other *H.*
 36 *cydno* races (Tukey's HSD test $p>0.01$), but significantly different from *H. m.*
 37 *malleti* and other *H. melpomene* races (Tukey's HSD test $p<0.01$). Results
 38 from the Tukey's test are presented next to colour code legend, where
 39 species/groups that present the same letter do not show significant
 40 differences in b' and LM indexes.



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43 **Figure 5SI. Host plant preference.** Egg laying percentage for each type of
 44 female: *H.m. malleti* (j_1), *H.t. florencia* (j_2) and *H.t. linaresi* (j_3). Labels,
 45 oerstedii: *Passiflora oerstedii*; Granadilla: *P. ligularis*; Passion fruit: *P. edulis*;
 46 other: *P. quadrangularis*, *P. arborea* and *P. maliformis* ; suberosa, rubra: *P.*
 47 *suberosa* and *P. rubra*. *Heliconius t. linaresi* females displayed a similar laying
 48 behaviour to *H. t. florencia*, ovipositing on many species, but different from *H.*
 49 *m. malleti* ($\text{LnL}=-638.749$ for a two parameter model, $j_1 \neq j_2 = j_3$).



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3 53 **Figure 6SI. *H. timareta* subspecies. *Heliconius timareta linaresi* (Holotype).**
4 54 Individual is lodged at the Colección de Mariposas del Instituto de
5 55 Investigación de Recursos Biológicos Alexander von Humboldt, Villa de
6 56 Leyva, Colombia (accession numbers: IAvH-E-163671). a. dorsal view, and b.
7 57 ventral view.
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Review Only

62 **Table 1SI. Individuals used in phylogenetic analyses.** Gene accession number and locality of individuals included in the
 63 phylogenetic analyses.

Species	subspecies	individual	Genbank <i>Tpi</i>	Genbank <i>Col-Coll</i>	Locality
<i>H.melpomene</i>	<i>rosina</i>	M544	AF413789	AF413674	Panamá
<i>H.melpomene</i>	<i>rosina</i>	M544	AF413790		Panamá
<i>H.melpomene</i>	<i>rosina</i>	M533	AF545457	AF512987	Panamá
<i>H.melpomene</i>	<i>rosina</i>	M532	AF545455	AF512988	Panamá
<i>H.melpomene</i>	<i>rosina</i>	M532	AF545456		Panamá
<i>H.melpomene</i>	<i>rosina</i>	M546	AF545452	AF512982	Panamá
<i>H.melpomene</i>	<i>rosina</i>	M811	AF413781	AF413673	Panamá
<i>H.melpomene</i>	<i>cythera</i>	8073	AF413779	AF413678	Ecuador
<i>H.melpomene</i>	<i>cythera</i>	8073	AF413780		Ecuador
<i>H.melpomene</i>	<i>cythera</i>	8074	AF413781	AF413677	Ecuador
<i>H.melpomene</i>	<i>cythera</i>	8074	AF413782	AF413677	Ecuador
<i>H.melpomene</i>	<i>cythera</i>	2856		KP074818	Ecuador
<i>H.melpomene</i>	<i>melpomene</i>	M415	AF545465	AF512975	French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	M415	AF545466		French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	C1384		JN897489	French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	M437	AF41391		French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	M437	AF41392		French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	M436	AF413774	AF413675	French Guyana
<i>H.melpomene</i>	<i>melpomene</i>	M436	AF413775		French Guyana

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7	<i>H.melpomene</i>	<i>melpomene</i>	507	AF45460		French Guyana
8	<i>H.melpomene</i>	<i>melpomene</i>	M115	DQ019228		Colombia
9	<i>H.melpomene</i>	<i>amaryllis</i>	JM1917	AY329833		Perú
10	<i>H.melpomene</i>	<i>amaryllis</i>	JM1271	-		Perú
11	<i>H.melpomene</i>	<i>amaryllis</i>	04-288		AM709831	Perú
12	<i>H.melpomene</i>	<i>amaryllis</i>	02x1850		HM052032	Ecuador
13	<i>H.melpomene</i>	<i>malleti</i>	8158	AY548152	AY548140	Perú
