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**BUTTERFLY FLIGHT MORPHOLOGY DIFFERS BETWEEN CANOPY
AND UNDERSTOREY IN A TROPICAL RAINFOREST COMMUNITY**

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1. ABSTRACT

1. Studies of altitudinal and latitudinal gradients in temperate areas often associate landscape structure and climate with animal morphology. In tropical rainforests there is a shift in conditions between the canopy and understorey. Butterfly communities have different compositions in these strata. This may imply that canopy and understorey butterflies may be under different selection pressures related to their flight morphology.
2. For riodinid butterflies in the Neotropics, it has been suggested that flight activities in the canopy and understorey are associated with different suites of morphological traits. However, this has rarely been tested in other groups of lepidopterans.
3. We examined data collected over five years in a long-term sampling project to describe the differences between canopy and understorey butterfly flight morphology in nymphalid species in the highly diverse Chocó rainforest. We measured variables including wing-area to thoracic-volume ratio, aspect ratio, and the relative distance to wing centroid. We explored whether canopy and understorey species had different allometric combinations of wing-areas and thoracic-volumes. Using independent contrasts based on a phylogeny of the Nymphalidae clade, we tested the hypothesis that low wing-area to thoracic-volume ratios were associated with a preference for living in the canopy in forest ecosystems. We also expected that understorey butterflies would have shorter distances to the wing centroid and higher aspect ratios as a possible compensation for a presumed higher wing-area to thoracic-volume ratios.
4. Butterfly species living in the canopy and understorey presented different combinations of wing-areas and thoracic-volumes. We confirmed the hypothesis that the preference for canopy of different butterfly species was significantly associated

with low wing-area to thoracic-volume ratios. However, the preference for canopy was also associated with higher aspect ratios and no association was found with the relative distance to the wing centroid.

5. Our results suggest that marked differences in the environmental conditions between the canopy and the understorey subsystems in the tropical rainforests may be a factor shaping flight morphology of Nymphalidae butterflies.

2. RESUMEN

1. Estudios en gradientes altitudinales y latitudinales han asociado cambios en la estructura del paisaje y el clima con la morfología de los animales. Los bosques tropicales presentan un cambio de condiciones entre el dosel y el sotobosque, y las comunidades de mariposas tienden a presentar composiciones diferentes en cada estrato. Esto implica que las mariposas de dosel y sotobosque podrían sufrir diferentes presiones selectivas en su morfología de vuelo.
2. Para mariposas Riodinidae en los neotrópicos, se ha sugerido que volar en dosel o sotobosque podría estar asociado a diferentes sets de características morfológicas. Sin embargo, esto casi no ha sido explorado en otros grupos de lepidópteros.
3. Examinamos datos colectados en cinco años en un proyecto de muestreo a largo plazo en para describir la relación entre las morfologías de vuelo de mariposas de dosel y sotobosque en mariposas Nymphalidae de una comunidad ecuatoriana en el bosque lluvioso del Chocó. Las variables que medimos incluyen la tasa área alar: volumen del tórax, la tasa de aspecto, y la distancia relativa al centroide alar. Exploramos si las mariposas de dosel y sotobosque difieren en sus combinaciones alométricas de áreas alares y volúmenes torácicos. Usando contrastes independientes basados en una filogenia del clado Nymphalidae, probamos la hipótesis de que la preferencia de las mariposas por dosel está asociada a tasas bajas de área alar:volumen del tórax. También probamos si las mariposas de sotobosque tienen distancias al centroide alar más reducidas y altas tasas de aspecto como posible compensación a una tasa alta de área alar: volumen del tórax.
4. Nuestros resultados muestran que las mariposas de dosel y sotobosque presentan combinaciones diferentes de áreas alares y volúmenes torácicos. Las mariposas de

dosel estuvieron significativamente asociadas a bajas tasas área alar: volumen del tórax. Sin embargo, la preferencia por sotobosque estuvo asociada a bajas tasas de aspecto, y no hubo asociación con la distancia relativa al centroide.

5. Este estudio sugiere que en los bosques tropicales las diferencias ambientales entre el sotobosque y el dosel podrían ser un factor explicando la distribución vertical de la morfología.

3. MANUSCRITO PARA LA PUBLICACIÓN

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Butterfly flight morphology differs between canopy and understorey in a tropical rainforest community

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El presente trabajo se presenta en el formato de la Revista *Journal of Animal Ecology* a partir de la siguiente página.

1 **Butterfly flight morphology differs between canopy and understorey**
2 **in a tropical rainforest community**

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7 **Summary**

- 8 1. Studies of altitudinal and latitudinal gradients in temperate areas often associate
9 landscape structure and climate with animal morphology. In tropical rainforests there
10 is a shift in conditions between the canopy and understorey. Butterfly communities
11 have different compositions in these strata. This may imply that canopy and
12 understorey butterflies may be under different selection pressures related to their flight
13 morphology.
- 14 2. For riodinid butterflies in the Neotropics, it has been suggested that flight activities in
15 the canopy and understorey are associated with different suites of morphological
16 traits. However, this has rarely been tested in other groups of lepidopterans.
- 17 3. We examined data collected over five years in a long-term sampling project to
18 describe the differences between canopy and understorey butterfly flight morphology
19 in nymphalid species in the highly diverse Chocó rainforest. We measured variables
20 including wing-area to thoracic-volume ratio, aspect ratio, and the relative distance to

21 wing centroid. We explored whether canopy and understorey species had different
22 allometric combinations of wing-areas and thoracic-volumes. Using independent
23 contrasts based on a phylogeny of the Nymphalidae clade, we tested the hypothesis
24 that low wing-area to thoracic-volume ratios were associated with a preference for
25 living in the canopy in forest ecosystems. We also expected that understorey
26 butterflies would have shorter distances to the wing centroid and higher aspect ratios
27 as a possible compensation for a presumed higher wing-area to thoracic-volume ratios.

28 4. Butterfly species living in the canopy and understorey presented different
29 combinations of wing-areas and thoracic-volumes. We confirmed the hypothesis that
30 the preference for canopy of different butterfly species was significantly associated
31 with low wing-area to thoracic-volume ratios. However, the preference for canopy
32 was also associated with higher aspect ratios and no association was found with the
33 relative distance to the wing centroid.

34 5. Our results suggest that marked differences in the environmental conditions between
35 the canopy and the understorey subsystems in the tropical rainforests may be a factor
36 shaping flight morphology of Nymphalidae butterflies.

37

38 **Key-words:** adaptive syndromes, allometry, Chocó, Canandé, ecomorphology, Ecuador,
39 Lepidoptera, stratification.

40 **Introduction**

41 Flight has been fundamental to dispersion, migration, and colonization events in insects (Hill,
42 Thomas & Blakeley 1999; Grimaldi & Engel 2005; Klowden 2007). In butterflies, morphological
43 parameters have been shown to affect flight performance (Ellington 1984a; Ellington 1984b;
44 Betts & Wootton 1988). For example, long and narrow wings are aerodynamically more efficient
45 than short and wide wings, because the former morphology reduces induced drag and associated
46 power expenditure (Vogel 1994; Berwaerts, Van Dyck & Aerts 2002). Therefore, aspect ratio is a
47 useful parameter to describe the proportion of wing length to the mean wing chord. Thorax
48 morphology (either biomass or biovolume) has also been shown to be relevant to flight. Since
49 more than 96% of thoracic mass consists of flight muscles (Marden 1987, 1989), larger thoraxes
50 have been associated with higher power outputs, leading to an increase in flight thrust (Vogel
51 1994). Because the power that a butterfly thorax can produce is limited by its size, parameters
52 such as wing loading (wing-area: body mass), flight muscle ratio (flight-muscle mass: total body
53 mass), and the wing-area to thoracic-volume ratio, correct thoracic masses for differences in total
54 body masses (Dudley 1990; Marden & Chai 1991; Srygley & Dudley 1993). Another useful
55 parameter for analysing flight performance in insects has been the distance of the wing centroid
56 to the wing base. This metric has been associated with a better body response in the pitching
57 plane and to increased manoeuvrability and force production. (Ellington 1984a; Srygley 1994;
58 DeVries, Penz & Hill 2010).

59 Morphological flight traits of insects have been studied in the context of spatial and
60 temporal variation as potential adaptations to landscape structure, weather, dispersal and
61 migration (e.g. Cárdenas et al. 2013, Schoville et al. 2013; Rauhamaki et al. 2014). In the
62 European butterflies *Melitaea cinxia* L. (Nymphalidae) and *Pararge aegeria* L. (Nymphalidae),
63 populations with larger thoracic masses and larger wing loadings were found principally in highly

64 fragmented landscapes, apparently because these morphologies improve flight performance,
65 necessary to fly between patches of forest (Norberg & Leimar 2002; Merckx & Van Dyck 2006).
66 In *Pararge aegeria* and *Danaus plexippus* L.(Nymphalidae), individuals that emerge during the
67 migration season had larger forewings and more angular wing shapes in comparison with
68 individuals that were not migrating, suggesting that these morphologies are advantageous to fly
69 long distances (Van Dyck & Wiklund 2002; Altizer & Davis 2010). In tropical butterflies,
70 morphological traits related to flight have been associated with palatability, mimicry, predator
71 avoidance and seasonality. Palatable and non-mimetic species had significantly more flight
72 muscle mass compared with unpalatable and mimetic butterflies, apparently as a consequence of
73 a higher predation pressure on the former (Srygley & Chai 1990; Marden & Chai 1991; Srygley
74 1994). Larger wings in fruit-feeding butterflies were associated with shorter seasonal periods of
75 flight in Brazilian rainforests. Presumably a large wing size limits length of flight period (Ribeiro
76 & Freitas 2011).

