1   **Running head**

2   THE AGE OF BUTTERFLIES REVISITED (AND TESTED)

3   **Title**

4   The Trials and Tribulations of Priors and Posteriors in Bayesian Timing of

5   Divergence Analyses: the Age of Butterflies Revisited.

6

7   **Authors**

8   NICOLAS CHAZOT[1*], NIKLAS WAHLBERG[1], ANDRÉ VICTOR LUCCI FREITAS[2],

9   CHARLES MITTER[3], CONRAD LABANDEIRA[3,4], JAE-CHEON SOHN[5], RANJIT KUMAR

10   SAHOO[6], NOEMY SERAPHIM[7], RIENK DE JONG[8], MARIA HEIKKILÄ[9]

11   **Affiliations**

12   [1]*Department of Biology, Lunds Universitet, Sölvegatan 37, 223 62, Lund, Sweden.*

13   [2]*Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de*

14   *Campinas (UNICAMP), Cidade Universitária Zeferino Vaz, Caixa postal 6109,*

15   *Barão Geraldo 13083-970, Campinas, SP, Brazil.*

16   [3]*Department of Entomology, University of Maryland, College Park, MD 20742, U.S.A.*

17   [4]*Department of Paleobiology, National Museum of Natural History, Smithsonian*

18   *Institution, Washington, DC 20013, USA; Department of Entomology and BEES*

19   *Program, University of Maryland, College Park, MD 20741; and Key Lab of Insect*

20   *Evolution and Environmental Change, School of Life Sciences, Capital Normal*

21   *University, Beijing 100048,*

22   [5]*Institute of Littoral Environment, Mokpo National University, Muan, Jeonnam 58554,*

23   *Republic of Korea*

24   [6]*IISER-TVM Centre for Research and Education in Ecology and Evolution (ICREEE),*

25   *School of Biology, Indian Institute of Science Education and Research,*

26   *Thiruvananthapuram, Kerala 695 551, India.*

27   [7]*Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Campus*

28   *Campinas, CTI Renato Archer - Av. Comendador Aladino Selmi, s/n - Amarais,*

29   *Campinas - SP, 13069-901, Brazil.*

30   [8] *Naturalis Biodiversity Center, Department of Entomology, PO Box 9517, 2300 RA*

31   *Leiden, The Netherlands.*

32   [9] *Finnish Museum of Natural History LUOMUS, Zoology Unit, P.O. Box 17, FI-*

33   *00014 University of Helsinki, Finland.*

34

35   **Corresponding author (*):**

36   Nicolas Chazot

37   *Department of Biology, Lunds Universitet, Sölvegatan 37, 223 62, Lund, Sweden.*

38   email: chazotn@gmail.com

39

40 **Abstract**

41 The need for robust estimates of times of divergence is essential for downstream

42 analyses, yet assessing this robustness is still rare. We generated a time-calibrated

43 genus-level phylogeny of butterflies (Papilionoidea), including 994 taxa, up to 10

44 gene fragments and an unprecedented set of 12 fossils and 10 host-plant node

45 calibration points. We compared marginal priors and posterior distributions to assess

46 the relative importance of the former on the latter. This approach revealed a strong

47 influence of the set of priors on the root age but for most calibrated nodes posterior

48 distributions shifted from the marginal prior, indicating significant information in the

49 molecular dataset. We also tested the effects of changing assumptions for fossil

50 calibration priors and the tree prior. Using a very conservative approach we estimated

51 an origin of butterflies at 107.6 Ma, approximately equivalent to the Early

52 Cretaceous–Late Cretaceous boundary, with a credibility interval ranging from 89.5

53 Ma (mid Late Cretaceous) to 129.5 Ma (mid Early Cretaceous). This estimate was

54 robust to alternative analyses changing core assumptions. With 994 genera, this tree

55 provides a comprehensive source of secondary calibrations for studies on butterflies.

56 **Keywords**

58 **INTRODUCTION**

59 An increasing amount of molecular information is allowing the inference of broad and

60 densely sampled phylogenetic hypotheses for species-rich groups. This effort,

61 combined with the emergence of a great number of methods investigating trait

62 evolution, historical biogeography, and the dynamics of diversification have increased

63 the need for time-calibrated trees. Estimating divergence times in molecular

64 phylogenetic work depends primarily on fossils to constrain models of heterogeneous

65 rates of substitutions. Consequently, the robustness of such estimates relies on the

66 quality of fossil information, involving age and taxonomic assignment (Parham et al

67 2012), the priors assigned to nodes that are calibrated in a Bayesian analysis

68 (Warnock et al 2012, Brown & Smith 2017), and the amount of information inherent

69 in the molecular dataset (Yang & Rannala 2006, Rannala & Yang 2007, dos Reis &

70 Yang 2013).

71 Fossils inform us of the minimum age of a divergence, imposing a temporal constraint

72 that is widely accepted. However, the constraint of a simple hard minimum age is

73 insufficient information for a proper analysis of times of divergence, particularly as

74 there is an absence of information about maximum ages for divergences, including the

75 root node. Often fossil information is modeled as a probability distribution, such as a

76 lognormal or exponential distribution, indicating our beliefs regarding how

77 informative a fossil is about the age of a divergence (Drummond et al 2006, Warnock

78 et al. 2015). The distributional shapes of these priors are often established without

79 justification (Warnock et al. 2012). Ideally, in node-based dating, fossil information is

80 used only as a minimum age constraint for a given divergence in the form of a

81 uniform prior with a minimum age equaling the fossil age and a maximum age

82   extending beyond the age of the clade in question. In such cases at least one

83   maximum constraint is needed, often also based on fossil information. Another

84   approach is use of extraneous additional information, such as using ages of host plant

85   families as maximum constraints for highly specialized phytophagous insect clades

86   (Wahlberg et al. 2009). In such cases, a uniform prior also can be used, with the

87   maximum set to the age of the divergence of the host plant family from its sister

88   group and the minimum set to the present time.

89   Brown & Smith (2017) recently have pointed out the importance of assessing the

90   relative influence of priors over the actual amount of information contained in the

91   molecular dataset. As noted above, users specify fossil calibrations using prior

92   distributions by modeling the prior expectation about the age of the node constrained.

93   However, the broader set of fossil constraints can interact with each other and with

94   the tree prior, leading to marginal prior distributions at nodes that usually differ from

95   the user's first intention (Warnock et al 2012). If relevant information were contained

96   within the molecular dataset, one would expect the posterior distribution to shift from

97   the marginal prior distribution. In the case of angiospermous plants, Brown & Smith

98   (2017) showed that the marginal prior resulting from the interaction of all priors

99   (fossils and the tree) excluded an Early Cretaceous origin, in effect giving such an

100   origin zero probability. In addition, many calibrated internal nodes showed nearly

101   complete overlap of marginal prior and posterior distributions, suggesting little

102   information in the molecular dataset but a potentially strong influence of the set of

103   priors.

104   With more than 18,000 species described and extraordinary efforts made to infer

105   phylogenetic hypotheses based on molecular data, butterflies (Lepidoptera:

106   Papilionoidea) have become a model system for insect diversification studies.

107   Nevertheless, the paucity of information available to infer times of divergence in

108   butterflies questions the reliability of the various estimates (e.g. Garzón-Orduña et al.

109   2015). Heikkilä et al. (2012) for example, used only three fossils to calibrate a higher-

110   level phylogeny of the superfamily Papilionoidea. The shortage of fossil information

111   for calibrating large-scale phylogenies also means that, most of the time, species-level

112   phylogenies at a smaller scale rely on secondary calibration points extracted from the

113   higher-level time-trees (e.g. Peña et al. 2011, Matos-Maravi et al. 2013, Kozak et al.

114   2015, Chazot et al. 2016, Toussaint & Balke 2016).

115   In a recent paper, de Jong (2017) revisited the butterfly fossil record, providing a

116   discussion about the quality of the different fossil specimens as well as their

117   taxonomic placement. Using this information, we established an unprecedented set of

118   12 fossil calibration points across all butterflies, which we use in this study to revisit

119   the timescale of butterfly evolution in a comprehensive phylogenetic framework, and

120   investigate the robustness of this new estimate. We complement the minimum age

121   constraints of clades based on fossils with maximum age constraints based on the ages

122   of host plant families. Some clades of butterflies have specialized on specific groups

123   of angiosperm hosts for larval development, such that one may assume that

124   diversification of the associated butterfly clade only occurred after the appearance of

125   the host plant clade. We use this assumption as additional information to calibrate the

126   molecular clock by setting the age of specific clades of butterflies to be younger than

127   the estimated age of their host plant lineage. We restrained these calibrations to

128   higher-level host plant clades.

