

1 **Adaptive shifts underlie the divergence in wing morphology in bombycoid moths**

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24 **Short Title:** Evolution of moth flight morphology

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30 B.R.A. designed research; B.R.A., U.B.S., K.C.K, and C.A.H. performed research; B.R.A., M.T.,
31 A.J.A., U.B.S., and K.C.K, analyzed data; B.R.A wrote the paper; all authors contributed towards
32 editing the paper.

33

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35

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37

38 **ABSTRACT**

39 The evolution of flapping flight is linked to the prolific success of insects. Across Insecta, wing
40 morphology diversified, strongly impacting aerodynamic performance. In the presence of
41 ecological opportunity, discrete adaptive shifts and early bursts are two processes hypothesized
42 to give rise to exceptional morphological diversification. Here, we use the sister-families
43 Sphingidae and Saturniidae to answer how the evolution of aerodynamically important traits is
44 linked to clade divergence and through what process(es) these traits evolve. Many agile
45 Sphingidae evolved hover-feeding behaviors, while adult Saturniidae lack functional mouth
46 parts and rely on a fixed energy budget as adults. We find that Sphingidae underwent an
47 adaptive shift in wing morphology coincident with life history and behavior divergence, evolving
48 small high aspect-ratio wings advantageous for power reduction that can be moved at high
49 frequencies, beneficial for flight control. In contrast, Saturniidae, which do not feed as adults,
50 evolved large wings and morphology which surprisingly does not reduce aerodynamic power,
51 but could contribute to their erratic flight behavior, aiding in predator avoidance. We suggest
52 that after the evolution of flapping flight, diversification of wing morphology can be potentiated
53 by adaptative shifts, shaping the diversity of wing morphology across insects.

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57 INTRODUCTION

58 The evolution of flight is thought to be a key innovation [1] foundational to the success of
59 insects, one of the most speciose clades of animals on Earth. In flying insects, flight is critical for
60 most aspects of life history including dispersal, migration, predator avoidance, feeding, and
61 courtship behaviors. The flight morphology of flying insects, therefore, likely faces strong
62 selective forces to meet the functional demands of a species [2, 3]. Selection can act on flight
63 morphology to significantly impact flight performance [4]. Indeed, flying insects show an
64 extraordinary diversity of wing and body sizes and shapes [2, 5, 6]. Revealing the phylogenetic
65 patterns of insect flight morphology and the processes driving its evolution is a prime
66 opportunity to examine how the evolution of aerodynamically important traits is linked to the
67 divergence of diverse clades.

68 Clade divergence and the subsequent diversification of lineages and morphology can
69 occur through different evolutionary processes. In the presence of an ecological opportunity,
70 the tempo of trait evolution can accelerate and its mode can deviate from a random Brownian
71 Motion (BM) process, the null model of trait evolution. Early bursts [7-9] and discrete
72 adaptative shifts [10-12] are two alternative processes hypothesized to give rise to exceptional
73 morphological diversity. An early burst is associated with the adaptative radiation of a clade
74 where morphological disparity is established early and followed by a subsequent slowdown in
75 diversification rate [8, 9]. Adaptive shifts are when discrete shifts occur along a single branch
76 and are not followed by a slowdown in diversification rate [10-12]. Traits with known functional
77 consequences (e.g. wing morphology) are more likely to reflect the ecology of a species [13],
78 and therefore are more likely to be associated with non-BM processes when ecologically

79 distinct clades evolve. Therefore, testing if insect wing and body morphology evolution deviates
80 from BM and shifts in tandem with life history and behavior will demonstrate the evolutionary
81 processes driving morphological diversification as clades diverge to occupy different biological
82 niches.

83 Wing size and shape, as well as body size have known aerodynamic consequences for
84 maneuverability, force production, and power requirements. Nearly any aspect of shape can
85 affect aerodynamics, but several metrics of wing morphology are common predictors of flight
86 performance, notably wing loading (W_s), aspect ratio (AR), and radius of the second moment of
87 area (\hat{r}_2). A lower W_s , the ratio between body mass (m_t) and wing area (S), typically enhances
88 maneuverability, increasing the wing force production to body mass ratio, as seen in birds [14-
89 16], bats [17-19], and moths and butterflies [20, 21]. Larger AR wings (long, slender) can reduce
90 the power requirements of flight [6, 19, 22], but can also reduce maneuverability [3, 21, 23].
91 High \hat{r}_2 wings will have more area concentrated distally, which increases force production
92 because more of the wing is moving more quickly. But high \hat{r}_2 can increase power
93 requirements and reduce maneuverability [24]. Finally, interspecific variation in wing and body
94 morphology will have direct consequences for wing beat frequency (n) [6, 25]. An increase in n
95 increases active force generation [26], but at the cost of increasing inertial power (P_{acc}), the
96 power required to oscillate the wing mass [27].

97 The moth superfamily Bombycoidea provides an opportunity to test hypotheses related
98 to the evolution of flight morphology within closely related, but divergent clades. Bombycoidea
99 is a globally distributed, diverse clade of more than 5,000 species [28]. The most diverse
100 families in the Bombycoidea are hawkmoths and wild silk moths (Sphingidae and Saturniidae,

101 respectively); sister families [29-31] of strikingly different life histories and flight behaviors.
102 Hawkmoths are active, fast flyers [32] known for their maneuverability and hover feeding
103 behavior [33, 34], where species can successfully track flower oscillations up to 14 Hz [33, 34].
104 However, hovering requires a high power output [35]. Wild silk moths (here forth “silkmoths”)
105 display a flight behavior that is often described as bobbing or erratic, but fast and agile when
106 escaping from predators [32, 36-38]. Silkmoths lack functional mouth parts and must rely on
107 the strictly finite energy stores, gathered during the larval period, throughout their entire,
108 albeit short, reproductive adult life stage [38]. The divergence in life history and flight behavior
109 between hawkmoths and silkmoths represent different niches, and would be expected to have
110 correlated changes in flight morphology.

111 Here, we focus on the hawkmoths and silkmoths to test if each clade has evolved
112 distinct flight morphology and determine what evolutionary processes led to extant
113 morphological disparity. We hypothesize that hawkmoths evolved morphology favorable for
114 maneuverability in order to rapidly track flower movements during hover feeding, while
115 silkmoths evolved morphology favorable for power reduction in order to conserve limited
116 energy as adult stage silkmoths do not feed. We next examine the morphological disparity
117 through time (DTT) and compare different models of trait evolution to determine the processes
118 that led to the diversity of extant flight morphology. We hypothesize that the distinct
119 transitions in life history and flight behavior between hawkmoths and silkmoths were
120 accompanied by distinct adaptive shifts in flight morphology.

121

122

123 MATERIALS AND METHODS

124 We created a time-calibrated Bombycoidea phylogeny, sampling representatives of all families,
125 following published methods [31]. In total, the phylogenetic dataset of 606 loci included 57
126 species and one outgroup. The tree was inferred using a maximum likelihood approach and
127 time calibrated based on the dates of corresponding nodes in a recently published Lepidoptera
128 phylogeny that relied on 16 fossil calibrations with uniform priors and uncorrelated rates [30].

129

130 *Morphometrics*

131 Body and wing morphology was digitized from museum images using StereoMorph
132 (V1.6.2) [39]. Male specimens were analyzed when available (53 of 57 species); males are
133 known to exhibit higher flight activity in comparison to females [5, 40]. Eight landmarks
134 characterized the body; Bézier curves outlined the right forewing and hindwing (Fig. S1).