77 In tropical rain forests, biotic and abiotic factors, such as temperature, light, humidity,
78 wind, and animal and plant communities, change significantly along a vertical gradient ranging
79 from ground level to the upper canopy (Basset et al. 2001; Fermon et al. 2005; Richards 1952;
80 Stork & Grimbacher 2006). Such variation results in a diversity of microhabitats which may
81 harbour assemblages of organisms adapted to each particular condition (Elton 1973; Schultze,
82 Linsenmair & Fiedler 2001). There is abundant evidence of community partitioning along
83 vertical gradients in Neotropical butterflies that tend to be stratified at the subfamily level
84 (DeVries 1988; Burd 1994; Beccaloni 1997; DeVries, Murray & Lande 1997; DeVries & Walla
85 2001; DeVries et al. 2012; Checa et al. 2014; Fordyce & DeVries 2016). Niche partitioning along
86 vertical scales may even be one of the explanations for the high biological diversity in tropical
87 rainforests (Richards 1952, 1969; Hallé et al. 2012).

88 Few studies have examined the morphological differences along vertical strata of butterfly
89 communities in the tropics. Hall & Willmott (2000) found that in Amazonian riodinids, the wing-
90 area to thoracic-volume ratio was grouped into two classes: (1) butterflies with a combination of
91 either small wings and large thoraxes (i.e. low WA:TV ratios) or, (2) butterflies with a
92 combination of large wings and small thoraxes (i.e. high WA:TV ratios). The authors
93 hypothesized that each class was related to the butterflies' strata preference as an adaptation to
94 different climatic conditions. Although field observations suggested a difference in morphology
95 among butterflies inhabiting canopy versus understorey (Hall & Willmott 2000; DeVries, Penz &
96 Hill 2010), Hall & Willmott's (2000) hypothesis has never been tested. The goal of this study was
97 to explore variation in flight morphology of Nymphalidae butterflies between the canopy and
98 understorey in a tropical rainforest in the Ecuadorian Chocó using data from a long-term
99 monitoring program.

100 We hypothesize (i) that canopy nymphalid butterflies present different allometric
101 combinations of flight morphological traits compared with those in the understorey, where the
102 wing-area to thoracic-volume ratio is expected to be higher for understorey butterflies and lower
103 for canopy butterflies (Hall & Willmott 2000). (ii) Since other morphological parameters have
104 been shown to vary among Neotropical Nymphalidae at different strata (e.g. DeVries, Penz &
105 Hill 2010; Alexander & DeVries 2012), we expected that understorey butterflies would have
106 shorter distances to the wing centroid and higher aspect ratios as a possible compensation for a
107 presumed higher wing-area to thoracic-volume ratios.

108

109

110

111

112 **Materials and Methods**

113 **Sampling**

114 We used data from a long-term butterfly monitoring project (2006-2007, 2011-2013) by Checa
115 (2016) in Reserva Río Canandé (00°28'S, 79°12'W), located in the Ecuadorian Chocó. The
116 sampling design consisted of 32 Van Someren-Rydon traps (Hughes, Daily & Ehrlich, 1998)
117 distributed in two transects about 320 m long. On each transect, eight sampling sites were
118 established and each site was separated by 40 m. At each site, one trap was positioned in each of
119 the two strata, understorey (1–1.5 m) and canopy (20–30 m). All sample sites were similar in
120 terms of altitude (around 500 masl) and topography (10% inclination, measured visually). The
121 baits used were rotting banana (two–three days of fermentation) and rotten shrimp (15 days of
122 fermentation), based on Checa et al. (2009) methodology. The traps were checked daily for six
123 days every two months. The collected butterflies were deposited at the Museum of Zoology,
124 Invertebrates Section (QCAZ), of the *Pontificia Universidad Católica del Ecuador*, Quito,
125 Ecuador, and identified using specialized literature (e.g. Lamas 2004; Warren et al. 2012).
126 Common and easy to recognize species were identified *in situ*, marked and released.

127

128 **Morphological traits**

129 We selected eight to 10 individuals of each species that were in the best physical conditions (i.e.
130 undamaged wings and thoraxes) to be measured. Due to a limited number of individuals
131 collected, we measured fewer individuals of the following species: *Adelpha naxia* (n= 5),
132 *Memphis aulica* (n= 6), *Siproeta stelenes* (n= 5), *Heliconius hecale* (n= 2), *Hamadryas arinome*
133 (n= 2), *Archaeoprepona demophon* (n= 6), *Catoblepia xanthicles* (n= 7) and *Magneuptychia*
134 *mycalesis* (n= 6) (see Warren et al. 2012 for a list of taxonomic authorities and full names of
135 these butterfly species).

136 The morphological variables measured were: wing-area, forewing length, thorax length,
137 thorax height, thorax breadth and distance to centroid (see Appendix S2 for details of each
138 measurement). Digital images of the individual specimens were taken with a Canon EOS-1100
139 digital reflex camera with an 18–55mm (at 55mm) lens placed on a tripod and orientated in the
140 same plane as the wings. Constant exposure parameters were used (shutter speed = 1/6; ISO =
141 100; diaphragm = f13). Measurements were made on the digital images using ImageJ software
142 (Schneider et al. 2012). Butterflies were placed in a light-box with the wings closed, with the
143 forewing and hindwing overlapped in a position similar to that seen during flight (DeVries, Penz
144 & Hill 2010; Altizer & Davis 2010). Thorax breadth was measured with a manual calliper to the
145 nearest 0.2 mm (Mitutoyo Corporation, Japan). Given the low frequency of females captured with
146 our sampling method (Checa et al. 2009) we only analysed males.

147 For calculation of wing area, the thoraxes were digitally removed using ImageJ. This trait
148 was calculated as the sum of the areas of both hindwing and forewing in the simulated flight
149 position as mentioned above. With these variables, we calculated the following flight-related
150 parameters: aspect ratio, wing-area to thoracic-volume ratio and relative distance to wing
151 centroid. Aspect ratio (AR) was calculated as: $4 * \text{forewing length}^2 * \text{wing area}^{-1}$ (Betts &
152 Wootton 1988; Dudley 1990). The thoracic volume (mm^3) was estimated as thoracic length *
153 thoracic breadth * thoracic height. The wing-area to thoracic-volume ratio (WA:TV) was
154 estimated as: $\text{wing area} * \text{thoracic volume}^{-2/3}$ (Hall & Willmott 2000). The wing centroid (WC)
155 was calculated using ImageJ as an average of the central pixels of x and y coordinates of all
156 pixels inside the wing area (Schneider et al. 2012). The distance of this vector to the wing base
157 was measured in millimetres using ImageJ (DWC). This distance was subtracted from the square
158 root of wing area to correct for differences in wing size. This value was named as “relative
159 distance to wing centroid” (RDC). A total of 606 individuals were measured, from 67 species

160 belonging to six subfamilies: Limenitidinae, Heliconinae, Nymphalinae, Satyrinae, Biblidinae
161 and Charaxinae (Appendix S1).

162

163 **Stratification Patterns**

164 To visualize differences between canopy and understorey butterfly assemblages, a non-metric
165 multidimensional scaling (NMDS) ordination was performed based on a Morisita distance
166 community dissimilarity matrix, which had the lowest stress value (stress= 0.1947). Because
167 traps were not placed in the exact same sites between 2006–2007 and 2011–2013, only data
168 between 2011 and 2013 was used for this particular analysis. To test if the differences among
169 canopy and understorey assemblages were significant, we used analysis of similarities
170 (ANOSIM). A SIMPER analysis was employed to compare the contribution of species to
171 separate the strata assemblages. These analyses were performed using PAST software v.3.14
172 (Hammer et al. 2001). 72 species were included in this analysis.

173 To provide a continuous measure of the vertical distribution, we created a stratification
174 index (SI). This index represents the difference between the canopy and understorey captures
175 divided by the total of captures for each species. The resulting values vary between -1 and 1
176 where an index close to -1 is a preference for the understorey, near 0 suggests no preference for
177 either strata and near to 1 is a preference for the canopy. To avoid uncertainty due to low sample
178 size (which is the case of rare species) we restricted our sample for analysis to species with more
179 than 10 captured specimens.

180 To test whether our nymphalid species had a similar WA and TV relationship to that
181 found by Hall & Willmott (2000), we plotted both variables in a graph. Since the two predicted
182 groups, canopy vs. understorey, were visually classified, we quantitatively assessed
183 discrimination by categorizing species as canopy (C) or understorey (U) according to a binomial

184 test, using 0.5 as the theoretical value of the expected distribution of observations. We excluded
185 the non-significant (NS) species and used an analysis of covariance (ANCOVA) to test if canopy
186 and understorey butterflies significantly differed in terms of their WA and TV values. To meet
187 the assumptions of this test, data were log transformed, linearity of data was confirmed using
188 least-square linear regressions and normality of residuals was checked using the Shapiro-Wilk
189 test ($P>0.05$). The binomial test was performed in using SPSS statistic software (IBM
190 Corporation, Chicago, USA), and the ANCOVA in PAST software v.3.14 (Hammer et al. 2001).

191

192 **Accounting for a phylogenetic independence of morphological traits**

193 When inferring adaptation, comparisons of characters across a range of species without
194 accounting for the effects of phylogenetical relationships may increase chances of making both
195 type I and II errors (Gittleman & Luh 1992), because related species do not represent independent
196 data, and are therefore considered pseudoreplicates (Felsenstein 1985). In the recent years,
197 comparative studies have become unpublishable unless analysed in a phylogenetical context
198 (Losos 2011). For example, Srygley's (1994) hypothesis of locomotor mimicry in Neotropical
199 butterflies was strongly criticized for not accounting phylogenetic inertia (Brower 1995). For this
200 reason, we analysed our data in a phylogenetic context to address the hypothesis of a relation
201 between morphology and stratification. To overcome the problem of species phylogenetic
202 dependence, we used independent contrasts (Felsenstein 1985). This technique estimates the
203 amount of difference in the character values between sister taxa or nodes relative to their
204 phylogenetic affinity (e.g. number of mutations per site), returning values (contrasts) useful in a
205 statistic analysis. This approach assumes a Brownian model of character evolution (see
206 Felsenstein 1985; Harvey & Purvis 1991, and Garland, Harvey & Ives 1992 for more details of
207 the algorithm). We assumed the presence of phylogenetic signal since this was found in

208 morphological traits (Ribeiro & Freitas, 2011) and stratum preference (Pardouret et al. 2013;
209 Fordyce & DeVries 2016) in similar communities of Neotropical nymphalid butterflies.