129   The most recent estimates of divergence times using representatives of all butterfly

130   families inferred a crown clade age of butterflies of 110 Ma (Heikkilä et al. 2012) and

131   104 Ma (Wahlberg et al. 2013). These two dates yield to a large discrepancy when

132   taking the fossil record into account, as the oldest known fossil butterfly is estimated

133   to be 55.6 Ma and can be confidently assigned to the extant family Hesperiidae (de

134   Jong 2016, 2017). Such discrepancy has been extensively debated for a similar case,

135   the origin of angiosperms, often estimated to have originated during the Triassic (252-

136   –201 Ma ago), while the oldest undisputed fossil is pollen dated at 136 Ma. Despite a

137   much more fragmentary fossil record for butterflies, the same questions remain. First,

138   are the previous estimates robust to a more comprehensive assemblage of fossils and

139   taxon sampling? Second, is the 50 million-year discrepancy between molecular clock

140   estimates and the fossil record accurate or the result of a lack of information

141   contained in the molecular dataset? In other words, how much does the set of priors

142   influence the results?

143   Here, we generated a genus-level phylogeny of Papilionoidea, including 994 taxa, in

144   order to maximize the number and position of fossil calibration points and increase

145   the potential amount of molecular information. By establishing the set of 12 fossils

146   and 10 host-plant calibration points, we time-calibrated the tree in order to provide a

147   revised estimate of the timing in diversification of butterflies. We then assessed the

148   robustness of these results to the assumptions made throughout the analysis, including

149   (i) different subsets of fossil constraints, (ii) the prior distributions of fossil constraints,

150   (iii) a different estimate for host plant ages, (iv) a Yule tree prior, (v) a reduced taxon

151   sampling and (vi) the addition of a mitochondrial gene fragment to the nine nuclear

152   gene regions.

153   Finally, we compared the user specified priors, marginal prior and posterior

154   distributions of different analyses, to assess the influence of our set of constraints on

155   the estimated timing of divergence.

156

157   **MATERIALS AND METHODS**

158   *Molecular Dataset*

159   When designing our dataset, we aimed at building a genus-level tree of Papilionoidea.

160   We assembled a dataset of 994 taxa from the database VoSeq

161   (http://www.nymphalidae.net/db.php, Peña & Malm 2012), with each taxon

162   representing a genus. We chose to include gene fragments that were available across

163   the whole tree in order to avoid large clade-specific gaps in the molecular dataset. In

164   addition, Sahoo et al. (2016) pointed out a conflicting signal in the family Hesperiidae

165   between nuclear and mitochondrial markers. Thus, we chose to primarily focus on

166   nuclear markers. Our final dataset included nine gene fragments: ArgKin (596bp),

167   CAD (850bp), EFI-□ (1240 bp), GAPDH (691bp), IDH (710 bp), MDH (733 bp),

168   RPS2 (411 bp), RPS5 (617 bp) and wingless (412 bp) for a total length of 6260 base

169   pairs. The list of taxa and Genbank accession codes are available in the

170   Supplementary Material S1.

171   *Set of Time-Calibrations for Timing Analyses*

172   Fossil calibrations – Previous studies estimating times of divergence of butterfly

173   lineages have largely relied on unverified fossil calibrations. The identifications of

174   these calibrations were often based on overall similarity with extant taxa, not

175   apomorphies. In the present study, we initially chose 14 fossil butterflies that were

176   recently critically reviewed by de Jong (2017) and displayed apomorphic characters

177   or character combinations diagnostic of extant clades, thereby allowing reliable

178   allocation of fossils on the phylogenetic tree to provide minimum ages to the

179   corresponding nodes. These fossils included three inclusions in Dominican Amber

180   and 11 compression/impression fossils. For the age of these fossils we have relied on

181   the most recent dates established from recent advances in Cenozoic

182   chronostragigraphy, geochronology, chemostratigraphy and the geomagnetic polarity

183   time scale (Walker et al., 2013). These improvements by geologists and specialists in

184   allied disciplines have provided an increased precision in age dates of stratigraphic

185   record (International Commission on Stratigraphy, 2012). The list of fossils and their

186   positions in the tree is given in Table 1 and Figure 1. For more detailed information

187   on the identification of these fossils, localities, preservation type and current

188   depositories, see de Jong (2017).

189   When a fossil was assigned to a clade, we calibrated the stem age of this clade,

190   specifically the time of divergence from its sister clade, instead of the crown age or

191   the first divergence event recorded in the phylogeny. As a consequence of this choice,

192   we removed two of the 14 fossils. We did not use *Praepapilio colorado* Durden &

193   Rose, 1978 (Papilionidae, 48.4 Ma) nor the less well-preserved *Praepapilio gracilis*

194   Durden & Rose, 1978 (Papilionidae) of the same age because its position at the root

195   of the tree was uninformative given the presence of the 55.6 million years old

196   *Protocoeliades kristenseni* de Jong, 2016 placed at the crown of the Hesperiidae. For

197   similar reasons, we did not use *Doxocopa wilmattae* Cockerell, 1907

198   (Nymphalinae+Biblidinae+Limenitidinae+Apaturinae, 33.8 Ma) because its position

199   was uninformative given the presence of *Vanessa amerindica* Miller & Brown, 1989

200   of the same age but placed lower in the tree.

201 Host plant calibrations – Butterflies are well known for their strict relationships with

202 specific groups of plants used by their larvae. Such associations have previously been

203 suggested as evidence for coevolution (Ehrlich & Raven, 1964, Janz & Nylin 1998,

204 Nylin & Janz 1999). In the present study, we selected nine calibration points based on

205 known information of host plant specificity by butterflies since the large revision of

206 Ackery (1988) (see also Beccalloni et al. 2008 for Neotropical species), and revised

207 for those host plant records listed as having spurious or occasional records (AVLF

208 unpublished data). Host plant clades used by single genera or a small group of

209 recently-derived genera were discarded, such as the use of Aristolochiaceae by

210 Troidini. In these cases the butterflies clearly are much more recent than their

211 associated plant clades, and consequently do not contribute relevant time information

212 to the tree. The ages of each plant group were defined as maximum ages for the

213 respective nodes (Table 1). For all host plant maximum constraints we used the

214 estimate from Magallón et al. (2015) using the upper boundary of the 95% credibility

215 interval of the stem age of the host plant clade. We also constrained the root of the

216 Papilionoidea with a maximum age corresponding to the crown age of angiosperms

217 from Magallón et al. (2015). The host plant calibrations were placed at the crown of

218 the butterfly clades as a conservative approach since we do not know when the host

219 plant shift occurred on the stem branch. However, we assume that the diversification

220 of the clade could not have begun earlier than the origin of the host plant family.

221

222 *Analyses Overview*

223 Given computational limitations for such a large dataset, we adopted the following

224 procedure (details given below). We ran PartitionFinder v. 1.1 (Lanfear et al. 2012) to

225   identify the best partition scheme. Using this result we performed a maximum

226   likelihood analysis to obtain a tree topology. This tree topology was transformed into

227   a time-calibrated ultrametric tree and used thereafter as a fixed topology and starting

228   tree in all our dating analyses. Branch lengths were estimated using BEAST v. 1.8.3

229   (Drummond et al. 2012) with a simpler partitioning scheme, a birth-death tree prior,

230   lognormal relaxed molecular clocks, and a combination of minimum (fossils) and

231   maximum (host plants) constraints for which all were set with uniform priors. This

232   constituted the core analysis. We then performed additional analyses to test the

233   robustness of our results to (i) different subsets of fossil constraints, (ii) the prior

234   distribution of fossil constraints, (iii) a different estimate for host-plant ages, (iv) a

235   Yule tree prior, (v) a reduced taxon sampling, and (vi) the addition of a mitochondrial

236   gene fragment.

237

238   *Core Analysis*

239   Tree topology – We started by running PartitionFinder v. 1.1 (Lanfear et al. 2012) on

240   the concatenated dataset, allowing all possible combinations of codon positions of all

241   genes. Substitution models were restricted to a GTR+G model and branch lengths

242   were linked. We then performed a maximum likelihood analysis using RAxML v8

243   (Stamatakis 2006) using the best partitioning scheme identified by PartitionFinder and

244   1000 ultrafast bootstraps. The resulting tree was set as a fixed topology for the dating

245   analyses. To do so, the tree was transformed into a time-calibrated ultrametric tree

246   using the package *ape* (Paradis et al. 2004) and all minimum and maximum

247   constraints in order to obtain a starting tree suitable for BEAST analyses.