135 Wing measurements for all species began by re-orienting each wing to a comparable
136 orientation consistent with known flight position. The forewing was rotated so its long axis was
137 perpendicular to the long axis of the body. In Sphingidae, the hindwing long axis was also
138 rotated perpendicular to the long axis of the body; the approximate orientation during flight.
139 The hindwing of Saturniidae and the “other bombycoid families” were kept in the same
140 orientation of dried museum specimens, which is the approximate orientation during flight and
141 provides a consistent and comparable orientation across species. A combined wing outline was
142 created from the non-overlapping portions of the rotated forewing and hindwing, resampled to
143 generate 75 evenly spaced points.

144 Analysis of wing shape traits was conducted in Matlab (R2018b–9.5.0.944444). Wing
145 parameters (R , \bar{c} , S , AR , \hat{r}_2 , and W_s) were calculated following Ellington [24]. n was estimated
146 from morphology [25].

147

148 *Phylogenetic comparisons*

149 A phylogenetic principal components analysis (pPCA) [41] was conducted on forewing,
150 hindwing, and combined shapes. The dominant pPC axes for wing shape were determined using
151 the broken stick method implemented in the bsDimension function of the PCDimension R
152 package V 1.1.11 [42].

153 For each trait, we performed a disparity through time (DTT) analysis [8] (1000
154 simulations); a maximum likelihood estimation of the presence of shifts and their positions
155 using PhylogeneticEM [43]; and compared the fit of 10 different models of trait evolution using
156 mvMORPH [44]. These analyses were conducted in RStudio (V1.1.383) using R (V4.0.2).
157 Unabridged methods are supplementary material. See Table S1 for list of all variables and
158 derivation. Data is available on Dryad [45].

159

160 **RESULTS**

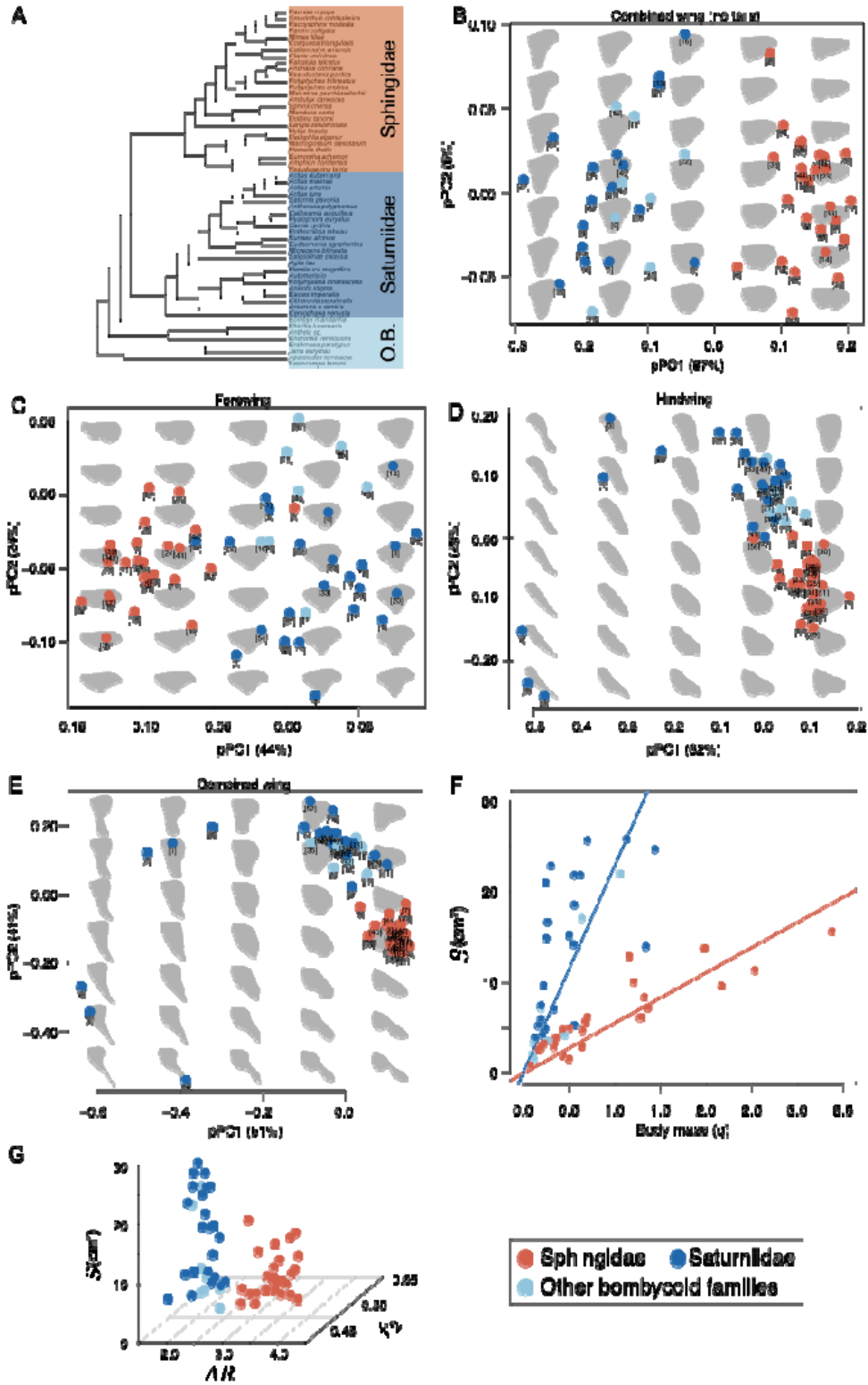
161 *Phylogeny*

162 Phylogenetic relationships of the 57 species in this study show a monophyletic, well-supported
163 clade of the Sphingidae and Saturniidae as sister-lineages, with the Bombycidae as the sister to
164 those two (Fig. 1A; S2). Relationships are congruent with previous studies [29, 31, 46, 47].

165

166 *Hawkmoths and silkmoths each have diverse, but clustered wing shapes in morphospace.*
167 We first used a phylogenetic principal components analysis to assess the variation in extant
168 wing shape in a data-driven, evolutionary framework. For all three wing shapes (forewing,
169 hindwing, and combined), most of the variation is explained by the first two pPC axes (Fig. 1B-E;
170 Table S2); pPC three or four explained no more than 14% of the variation (Fig. S3A-D; Table S2).
171 Hindwing and combined wing morphospaces capture the evolution of hindwing tails in some
172 silkmoth species, but hawkmoths and silkmoths remain clustered (Fig. 1C-D). When tailed
173 species (#1, 2, 3, 4, 20, 28) are removed (Fig. 1B), families remain clustered in combined-wing
174 shape space; variation along pPC1 generally corresponds to AR.

175 The wing shapes of hawkmoths and silkmoths are well separated in morphospace. We
176 conducted a MANOVA on each wing shape; pPC1-4 scores were the response variables and
177 clade (hawkmoth; silkmoth; Other Bombycoïd Families, abbreviated O.B.) was the factor. Each
178 wing shape is significantly separated between clades (Forewing: $F=14.91, p<10^{-13}$; hindwing:
179 $F=10.84, p<10^{-10}$; combined wing: $F=14.96, p<10^{-13}$). Separation persists when considering only
180 hawkmoths and silkmoths (Forewing: $F=44.42, p<10^{-14}$; hindwing: $F=10.84, p<10^{-10}$; combined
181 wing: $F=101.17, p<10^{-15}$), and for the combined wing when tailed silkmoths are removed from
182 the analysis (All families: $F=16.19, p<10^{-13}$; hawkmoths-vs-silkmoths: $F=144.06, p<10^{-15}$).