210 To reconstruct the phylogeny of the Nymphalidae clade that was used for calculating the
211 independent contrasts, we obtained sequences of the cytochrome oxidase subunit 1 (COI) (see
212 Checa (2016) for details on DNA extraction, marker amplification and sequencing). For
213 *Fountainea ryphea*, *Adelpha cytherea*, *Eresia clara*, *Heliconius eleuchia*, *Heliconius hecale*,
214 *Heliconius hecalesia* *Opsiphanes cassiae*, *Opsiphanes cassina* and *Pierella helivina*, sequences
215 were obtained from the NCBI Nucleotide database (NCBI Resource Coordinators 2016). Because
216 sequences for *Eunica pomona*, *Heliconius erato* and *Opsiphanes cassiae* were not available, these
217 species were excluded from the analyses. A phylogeny was traced with the Phylogenerator
218 package in R (R Development Core Team 2013) using maximum-likelihood inference and
219 bootstrapping tests to determine the reliability of branches. The phylogeny, means of
220 morphological data, and SI of each species were plotted in PHYLIP 3.695 software (Felsenstein
221 2005) to calculate standardized independent contrasts using the PHYLIP Contrasts program.
222 Least-square linear regressions were calculated with PAST software v.3.14 (Hammer et al. 2001)
223 to test whether there were any significant associations between the standardized contrasts in
224 stratification index and the morphological traits: WA:TV, RWC and AR. Regression lines were
225 forced through the origin, following Garland, Harvey & Ives (1992).

226

227 **Results**

228 The composition and abundance of the butterfly species was different between canopy and
229 understorey (NMDS, Fig. 1). These differences between the strata were highly significant
230 (ANOSIM, $P=0.0001$), and 30 species explained 75% of the variation (SIMPER, Appendix S3).

231 Nine species, *Adelpha cytherea*, *Antirrhea philaretus*, *Dulcedo polita*, *Haetera piera*,
232 *Cithaeris pireta*, *Caligo atreus*, *Caligo brasiliensis*, *Caligo zeuxippus* and *Pierella helvina*, had
233 the highest preference for the understorey as indicated by the stratification index (SI = -1).
234 *Callicore atacama* was the only species that showed complete preference for the canopy (SI = 1)
235 (Fig. 2, Table 1).

236 Twenty-nine species were significantly associated to canopy (Table 2, binomial test). Of
237 these, 11 belong to the Biblidinae and 9 to the Charaxinae. Five of 6 Limenitidinae were
238 categorized as canopy species. Twenty species were significantly associated to understorey, of
239 which 13 belong to the Satyrinae . Five out of 7 Nymphalinae showed non-significant
240 stratification (Table 2).

241 Two groups with significantly different combinations of WA and TV were clearly
242 distinguished between canopy and understorey butterflies (ANCOVA, $P < 0.001$). At any given
243 WA, a canopy butterfly had a larger TV than its understorey counterpart, or, at any given TV, an
244 understorey butterfly had a larger WA than its canopy counterpart (Fig. 3). *Caerois gerdrudtus*
245 (SI=-0.48 U), *Cissia confusa* (SI=0.17 NS) and *Magneptychia mycalesis* (SI=0.80 C) had the
246 largest WA:TV ratio (62.0, 48.0 and 47.5 respectively). *Memphis cleomestra* (SI=0.48 C),
247 *Archaeoprepona demophon* (SI=-0.01 NS) and *Prepona philipponi* (SI=0.88 C) had the smallest
248 ratios (11.5, 11.6 and 14.4 respectively) (Fig. 2).

249 The CO1 phylogeny for the captured species is shown in Fig. 2. According to the
250 bootstrap values, further support was found at the tip of the branches (species level) rather than at
251 the base of the branches.

252 The relationships between the standardized contrasts for SI vs. WA:TV and AR were
253 highly significant (WA:TV: $P = 0.002$, slope = -3.54, $R^2 = 0.135$; AR: $P = 0.0089$, slope = 0.15,

254 $R^2=0,119$) (Table 3, Fig. 4). The relationship between SI and RDC was not significant ($P=0.229$)
255 (Fig. 4).

256

257 **Discussion**

258 There were significant differences between canopy and understorey assemblages of butterfly
259 species. This is in agreement with other studies of vertical stratification in Neotropical rainforest
260 butterflies (DeVries 1988; DeVries, Murray & Lande 1997; DeVries & Walla 2001; Alexander &
261 DeVries 2012; DeVries et al. 2012; Fordyce & DeVries 2016).

262 Our results confirm the hypothesis that nymphalid butterflies exhibit a morphological
263 pattern comprising two groups with different ratios of WA and TV, consistent with that described
264 by Hall & Willmott (2000) for the Riodinidinae. Furthermore, when corrected for the effects of
265 phylogeny, species with a higher preference for flying in the canopy have lower WA:TV ratios
266 compared with butterflies with a higher preference for flying in the understorey. This relation
267 probably arises because of the differences in temperature between canopy and understorey. We
268 suggest that canopy butterflies do not need large wings with respect to their body sizes, probably
269 because in this stratum, there is more warm air that helps them glide in space and fly. On the
270 other hand, understorey butterflies may probably need larger wings with respect to their body size
271 because in this stratum there is relatively colder air, making it more difficult and energetically
272 more demanding to fly (Wegner 1997; Noth 2008). It is widely known that in tropical rainforests,
273 there are considerable differences in available light and temperature between the canopy and the
274 understorey (DeVries 1988; Scheffers et al. 2013; Fordyce & DeVries 2016). For example, in El
275 Durango (P. Salazar, unpublished data), located 100 km north-west from our study area, there
276 was an average difference of 2.29 °C in the average ambient temperature during the daytime
277 between the canopy and understorey, with a maximum difference of 6.8 °C near noon, when

278 butterflies are more active. Before 9h00 and after 17h00 differences were less than one degree
279 Celsius. It is known that insect wing beat frequencies increase with ambient temperature
280 (Hargrove 1980; Curtsinger & Laurie-Ahlberg 1981; Azevedo et al. 1998). If we consider that
281 metabolic rates during flight are 50–100 times the resting level (Ellington 1985), the negative
282 correlation between the WA:TV ratio and metabolic rate presented by Hall & Willmott (2000), it
283 is probable that butterflies that fly in the canopy operate at higher wing beat frequencies than
284 their understorey counterparts. This higher wing beat frequency may help butterflies to escape
285 from predators, in the canopy where is significantly more predation pressure (Schultze,
286 Linsenmann & Fiedler 2001; see discussion below). Given that wing beat frequency is a major
287 factor of power expenditure (Ellington 1984b), a large, strong thorax might be helpful to sustain
288 this kind of flight. For understorey butterflies operating at low wing beat frequencies, a large
289 thorax would be unpractical. However, if we consider that acceleration is dependent on wing size
290 and wing beat frequency (Ellington 1984b), an increase in the wing size may help to compensate
291 for a decrease in thorax size. In *Drosophila*, an increased wing:thorax size ratio has also been
292 proposed to be adaptive for flight at cold temperatures (David et al. 1994; Azevedo et al. 1998).

293 Hall & Willmott (2000) analysed previously reported data and showed a negative relation
294 between the WA:TV ratio and flight speed suggesting that morphological traits influence flight
295 performance. Likewise, Berwaerts, Van Dyck & Aerts (2002) found positive correlations
296 between the relative thoracic mass (analogue to the inverse of WA:TV) with the maximal
297 acceleration capacity in *P. aegeria*. Dudley & Srygley (1994) found in neotropical butterflies that
298 greater flight speeds were associated with large relative thoracic size. Because canopy butterflies
299 had low WA:TV ratios, understorey butterflies were expected to have high ARs and low RDCs
300 as a possible compensation for a presumed decrease flight performance (Dudley & Srygley 1994;
301 Vogel 1994; Betts & Wootton 1989; Alitzer & Davis 2010). However, AR was higher for canopy

302 butterflies and no consistent relation was found for RDC. Therefore, we reject our second
303 hypothesis. Instead, we suggest that canopy butterflies in our study site, presenting high values of
304 AR and low WA:TV ratios, might potentially be fast flyers in comparison to understorey
305 butterflies, but this is still to be tested.

306 Pardonnet et al. (2013) found morphological differences between gap and understorey
307 fruit-feeding nymphalids in a Peruvian rainforest. The understorey butterflies had higher WA:TV
308 ratios compared to their gap counterparts. Some of the species in forest gaps were typically
309 considered as canopy butterflies in other studies (e.g. DeVries & Walla 2001), suggesting that
310 these are canopy butterflies that visit gaps for feeding. The factors contributing to this
311 differentiation were light levels as well as vine and herb densities.

312 An explanation for the low R^2 observed in the regressions of the standardized contrasts of
313 SI versus WA:TV and AR could be the low level of resolution of our phylogeny above the
314 subfamily level. Due to their fast rate of evolution, mitochondrial genes are more useful to
315 resolve relationships among species, but may be less informative regarding information for
316 higher taxonomic levels (subfamilies, families, orders), because the phylogenetic signal at these
317 resolutions is eroded (Garamszegi & Gonzalez-Voyer 2014). Compared to the Wahlberg et al.
318 (2009) phylogeny of Nymphalidae, our topology had two main differences. Nymphalinae and
319 biblidinae were merged into a single clade and species of both subfamilies were intermingled.
320 Limenitidinae should belong to the Heliconiinae clade. In addition, *P. helvina*, a member of
321 Satyrinae, was incorrectly placed within the Charaxinae. This might have led to several errors in
322 calculations of contrast values. This is difficult to assess, because independent contrasts do not
323 provide data concerning the identity of comparisons. However, a range of new analyses is
324 becoming increasingly available to overcome this restriction (Garamszegi & Gonzalez-Voyer
325 2014). Regardless of the limitation related to a phylogeny based solely on one gene as the one

326 used here, phylogenies are fundamental in comparative biology and must be accounted for, as
327 noted by Felstestein (1985). This is particularly true for ecological studies focused on rich
328 multispecies communities that include many closely related species as in our study where each
329 species is not an independent replicate but a pseudoreplicate due to the shared variation of related
330 taxa.