248   Time tree – We used BEAST v. 1.8.3 (Drummond et al. 2012) to perform our time-

249   calibration analysis. Given the size of our dataset, we reduced the number of

250   partitions in our dating analysis to three partitions, each partition being one codon

251   position of all genes pooled together. Substitution rate for each partition was modeled

252   by GTR+G and an uncorrelated lognormal relaxed molecular clock. We used a Birth-

253   Death process as branching process prior. In order to have a fixed topology we turned

254   off the topology operators in BEAUTi and we specified the topology obtained with

255   RAxML made ultrametric with the *ape* package.

256   Setting the priors for calibration points is always an important matter of discussion.

257   Non-uniform priors are often used, yet in the majority of studies the choice of

258   parameters defining the shape of the prior distribution is not justified (Warnock et al.

259   2012). For the core analysis we followed a conservative approach – considering that

260   fossils only provide a minimum age, while host plant calibrations only provide a

261   maximum age for the nodes they were assigned to – and we used uniform prior

262   distributions for all calibration points (Table 1). When a node was calibrated with

263   fossil information, the distribution ranged from the estimated age of the fossil to the

264   age of angiosperm origin (extracted from Magallón et al. 2015). When a node was

265   calibrated using host-plant age, the prior distribution ranged from 0 (present) to the

266   age of the host plant clade origin. When a node was calibrated with both types of

267   information, the distribution ranged for the age of the fossil to the age of host plant

268   clade origin. We also used a uniform prior for the tree root height, ranging between

269   the oldest fossil used in the analysis and the age of angiosperm origin. Host plant

270   calibrations, as well as the origin of angiosperms were extracted from Magallón et al.

271   (2015), using the upper boundary of the 95% credibility interval of the stem age of the

272   host plant clade. Our choice of combining (1) uniform prior distributions, (2) fossil

273 calibration of stem nodes, (3) the oldest stem age of the host plant clades and (4) host

274 plant calibration of crown nodes has important implications. On the one hand these

275 choices are the most conservative options, cautiously using the information given by

276 each type of calibration point and taking into account uncertainty surrounding the

277 information used. On the other hand, they are also the least informative.

278 We performed four independent runs of 30 million generations, sampling every 30

279 000 generations. We checked for a satisfactory convergence of the different runs

280 using Tracer v. 1.6.0 (Rambaut et al. 2014) and the effective sample size values in

281 combination. Using LogCombiner v. 1.8.3 (Drummond et al. 2012), we combined the

282 posterior distributions of trees from the three runs, discarding the first 100 trees (10%

283 burn-in) of each run. Using TreeAnnotator v. 1.8.3 (Drummond et al. 2012) we

284 extracted the median and the 95% credibility interval of the posterior distribution of

285 node ages.

286

287 *Alternative Analyses*

288 We tested the effect of making alternative choices along the core analysis on our

289 estimates of divergence times. Unless stated otherwise, we made only one

290 modification at a time; all other parameters remained identical to that described for

291 the core analysis. We performed at least two independent runs of 30 million

292 generations per alternative parameter set and more if convergence was not reached.

293 Different subset of fossils – We aimed at testing whether using only a fraction

294 of the fossil information affected the estimation of divergence times and whether the

295 position of calibrations (close to the root or close to the tips) also changed the results.

296   Thus, we divided our set of fossil constraints into two subsets depending on their

297   position in the tree. One subset included fossil calibration points assigned at a deep

298   level in tree (hereafter: higher-level fossils): *Lethe*, *Mylothrites*, *Neorinella*,

299   *Pamphilites*, *Prolibythea*, *Protocoeliades* and *Vanessa* (Table 1). The other subset

300   included fossil calibration points close to the tips of our phylogeny (hereafter: lower-

301   level fossils): *Doritites*, *Thaites*, *Dynamine*, *Theope* and *Voltinia* (Table 1). In both

302   cases the full set of maximum constraints was used. We performed one analysis for

303   each subset.

304   Exponential fossil priors – In the core analysis we used uniform distributions

305   for calibration points, which is a conservative option but also the least informative. As

306   an alternative, we designed exponential priors for fossil calibration points.

307   Exponential priors use the age of a fossil as minimum age for the node it has been

308   assigned to, but also assume that the probability for the age of the node decreases

309   exponentially as time increases. In BEAUTi, we set the offset of exponential

310   distributions with the age of the fossil. The distribution was truncated at the maximum

311   age used in the uniform priors. The shape of the exponential distribution is controlled

312   by a mean parameter, which has to be arbitrarily chosen by the users. The choice of

313   mean parameter can be found in Table 1. Priors for host plant calibration points were

314   not changed (i.e., uniform priors).

315   Alternative host plant ages –The origin and timing of diversification of

316   angiosperms is controversial. While the oldest undisputed fossil of Angiospermae is

317   from the early Cretaceous (136 Ma, Brenner 1996), most divergence time estimations

318   based on molecular clocks have inferred a much older origin. In the core analysis, we

319   chose to use host plant ages derived from the tree of angiosperms time-calibrated by

320   Magallón et al. (2015), who imposed a constraint on the origin of angiosperms based

321   on this fossil information. They found a crown age for angiosperms of ~ 140 Ma. As

322   an alternative consistent with an older origin of angiosperms we used ages recently

323   inferred by Foster et al. (2017), who recovered a crown age of angiosperms of ~ 209

324   Ma. All maximum constraints were replaced by those inferred by Foster et al. (2017).

325   The origin of angiosperms was used as a maximum constraint was set to the upper

326   boundary of the 95% credibility interval of the crown age of the angiosperms i.e.,

327   252.8 Ma. Because the posterior distributions of node ages for this analysis were very

328   skewed, we extracted the median of the distribution, the 95 % credibility interval and

329   the mode of the kernel density estimate of nodes using the R package *hdrcde*. For

330   comparison, we also estimated the mode of posterior distributions for the core

331   analysis and all alternative tests.

332   Yule branching process prior – Condamine et al. (2015) showed that the prior

333   for the tree growth can a have a great impact on the estimated divergence times. In the

334   core analysis we used a Birth–Death prior, which models the tree formation with a

335   constant rate of lineage speciation and a constant rate of lineage extinction. As an

336   alternative, we used a Yule prior, which involved a constant rate of speciation and no

337   extinction to assess whether age estimates changed or not.

338   Reduced dataset – In our core analysis, we chose to maximize the taxon

339   sampling – increasing the number of lineages – which increased the fraction of

340   missing data in the molecular dataset. We tested whether increasing the molecular

341   dataset completion to the detriment of taxon sampling changed the results. In this

342   reduced dataset, we included all the genera for which a specific minimum number of

343   genes were available. The missing data in the molecular dataset are not uniformly

344 distributed across the tree; for example, Lycaenidae have more missing data than the

345 Nymphalidae. Therefore, a different cut-off value was chosen for each family in order

346 to keep a good representation of the major groups (Papilionidae: 5 genes, Hedylidae:

347 8 genes, Hesperiidae: 9 genes, Pieridae: 8 genes, Lycaenidae: 4 genes, Riodinidae: 8

348 genes, Nymphalidae: 9 genes). In order to allow assignment of all fossils to the same

349 place as in the core analysis, nine taxa having a number of genes below the cut-off

350 value had to be added. We ended up with a dataset reduced to only 364 taxa instead of

351 994 in the core analysis. Accordingly, the fraction of missing data decreased from

352 39.5% in the core analysis to 21.4%. Given this important modification of the dataset

353 we generated a new topology with RAxML, which was then calibrated identically to

354 the core analysis.

355   *Mitochondrial gene fragment* – We tested whether adding mitochondrial

356 information in the dataset would affect our results. To do so, we added the

357 cytochrome-oxydase-subunit 1 gene to the molecular dataset. Given the conflicting

358 signal in Hesperiidae between nuclear and mitochondrial information (Sahoo et al.

359 2016), the COI was not added to the Hesperiidae. We performed a new RAxML

360 analysis in order to obtain a new topology. This new tree was calibrated with BEAST

361 identically to the core analysis, with one difference. The mitochondrial gene was

362 added as two partitions separated from the nuclear partitions: the first and second

363 positions of COI were pulled together and the third position had its own partition.

364 Therefore this analysis had five partitions.

365

366 *Comparing Prior and Posterior Distributions*

367   When performing a Bayesian analysis, comparing prior and posterior parameter

368   distributions can be informative about the amount of information contained by our

369   data compared to the influence of prior information. As exemplified by Brown &

370   Smith (2017), such a comparison can shed light on the discrepancies observed in the

371   fossil record and the divergence times estimated from a time-calibrated molecular

372   clock. It may also help to disentangle the effect of interaction among calibration

373   points. For each calibrated node we can compare the user-designed prior distribution

374   (e.g., uniform distributions in the case of the core analysis), the marginal prior

375   distribution that is the result of the interaction between the user priors and the tree

376   prior, and the posterior distribution that is the distribution after observing the data.