184 Figure 1. The evolution and trajectory of wing shape diversity.

185 (A) The phylogenetic relationships of bombycoids and outgroups (node labels in Fig. S2B).
186 O.B. refers to Other Bombycoid families (the name we give to all long-branched species that do
187 not belong to either the Saturniidae or Sphingidae clades). Clade color is consistent across
188 figures. Projections of shapes from (B) combined wing without tails, (C) forewing, (D) hindwing,
189 and (E) combined wing onto the first two pPCs demonstrates the separation between extant
190 hawkmoths and silkmoths (pPC 3 and 4 and species number key in Fig. S3). (F) Wing size and (G)
191 combined wing functional shape metrics also diverge between hawkmoths and silkmoths.
192

193 *Wing area is greater in silkmoths than hawkmoths*

194 In addition to shape, we determined if wing size is larger for a given body size between the two
195 clades. We conducted a linear regression between S and m_t (Fig. 1F), constraining the y-
196 intercept for each family to zero (Hawkmoths: $r^2=0.90$, $F=234.4$, $p<10^{-13}$; Silkmoths: $r^2=0.75$,
197 $F=66.8$, $p<10^{-7}$). An ANCOVA with family as a factor reveals significant differences in regression
198 slope ($F=8.732$, $p=0.0005$), indicating wing area is larger for a given body size in silkmoths than
199 hawkmoths. Next, before accounting for phylogeny, the relative wing area of each species
200 (S/m_t) is significantly different between hawkmoths and silkmoths (2-tailed t-test, $p<10^{-9}$). A
201 comparison of absolute wing area between the clades reinforces these differences (Fig. 1F,G;
202 S4A,B).

203

204 *Aerodynamic features of the wing and body also separate between clades*

205 To complement the data-driven pPCA and relate variation in wing and body shape and size to
206 aerodynamic metrics, we next quantified several specific morphological variables:
207 nondimensional radius of second moment of area (\hat{r}_2), aspect ratio (AR), wing loading (W_s), and
208 the fraction of body length occupied by the abdomen (\hat{l}_{abd}) and thorax (\hat{l}_{tho}). Before
209 accounting for phylogeny, combined wing AR, W_s , and \hat{r}_2 are all significantly greater in

210 hawkmoths than in silkmoths (Fig. 1G; Table S3). Finally, while variation in total body length (l_b)
211 spans a similar range within each family, clade average \hat{l}_{abd} is significantly longer in hawkmoths
212 than silkmoths and \hat{l}_{tho} is generally greater in silkmoths than in hawkmoths (Table S3). To
213 further ensure these multiple comparisons did not bias our statistics, we conduct a separate
214 MANOVA of the wing (\hat{r}_2 , AR, W_s) and body (\hat{l}_{abd} , \hat{l}_{tho}) traits between hawkmoths and
215 silkmoths and, in both cases, find significant separation between the clades (wing: $F=107.15$,
216 $p<10^{-15}$; body: $F=11.432$, $p<10^{-5}$).

217

218 *Wing beat frequency diverges between hawkmoths and silkmoths.*

219 Wing beat frequency (n) is also an important feature of flight that depends on wing and body
220 size. n , estimated from scaling relationships (Table S1; Deakin, 2010), is distinct from wing
221 shape, but not independent of wing and body size (total body mass, m_t , and the mass of the
222 wing pair, m_w , were estimated from museum specimens; see supplemental Fig. S6; Table S5).
223 Based on morphological differences, n is significantly greater in hawkmoths (n : mean \pm SD:
224 29.37 ± 9.89 Hz) compared to silkmoths (n : mean \pm SD: 14.34 ± 5.21 Hz, $p<0.0001$; Table S3).

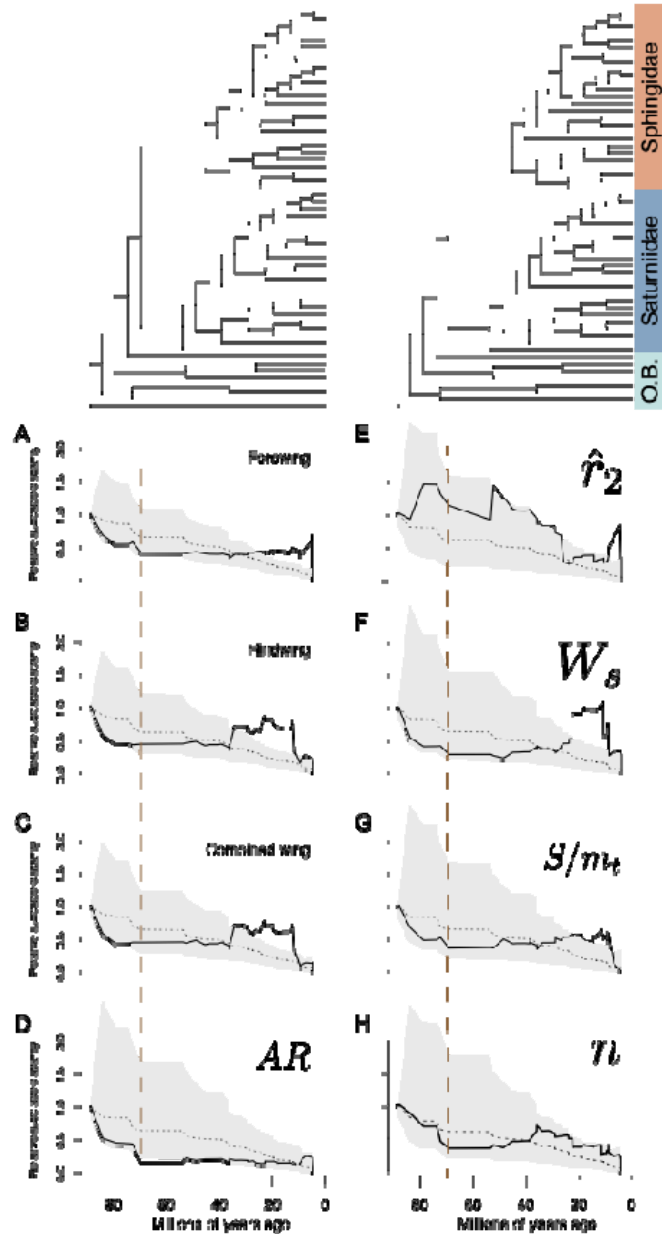
225

226 *Relative subclade disparity through time (DTT) shows both an early and recent accumulation of*
227 *morphological diversity*

228 A DTT analysis determines how morphological disparity accumulated over time. The relative
229 subclade disparity of each shape is similar through time. Early in evolutionary history, relative
230 subclade disparity is less than expected by BM for all three wings; the lowest values fall just
231 inside the 95% confidence interval of BM trait simulation at the point when hawkmoths and
232 silkmoths split (~66 MYA; Fig. 2A-C). From that time, subclade disparity remained relatively

233 static until sharply and significantly rising above BM expectations ~38 MYA (Fig. 2A-C),
234 indicating younger subclades evolved a greater proportion of modern disparity than expected
235 under BM. Removing tailed species from the analysis produces a similar result, but the rise in
236 relative subclade disparity above the BM expectation now occurs more recently (Fig. S4C). The
237 DTT of combined wing metrics (W_s , n , S/m_t) follow similar patterns (Fig. 2D-H), with the
238 exception of \hat{r}_2 (Fig. 2E). Notably, relative subclade disparity of AR significantly deviates below
239 the BM expectation coincident with the divergence of the two sister-clades (Fig. 2D). Again, at
240 approximately 38 MYA, the disparity of these wing traits begins to rise above the BM
241 expectation, but only S/m_t and W_s significantly rise above the expectation under a BM process
242 (Fig. 2F-G). A multivariate DTT of normalized functional wing metrics reveals a similar overall
243 trend (Fig. S5).