331 In summary, our results support Hall & Willmott's (2000) hypothesis that habitat
332 preference for canopy and understorey is associated to a set of butterflies morphological traits.
333 Nymphalidae stratification was associated to WA:TV ratios and AR in a Neotropical butterfly
334 community. Future research involving the integration of ecological and biomechanical data in
335 experimental studies and computational modelling may help elucidating the causes and
336 consequences of forests vertical distribution of morphology in butterflies.

337

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Figures

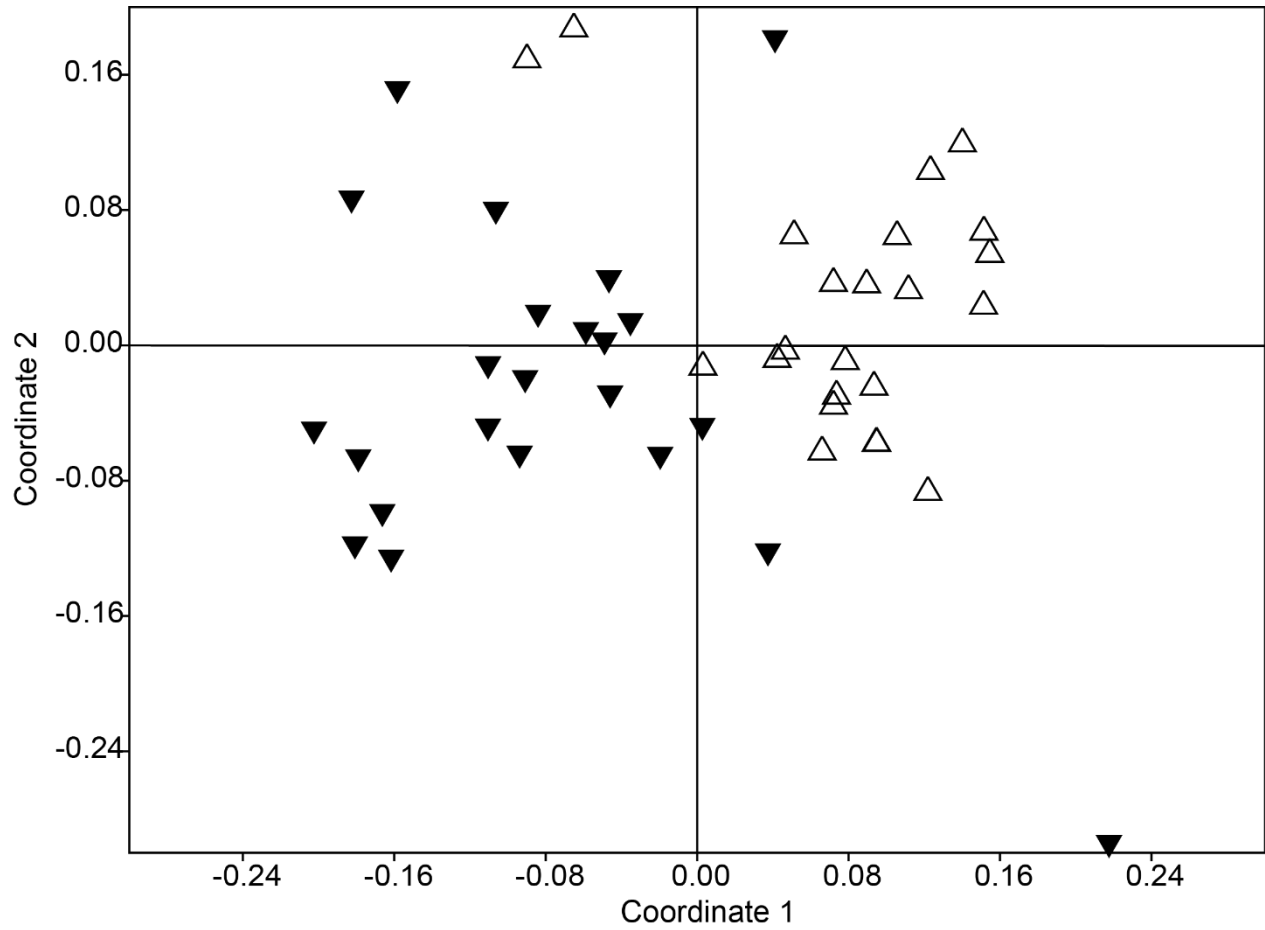


Figure 1. Non-metric multidimensional scaling plot. Each triangle represents a sampling period (one week) between 2011 and 2013. Canopy assemblages are represented by open triangles, and black inverted triangles represent understorey assemblages. Stress= 0.1947 under Morisita distance similarity matrix.

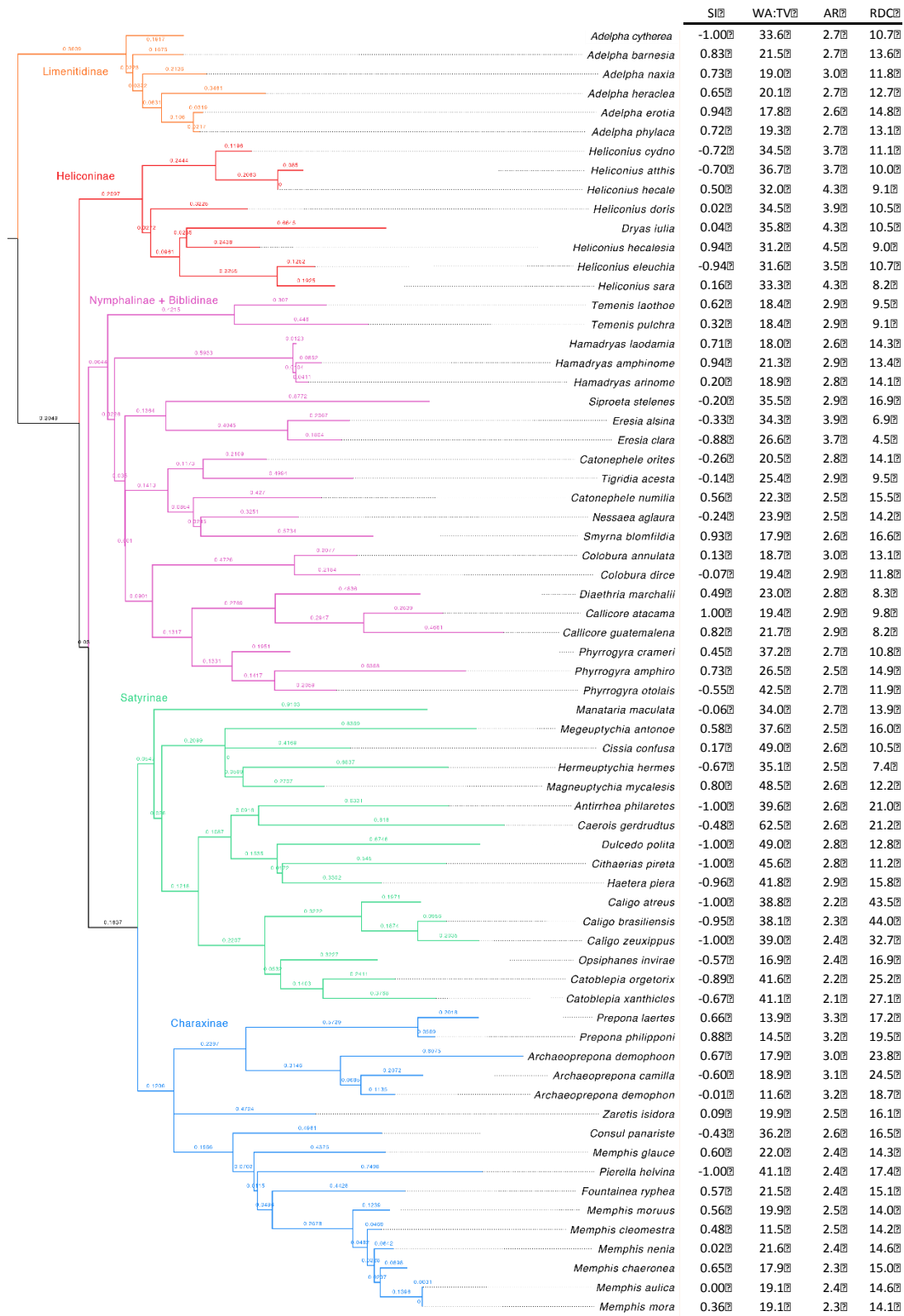


Figure 2. Maximum-likelihood Phylogeny for 67 nymphalid species collected at Reserva Río Canandé. The stratification index (SI), and means of the wing-area to thoracic-volume ratio (WA:TV), relative distance to wing centroid (RDC) and aspect ratio (AR) are denoted. Values above branches represent bootstrap values. Clades representing subfamilies are coloured.