377   For the core analysis, the two different subsets of fossils and the alternative host plant

378   ages analyses were re-run without any data to sample from the marginal prior. In each

379   case we performed two independent runs of 50 million generations, sampling every

380   50 000 generations. The results were visualized with Tracer. When necessary, we

381   performed an additional run. Using LogCombiner, the runs were combined after

382   deleting the first 10% as burn-in. The results of the analyses with and without the

383   molecular dataset were imported into R (R Development Core Team 2008) and for

384   each calibrated node as well as the root height we compared the kernel density

385   estimates of the marginal prior and the posterior distributions (R package *hdrcde*).

386   *Comparison with Previous Studies*

387   For the root of all Papilionoidea and the seven families we compared the estimates

388   obtained in the core analysis to previous studies that also used fossil information.

389

390   **RESULTS**

391   *Core Analysis*

392   The core analysis performed with BEAST used the full set of fossils and host plant

393   constraints from Magallón et al. (2015) on the topology found with RAxML. This

394   analysis resulted in a root estimate for all Papilionoidea of 107.6 Ma (Fig.1,

395   Supplementary Material S2). The 95% credibility interval of the posterior distribution

396   ranged from 88.5 to 129.5 Ma. The lineage leading to Papilionidae diverged first at

397   the root of Papilionoidea and the crown age of Papilionidae was inferred to be 68.4

398   Ma (95%CI=53.5–84.3). Hedylidae and Hesperiidae diverged from Pieridae–

399   Lycaenidae–Riodinidae–Nymphalidae at 106.5 Ma (95%CI=88.0–127.2) and

400   diverged from each other at 99.2 Ma (95%CI=80.7–119.2). The crown age of the

401   sampled Hedylidae was 32.8 Ma (95%CI=23.4–43.6) and crown age of Hesperiidae

402   was 65.2 Ma (95%CI=55.8–78.1). Pieridae diverged from Lycaenidae–Riodinidae–

403   Nymphalidae at 101.1 Ma (95%CI=83.0–120.3) and extant lineages started

404   diversifying around 76.9 Ma (95%CI=63.1–92.4). Lycaenidae and Riodinidae

405   diverged from Nymphalidae at 97.4 Ma (95%CI=80.4–116.5) and diverged from each

406   other at 87.8 Ma (95%CI=73.2–106.1). The crown age of Lycaenidae was 71.0 Ma

407   (95%CI=57.2–85.2) and crown age of Riodinidae was 73.4 Ma (95%CI=60.3–88.1).

408   Finally, the crown age of Nymphalidae was inferred to be 82.0 Ma (95%CI=68.1–

409   98.3).

410

411   *Alternative Analyses*

412   In most cases the seven alternative parameters tested yielded very similar results (Fig.

413   2, Supplementary Material S3-S8). Reducing the number of taxa in order to decrease

414   the fraction of missing data, using higher-level calibration points only, or using a Yule

415   process tree prior (instead of a Birth–Death prior), gave virtually identical results as

416   the core analysis above. Using only lower-level fossil constraints (close to the tips of

417   the phylogeny) resulted in the youngest estimates of all alternative runs, with a crown

418   age of Papilionoidea of 94.5 Ma (mode=83.8, 95%CI=67.8–126.6). Using exponential

419   fossil priors mainly resulted in a narrower credibility interval, while the mode and

420   median age estimates were only 7–8 million years younger than the core analysis

421   mode estimate (Fig. 2, Supplementary Material S6). Adding mitochondrial

422   information also lead to a 7–8 million-year younger estimate for the crown age of

423   Papilionoidea, but the credibility interval remained comparable to the core analysis

424   (Supplementary Material S7). Finally, using a hypothesis of older host plant ages

425   extracted from Foster et al. (2017), we obtained the greatest difference. The upper

426   boundary of the credibility interval largely shifted toward much older ages (95%C

427   I=88.5–167.2) as well as the median (119.5 Ma). The posterior distribution was,

428   however, very skewed, with a mode of 101.0 Ma, and converged to the same age as

429   the core analysis (Fig. 2, Supplementary Material S8).

430   These variations for the root age among different alternative analyses were recovered

431   for the ages of the different subfamilies. For example, all lower-level fossils always

432   led to younger estimates while older ages from Foster et al. (2017) always led to older

433   estimates (Fig. 2).

434   *Comparing Prior and Posterior Distributions*

435   We compared the posterior distributions to the marginal prior distributions for the

436   different calibrated nodes in the core analysis. We set all fossil and host plant

437   constraints with uniform prior distributions as we considered this as the most

438   conservative approach. However, it is important to note that the marginal prior

439   distributions at these nodes, which result from the interactions between all calibration

440   priors and tree prior, are not uniform (Fig. 3).

441   Across all calibrated node points, many of them showed shifts of posterior

442   distributions from the marginal priors, indicating that the results of the core analysis

443   was not a simple outcome of our set of priors (Fig. 3). Interestingly, the nodes

444   calibrated by *Doritites*, *Dynamine*, *Thaites*, *Theope* and *Voltinia*, which are all the

445   fossils placed close to the tips of our phylogeny, tended to shift away from the

446   minimum boundary, toward older ages than the marginal prior distribution.

447   Alternative analyses performed with only these lower-level fossils yielded the

448   youngest tree for butterflies. This suggests that higher-level fossils bring important

449   additional information, leading posterior distributions of lower-level nodes to shift

450   away from the prior distributions in the core analysis.

451   The nodes calibrated with the higher-level fossils *Mylothrites*, *Prolibythea*, *Neorinella*

452   and *Vanessa* showed posterior distributions largely overlapping with their marginal

453   prior distributions. Many host plant calibrated points showed a shift from the marginal

454   prior distribution (Fig. 3). In all cases, except the node also calibrated with the fossil

455   *Lethe* the crown age of the butterfly clade inferred was much younger than the age of

456   the corresponding host plant clade.

457   For the root of Papilionoidea, the marginal prior and posterior distributions largely

458   overlapped in the core analysis, therefore not indicating whether our molecular

459   dataset contained significant information about the root age or not. We also compared

460   the posterior and the marginal prior distributions for alternative analyses performed

461   with different subsets of fossil calibrations (Fig. 4). When using only higher-level

462   fossils, the posterior distribution was almost identical to the core analysis, but the

463   marginal prior slightly shifted from the marginal prior of the core analysis toward a

464   younger age. The use of only lower-level fossils had more profound effects. In such a

465   case, prior distributions of the core analysis and the lower-level fossils alternative

466   completely overlapped. The posterior distribution, however, shifted toward younger

467   ages, yielding the most recent estimate for the root age among all analyses

468   (mean=94.5, mode=83.8, 95%CI=67.8–126.5). We also looked at the effect of using

469   relaxed maximum ages (based on Foster et al. 2017). In this case, marginal prior

470   distribution for the root age shifted to a mean of ~148 Ma (Fig. 4) and a credibility

471   interval spanning 100 Ma (95%CI=99.9–205.8). The posterior distribution was very

472   skewed, retaining a wider credibility interval than the core analysis (95%CI=88.5–

473   167.5), but significantly shifted from the prior distribution toward the posterior

474   distribution of the core analysis (median=119.5, mode=101.0).

475   *Comparison with Previous Studies*

476   For the root of Papilionoidea, our estimate in the core analysis using the mode age of

477   the distribution was very similar to Wahlberg et al. (2013) and Heikkilä et al. (2012),

478   with a mean age estimate of 104.6 and 110.8 Ma, respectively (107.6 Ma in the core

479   analysis, Fig. 5). For the crown age of families our estimates were often consistent

480   with most of previous studies. For Papilionidae, our crown age estimate (68.4,

481   95%CI=53.5–84.3) was very similar to Wahlberg et al. (2013) and Heikkilä et al.

482   (2012), while Condamine et al. (2012) in a study focusing primarily on this family

483   found younger ages of about 15 million years. For Hedylidae, only the study by

484   Heikkilä et al. (2012) had an estimate for the crown age, whose mean age was 45.3

485   Ma, which is older than our result (32.8, 95%CI=23.4–43.6). The age of Hesperiidae

486   (65.2, 95%CI=55.8–78.1) was similar to Wahlberg et al. (2013) and Heikkilä et al.

487   (2012), but much younger than Sahoo et al. (2017) with an estimate of 82 Ma.