244 As relative subclade disparity shifts from consistently low values below the BM
245 expectation to high values above the BM expectation in recent evolutionary history,
246 morphological disparity index (MDI) values for each trait are near zero and not statistically
247 significant (other than AR: -0.221 ± 0.202 ; $p=0.022$; Table S6). While MDI values of
248 approximately zero typically indicate a BM process, here, wing morphology deviates from the
249 BM simulation in both deep and recent time. Instead, our findings suggest that morphological
250 disparity was established *between* subclades early in the evolutionary history of the group
251 (indicated by values below the BM expectation) and additional disparity was established within
252 each subclade in recent time (indicated by values above the BM expectation). While this
253 pattern deviates from the BM expectation, the two deviations are in opposite directions, which
254 is why we find an MDI near zero.



255
 256 Figure 2. Disparity through time reveals that wing morphology diverged early between the
 257 clades and additional variation accumulated within each clade in more recent time.
 258 In each panel, the dashed line represents the median simulated subclade disparity under a
 259 single rate BM process and includes the 95% confidence interval in grey. The observed relative
 260 subclade disparity is presented as a solid black line. All traits other than r_2 show a similar trend
 261 in relative subclade disparity with low values deviating below the BM expectation in the early
 262 evolutionary history of the clade and high values in recent time. The low values in the early
 263 history of the clade indicate the disparity between clades was established early and the high
 264 values in recent history indicate disparity within each clade was established in more recent
 265 time. The brown vertical dashed line represents the time at which hawkmoths and silkmoths
 266 split.
 267

268 *Adaptive shifts account for differences in the evolution of several traits of wing morphology.*

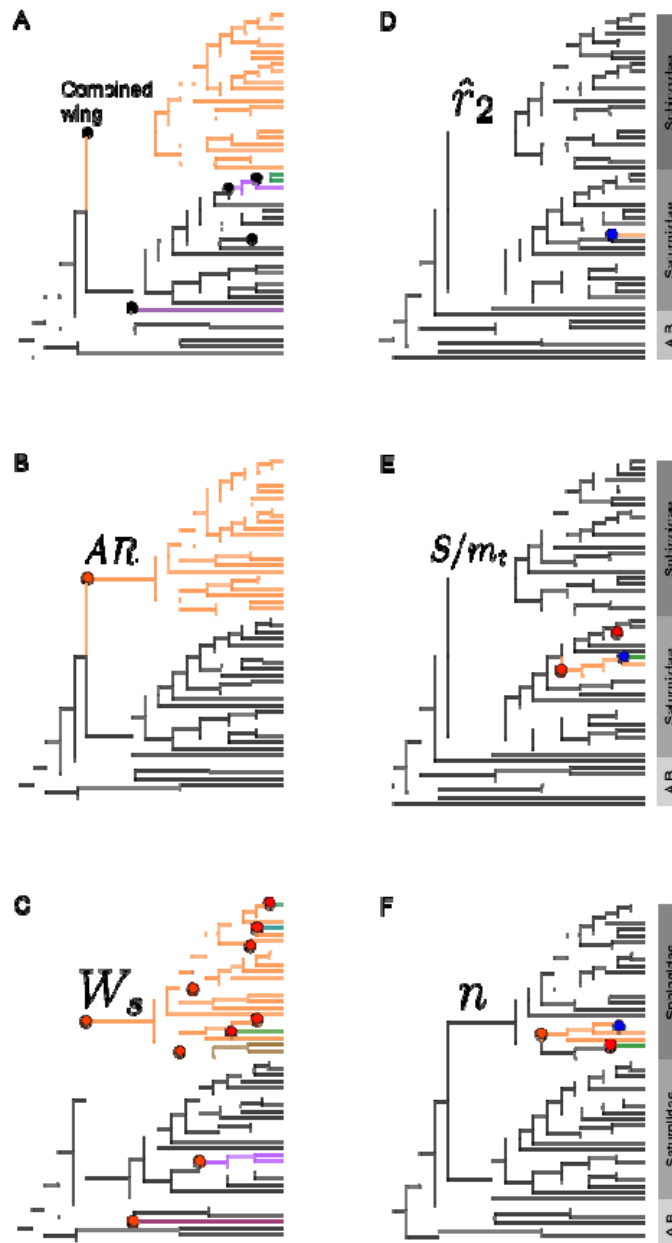
269 Next, we tested whether an adaptive shift is responsible for the divergence in wing shape and
270 its associated traits between hawkmoths and silkmoths without *a priori* hypotheses of shift
271 location(s). We found support for an adaptive shift at the ancestral node of the hawkmoth
272 clade for combined wing shape, AR, and W_s (Fig. 3A-C). More recent adaptive shifts also
273 occurred for combined wing shape, W_s , \hat{r}_2 , S/m_t , and n (Fig. 3A-F). The recent adaptive shifts in
274 silkmoth combined wing shape are associated with the independent tail evolutions (Fig. 3A).
275 The recent adaptive shift for n occurs in the hawkmoth subfamily, Macroglossinae, known for
276 its particularly high n (Fig. 3F). Adaptive shifts did not occur at the ancestral node for either
277 sister family for combined wing \hat{r}_2 , S/m_t , or n . In the absence of an adaptive shift, a trait can
278 still have diverged between the sister-clades through other evolutionary processes. However, a
279 single adaptive shift is inferred at the ancestral hawkmoth node when all functional
280 (normalized) wing metrics are analyzed together, supporting the findings that hawkmoth wing
281 morphology undergoes an adaptive shift (Fig. S5).

282

283 *Wing morphology does not evolve under a single-rate Brownian motion process*

284 Next, we determined which model best fit the evolution of combined wing shape and its
285 associated morphological features. For all traits, the model representing the adaptive shifts
286 detected in the PhylogeneticEM analysis always fit best (Table S7). However, an adaptive shift
287 was not detected in the previous analysis at the node for either sister family for \hat{r}_2 , S/m_t , or n ,
288 and this study is focused on the sister-clade divergence; the absence of an adaptive shift is

289 likely due to the complex selective pressures on these traits that depend on both body and
290 wing morphology.



291
292 Figure 3. An adaptive shift is responsible for divergence in wing shape (A), aspect ratio (B), and
293 wing loading (C) between hawkmoths and silkmoths. Each branch color indicates a separate
294 regime (a set of branches evolving under a different set of model parameters). All branches
295 sharing the same color also share the same evolutionary mode. Shifts to new regimes are
296 indicated by dots. For univariate traits, red dots indicate shifts to a larger trait value optima and
297 blue dots indicate shifts to a smaller trait value. Black dots are used for shifts in multivariate
298 traits, but do not indicate a direction.

299

300 DISCUSSION

301 Flight morphology can have a strong influence on the aerodynamic performance of flying
302 animals. We find that early in the evolutionary history of the moth superfamily Bombycoidea,
303 wing shape and size were generally conserved until the ancestors of the hawkmoth and
304 silkmoth sister clades rapidly diverged (Fig. 3A-C), which is consistent with the early
305 establishment of morphological disparity *between* clades (Fig. 2).