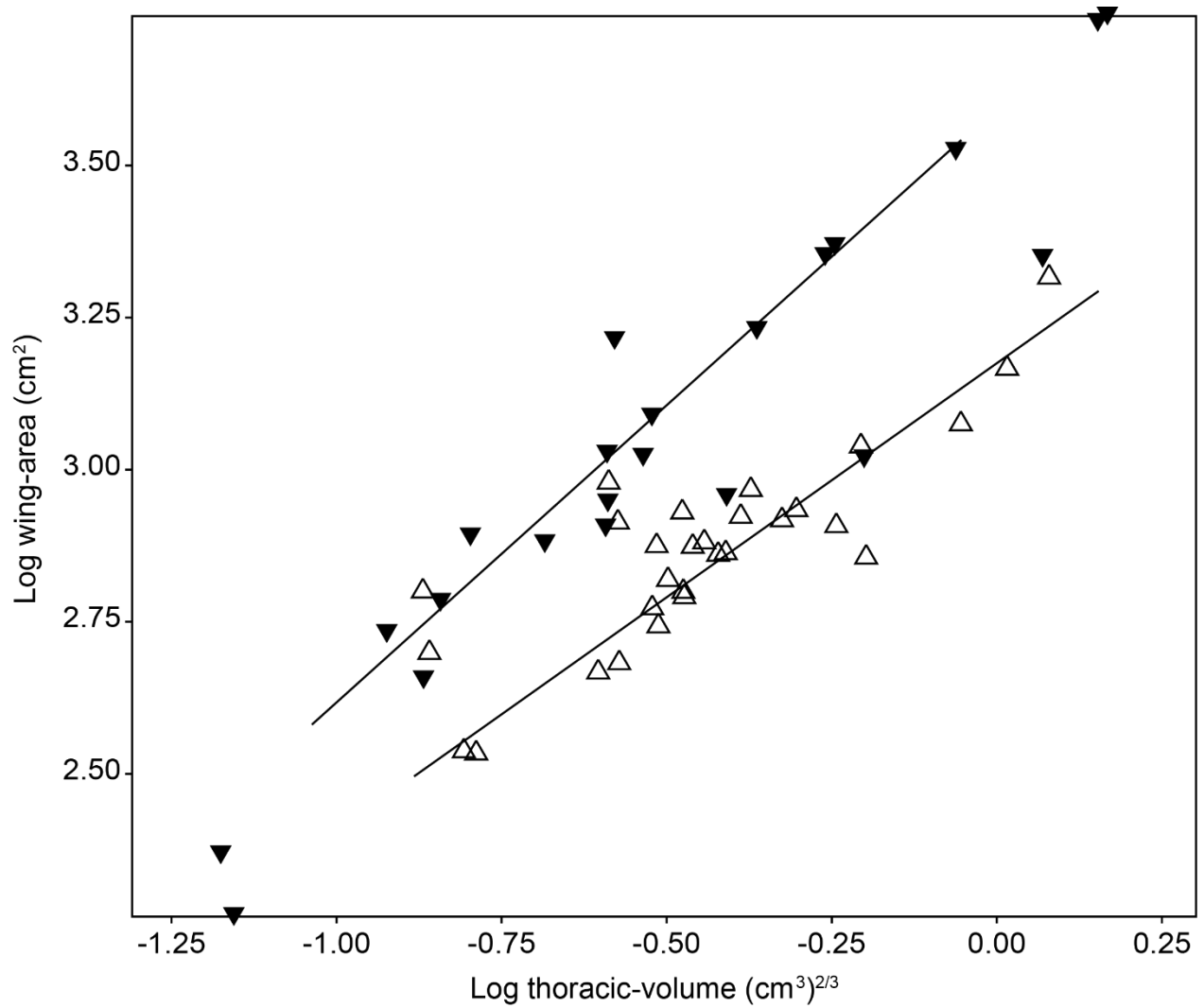


Figure 3. Relationship between thoracic-volume and wing-area and of 67 nymphalid species collected at Reserva Río Canandé. Open triangles represent canopy butterflies and inverted black triangles represent understory butterflies, categorized according to the binomial test. Two groups were significantly separated (ANCOVA, $P < 0.001$). Non-significant species were excluded.

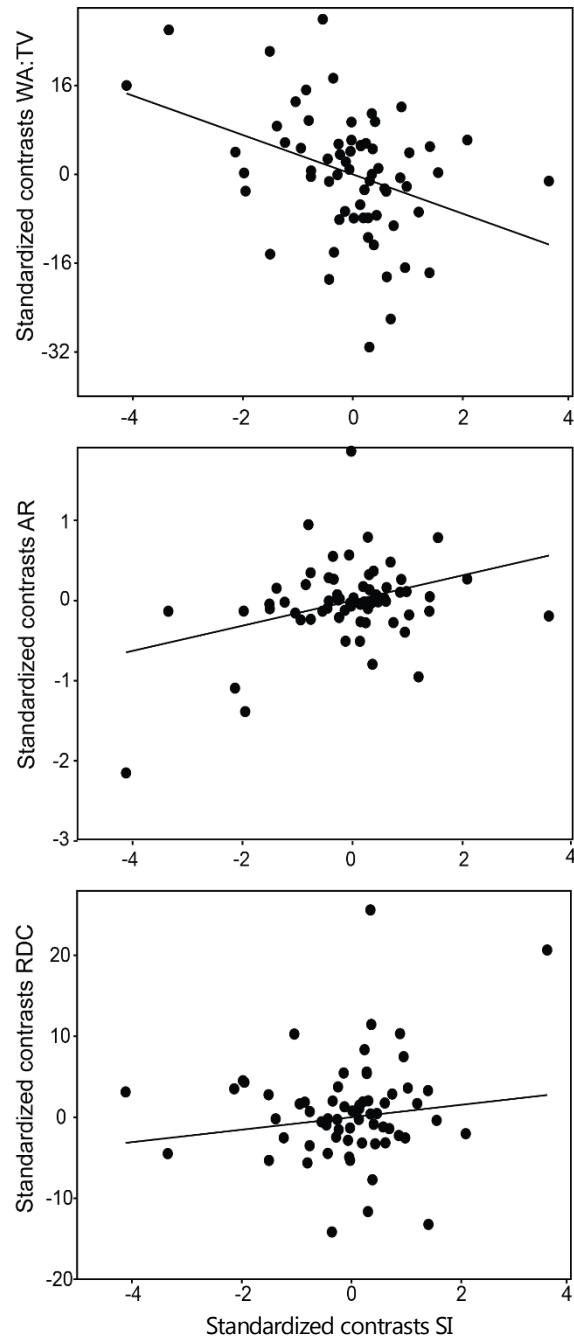


Figure 4. Plotted standardized contrasts for the relations between the Stratification Index and morphological traits: wing-area to thoracic-volume (WA:TV), aspect ratio (AR) and relative distance to wing centroid (RDC). Regression lines were forced through the origin. Regression statistics need to be cited here for both figures.

Table 1. Vertical distributions of the 67 most abundant nymphalid species in Reserva Río Canandé, captured in the years 2006-2007 and 2011- 2013, tested against a null hypothesis of equal abundance in the canopy and the understorey.

Species	Number of captures			SI	p-value	significance	C/U
	Canopy	Understorey	Total				
Limnithidinae							
<i>Adelpha barnesia</i>	33	3	36	0.83	<0.001	**	C
<i>Adelpha cytherea</i>	0	12	12	-1.00	<0.001	**	U
<i>Adelpha erotia</i>	34	1	35	0.94	<0.001	**	C
<i>Adelpha heraclea</i>	19	4	23	0.65	0.003	**	C
<i>Adelpha naxia</i>	19	3	22	0.73	0.001	**	C
<i>Adelpha phylaca</i>	308	51	359	0.72	<0.001	**	C
Heliconiinae							
<i>Dryas iulia</i>	28	26	54	0.04	0.892	NS	CU
<i>Heliconius atthis</i>	3	17	20	-0.70	0.003	**	U
<i>Heliconius cydno</i>	11	68	79	-0.72	<0.001	**	U
<i>Heliconius eleuchia</i>	1	33	34	-0.94	<0.001	**	U
<i>Heliconius hecale</i>	9	3	12	0.50	0.146	NS	CU
<i>Heliconius hecalesia</i>	31	1	32	0.94	<0.001	**	C
<i>Heliconius sara</i>	25	18	43	0.16	0.360	NS	CU
<i>Heliconius doris</i>	21	20	41	0.02	1.000	NS	CU
Nymphalinae							
<i>Colobura annulata</i>	9	7	16	0.13	0.804	NS	CU
<i>Colobura dirce</i>	13	15	28	-0.07	0.851	NS	CU
<i>Eresia alsina</i>	6	12	18	-0.33	0.238	NS	CU
<i>Eresia clara</i>	2	32	34	-0.88	<0.001	**	U
<i>Siproeta stelenes</i>	6	9	15	-0.20	0.607	NS	CU
<i>Smyrna blomfieldia</i>	82	3	85	0.93	<0.001	**	C
<i>Tigridia acesta</i>	38	50	88	-0.14	0.241	NS	CU
Biblidinae							
<i>Callicore atacama</i>	17	0	17	1.00	<0.001	**	C
<i>Callicore guatemalena</i>	10	1	11	0.82	0.012	*	C
<i>Catonephele numilia</i>	79	22	101	0.56	<0.001	**	C
<i>Catonephele orites</i>	7	12	19	-0.26	0.359	NS	CU
<i>Diaethria marchalii</i>	947	324	1271	0.49	<0.001	**	C
<i>Hamadryas amphinome</i>	188	6	194	0.94	<0.001	**	C
<i>Hamadryas arinome</i>	6	4	10	0.20	0.754	NS	CU
<i>Hamadryas laodamia</i>	24	4	28	0.71	<0.001	**	C
<i>Nessaia aglaura</i>	55	89	144	-0.24	0.006	**	U
<i>Pyrrhogyra amphiro</i>	19	3	22	0.73	0.001	**	C
<i>Pyrrhogyra crameri</i>	94	36	130	0.45	<0.001	**	C
<i>Pyrrhogyra otolais</i>	18	62	80	-0.55	<0.001	**	U