488   Pieridae is the family that showed highest variation in age estimates among different

489   studies. Our estimate (76.9 Ma, 95%CI=63.1–92.4 Ma) falls in between the youngest

490   estimate from Wahlberg et al. (2013), in which the credibility interval goes down to

491   39 Ma, and the oldest estimate from Braby et al. (2006), in which the oldest boundary

492   of the credibility interval was 111.6 Ma. For Lycaenidae, which contain no fossils

493   calibrations, the results between our core analysis (73.4, 95%CI=60.3–88.1),

494   Wahlberg et al. (2013) and Heikkilä et al. (2012) were virtually identical. For the

495   crown age of Riodinidae, our core analysis (70.9, 95%CI=57.2–85.2) gave identical

496   results to Heikkilä et al. (2012). Espeland et al. (2015), in a study focusing

497   specifically on this family found about 10 million-year older ages. Wahlberg et al.

498   (2013), however, found a much younger estimate, about 20 Ma younger. For

499   Nymphalidae, we have the greatest number of time calibrations, but they all tend to

500   find very similar results. Our estimation (82.0, 95%CI=68.1–98.3) was very close to

501   Wahlberg et al. (2013) and Heikkilä et al. (2012). This estimation was about 12

502   million years younger than the study by Wahlberg et al. (2009) focusing on

503   Nymphalidae.

504   **DISCUSSION**

505   We generated a genus-level phylogeny of the superfamily Papilionoidea, including

506   994 taxa. Taking advantage of a recent revision of the lepidopteran fossil record we

507   established a new set of 12 fossil calibration points, which were combined to 10

508   secondary calibrations from host plant ages.

509   *Fossils and Minimum Ages*

510   In the core analysis we adopted a very conservative approach. This choice involves

511   taking into account the uncertainty surrounding the information available for each

512   calibration point, although at the expense of the amount of useful information

513   available. For fossil constraints, this had two consequences. First, we calibrated the

514   stem of the focal clade a fossil was assigned to, by calibrating the divergence from its

515   sister group instead of the first divergence recorded in the phylogeny within the focal

516   clade itself. Calibrating the crown age of the focal clade – meaning that we assume

517   that the fossil is "nested" within the clade – may lead to an overestimation of the

518   crown age. Such would be the case if lineages are undersampled at the root, or if

519   extinction occurred, or if the fossil belongs to a lineage that actually diverges

520   somewhere along the stem. Calibrating a higher node with the age of the fossil, which

521   involves loss of some information, is considered to be the best way to avoid these

522   problems. Second, we used uniform prior distributions bounded by the age of the

523   fossil and the age of angiosperms. We considered that fossils provide only a minimum

524   age for a node, a condition that is especially exacerbated by the exceptionally poor

525   fossil record of Lepidoptera in general (Labandeira and Sepkoski, 1993) and

526   Papilionoidea in particular (Sohn et al. 2015) when compared to the four other major

527   hyperdiverse insect lineages (Coleoptera, Hymenoptera, Diptera and Hemiptera).

528   Prior expectation on the age of the node cannot be modeled more accurately without

529   additional information. However, the marginal priors resulting from the interactions

530   among the different priors actually strongly differ from this assumption.

531

532   *Higher-* versus *Lower-Level Calibrations*

533   Generally, favoring multiple calibrations placed at various positions in a tree instead

534   of a single or few calibrations, seem to produce more reliable estimates of molecular

535   clocks (Conroy & Van Tuinen 2003, Smith & Peterson 2002, Soltis et al. 2002,

536   Duchêne et al. 2014). Calibrations distributed across a tree may allow a better

537   estimation of substitution rates and their pattern of variation among lineages

538   (Duchêne et al. 2014), and improve age estimates in cases of taxon undersampling

539   (Linder et al. 2005).

540   Calibrations placed at deep levels in the tree are usually favored (Sauquet 2012, Hug

541   & Roger 2007) over calibrations at lower levels for better capturing the overall

542   genetic variation (Duchêne et al. 2014). Yet, deep calibrations also tend to

543   underestimate the mean substitution rate and lead to an overestimation of shallow

544   nodes, referred to as "tree extension" by Phillips (2009). For the butterflies, we

545   investigated the consequences of using different subsets of fossil calibrations

546   according to their positions in the tree (higher *versus* lower-level calibrations),

547   compared to the full set of fossil constraints. With a subset of fossils placed only at

548   higher levels in the phylogeny we obtained results similar to the full set of fossils in

549   the core analysis, either at the deep nodes or shallow nodes, indicating no tree

550   extension effect. This effect may also indicate that the lower level calibration points

551   that are close to the tips are uninformative, and when included in the core analysis, do

552   not affect the timescale but clearly affected the priors (see below).

553   Alternatively, lower-level calibrations can lead to an overestimation of the mean

554   substitution rate across the tree, thereby underestimating the timescale (Phillips 2009).

555   Interestingly, when only a subset of fossils were used and placed close to the tips, it

556   led to the youngest estimates, including the credibility intervals. This potentially

557   indicates an effect of mean substitution rate overestimation. Also, we noticed in the

558   core analysis that the nodes calibrated by *Protocoeliades* and *Vanessa* (two deep node

559   constraints) showed posterior distributions abutting against the minimum boundaries

560   defined by the age of the fossils, therefore preventing the tree (or at least these nodes)

561   to be younger.

562

563   *Host Plants and Maximum Ages*

564   For calibration points constrained by the age of the host plant group, we considered

565   that only the crown of the focal clade could be assigned confidently to the host plant

566   group, as the stem or part of the stem could be older than the host plant (the host plant

567   shift would be happening somewhere along the stem). Support arises from molecular

568   biological and paleobiological evidence that the establishment of specialized insect-

569   herbivore associations can considerably postdate the origins of their hosts, as in a

570   Bayesian analysis of 100 species of leaf-mining *Phyllonorycter* moths (Lepidoptera:

571   Gracillariidae) and their dicot angiosperm hosts (Lopez-Vaamonde et al. 2006).

572   Relying on host plant ages for calibrating a butterfly tree is questionable while the

573   timing of the divergence of angiosperms is still highly controversial (e.g. Magallón et

574   al. 2015, Foster et al. 2017). As a result, first we calibrated our tree using the oldest

575   boundary of 95% CI of the stem age of a host plant clade. This allowed us to take into

576   account the uncertainty surrounding the timing of the first appearance of the host

577   plant but consequently, it also relaxed the prior hypothesis for the calibrations.

578   Secondly, we compared two alternative timescales for the angiosperms: a

579   paleontological estimate, which infers an Early Cretaceous origin of angiosperms

580   (Magallón et al. 2015), and a molecular clock estimate that we extracted from Foster

581   et al. (2017), which infers a stem age for angiosperms during the Early Triassic about

582   100 million years older. These two alternative scenarios affected the size of the

583   credibility intervals and the shape of the posterior distributions. For the crown of

584   Papilionoidea, the upper boundary of the 95%CI was ~37 million years older when

585   using the molecular clock estimate. However, the shape of the distribution was very

586   asymmetrical, with a mode of the distribution very close to the core analysis (101.0

587   Ma), suggesting that the estimation for the age of the root still concentrated around

588   the same ages. Using the hypothesis of an Early Triassic origin of angiosperms

589   implied very permissive priors toward old ages, which are most likely responsible for

590   the very wide credibility intervals and asymmetrical posterior distributions recovered

591   in the alternative analysis of using ages from Foster et al. (2017). Therefore, it is

592   tempting to use the time-scale inferred using Magallón et al. (2015)'s ages of

593   angiosperms, as it greatly narrows down the uncertainty surrounding butterfly ages,

594   and aligns more realistically with the fossil angiosperm record. However, as long as

595   there is no consensus on the timing of angiosperm diversification there is no reason to

596   favor one or the other.

597

598   *Priors and Posterior Distributions*

599   We compared the marginal priors to the posterior distributions for different analyses

600   for the root of Papilionoidea and for the different calibration points in the core

601   analysis. We found several calibration points showing a substantial shift of posterior

602   distribution. This indicates that our age estimates are not entirely driven by the set of

603   constraints, but instead that the molecular dataset brings additional information about

604   the age of the calibrated nodes. An interesting pattern we found in the core analysis is

605   the consistent trend of posterior distributions of the lower-level calibrated nodes to

606   shift toward older ages than the priors. Meanwhile, some higher-level node

607   calibrations shifted toward younger ages than the prior but most of them largely

608   overlapped with their prior distribution. Consequently, posterior estimates tend to

609   contract the middle part of tree compared to the prior estimates.

610   There are at least three reasons for the anomalous gap between the earliest fossil

611   papilionoid occurring at 55.6 Ma and its corresponding Bayesian median age of 110

612   Ma, that represents a doubling of the lineage duration. First, it long has been known

613   that the lepidopteran fossil record is extremely poor when compared to the far more

614   densely and abundantly occurring fossils of the four other hyperdiverse, major insect

615   lineages of Hemiptera, Coleoptera, Diptera and Hymenoptera (Labandeira and

616   Sepkoski, 1993). Second, particularly large-bodied apoditrysians such as

617   Papilionoidea, have even a poorer fossil record than other Lepidoptera in general,

618   particularly as they bear a fragile body habitus not amenable to preservation.