306 The evolutionary split between these two families has been dated to have occurred
307 approximately 66 (confidence interval: 56.9 to 75.4) million years ago [30], suggesting that
308 these wing morphology trajectories may have been evolving since then. The initial divergence
309 in wing morphology between hawkmoths and silkmoths was followed by subsequent
310 diversification within each group, indicated by the rise in relative subclade disparity above a BM
311 expectation coinciding with the more recent speciation events occurring within each family (Fig.
312 2). However, despite recent diversification, wing morphology did not converge between the
313 two sister-families, indicated by the strong separation between the families in phylogenetic
314 morphospace (Fig. 1).

315 Even specific species that converged in life history did not fully converge to employ
316 overlapping wing shapes. For example, while the majority of hawkmoths are known for their
317 hovering nectaring behavior as adults, members of the hawkmoth subfamily, Smerinthinae
318 (Node 67; Fig. 1A, S2B), have lost the ability to feed as adults [38], convergent with silkmoths.
319 However, the combined wing morphology (shape, size, and most associated traits) of
320 Smerinthinae species (Node 67 in Fig. S2B) remains divergent from silkmoths, implying that
321 Smerinthinae wing morphology is constrained by its evolutionary history. Finally, while we

322 chose species to broadly cover the groups within bombycoids, sampling is far from complete.
323 Therefore, we remain conservative in our interpretation, focusing on the split between
324 hawkmoths and silkmoths for which we were able to accumulate broad sampling for our
325 analysis. In sum, these data provide phylogenetic evidence supporting our hypothesis that
326 distinct flight morphology evolved in each sister clade.

327

328 *The evolutionary divergence of wing morphology has implications for flight performance.*

329 Given that the hawkmoth and silkmoth clades diverged in wing morphology, we can
330 explore the consequences of these two morphologies for flight performance. While flight
331 performance depends on many other factors, most notably wing movement, shape and size do
332 have implications for aerodynamics. Contrary to our expectations, we did not observe
333 morphological changes that were consistent with extreme maneuverability in hawkmoths and
334 extreme power reduction in silkmoths. Hawkmoths, known to be maneuverable hover feeders,
335 have evolved small wings of high AR, W_s , and \hat{r}_2 ; all metrics typically associated with power
336 reduction, efficient force production, and lower degrees of maneuverability. In contrast,
337 silkmoths, a group that does not feed as adults and is known for its bobbing (erratic) flight
338 behavior, have evolved large wings of low AR, W_s , and \hat{r}_2 .

339

340 *Hawkmoth wing morphology likely reduces power without sacrificing maneuverability*

341 The high AR and \hat{r}_2 wings of hawkmoths might act to reduce power and increase force
342 production efficiency while *not* sacrificing maneuverability in comparison to silkmoths that are
343 employing wings of lower AR and \hat{r}_2 . All else being equal, high AR and \hat{r}_2 wings will reduce the

344 induced power (P_{ind}) requirements of flight [6, 19, 22] and increase force production efficiency
345 [5, 48, 49], respectively. However, both traits could come at the cost of reduced
346 maneuverability due to an increase in the moments of inertia of the wing pair [3, 5, 6, 21, 23].
347 For a wing of constant area, uniform thickness, and density, a larger AR and \hat{r}_2 will necessarily
348 make the wing longer (increasing AR) while also concentrating more area distally along the
349 span of the wing (increasing \hat{r}_2). Both scenarios correspond to an increase in wing moments of
350 inertia, suggesting silkmoths should be more maneuverable than hawkmoths [5, 24]. However,
351 wing size will also have a strong impact on wing moment of inertia, and silkmoths have evolved
352 larger wings (per body size) than hawkmoths (Fig. 1F-G; Table S3). Hawkmoths evolved high AR
353 by reducing mean chord length, \bar{c} , rather than through an increase in wing span, R (Fig. 1B;
354 Table S4). Therefore, while selection for economical flight (increased AR) might often reduce
355 maneuverability, the evolution of small, high AR wings in the hawkmoth clade (achieved
356 through a reduction in \bar{c}) could act to increase economy while not necessarily sacrificing
357 maneuverability.

358 The potential cost of small wing size is that proportionally smaller wings could reduce
359 wing stroke-averaged aerodynamic force production, if wing movement remains constant.
360 However, in flapping or revolving wings, when all other things are equal, the greater \hat{r}_2 and n
361 (inferred through scaling relationships) of hawkmoths would increase their magnitude of
362 torque production relative to silkmoths. The velocity of a wing section increases with its
363 distance from the axis of rotation, and aerodynamic force production is proportional to velocity
364 squared. Therefore, shifting more area distally (increasing \hat{r}_2) and moving the wing at higher
365 speeds (increasing n) will increase aerodynamic force production [e.g. 26, 48, 49]. Additionally,

366 increasing n allows for more frequent modification of force vectors, which could enhance flight
367 control and maneuverability. Natural selection could thus act on wing shape, size, and
368 frequency (tradeoffs through scaling relationships) to modify the means of force production,
369 power, and flight control across species.

370

371 *Lower wing loading (W_s) in silkmoths could contribute to maneuverability and erratic flight*

372 It is possible that inter-clade differences in W_s contribute to inter-clade differences in
373 flight behavior between families. A lower W_s increases both maneuverability [14-21] and flight
374 path unpredictability [50]. Silkmoths, which evolved significantly lower W_s in comparison to
375 hawkmoths (Fig. 1G, Table S3), are well known for their erratic flight patterns [32, 38] where
376 vertical position is regularly changing throughout their flight bout. An erratic, or unpredictable
377 flight path, can enhance predator avoidance [15, 51], and therefore, survival and fitness. In
378 hummingbird flight, positional predictability and W_s are positively correlated where
379 hummingbirds with lower wing loading are less predictable [50]. If the relationship between W_s
380 and predictability is true in other systems, then the divergence in W_s between hawkmoths and
381 silkmoths is precisely the expectation based on the divergence in flight behavior between the
382 two clades. Therefore, it's likely that evolution of silkmoth wing morphology, particularly low
383 W_s , is directly tied to the production of erratic flight patterns and the ability to avoid predation.

384

385 *Body shape evolution might aid predator avoidance in silkmoths*

386 Next, we examined the implications of body size evolution for flight performance. In
387 comparison to hawkmoths, silkmoths have a shorter l_b and a longer thorax compared to the

388 abdomen, thereby decreasing I_{yy} and I_{zz} of the body and likely increasing maneuverability.
389 These patterns could allow silkmoths greater angular accelerations during pitch and yaw
390 maneuvers and might be complemented by a reduction in the distance between the center of
391 mass and wing hinge [52]. Indeed, species of neotropical butterflies equipped with a shorter
392 abdomen and larger thorax were more successful at evading predators than species with
393 shorter thoraces and longer abdomens [52]. Therefore, in addition to wing elaborations [32, 38,
394 46] and bobbing flight behavior [32, 36-38], our data suggest that the evolution of a large
395 thorax and short abdomen is an additional mechanism contributing to predator avoidance in
396 silkmoths.