<i>Temenis laothoe</i>	76	18	94	0.62	<0.001	**	C
<i>Temenis pulchra</i>	87	45	132	0.32	<0.001	**	C
Charaxinae							
<i>Archaeoprepona camilla</i>	9	36	45	-0.60	<0.001	**	U
<i>Archaeoprepona demophon</i>	46	47	93	-0.01	1.000	NS	CU
<i>Archaeoprepona demophoon</i>	10	2	12	0.67	0.039	*	C
<i>Consul panariste</i>	12	30	42	-0.43	0.008	**	U
<i>Fountainea ryphea</i>	259	71	330	0.57	<0.001	**	C
<i>Memphis aulica</i>	10	10	20	0.00	1.000	NS	CU
<i>Memphis chaeronea</i>	90	19	109	0.65	<0.001	**	C
<i>Memphis cleomestra</i>	395	138	533	0.48	<0.001	**	C
<i>Memphis glauca</i>	40	10	50	0.60	<0.001	**	C
<i>Memphis mora</i>	49	23	72	0.36	0.003	**	C
<i>Memphis moruus</i>	111	31	142	0.56	<0.001	**	C
<i>Memphis nenia</i>	52	50	102	0.02	0.921	NS	CU
<i>Prepona laertes</i>	24	5	29	0.66	0.001	**	C
<i>Prepona philipponi</i>	16	1	17	0.88	<0.001	**	C
<i>Zaretis isidora</i>	36	30	66	0.09	0.539	NS	CU
Satyrinae							
<i>Antirrhea philaretus</i>	0	12	12	-1.00	<0.001	**	U
<i>Caerois gerdrudtus</i>	6	17	23	-0.48	0.035	*	U
<i>Caligo atreus</i>	0	50	50	-1.00	<0.001	**	U
<i>Caligo brasiliensis</i>	1	43	44	-0.95	<0.001	**	U
<i>Caligo zeuxippus</i>	0	41	41	-1.00	<0.001	**	U
<i>Catoblepia orgetorix</i>	2	36	38	-0.89	<0.001	**	U
<i>Catoblepia xanthicles</i>	2	10	12	-0.67	0.016	*	U
<i>Cissia confusa</i>	17	12	29	0.17	0.458	NS	CU
<i>Cithaerias pireta</i>	0	28	28	-1.00	<0.001	**	U
<i>Dulcedo polita</i>	0	15	15	-1.00	<0.001	**	U
<i>Haetera piera</i>	1	55	56	-0.96	<0.001	**	U
<i>Hermeuptychia hermes</i>	10	50	60	-0.67	<0.001	**	U
<i>Magneuptychia mycalesis</i>	9	1	10	0.80	0.021	*	C
<i>Manataria maculata</i>	22	25	47	-0.06	0.771	NS	CU
<i>Megeuptychia antonoe</i>	123	33	156	0.58	<0.001	**	C
<i>Opsiphanes invirae</i>	16	59	75	-0.57	<0.001	**	U
<i>Pierella helvina</i>	0	12	12	-1.00	<0.001	**	U

SI= stratification index. C= canopy. U= understorey. Significant levels of two-tailed probabilities for the binomial tests are: **P <0.01, *P<0.05, NS=not significant at $\alpha=0.05$.

Table 2. Number of species in canopy, understorey and non-significant stratification (NS), categorized according to the binomial test.

Subfamily	Canopy	Understorey	NS	Total
Biblidinae	11	2	2	15
Charaxinae	9	2	4	15
Heliconiinae	1	3	4	8
Limenitidinae	5	1	0	6
Nymphalinae	1	1	5	7
Satyrinae	2	13	3	18
Total	29	22	18	

Significant levels of two-tailed probabilities for the binomial tests are: **P < 0.01, *P < 0.05, NS = not significant, at $\alpha = 0.05$

Table 3. Least-square linear regressions for standardized contrasts for stratification index (SI) vs. wing-area to thoracic-volume (WA:TV), aspect ratio (AR) and relative distance to wing centroid (RDC). Regression lines were forced through the origin.

Variable	Slope	R ²	P
WA:TV	-3.543	0.1354	0.002**
AR	0.157	0.1192	0.0089**
RDC	0.768	0.0221	0.229 NS

Supporting information

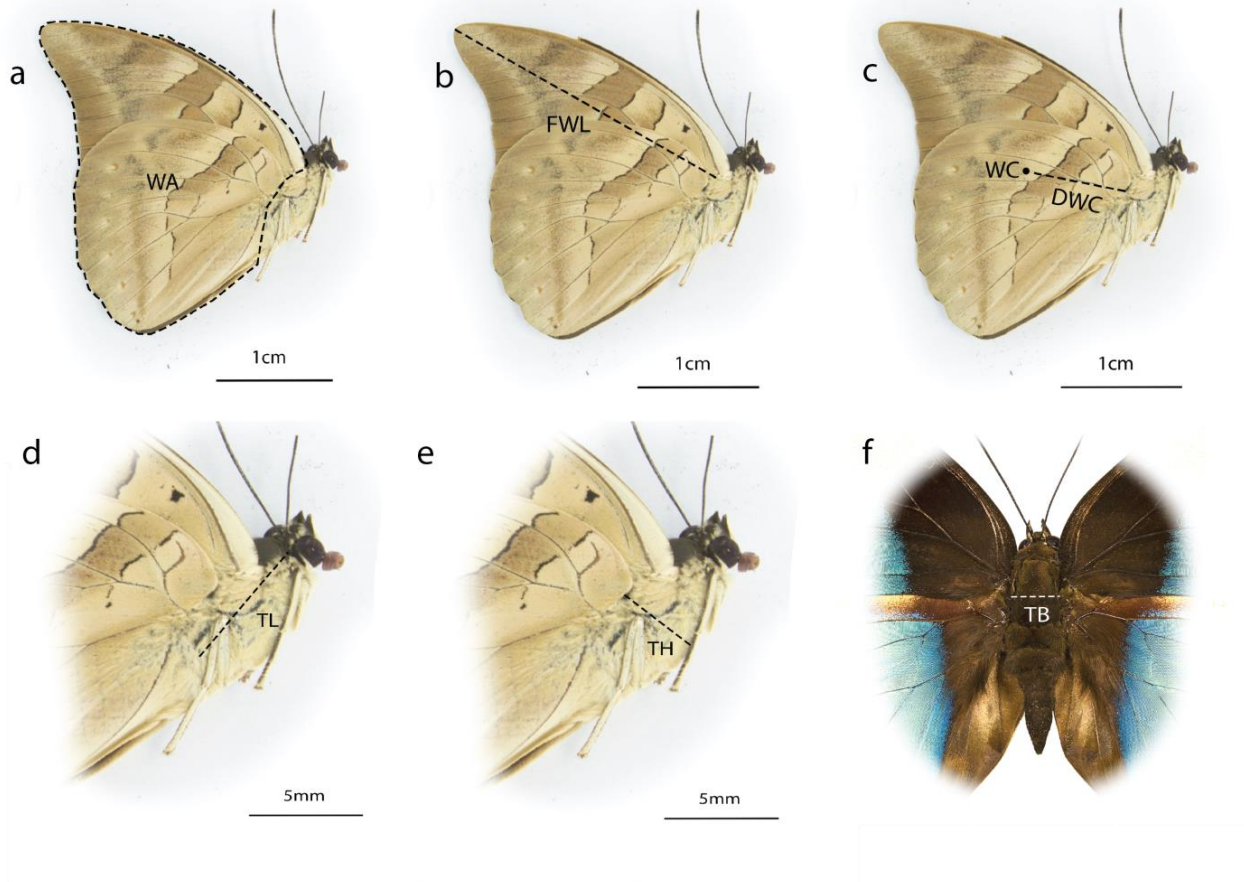
Appendix S1. List of morphological variables for each species measured in this study. Species means with standard deviations are shown.