619   Additionally, as external feeders papilionoids lack a distinctive, identifiable trace

620   fossil record such as leaf mines, galls and cases (Sohn et al. 2015). Third, there are

621   very few productive terrestrial compression or amber deposits spanning the Upper

622   Cretaceous, from 100 Ma to the Cretaceous–Paleogene boundary of 66.0 Ma, and the

623   part of the Paleogene Period from 66.0 Ma to the earliest papilionoid fossil of 55.6

624   Ma (Labandeira, 2014; Sohn et al., 2015). Some of these deposits have recorded very

625   rare small moth fossils, but to date no papilionoid, or for that matter, other large

626   lepidopteran taxa such as saturniids or pyraloids have been found.

627   The root of the tree was only calibrated with the oldest fossil in our dataset, a 55.6

628   million-year-old papilionoid, and the crown age of the angiosperms. However, the

629   prior distribution for the root in the core analysis clearly excluded an origin of

630   butterflies close to 55.6 Ma, but rather a distribution centered on a median of 110 and

631   ranging between 86.4 and 136.2 Ma. The posterior distribution for the root in the core

632   analysis largely overlapped with the prior. However, when we used alternative ages

633   for the angiosperms (older ages), the marginal prior for the root shifted to

634   substantially older ages. Nevertheless, the posterior distribution showed a significant

635   shift toward younger ages, albeit highly skewed, toward ages similar to the core

636   analysis. This suggests that our estimate of the root age in the core analysis is not

637   simply driven by our set of priors, even if we do not actually observe a shift between

638   marginal prior and posterior distributions.

639   We observed some differences in prior and posterior distributions at the root when

640   considering only subsets of fossils. When using only the subset of higher-level fossils,

641   the marginal prior for the root showed very little difference from the core analysis

642   prior and the posterior distributions completely overlapped. When using the subset of

643   lower-level fossils the marginal prior remained similar to the core analysis but the

644   posterior distribution showed a substantial shift toward younger ages, yielding the

645   youngest estimation of the age of Papilionoidea among all our analyses. As such, it

646   seems that the choice of fossils did not change the prior estimation of the root, but the

647   posterior distribution was largely influenced by higher-level fossils. As we suggested

648   earlier, lower-level fossils only may be overestimating the mean substitution rate

649   across the tree, and therefore underestimating the time scale, while the

650   implementation of higher-level fossils seems to be correcting for this.

651   *Timescale of Butterflies Revisited*

652   We propose a new estimate for the timing of diversification of butterflies, based on an

653   unprecedented set of fossil and host-plant calibrations. We estimated the origin of

654   butterflies between 89.5 and 129.5 Ma, the median of this posterior distribution being

655   107.6 Ma, which corresponds to the Early Cretaceous–Late Cretaceous boundary

656   interval. The result of our core analysis for the root is very close to previous estimates

657   by Wahlberg et al. (2013) and Heikkilä et al. (2012). The comparisons of alternative

658   analyses, the prior and posterior distributions showed that this result is robust to

659   almost all the choices made throughout the core analysis and that our molecular

660   dataset contains significant information in addition to the time constraints. This

661   estimation means that there is a 45 million-year-long gap between the oldest known

662   butterfly fossil and the molecular clock estimate. Accordingly, as Brown & Smith

663   (2017) stated for the case of angiosperms, we do not know whether a larger molecular

664   dataset – implying potentially more information for estimating the molecular clock–

665   would allow the root to become younger. Alternatively, the fossil record for

666   butterflies is so sparse that an intervening fossil gap is very likely. Besides, the fossil

667   *Protocoeliades kristenseni*, which is 55.6 Ma can be assigned confidently to the

668   crown of the family Hesperiidae and the stem of Coeliadinae well within the

669   Papilionoidea. For angiosperms, a very rich fossil record is available compared to

670   butterflies (e.g., Magallón et al. (2015), which used 137 fossils to calibrate a

671   phylogeny of angiosperms), rendering the absence of angiosperms, either as pollen or

672   macrofossils, that are older than 136 Ma much more puzzling.

673

674   **ACKNOWLEDGMENTS**

684

685   **SUPPORTING INFORMATION**

686   S1. List of taxa and Genbank accession codes.

687   S2. Tree obtained from the core analysis. Node ages are the median of node age

688   posterior distributions.

689   S3. Tree obtained from the reduced dataset. Node ages are the median of node age

690   posterior distributions.

691   S4. Tree obtained when using only higher-level fossil calibrations. Node ages are the

692   median of node age posterior distributions.

693   S5. Tree obtained when using only lower-level fossil calibrations. Node ages are the

694   median of node age posterior distributions.

695   S6. Tree obtained when using exponential fossil calibration priors. Node ages are the

696   median of node age posterior distributions.

697   S7. Tree obtained when adding a mitochondrial gene fragment. Node ages are the

698   median of node age posterior distributions.

699   S8. Tree obtained when using the host-plant ages obtained from Foster et al. (2017).

700   In S8a node ages are the median of node age posterior distributions, while in S8b the

701   node ages are the mode the mode of the kernel density estimate of the posterior

702   distribution.

703

704   **LITERATURE CITED**

705   Braby, M.F., Vila, R., Pierce, N.E. 2006. Molecular phylogeny and systematics of the

706   Pieridae (Lepidoptera: Papilionoidea): higher classification and biogeography. Zool. J.

707   Linn. Soc. 147:239–275.

708   Brenner, G.J. 1996. Evidence for the earliest stage of angiosperm pollen evolution: a

709   paleoequatorial section from Israel. In: Taylor, D.W., Hickey, L.J., editors, Flowering

710   plant origin, evolution & phylogeny. Boston, MA: Springer. P. 91–115.

711   Brown, F.M. 1976. †*Oligodonta florissantensis*, gen. n., sp. nov. (Lepidoptera:

712   Pieridae). Bull. Allyn Mus., 37:1–4.

713   Brown, J.W., and Smith, S.A. 2017. The past sure is tense: On interpreting

714   phylogenetic divergence time estimates. Syst. Biol., 66, doi: 10.1093/sysbio/syx074

715   Chazot, N., Wilmott, K.R., Freitas, A.V.L., de Silva, D.L., Pellens, R., Elias, M. 2016.

716   Patterns of species, phylogenetic and mimicry diversity of clearwing butterflies in the

717   Neotropics. In: Pellens, R., Grandcolas, P., editors, Biodiversity conservation and

718   phylogenetic systematics. Topics in Biodiversity and Conservation, 14:333–353

719   Cockerell, T.D.A. 1907. A fossil butterfly of the genus *Chlorippe*. Canad. Entomol.,

720   39:361–363,

721   Condamine, F.L., Toussaint, E.F.A., Clamens, A.L., Genson, G., Sperling, F.A.,

722   Kergoat, G.J. 2015. Deciphering the evolution of birdwing butterflies 150 years after

723   Alfred Russel Wallace. Sci. Rept., 5:11860.

724   Conroy, C.J., and van Tuinen, M. 2003. Extracting time from phylogenies: positive

725   interplay between fossil and genetic data. J. Mammal. 84:444–455.

726   de Jong, R. 2016. Reconstructing a 55-million-year-old butterfly (Lepidoptera:

727   Hesperiidae). Eur. J. Entomol. 113:423–428.

728   de Jong, R. 2017. Fossil butterflies, calibration points and the molecular clock

729   (Lepidoptera: Papilionoidea). Zootaxa 4270:1–63.

730   dos Reis, M. and Yang, Z., 2013. The unbearable uncertainity of Bayesian divergence

731   time estimation. J. Syst. Evol. 51:30–43.

732   Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. 2012. Bayesian phylogenetics

733   with BEAUTi and BEAST 1.7. Mol. Biol. Evol. 29:1969–1973.

734   Duchêne, S., Lanfear, Ho, S.Y.W., 2014. The impact of calibration and clock-model

735   choice on molecular estimates of divergence times. Mol. Phylogen. Evol., 78:277–289.

736   Durden, C.J., and Rose, H. 1978. Butterflies from the Middle Eocene: the earliest

737   occurrence of fossil Papilionoidea (Lepidoptera). Pearce-Sellards Ser. 29:1–25,

738   Foster, C.S.P., Sauquet H., van der Merwe, M., McPherson, H., Rosette, M., Ho,

739   S.Y.W. 2017. Evaluating the impact of genomic data and priors on Bayesian

740   estimates of the angiosperm evolutionary timescale. Syst. Biol., 66:338–351.