397

398 *Adaptive shifts are responsible for the divergence in wing morphology between hawkmoths and*
399 *silkmoths*

400 An adaptive shift is found at the stem of hawkmoths for both wing shape, AR, and W_s (Fig. 3A-
401 C), indicating that the shape and relative size of hawkmoth wings are evolving around an
402 adaptive peak. Although disparity was established early in the evolutionary history of the clade
403 (Fig. 2), rather than slow down in diversification rate, which would occur in an early burst [8, 9],
404 the initial divergence in life history and flight morphology gives rise to the accumulation of
405 additional disparity within each clade in recent time (Fig. 2). Indeed, the recent accumulation of
406 disparity within a subclade is associated with evolution around an adaptive peak [53], and the
407 absence of evidence for an early burst in the diversification of wing morphology is consistent
408 with major inter-continental radiations in other systems [7, 10, 11].

409 The discrete adaptive shift in hawkmoth wing morphology parallels the evolution of the
410 hover feeding behavior in hawkmoths and the loss of adult-stage feeding in silkmoths. The
411 adaptive shift in hawkmoth wing morphology to small, slender wings of high AR that can be
412 moved at high frequencies might be directly related to the evolution of hover feeding, which
413 requires enhanced flight control and high power output [35], as high AR wings are known to
414 reduce flight power requirements [6].

415 An adaptive shift at the stem of hawkmoths was not found for all wing morphology
416 traits, suggesting a potential decoupling of the processes, and, therefore, selective pressures,
417 driving the evolution of overall wing shape, size, and specific features. It should not be expected
418 that all features of wing morphology evolve under the same process. Wing metrics, like \hat{r}_2 ,
419 which is related to force production efficiency [24], appear to be more conserved, and those
420 related to both wing and body size, like n and S/m_t , might be under particularly complex
421 selective pressures.

422 Differently, an adaptive shift was never found for any trait at the stem of the silkmoth
423 clade, which could be expected given the less drastic separation in wing morphology traits
424 between silkmoths and the other bombycoid families (Fig. 2). In contrast, more recent adaptive
425 shifts were detected and associated with the evolution of hindwing tails in silkmoths (Fig. 3A)
426 and high n in diurnal hawkmoths (Fig. 3F). While these recent shifts need to be supported
427 through further sampling within these specific groups, it is exciting that they might be indicative
428 of recent shifts in flight morphology within these clades, providing a potential opportunity to
429 identify specialized species or subclades for future functional studies in live animals.

430 The overall combined wing morphology is derived from two functionally linked and
431 overlapping wing structures (forewing and hindwing) that can each potentially evolve
432 independently in size and shape, unlocking additional complexities unachievable by a single
433 wing alone. While forewing and hindwing morphology also diverge between groups, the
434 absolute values of these traits are different between the fore- and hindwing (Fig. S4). Different
435 components of the same functional system often evolve at different tempos and modes [54],
436 raising questions of whether or not certain aspects of wing morphology constitute evolutionary
437 modules. The integration of techniques from developmental and evolutionary biology will be
438 particularly fruitful when investigating the modularity of insect wing units.

439

440 *Conclusion*

441 Silkmoths and hawkmoths evolved distinct flight morphology through an adaptive shift
442 in hawkmoth wing morphology, which occurred in parallel to the evolution of the hover feeding
443 behavior in hawkmoths. The sister-clade divergence of wing morphology metrics, which are
444 historically derived for fixed-winged aircrafts, is not totally consistent with initial expectations
445 of flight performance based on the life history of species in each clade. However, aerodynamic
446 performance emerges from the interaction of wing shape, size, and movement [6, 55], and it is
447 likely that hawkmoths achieve high levels of flight control through high n and other kinematic
448 adjustments. Our findings indicate that aerodynamically important morphological traits can
449 experience drastic shifts in parallel to the divergence in life history and flight behavior. While
450 the evolution of flapping flight in insects is thought to be a key innovation [1], diversification

451 can be further potentiated by more recent adaptive shifts, helping to shape the diversity of
452 wing morphology seen across extant aerial animals.