Species	FWL[mm]		WA[mm ²]		DC[mm]		TV[mm ³]		WA:TV		AR		RDC	
	mean	std	Mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
Limnitiidae														
<i>Adelpha barnesia</i>	29.77	0.93	667.36	59.35	12.24	0.91	0.18	0.04	21.51	3.18	2.67	0.14	13.57	0.80
<i>Adelpha cytherea</i>	24.56	0.83	451.28	32.01	10.52	0.80	0.05	0.01	33.62	2.78	2.68	0.11	10.71	0.89
<i>Adelpha erotia</i>	32.63	0.94	818.41	56.12	13.81	0.74	0.43	0.43	17.77	5.47	2.61	0.16	14.78	1.29
<i>Adelpha heraclea</i>	28.24	1.53	599.49	81.25	11.73	0.91	0.16	0.02	20.11	2.99	2.68	0.13	12.71	0.97
<i>Adelpha naxia</i>	30.65	0.69	625.27	31.14	13.23	0.43	0.19	0.05	18.99	2.50	3.01	0.17	11.77	0.82
<i>Adelpha phylaca</i>	29.34	0.68	637.87	26.30	12.20	0.45	0.19	0.04	19.33	1.97	2.70	0.08	13.05	0.43
Heliconiinae														
<i>Dryas iulia</i>	41.47	1.56	803.43	66.03	17.79	0.66	0.11	0.01	35.77	3.14	4.29	0.21	10.54	0.85
<i>Heliconius atthis</i>	37.24	3.35	755.49	143.65	17.36	2.07	0.09	0.02	36.72	5.95	3.71	0.14	10.02	0.61
<i>Heliconius cydno</i>	40.36	1.05	881.30	66.35	18.56	1.06	0.13	0.02	34.50	4.21	3.71	0.21	11.11	1.38
<i>Heliconius eleuchia</i>	37.67	1.27	801.96	60.66	17.65	0.98	0.13	0.02	31.62	3.42	3.54	0.07	10.65	0.48
<i>Heliconius hecale</i>	42.19	0.44	821.95	28.92	19.56	0.95	0.13	0.01	32.00	0.93	4.33	0.06	9.11	0.44
<i>Heliconius hecalesia</i>	43.23	1.19	829.38	65.81	19.81	0.90	0.14	0.02	31.20	2.18	4.52	0.24	8.97	1.47
<i>Heliconius sara</i>	35.96	1.24	599.09	26.39	16.26	0.81	0.08	0.01	33.25	2.42	4.33	0.31	8.21	0.97
<i>Heliconius doris</i>	39.76	1.29	818.35	76.38	18.13	0.76	0.12	0.03	34.49	4.55	3.88	0.19	10.45	1.05
Nymphalinae														
<i>Colobura annulata</i>	35.18	1.91	820.08	69.95	15.49	0.77	0.30	0.06	18.67	2.11	3.02	0.11	13.13	0.81
<i>Colobura dirce</i>	31.27	0.86	667.94	43.88	14.03	0.59	0.21	0.04	19.41	1.86	2.93	0.12	11.80	0.63
<i>Eresia alsina</i>	27.38	0.77	386.55	26.80	12.76	0.56	0.04	0.01	34.31	4.33	3.89	0.24	6.90	0.65
<i>Eresia clara</i>	18.45	0.62	184.24	18.69	9.08	0.30	0.02	0.00	26.56	3.51	3.73	0.39	4.47	0.72
<i>Siproeta stelenes</i>	41.52	1.14	1204.68	82.10	17.78	0.72	0.20	0.03	35.51	2.97	2.87	0.13	16.91	1.10
<i>Smyrna blomfieldia</i>	37.56	1.04	1106.64	88.69	16.64	0.79	0.49	0.08	17.90	1.40	2.56	0.14	16.61	1.27
<i>Tigridia acesta</i>	26.26	1.31	483.97	44.14	12.50	0.87	0.08	0.02	25.43	2.24	2.86	0.18	9.48	0.96
Biblidinae														
<i>Callicore atacama</i>	25.93	0.28	470.27	25.41	11.89	0.69	0.12	0.03	19.44	3.19	2.87	0.14	9.79	0.89
<i>Callicore guatemalena</i>	22.26	0.91	346.11	35.12	10.42	0.75	0.07	0.01	21.72	3.52	2.88	0.25	8.16	1.00
<i>Catonephele numilia</i>	34.44	1.12	938.14	49.67	15.17	0.62	0.28	0.04	22.34	2.20	2.53	0.15	15.45	0.91
<i>Catonephele orites</i>	34.52	1.74	868.56	103.53	15.34	0.47	0.30	0.08	20.52	6.16	2.76	0.11	14.09	1.80
<i>Diaethria marchalii</i>	21.99	0.97	348.84	27.94	10.39	0.27	0.06	0.02	23.04	2.95	2.78	0.14	8.28	0.61
<i>Eunica pomona</i>	33.68	0.66	758.75	38.11	14.95	1.06	0.17	0.01	24.87	1.77	3.00	0.27	12.59	1.75
<i>Hamadryas amphinome</i>	34.92	1.16	847.45	90.69	15.70	1.05	0.26	0.05	21.26	4.60	2.89	0.15	13.38	0.80
<i>Hamadryas arinome</i>	34.37	0.26	844.50	4.53	14.96	0.51	0.31	0.08	18.87	3.29	2.80	0.06	14.10	0.59
<i>Hamadryas laodamia</i>	33.01	1.71	836.65	78.61	14.65	0.59	0.33	0.07	18.02	2.25	2.61	0.17	14.25	1.24
<i>Nessaea aglaura</i>	33.74	1.38	900.02	61.42	15.78	0.78	0.24	0.06	23.91	4.53	2.54	0.21	14.20	0.66
<i>Pyrrhogyra amphiro</i>	33.09	2.09	861.94	64.23	14.47	1.10	0.19	0.05	26.48	4.48	2.54	0.18	14.87	0.91

<i>Pyrrhogyra crameri</i>	26.20	0.80	506.27	24.25	11.69	0.34	0.05	0.01	37.23	5.41	2.71	0.12	10.80	0.57
<i>Pyrrhogyra otolais</i>	28.38	1.43	605.06	54.11	12.72	0.57	0.05	0.01	42.47	3.16	2.67	0.07	11.85	0.76
<i>Temenis laothoe</i>	28.46	1.19	559.95	65.97	14.16	1.13	0.17	0.03	18.40	2.35	2.91	0.24	9.47	1.61
<i>Temenis pulchra</i>	26.59	1.52	486.94	69.09	12.90	0.43	0.14	0.03	18.38	1.93	2.93	0.18	9.12	1.26
Charaxinae														
<i>Archaeoprepona camilla</i>	58.11	5.34	2223.55	426.98	22.50	2.55	1.27	0.19	18.93	2.43	3.07	0.25	24.47	2.02
<i>Archaeoprepona demophon</i>	52.77	1.94	1767.34	146.07	23.35	1.72	1.89	0.26	11.63	1.16	3.16	0.11	18.66	0.91
<i>Archaeoprepona demophoon</i>	55.53	6.37	2095.93	443.17	21.74	2.78	1.31	0.33	17.88	3.86	2.97	0.26	23.84	2.02
<i>Consul panariste</i>	36.80	1.30	1047.67	53.96	15.83	0.66	0.16	0.02	36.21	2.64	2.59	0.16	16.53	0.91
<i>Fountainea ryphea</i>	30.40	0.77	769.48	30.00	12.60	0.77	0.22	0.03	21.48	1.46	2.40	0.13	15.14	1.05
<i>Memphis aulica</i>	31.26	0.99	831.00	71.16	14.19	0.69	0.29	0.04	19.12	2.18	2.36	0.16	14.62	1.52
<i>Memphis chaeronea</i>	31.76	0.81	869.47	43.73	14.49	0.41	0.35	0.07	17.93	3.15	2.32	0.12	14.99	0.80
<i>Memphis cleomestra</i>	30.16	0.74	726.16	51.86	12.77	0.44	0.50	0.06	11.54	1.26	2.51	0.11	14.16	1.20
<i>Memphis glauce</i>	30.21	0.66	756.64	88.91	13.16	1.13	0.20	0.03	22.02	2.80	2.44	0.25	14.31	1.17
<i>Memphis mora</i>	29.30	0.83	738.47	41.37	13.02	0.42	0.24	0.03	19.14	2.02	2.33	0.10	14.14	0.82
<i>Memphis moruus</i>	29.95	0.78	733.92	56.72	13.10	0.95	0.23	0.05	19.87	3.50	2.45	0.14	13.98	1.15
<i>Memphis nenia</i>	31.54	0.87	836.30	32.78	14.33	0.79	0.25	0.04	21.56	1.92	2.38	0.14	14.58	0.87
<i>Prepona laertes</i>	44.63	1.63	1202.99	69.83	17.44	1.12	0.83	0.19	13.89	1.56	3.32	0.17	17.23	1.32
<i>Prepona philipponi</i>	48.46	3.93	1484.95	202.17	18.96	2.31	1.06	0.15	14.48	2.51	3.17	0.14	19.50	0.83
<i>Zaretis isidora</i>	33.26	0.77	890.87	79.65	13.70	0.96	0.30	0.03	19.91	1.58	2.50	0.26	16.12	2.08
Satyrinae														
<i>Antirreha philaretos</i>	46.79	2.24	1692.84	132.79	20.16	1.20	0.28	0.06	39.64	4.18	2.59	0.12	20.95	1.34
<i>Caerois gerdrudtus</i>	45.86	2.14	1630.06	221.03	19.11	1.64	0.14	0.03	62.50	7.96	2.60	0.14	21.19	1.92
<i>Caligo atreus</i>	77.83	1.67	5431.78	302.96	30.22	1.89	1.70	0.29	38.79	5.03	2.24	0.11	43.45	3.22
<i>Caligo brasiliensis</i>	79.75	3.30	5565.73	452.25	30.51	1.17	1.78	0.26	38.09	3.03	2.29	0.07	44.03	2.70
<i>Caligo zeuxippus</i>	63.15	1.20	3334.48	173.43	24.98	0.76	0.81	0.14	39.02	4.47	2.40	0.09	32.74	1.33
<i>Catoblepia orgetorix</i>	49.63	1.34	2238.72	81.16	22.09	0.76	0.41	0.08	41.61	5.88	2.20	0.06	25.22	1.05
<i>Catoblepia xanthicles</i>	49.87	1.40	2325.93	142.10	21.07	0.83	0.43	0.05	41.08	2.48	2.14	0.12	27.14	1.34
<i>Cissia confusa</i>	24.69	1.46	474.58	50.86	11.26	0.70	0.03	0.01	49.02	8.78	2.59	0.32	10.49	1.11
<i>Cithaerias pireta</i>	27.58	0.99	537.62	43.43	11.99	0.94	0.04	0.01	45.56	3.85	2.84	0.08	11.18	0.67
<i>Dulcedo polita</i>	32.81	1.56	775.01	85.39	15.01	0.82	0.06	0.01	48.95	6.18	2.79	0.10	12.79	1.09
<i>Haetera piera</i>	38.93	1.33	1060.11	76.11	16.76	1.28	0.13	0.02	41.78	5.23	2.86	0.11	15.78	1.29
<i>Hermeuptychia hermes</i>	17.08	0.92	232.71	22.11	7.86	0.37	0.02	0.00	35.10	2.39	2.51	0.13	7.38	0.52
<i>Magneuptychia mycalesis</i>	28.83	1.19	638.85	60.24	13.06	0.66	0.05	0.01	48.54	8.88	2.61	0.18	12.19	1.47
<i>Manataria maculata</i>	38.41	1.00	1081.24	54.57	18.98	0.76	0.18	0.03	34.00	3.96	2.73	0.14	13.89	1.24
<i>Megeuptychia antonoe</i>	34.60	1.18	964.37	70.76	15.02	0.86	0.13	0.02	37.61	4.13	2.49	0.06	16.02	0.89
<i>Opsiphanes cassiae</i>	48.10	0.87	1763.00	83.01	20.55	0.88	1.12	0.22	16.62	2.99	2.63	0.03	21.43	1.87
<i>Opsiphanes invirae</i>	35.56	1.27	1041.80	64.07	15.39	0.80	0.50	0.11	16.93	2.49	2.43	0.11	16.87	1.33
<i>Pierella helvina</i>	38.01	2.28	1219.52	157.70	17.49	1.31	0.16	0.03	41.07	6.75	2.38	0.15	17.36	1.28

FWL=forewing length, WA=wing area, DC=distance to centroid, TV=thoracic volume, WA:TV=wing-area to thoracic-volume, AR= aspect ratio, RDC= relative distance to centroid.