14	<i>H.melpomene</i>	<i>malleti</i>	11444	EU852063		Perú
15	<i>H.melpomene</i>	<i>aglaope</i>	JM1178	AY329831		Perú
16	<i>H.melpomene</i>	<i>aglaope</i>	JM1174	AY329829		Perú
17	<i>H.melpomene</i>	<i>aglaope</i>	04-286		AM709829	Perú
18	<i>H.melpomene</i>	<i>aglaope</i>	04-288		AM709830	Perú
19	<i>H.melpomene</i>	<i>aglaope</i>	E-8-1		U08487	Ecuador
20	<i>H.melpomene</i>	<i>malleti</i>	M272	EU852064	EU848500	Sucre, Florencia, Colombia
21	<i>H.melpomene</i>	<i>malleti</i>	M430	EU852065	EU848501	Sucre, Florencia, Colombia
22	<i>H.melpomene</i>	<i>malleti</i>	M437	EU852066	EU848502	Sucre, Florencia, Colombia
23	<i>H.melpomene</i>	<i>malleti</i>	M438	EU852067	EU848503	Sucre, Florencia, Colombia
24	<i>H.melpomene</i>	<i>malleti</i>	M454	EU852068	EU848504	Sucre, Florencia, Colombia
25	<i>H.melpomene</i>	<i>malleti</i>	M459	EU852069	EU848505	Sucre, Florencia, Colombia
26	<i>H.melpomene</i>	<i>malleti</i>	M470		EU848506	Sucre, Florencia, Colombia
27	<i>H.melpomene</i>	<i>malleti</i>	M502	EU852070	EU848507	Sucre, Florencia, Colombia
28	<i>H.melpomene</i>	<i>malleti</i>	M503		EU848508	Sucre, Florencia, Colombia
29	<i>H.melpomene</i>	<i>malleti</i>	M510	EU852071	EU848509	Sucre, Florencia, Colombia
30	<i>H.melpomene</i>	<i>malleti</i>	M512	EU852072	EU848510	Sucre, Florencia, Colombia
31	<i>H.melpomene</i>	<i>malleti</i>	M579	EU852073	EU848511	Sucre, Florencia, Colombia
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7	<i>H.melpomene</i>	<i>malleti</i>	M594		EU848512	Sucre, Florencia, Colombia
8	<i>H.melpomene</i>	<i>malleti</i>	M702	EU852074	EU848513	Sucre, Florencia, Colombia
9	<i>H.melpomene</i>	<i>malleti</i>	M704	EU852075	EU848514	Sucre, Florencia, Colombia
10	<i>H.melpomene</i>	<i>malleti</i>	M707	EU852076	EU848515	Sucre, Florencia, Colombia
11	<i>H.melpomene</i>	<i>malleti</i>	M1002		EU848516	Sucre, Florencia, Colombia
12	<i>H.melpomene</i>	<i>malleti</i>	STRIB8158		AY748026	Sucre, Florencia, Colombia
13	<i>H.melpomene</i>	<i>malleti</i>	8230		-	Sucre, Florencia, Colombia
14	<i>H.melpomene</i>	<i>malleti</i>	8159		-	Ecuador
15	<i>H.melpomene</i>	<i>malleti</i>	M211		-	Sucre, Florencia, Colombia
16	<i>H.melpomene</i>	<i>malleti</i>	M243		-	Sucre, Florencia, Colombia
17	<i>H.melpomene</i>	<i>malleti</i>	M244		-	Sucre, Florencia, Colombia
18	<i>H.melpomene</i>	<i>malleti</i>	M243		-	Sucre, Florencia, Colombia
19	<i>H.melpomene</i>	<i>malleti</i>	M244		-	Sucre, Florencia, Colombia
20	<i>H.melpomene</i>	<i>malleti</i>	M244		-	Sucre, Florencia, Colombia
21	<i>H.melpomene</i>	<i>bellula</i>	C-14-8		U08476	mocoa, Colombia
22	<i>H.melpomene</i>	<i>bellula</i>	M155	DQ019241	AY548136	mocoa, Colombia
23	<i>H.melpomene</i>	<i>bellula</i>	M155	DQ019242		mocoa, Colombia
24	<i>H.melpomene</i>	<i>bellula</i>	M67	AY548146	AY548120	mocoa, Colombia
25	<i>H.melpomene</i>	<i>bellula</i>	M67	AY548149		mocoa, Colombia
26	<i>H.melpomene</i>	<i>bellula</i>	M67	AY548149		mocoa, Colombia
27	<i>H.melpomene</i>	<i>bellula</i>	M74	AY548147	AY548125	mocoa, Colombia
28	<i>H.melpomene</i>	<i>bellula</i>	M74	AY548147	AY548125	mocoa, Colombia
29	<i>H.melpomene</i>	<i>bellula</i>	M74	AY548141		mocoa, Colombia
30	<i>H.melpomene</i>	<i>bellula</i>	M78		AY548128	mocoa, Colombia
31	<i>H.melpomene</i>	<i>bellula</i>	M158		AY548138	mocoa, Colombia
32	<i>H.melpomene</i>	<i>bellula</i>	M156		AY548137	mocoa, Colombia
33	<i>H.melpomene</i>	<i>bellula</i>	M156		AY548137	mocoa, Colombia