741   Garzón-Orduña, I.J., Silva-Brandão, K.L., Wilmott, K.R., Freitas, A.V.L., Brower,

742   A.V.Z. 2015. Incompatible ages for clearwing butterflies based on alternative

743   secondary calibrations. Syst. Biol., 64:752–767.

744   Hall, J.P.W., Robins, R.K., Harvey, D.J. 2004. Extinction and biogeography in the

745   Caribbean: new evidence from a fossil riodinid butterfly in Dominican amber. Proc. R.

746   Soc. Lond. B, 271:797–801.

747   Heer, O. 1849. Die Insektenfauna der Tertiärgebilde von Oeningen und von Radoboj

748   in Croatien. Vol. 2. Wilhelm Engelsmann, Leipzig, 264 pp.

749   Heikkilä, M., Kaila, L., Mutanen, M., Peña, C., Wahlberg, N. 2012, Cretaceous origin

750   and repeated Tertiary diversification of the redefined butterflies. Proc. R. Soc. Lond.

751   B, 279:1093–1099.

752   Ho, S.Y., and Phillips, M.J. 2009. Accounting for calibration uncertainty in

753   phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58: 367–380.

754   Hug, L.A., and Roger, A.J. 2007. The impact of fossils and taxon sampling on ancient

755   molecular dating analyses. Mol. Biol. Evol. 24:1889–1897.

756   International Commission on Stratigraphy. 2012. International Chronostratigraphic

757   Chart: http://www.stratigraphy.org/ICSchart/ChronostratChart2012.pdf

758   Kozak, K.M., Wahlberg, N., Neild, A.F.E., Dasmahapatra, K.J.K., Jiggins, C.D. 2015.

759   Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius*

760   butterflies. Syst. Biol., 64:505–524,

761   Labandeira, C.C. 2014. Amber. In: Laflamme, M., Schiffbauer, J.D., Darroch, S.A.F.,

762   editors, Reading and writing of the fossil record: Preservational pathways to

763   exceptional fossilization. Paleontol. Soc. Pap., 20:163–216.

764   Labandeira, C.C. and Sepkoski, J.J., Jr. 1993. Insect diversity in the fossil record.

765   Science, 261:310–315.

766   Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S. 2012. PartitionFinder: combined

767   selection of partitioning schemes and substitution models for phylogenetic analysis.

768   Mol. Biol. Evol. 29:1695–1701.

769   Linder, H.P., Hardy, C.R., Rutschmann, F. 2005. Taxon sampling effects in molecular

770   clock dating: An example from the African Restionaceae. Mol. Phylogen. Evol.

771   35:569–582.

772   Lopez-Vaamonde, C., Wikström, N., Labandeira, C., Godfray, H.C.J., Goodman, S.J.,

773   Cook, J.M. 2006. Fossil-calibrated molecular phylogenies reveal that leaf-mining

774   moths radiated millions of years after their host plants. J. Evol. Biol., 19:1314–1326,

775   Magallón, S. Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T.

776   2015. A metacalibrated time-tree documents the early rise of flowering plant

777   phylogenetic diversity. New Phytol., 207:437–453.

778 Martins-Neto, R.G., Kucera-Santos, J.C., Vieira, F.R. de M., Fragoso, L.M. de C.

779 1993. Nova espécie de borboleta (Lepidoptera: Nymphalidae: Satyrinae) da Formação

780 Tremembé, Oligoceno do Estado de São Paulo. Acta Geol. Leopold., 37:5–16.

781 Matos-Maravi, P.F., Peña, c.., Wilmott, K.R., Freitas, A.V.L., Whalberg, N. 2013.

782 Systematics and evolutionary history of butterflies in the "*Taygetis* clade"

783 (Nymphalidae: Satyrinae: Euptychiina): towards a better understanding of

784 Neotropical biogeography. Mol. Phylogenet. Evol. 66:54–68.

785 Miller, J.Y. and Brown, F.M. 1989. A new Oligocene fossil butterfly, *Vanessa*

786 †*amerindica* (Lepidoptera: Nymphalidae), from the Florissant Formation, Colorado.

787 Bull. Allyn Mus., 126:1–9.

788 Nel, A. and Descimon, H. 1984. Une nouvelle espèce de Lépidoptère fossile du

789 Stampien de Cereste (Lepidoptera Satyridae). Géol. Méditerran. 11:287–293.

790 Nel, A., Nel, J, Balme, C. 1993. Un nouveau Lépidoptère Satiyrinae fossile de

791 l'Oligocene du Sud-Est de la France (Insecta, Lepidoptera, Nymphalidae). Linn. Belg.,

792 14:20–36.

793 Paradis, E., Claude, J., & Strimmer, K. 2004. APE: analyses of phylogenetics and

794 evolution in R language. *Bioinformatics*, 20(2), 289-290.

795 Parham, J.F., Donoghue, P., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue,

796 J.G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E.,

797 van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs,

798 L., Mackovicky, P.J., Miller, J., Smith, K.T., Theodor, J.M., Warnock, R.C.M.,

799 Benton, M.J. 2012. Best practices for justifying fossil calibrations. Syst. Biol.,

800 61:346–359.

801 Peña, C., and Malm, T. 2012. VoSeq: A voucher and DNA sequence web application.

802 PLoS ONE, 7(6):e39071.

803 Peñalver, E., and Grimaldi, D.A. 2006. New data on Miiocene butterflies in

804 Dominican Amber (Lepidoptera: Riodinidae and Nymphalidae) with the description

805 of a new nymphalid. Am. Mus. Novit. 3591:1–17.

806 Phillips, M.J. 2009. Branch-length estimation bias misleads molecular dating for a

807 vertebrate mitochondrial phylogeny. Gene, 441:132–140.

808 R Development Core Team (2008). R: A language and environment for statistical

809 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-

810 900051-07-0, URL http://www.R-project.org.

811 Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. 2014. Tracer v1.6,

812 Available from http://tree.bio.ed.ac.uk/software/tracer/.

813 Rannala, B. and Yang, Z. 2007. Inferring speciation times under an episodic

814 molecular clock. Syst. Biol., 56:453–466.

815 Rebel, H. 1898. Fossile Lepidopteren aus der Miocän-Formation von Gabbro.

816 Sitzungsber. Akad. Wiss. Wien, 107:731–745.

817 Sahoo, R. K., Warren, A. D., Collins, S. C., & Kodandaramaiah, U. 2017. Hostplant

818 change and paleoclimatic events explain diversification shifts in skipper butterflies

819 (Family: Hesperiidae). BMC Evolutionary Biology, 17: 174. Doi: 10.1186/s12862-

820 017-1016-x

821 Sahoo, R.K., Warren, A.D., Wahlberg, N., Brower, A.V.Z., Lukhtanov, V.A.,

822 Kodandaramaiah, U. 2016. Ten genes and two topologies: an exploration of higher

823   relationships in skipper butterflies (Hesperiidae). Peer J, 4:e2653, doi:

824   10.7717/peerj.2653

825   Sauquet, H., Ho, S.Y.W., Gandolfo, M.A., Jordan, G.I., Wilf, P., Cantrill, D.J., Bayly,

826   M.J., Bromham, L., Brown, G.K., Carpenter, R.J., Lee, D.M., Murphy, D.J.,

827   Sniderman, J.M.K., Udovice, F. 2012. Testing the impact of calibration on molecular

828   divergence times using a fossil-rich group: The case of *Nothofagus* (Fagales). Syst.

829   Biol., 61:289–313.

830   Scudder, S.H. 1875. Fossil butterflies. Mem. Am. Assoc. Adv. Sci. 1:1–99.

831   Scudder, S.H. 1889. The fossil butterflies of Florissant. United States Geological

832   Survey, 8th Annual Report, pp. 439–472.

833   Scudder, S.H. 1892. Some insects of special interest from Florissant, Colorado, and

834   other points in the Tertiaries of Colorado and Utah. Bull. U.S. Geol. Surv., 93:1–35.

835   Smith, A.B., and Peterson, K.J. 2002. Dating the time of origin of major clades:

836   Molecular clocks and the fossil record. Annu. Rev. Earth Planet. Sci. 30:65–88.

837   Sohn, J.C., Labandeira, C., Davis, D., Mitter, C. 2012. An annotated catalog of fossil

838   and subfossil Lepidoptera (Insecta: Holometabola) of the world. Zootaxa 3286:1–132.

839   Sohn, J.C., Labandeira, C.C., Davis, D.R. 2015. The fossil record and taphonomy of

840   butterflies and moths (Insecta, Lepidoptera) and implications for evolutionary

841   diversity and divergence-time estimates. BMC Evol. Biol. 15:12.