Appendix S2. Parameters used for the calculation of body morphometrics. See Warren et al. (2012) for a guide to butterfly anatomy.



- a. Wing area (WA) calculation corresponded to the area of both the forewing and the hindwing overlapped in a position similar to that in flight, simulating the aerodynamic active surface.
- b. The forewing length (FWL) corresponded to the distance from the base of the costal vein to the apex (between R4 and R5).
- c. Wing centroid (WC) was located by ImageJ software as an average of x and y pixels inside the wing area. Relative distance to centroid (RDC) was measured as the distance between WC and the base of 1A.
- d. Thoracic length (TL) corresponded to the distance from the patagium to the scutellum 3.
- e. Thoracic height (TH) corresponded to the distance from the coxa 2 to the scutum 2.
- f. Thoracic breadth (TB) corresponded to the space between left and right postmedian wing levers.

Appendix S3. Results of the SIMPER analysis using Morisita distance matrix.

Species	Average dissimilitude	Contribution %	Cumulative %	Mean Understorey	Mean Canopy
<i>Hamadryas amphinome</i>	0.4821	3.832	3.832	2.15E+09	7.47E+08
<i>Haetera piera</i>	0.4776	3.797	7.629	7.47E+08	2.15E+09
<i>Memphis cleomestra</i>	0.4764	3.787	11.42	1.49E+09	1.87E+08
<i>Diaethria marchalii</i>	0.4707	3.742	15.16	2.05E+09	7.47E+08
<i>Smyrna blomfieldia</i>	0.4576	3.637	18.79	1.96E+09	7.47E+08
<i>Phyrhogyra crameri</i>	0.4307	3.424	22.22	1.96E+09	8.40E+08
<i>Archaeoprepona demophon</i>	0.4248	3.376	25.59	1.68E+09	7.47E+08
<i>Megeuptychia antonoe</i>	0.4036	3.208	28.8	1.87E+09	9.34E+08
<i>Tigridia acesta</i>	0.3716	2.954	31.76	1.21E+09	8.40E+08
<i>Cissia confusa</i>	0.3603	2.864	34.62	1.31E+09	1.12E+09
<i>Opsiphanes cassina</i>	0.36	2.861	37.48	1.12E+09	1.21E+09
<i>Archaeoprepona camilla</i>	0.3569	2.837	40.32	1.12E+09	1.49E+09
<i>Cithaerias pireta</i>	0.3504	2.785	43.1	1.12E+09	2.15E+09
<i>Catoblepia orgetorix</i>	0.349	2.774	45.88	1.12E+09	2.15E+09
<i>Callicore atacama</i>	0.3243	2.577	48.45	2.15E+09	1.21E+09
<i>Nessaea aglaura</i>	0.2943	2.34	50.79	7.47E+08	4.67E+08
<i>Prepona laertes</i>	0.293	2.329	53.12	2.05E+09	1.31E+09
<i>Zaretis isidora</i>	0.292	2.321	55.44	2.15E+09	1.31E+09
<i>Memphis glauce</i>	0.2602	2.068	57.51	2.15E+09	1.40E+09
<i>Memphis mora</i>	0.2385	1.896	59.41	2.05E+09	1.49E+09
<i>Chloreuptychia arnaca</i>	0.219	1.741	61.15	1.49E+09	2.15E+09
<i>Caerois gerdrutus</i>	0.2139	1.7	62.85	1.87E+09	1.68E+09
<i>Colobura dirce</i>	0.2122	1.687	64.54	2.05E+09	1.59E+09
<i>Colobura annulata</i>	0.1991	1.583	66.12	1.96E+09	1.68E+09
<i>Phyrrogyra otolais</i>	0.1949	1.549	67.67	1.59E+09	2.15E+09
<i>Pierella helvina</i>	0.1947	1.547	69.21	1.59E+09	2.15E+09
<i>Antirrhoea philaretus</i>	0.1931	1.535	70.75	1.59E+09	2.15E+09
<i>Dulcedo polita</i>	0.1926	1.531	72.28	1.59E+09	2.15E+09
<i>Temenis laothoe</i>	0.1924	1.529	73.81	2.15E+09	1.59E+09
<i>Callicore guatemalena</i>	0.1842	1.464	75.27	2.05E+09	1.68E+09
<i>Hamadryas laodamia</i>	0.1785	1.419	76.69	2.05E+09	1.68E+09
<i>Archaeoprepona demophon</i>	0.1634	1.299	77.99	2.15E+09	1.68E+09
<i>Pierella luna</i>	0.1595	1.268	79.26	1.68E+09	2.15E+09
<i>Heliconius doris</i>	0.1302	1.035	80.3	2.15E+09	1.77E+09
<i>Phyrrogyra amphiro</i>	0.128	1.018	81.31	2.15E+09	1.77E+09
<i>Consul panariste</i>	0.1264	1.005	82.32	1.77E+09	2.15E+09
<i>Caligo zeuxippus</i>	0.09931	0.7893	83.11	1.87E+09	2.15E+09
<i>Archaeoprepona amphimachus</i>	0.09895	0.7865	83.89	2.15E+09	1.87E+09
<i>Caligo brasiliensis</i>	0.0986	0.7837	84.68	1.87E+09	2.15E+09
<i>Zaretis isidora</i>	0.09582	0.7617	85.44	1.87E+09	2.15E+09
<i>Memphis moruus</i>	0.09401	0.7472	86.19	2.15E+09	1.87E+09
<i>Catoblepia xanthicles</i>	0.0933	0.7416	86.93	1.87E+09	2.15E+09
<i>Adelpha leuceria</i>	0.06785	0.5393	87.47	2.15E+09	1.96E+09
<i>Adelpha phylaca</i>	0.06689	0.5317	88	1.96E+09	2.15E+09
<i>Adelpha erotia</i>	0.06561	0.5215	88.52	2.15E+09	1.96E+09
<i>Eresia alsina</i>	0.06555	0.5211	89.04	2.15E+09	1.96E+09
<i>Adelpha messana</i>	0.06513	0.5177	89.56	2.05E+09	2.05E+09
<i>Eunica chlororhoa</i>	0.06412	0.5097	90.07	1.96E+09	2.15E+09
<i>Catobephele orites</i>	0.06412	0.5097	90.58	1.96E+09	2.15E+09
<i>Hmadryas amphichloe</i>	0.06385	0.5075	91.09	2.15E+09	1.96E+09
<i>Pareuptychia occirhoe</i>	0.06341	0.504	91.59	1.96E+09	2.15E+09
<i>Callicore lyca</i>	0.06292	0.5001	92.09	2.15E+09	1.96E+09
<i>Heliconius cydno</i>	0.06281	0.4992	92.59	2.05E+09	2.05E+09
<i>Memphis nenia</i>	0.0614	0.488	93.08	1.96E+09	2.15E+09
<i>Manataria maculata</i>	0.06104	0.4852	93.56	2.05E+09	2.05E+09
<i>Fountainea ryphea</i>	0.03448	0.274	93.84	2.05E+09	2.15E+09
<i>Heliconius atthis</i>	0.03446	0.2739	94.11	2.15E+09	2.05E+09
<i>Adelpha barnesia</i>	0.03392	0.2696	94.38	2.15E+09	2.05E+09
<i>Ectyma erycinoides</i>	0.03392	0.2696	94.65	2.15E+09	2.05E+09

<i>Adelpha heraclea</i>	0.0334	0.2654	94.92	2.15E+09	2.05E+09
<i>Hermeuptychia hermes</i>	0.0334	0.2654	95.18	2.15E+09	2.05E+09
<i>Hamadryas chaeronea</i>	0.03264	0.2595	95.44	2.15E+09	2.05E+09
<i>Adeplha fabricia</i>	0.03264	0.2595	95.7	2.15E+09	2.05E+09
<i>Hyposcada illinissa</i>	0.03242	0.2577	95.96	2.05E+09	2.15E+09
<i>Antirrhea pterocopha</i>	0.03242	0.2577	96.47	2.05E+09	2.15E+09
<i>Memphis aulica</i>	0.03242	0.2577	96.73	2.05E+09	2.15E+09
<i>Adelpha cocala</i>	0.03216	0.2556	96.99	2.15E+09	2.05E+09
<i>Memphis aureola</i>	0.03192	0.2537	97.24	2.15E+09	2.05E+09
<i>Prepona dexamenus</i>	0.03192	0.2537	97.49	2.15E+09	2.05E+09
<i>Prepona philipponi</i>	0.0317	0.252	97.75	2.05E+09	2.15E+09
<i>Magneuptychia inani</i>	0.0317	0.252	98	2.05E+09	2.15E+09
<i>Siproeta stelenes</i>	0.0317	0.252	98.25	2.05E+09	2.15E+09
<i>Caligo atreus</i>	0.0317	0.252	98.5	2.05E+09	2.15E+09
<i>Eresia eunice</i>	0.03169	0.2519	98.75	2.15E+09	2.05E+09
<i>Morpho cypris</i>	0.03169	0.2519	99	2.15E+09	2.05E+09
<i>Hmadryas arinome</i>	0.03169	0.2519	99.26	2.15E+09	2.05E+09
<i>Taygetis puritana</i>	0.03125	0.2484	99.51	2.05E+09	2.15E+09
<i>Opsiphanes tamarindi</i>	0.03125	0.2484	99.75	2.05E+09	2.15E+09
<i>Historis acheronta</i>	0.03101	0.2465	100	2.15E+09	2.05E+09

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