34	<i>H.melpomene</i>	<i>bellula</i>	M82		AY548129	mocoa, Colombia
35	<i>H.melpomene</i>	<i>bellula</i>	M31	AY548144	AY548119	mocoa, Colombia
36	<i>H.melpomene</i>	<i>bellula</i>	M31	AY548145		mocoa, Colombia
37	<i>H.melpomene</i>	<i>bellula</i>	M31	AY548145		mocoa, Colombia
38	<i>H.melpomene</i>	<i>bellula</i>	M70		AY548123	mocoa, Colombia
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7	<i>H.melpomene</i>	<i>bellula</i>	M72	AY548124	mocoa, Colombia
8	<i>H.melpomene</i>	<i>bellula</i>	M151	AY548133	mocoa, Colombia
9	<i>H.melpomene</i>	<i>bellula</i>	M156	AY548137	mocoa, Colombia
10	<i>H.melpomene</i>	<i>bellula</i>	M152	AY548134	mocoa, Colombia
11	<i>H.melpomene</i>	<i>bellula</i>	M149	AY548132	mocoa, Colombia
12	<i>H.melpomene</i>	<i>bellula</i>	M129	AY548131	mocoa, Colombia
13	<i>H.melpomene</i>	<i>bellula</i>	M30	AY548118	mocoa, Colombia
14	<i>H.melpomene</i>	<i>bellula</i>	M76	AY548126	mocoa, Colombia
15	<i>H.melpomene</i>	<i>bellula</i>	M68	AY548121	mocoa, Colombia
16	<i>H.melpomene</i>	<i>bellula</i>	M26	AY548117	mocoa, Colombia
17	<i>H.melpomene</i>	<i>bellula</i>	M78	AY548128	mocoa, Colombia
18	<i>H.melpomene</i>	<i>bellula</i>	M77	AY548127	mocoa, Colombia
19	<i>H.melpomene</i>	<i>bellula</i>	M69	AY548122	mocoa, Colombia
20	<i>H.melpomene</i>	<i>bellula</i>	M154	AY548135	mocoa, Colombia
21	<i>H.melpomene</i>	<i>bellula</i>	M75	AY548149	mocoa, Colombia
22	<i>H.melpomene</i>	<i>bellula</i>	M75	AY548148	mocoa, Colombia
23	<i>H.melpomene</i>	<i>plesseni</i>	9156	KP074823	Ecuador
24	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-569	AF545441	Panamá
25	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-559	AF545439	Panamá
26	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-566	AF545445	Panamá
27	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-566	AF545446	Panamá
28	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-567	AF545443	Panamá
29	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-567	AF545444	Panamá
30	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-553	AF413785	Panamá
31	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-553	AF413786	Panamá
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7	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-570	AF413787	AF413707	Panamá
8	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-570	AF413788		Panamá
9	<i>H.cydno</i>	<i>chioneus</i>	M90		AY548130	Panamá
10	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-557		KP074768	Panamá
11	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-560	AF545447	AF512992	Panamá
12	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-813		AF512978	Panamá
13	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-809		AF512980	Panamá
14	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-552	AF545448	AF512985	Panamá
15	<i>H.cydno</i>	<i>chioneus</i>	STRI-B-552	AF545449		Panamá
16	<i>H.cydno</i>	<i>weymeri</i>	M19	AY548142	AY548115	Valle del Cauca, Colombia
17	<i>H.cydno</i>	<i>weymeri</i>	M18	AY548143	AY548114	Valle del Cauca, Colombia
18	<i>H.cydno</i>	<i>weymeri</i>	M20		AY548116	Valle del Cauca, Colombia
19	<i>H.cydno</i>	<i>galanthus</i>	TX511	DQ448499		Costa Rica
20	<i>H.cydno</i>	<i>galanthus</i>	TX514	DQ448500		Costa Rica
21	<i>H.cydno</i>	<i>galanthus</i>	TX515	DQ448501		Costa Rica
22	<i>H.cydno</i>	<i>cordula</i>	M101	DQ019234	DQ019244	San Cristobal, Venezuela
23	<i>H.cydno</i>	<i>cordula</i>	M101	DQ019235		San Cristobal, Venezuela
24	<i>H.cydno</i>	<i>cordula</i>	M104	DQ019236	DQ019245	San Cristobal, Venezuela