453

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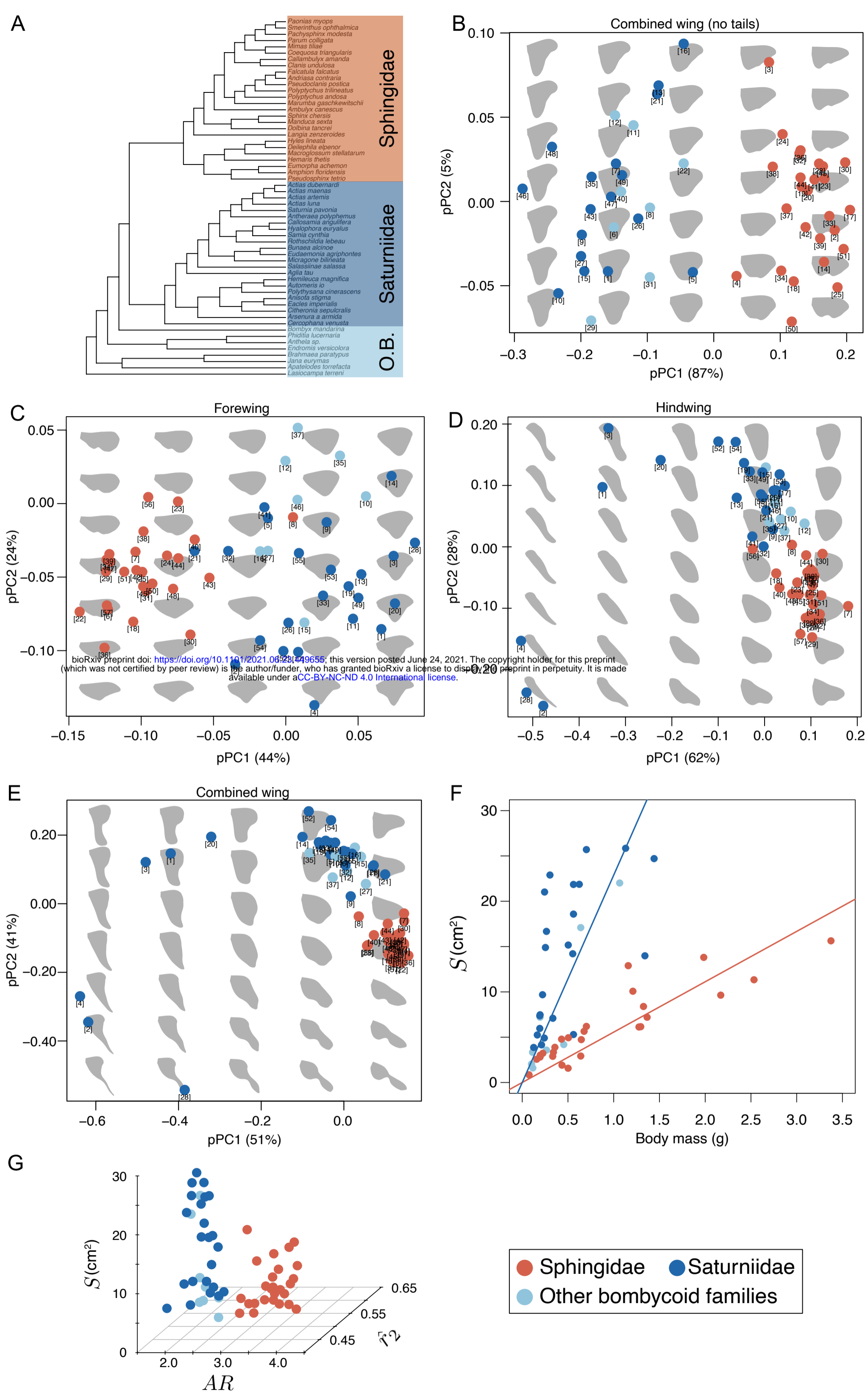
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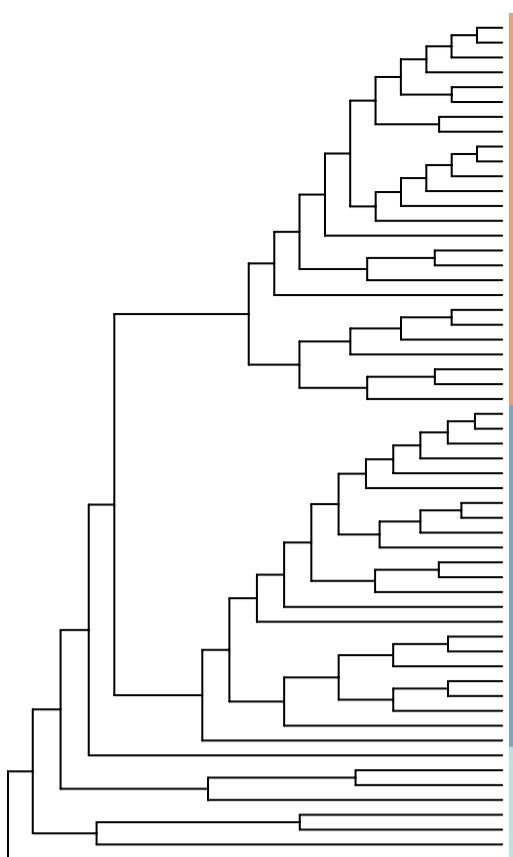
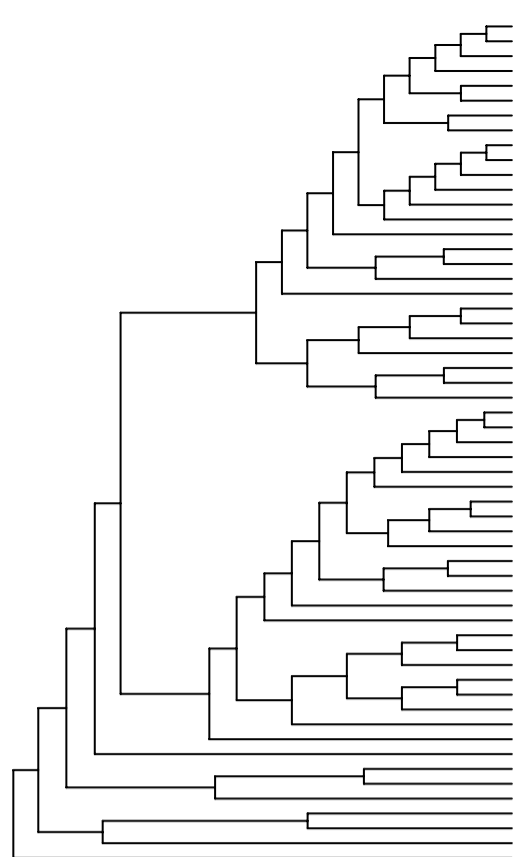
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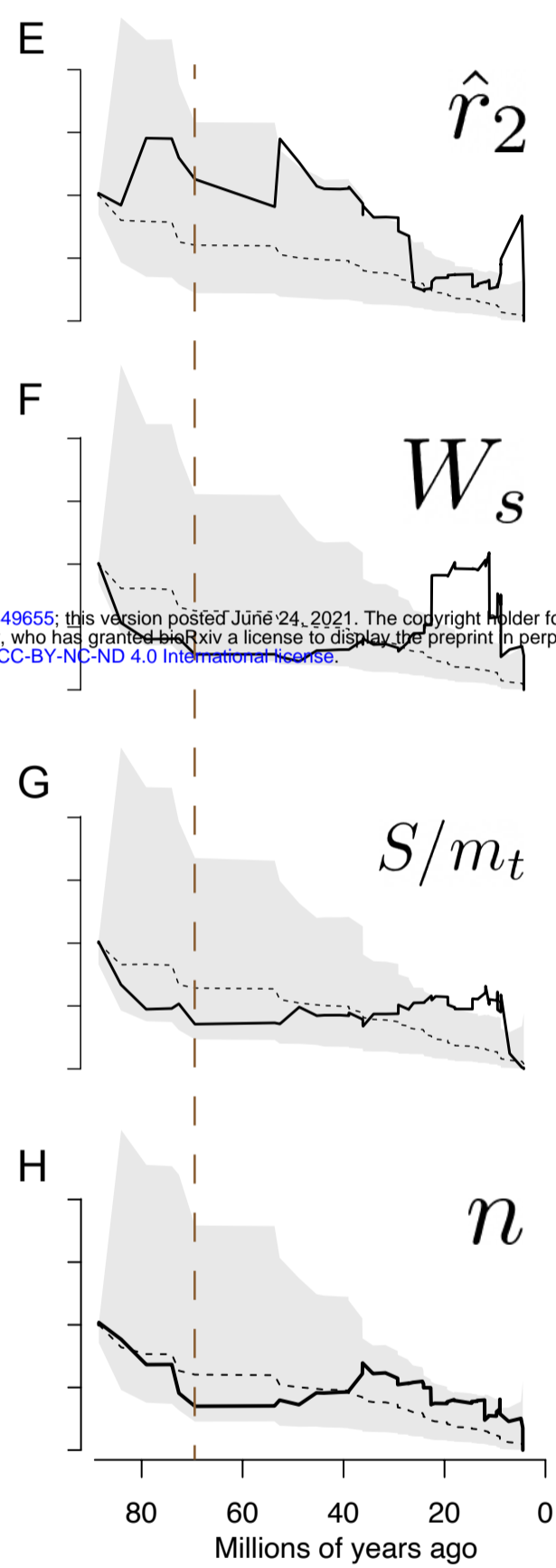