25	<i>H.cydno</i>	<i>cordula</i>	M104	DQ019237		San Cristobal, Venezuela
26	<i>H.cydno</i>	<i>cordula</i>	M187	DQ019238	DQ019251	San Cristobal, Venezuela
27	<i>H.cydno</i>	<i>cordula</i>	M182	DQ019239	DQ019250	San Cristobal, Venezuela
28	<i>H.cydno</i>	<i>cordula</i>	M105		DQ019246	San Cristobal, Venezuela
29	<i>H.cydno</i>	<i>cordula</i>	SC4		-	San Cristobal, Venezuela
30	<i>H.cydno</i>	<i>cordula</i>	SC27		-	San Cristobal, Venezuela
31	<i>H.cydno</i>	<i>cordula</i>		2096	KP074769	San Cristobal, Venezuela
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<i>H.heurippa</i>	8		KP074806	Cundinamarca, Colombia
<i>H.heurippa</i>	H8		DQ674448	Cundinamarca, Colombia
<i>H.heurippa</i>	H3		DQ674443	Cundinamarca, Colombia
<i>H.heurippa</i>	H7		DQ674447	Cundinamarca, Colombia
<i>H.heurippa</i>	H5		DQ674445	Cundinamarca, Colombia
<i>H.heurippa</i>	H4		DQ674444	Cundinamarca, Colombia
<i>H.heurippa</i>	H1		DQ674441	Cundinamarca, Colombia
<i>H.heurippa</i>	H2		DQ674442	Cundinamarca, Colombia
<i>H.heurippa</i>	H6		DQ674446	Cundinamarca, Colombia
<i>H.heurippa</i>	H9		DQ674449	Cundinamarca, Colombia
<i>H.heurippa</i>	H10		DQ674450	Cundinamarca, Colombia
<i>H.heurippa</i>	H11		DQ674451	Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-40	DQ674430	AY748059	Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-8	AF413777	AF413680	Cundinamarca, Colombia
<i>H.heurippa</i>	2417		KP074805	Cundinamarca, Colombia
<i>H.heurippa</i>	M4	DQ674440		Cundinamarca, Colombia
<i>H.heurippa</i>	M141	DQ674439		Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-34	DQ674438		Cundinamarca, Colombia
<i>H.heurippa</i>	M8	DQ674437		Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-39	DQ674436		Cundinamarca, Colombia
<i>H.heurippa</i>	M12	DQ674435		Cundinamarca, Colombia
<i>H.heurippa</i>	M17	DQ674434		Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-44	DQ674433		Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-44	DQ674432		Cundinamarca, Colombia
<i>H.heurippa</i>	STRI-B-51	DQ674431		Cundinamarca, Colombia

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7	<i>H.pachinus</i>		STRI-B-8036	AF413778	Costa Rica
8	<i>H.pachinus</i>		clone 10	DQ448511	Costa Rica
9	<i>H.pachinus</i>		clone 9	DQ448510	Costa Rica
10	<i>H.pachinus</i>		clone 8	DQ448509	Costa Rica
11	<i>H.pachinus</i>		clone 7	DQ448508	Costa Rica
12	<i>H.pachinus</i>		clone 6	DQ448507	Costa Rica
13	<i>H.pachinus</i>		clone 5	DQ448506	Costa Rica
14	<i>H.pachinus</i>		clone 4	DQ448505	Costa Rica
15	<i>H.pachinus</i>		clone 3	DQ448504	Costa Rica
16	<i>H.pachinus</i>		clone 2	DQ448503	Costa Rica
17	<i>H.pachinus</i>		clone 1	DQ448502	Costa Rica
18	<i>H.pachinus</i>		STRI-B-8023	AY748058	Costa Rica
19	<i>H.pachinus</i>		STRI-B-8036	AF413679	Costa Rica
20	<i>H.pachinus</i>		STRI-B-3035	KP074829	Costa Rica
21	<i>H.pachinus</i>		STRI-B-8021	-	Costa Rica
22	<i>H.pachinus</i>				
23	<i>H.pachinus</i>				
24	<i>H.pachinus</i>				
25	<i>H.pachinus</i>				
26	<i>H.timareta</i>	<i>tristero stat</i>			
27		<i>nov</i>	C-13-4	U08475	Colombia
28	<i>H.timareta</i>	<i>tristero stat</i>			
29		<i>nov</i>	C-15-4	U08477	Colombia
30	<i>H.timareta</i>	<i>thelxinoe</i>	02x2146	KC435427	Peru
31	<i>H.timareta</i>	<i>thelxinoe</i>	02x1598	KC435428	Peru
32	<i>H.timareta</i>	<i>thelxinoe</i>	mel2173	KC435429	Peru
33	<i>H.timareta</i>	<i>thelxinoe</i>	mel922	KC435430	Peru
34	<i>H.timareta</i>	<i>thelxinoe</i>	mel1517	KC435431	Peru
35	<i>H.timareta</i>	<i>thelxinoe</i>	mel1927	KC435432	Peru
36	<i>H.timareta</i>	<i>thelxinoe</i>	mel1929	KC435433	Peru
37	<i>H.timareta</i>	<i>thelxinoe</i>			
38	<i>H.timareta</i>	<i>thelxinoe</i>			
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7	<i>H.timareta</i>	<i>thelxinoe</i>	mel1937		KC435434	Peru
8	<i>H.timareta</i>	<i>thelxinoe</i>	mel1934		KC435435	Peru
9	<i>H.timareta</i>	<i>thelxinoe</i>	mel1933		KC435436	Peru
10	<i>H.timareta</i>	<i>florencia</i>	M326	EU852077		Sucre, Florencia, Colombia
11	<i>H.timareta</i>	<i>florencia</i>	M399	EU852078	EU848517	Sucre, Florencia, Colombia
12	<i>H.timareta</i>	<i>florencia</i>	M419	EU852079	EU848518	Sucre, Florencia, Colombia
13	<i>H.timareta</i>	<i>florencia</i>	M436	EU852080	EU848519	Sucre, Florencia, Colombia
14	<i>H.timareta</i>	<i>florencia</i>	M473	EU852081	EU848520	Sucre, Florencia, Colombia
15	<i>H.timareta</i>	<i>florencia</i>	M486		EU848521	Sucre, Florencia, Colombia
16	<i>H.timareta</i>	<i>florencia</i>	M504	EU852082		Sucre, Florencia, Colombia
17	<i>H.timareta</i>	<i>florencia</i>	M684	EU852083		Sucre, Florencia, Colombia
18	<i>H.timareta</i>	<i>florencia</i>	M703		EU848522	Sucre, Florencia, Colombia
19	<i>H.timareta</i>	<i>florencia</i>	M705	EU852084		Sucre, Florencia, Colombia
20	<i>H.timareta</i>	<i>florencia</i>	M1009		EU848523	Sucre, Florencia, Colombia
21	<i>H.timareta</i>	<i>timareta</i>	STRI-B-8520	EU852085	AY748060	Ecuador
22	<i>H.timareta</i>	<i>timareta</i>	STRI-B-8521	EU852086	AY748028	Ecuador
23	<i>H.timareta</i>	<i>timareta</i>	STRI-B-8521	EU852087		Ecuador
24	<i>H.timareta</i>	<i>timareta</i>	STRI-B-11436	EU852088	EU848524	Ecuador
25	<i>H.timareta</i>	<i>timareta</i>	STRI-B-11413	EU852089	EU848525	Ecuador
26	<i>H.timareta</i>	<i>timareta</i>	STRI-B-8529	EU852090	EU848526	Ecuador
27	<i>H.timareta</i>	<i>timareta</i>	STRI-B-11432	EU852091	EU848527	Ecuador
28	<i>H.timareta</i>	<i>timareta</i>	STRI-B-11439	EU852092	EU848528	Ecuador
29	<i>H.timareta</i>	<i>timareta</i>	STRI-B-8533	EU852093	EU848529	Ecuador
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<i>H.timareta</i>	<i>timareta</i>	STRI-B-8533	EU852094		Ecuador
<i>H.timareta</i>	<i>timareta</i>	STRI-B-8523	EU852095	EU848530	Ecuador
<i>H.timareta</i>	<i>linaresi</i>	M923	KU877723	KU877736	Puerto Rico, Caqueta, Colombia
<i>H.timareta</i>	<i>linaresi</i>	M1320	KU877724	KU877726	Puerto Rico, Caqueta, Colombia
<i>H.timareta</i>	<i>linaresi</i>	M1692	KU877714	KU877727	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M1839	KU877715	KU877737	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M1841	KU877716	KU877728	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2220	KU877717	KU877729	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2409	KU877718	KU877730	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2422	KU877725	KU877731	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2429	KU877719	KU877732	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2434	KU877720	KU877733	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2435	KU877721	KU877734	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.timareta</i>	<i>linaresi</i>	M2436	KU877722	KU877735	Las Morras, Puerto Amor, Caqueta ,Colombia
<i>H.numata</i>		2x38		HM052009	Peru
<i>H.numata</i>		STRI-B-8130		AY748062	Ecuador
<i>H.numata</i>		STRI-B-346	AF413773	AF413681	French Guyana

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For Review Only

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