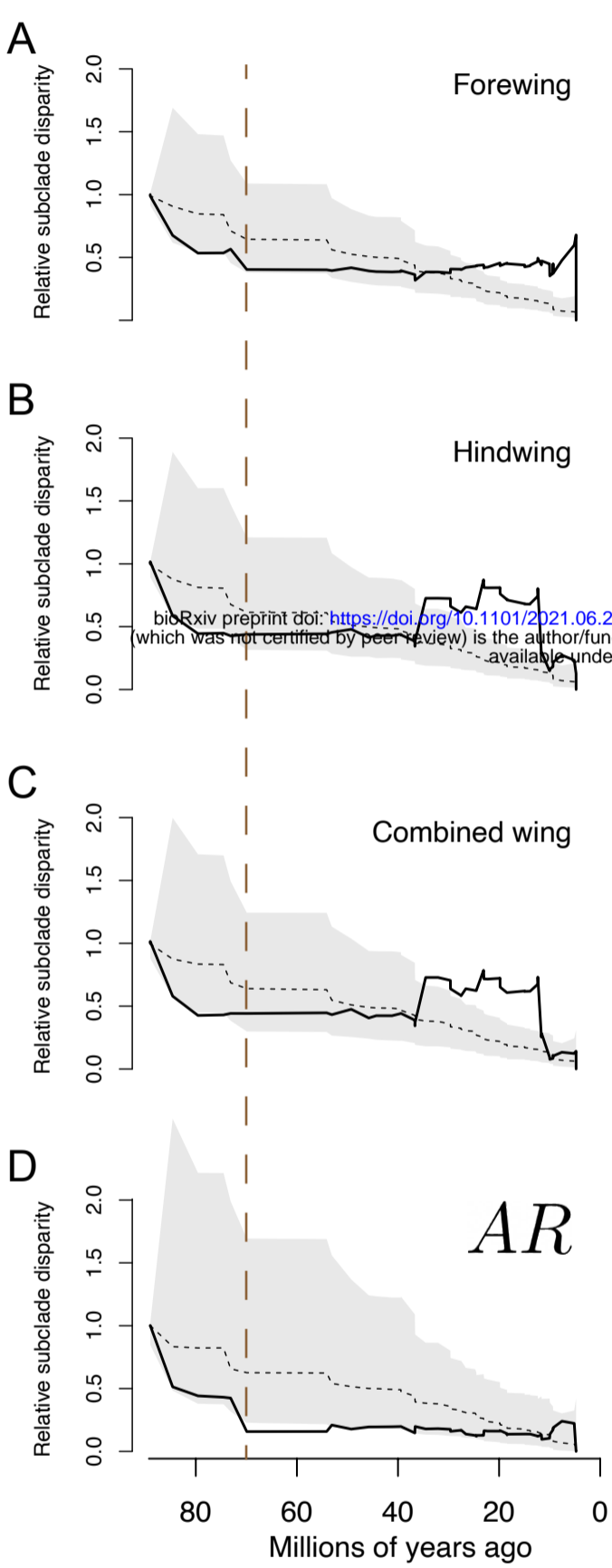
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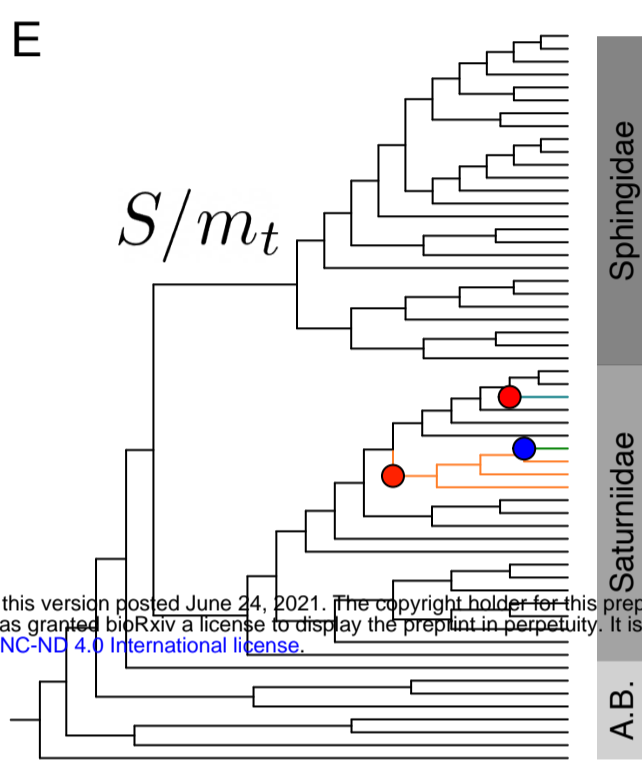
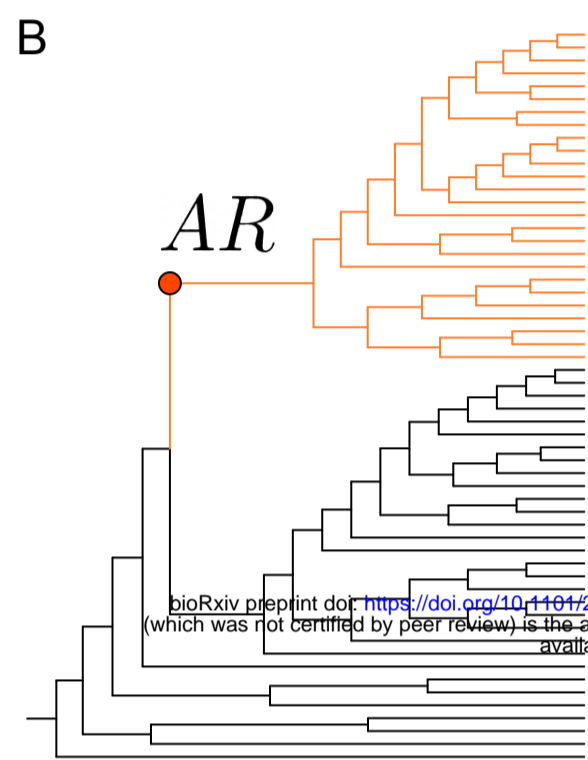
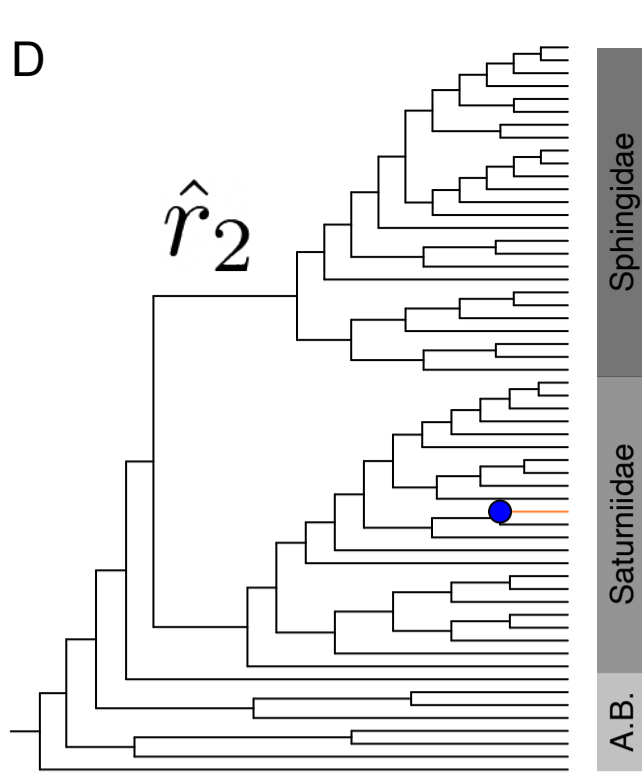
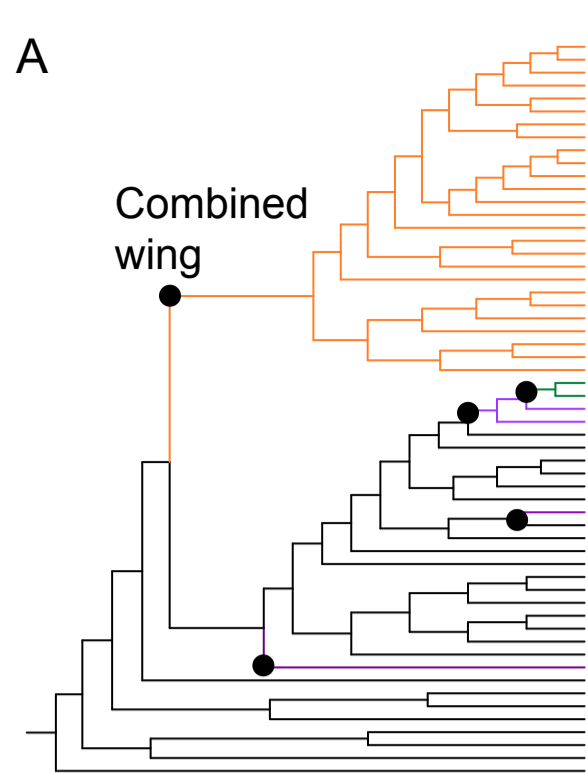




O.B. Saturniidae Sphingidae



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