842   Soltis, D.E., Soltis, P.S., Zanis, M.J. 2002. Phylogeny of seed plants based on

843   evidence from eight genes. Am. J. Bot. 89:1670–1681.

844   Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic

845   analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

846   Toussaint, E.F.A. and Balke, M. 2016. Historical biogeography of *Polyura* butterflies

847   in the oriental Palaeotropics: trans-archipelagic routes and South Pacific island

848   hopping. J. Biogeogr. 43:1560–1572.

849   Wahlberg, N., Leneveu, J., Kodandaramaiah, U., Peña, C., Nylin, S., Freitas, A.V.L.,

850   Brower, A.V.Z. 2009. Nymphalid butterflies diversify following near demise at the

851   Cretaceous/Tertiary boundary. Proc. R. Soc. Lond. B, 276:4295–4302.

852   Wahlberg, N., Wheat, C.W., Peña, C. 2013. Timing and patterns in the taxonomic

853   diversification of Lepidoptera (butterflies and moths). PLoS ONE, 8(11):e80875.

854   Walker, J.D., Geissman, J.W., Bowring, S.A., Babcock, L.E. 2013. The Geological

855   Society of America geologic time scale. GSA Bull., 125:259–272.

856   Warnock, R.C.M., Yang, Z., Donoghue, P.C.J. 2012. Exploring uncertainty in the

857   calibration of the molecular clock. Biol. Lett., 9:156–159.

858   Warnock, R.C.M., Parham, J.F., Joyce, W.G., Lyson, T.R., Donoghue, P.C.J. 2015.

859   Calibration uncertainty in molecular dating analyses: there is no substitute for the

860   prior evaluation of time priors. Proc. R. Soc. Lond. B, 282:20141013.

861   Yang, Z, and Rannala, B. 2006. Bayesian estimation of species divergence times

862   under a molecular clock using multiple fossil calibrations with soft bounds. Mol. Biol.

863   Evol., 23:212–226.

864

865   TABLE 1. (a) Fossil calibration points used to calibrate the tree as a minimum age for

866   the *Clade calibrated*. Unless stated otherwise, the fossil calibrations were placed at

867   the stem of the clade calibrated. *Lower* and *upper* values indicate the prior truncation

868   for both the uniform and exponential priors. The 140 Ma year upper truncation

869   corresponds to the age of Angiosperms from Magallón et al. 2015. A different upper

870   truncation value results from a fossil prior interacting with a host plant prior placed at

871   the same node or a lower node. *Mean* and *offset* are parameter values for the

872   exponential prior distribution. (b) Host-plant clades used to calibrate the tree as a

873   maximum age for the *Calibrated node*. Host plant calibrations were placed at the

874   crown of the clade calibrated. Ages from both Magallón et al. (2015) and Foster et al.

875   (2017) are indicated.

876   a)

Fossils	Clade calibrated	lower	upper	mean	offset
Doritites bosniaskii Rebel, 1898	Papilionidae: Parnassiinae: Luehdorfiini	5.3	140	25	5.3
Dynamine alexae Peñalver & Grimaldi, 2006	Nymphalidae: Biblidinae: *Dynamine*	15.9	89	20	15.9
Lethe corbieri Nel, Nel & Balme, 1993	Nymphalidae: Satyrinae: Satyrini	28.3	65	25	28.3
Mylothrites pluto Heer, 1849	Pieridae: Coliadinae+Pierinae	15.9	100	50	15.9
Neorinella garciae Martins-Neto et al., 1993	Crown of Amathusiini	23.0	65	20	23.0
Pamphilites abdita Scudder, 1875	Hesperiidae: Hesperiinae	23.0	140	30	23.0
Prolibythea vagabunda Scudder, 1889	Nymphalidae: Libytheinae	33.8	140	40.0	33.8
Protocoeliades kristenseni de Jong, 2016	Hesperiidae: Coeliadinae	55.6	140	35	55.6
Thaites ruminiana Scudder, 1875	Papilionidae: Parnassiinae: Parnassiini	23.0	140	25	23.0
Theope sp	Riodinidae: Riodininae: Nymphidiini: *Theope*	15.9	140	25	15.9
Voltinia dramba Hall, Robinson & Harvey, 2004	Riodinidae: Riodininae: Mesosemiini: *Voltinea*	15.9	140	30	15.9
Vanessa amerindica Miller & Brown, 1989	Nymphalidae: Nymphalinae: Nymphalini	33.8	140	30	33.8
Doxocopa wilmattae Cockerell, 1907	Nymphalidae: Nymphalinae+Biblidinae+ Limenitidinae+Apaturinae		Not used		
Praepapilio colorado Durden & Rose, 1978	Papilionidae		Not used		

877
878
879   b)

Host plant clade	Clade calibrated	Magallón et al. 2015	Foster et al. 2017
Angiospermae	root	140	252
Poaceae	Hesperiidae: Hesperiinae	65	112
Poaceae	Nymphalidae: Satyrinae	65	112
Fabaceae	Pieridae	100	123
Brassicaeae	Pieridae: Pierinae	103	97
Rubiaceae	Riodinidae: Leucochimona+Mesophtalma+ Mesosemia+Perophthalma +Semomesia	87	85
Apocynaceae	Nymphalidae: Danainae	69	85
Solanaceae	Nymphalidae: Ithomiini	87	68
Euphorbiaceae	Nymphalidae: Biblidinae	89	104
Sapindaceae	Nymphalidae: Biblidinae: Epiphilini+ Callicorini	87	91

880
881
882

883   FIGURE 1. Time-calibrated tree obtained from the core analysis. a) The relationships

884   and age estimates among the subfamilies of Papilionoidea. b) The relationships and

885   age estimates among the genera across the different families. Age estimates are

886   indicated at the nodes (Ma). Node bars represent the 95% credibility intervals.

887

888   FIGURE 2. Comparison of node age estimates for the root of Papilionoidea and the

889   seven families between the core analysis and the seven alternative analyses. Mode,

890   median and 95% credibility interval are presented.

891

892   FIGURE 3. Marginal prior (grey) and posterior distributions (orange) for the nodes

893   calibrated in the core analysis. Blue dashed lines represent minimum boundaries;

894   green dashed lines represent maximum boundaries.

895

896   FIGURE 4. Marginal prior and posterior distributions for the root age in the core

897   analysis using either a) alternative host-plant ages or b) alternative subsets of fossil

898   calibrations.

899

900   FIGURE 5. Comparison of node age estimates for the root of Papilionoidea and the

901   seven families between this study (core analysis) and estimates from previous studies.

902   Mode and 95%CI for the core analysis are presented. For the other studies the values

903   reported in the original study are used.

	Baroniinae	
68.45	Parnassiinae	PAPILIONIDAE
58.91	Papilioninae	

| 32.81 | Macrosoma | HEDYLIDAE |

99.19	Coeliadinae	
65.25	Pyrginae 1	
51.9 45.68	Pyrginae 2	
	Pyrrhopyginae	
50.33	Pyrginae 3	
48.53 43.82	Euschemoninae	HESPERIIDAE
	Eudaminae	
47.19	Heteropterinae	
43.58	Trapeziitinae	
41.4	Hesperiinae	

76.9	Dismorphinae	
71.03	Pseudopontiinae	
66.72	Coliadinae	PIERIDAE
	Pierinae	

70.99 57.61	Curetinae	
63.89	Poritiinae	
	Miletiinae	
56.45	Lycaeninae	LYCAENIDAE
54.61	Theclinae	
	Polyommatinae	

58.47	Euselasiini	
73.41	Nemeobiini	RIODINIDAE
	Riodininae	

61	Heliconiinae	
72.43	Limenitidinae	
62.4	Pseudergolinae	
52.55	Apaturinae	
56.42	Biblidinae	
51.67	Cyrestinae	NYMPHALIDAE
	Nymphalinae	
70.27	Libytheinae	
79.04	Danainae	
68.14	Calinaginae	
63.78	Charaxinae	
	Satyrinae	

107.6   99.19   106.46   101.15   87.77   97.36   82.04

| Cretaceous | Paleogene | Neogene | |

DISMORPHIINAE

43.09 — Leptidea
25.37 — Pseudopieris
Moschoneura
14.58 / 8.86 — Patia
11.09 — Enantia
9.16 — Lieinix
Dismorphia

⑦ 76.9 — Pseudopontia

PSEUDOPONTIINAE

33.16 — Nathalis
41.33 — Kricogonia
Pyrisitia
28.57 — Teriocolias
19.91 — Leucidia
16.26 — Eurema

47.95

⑧ 71.03 — Gandaca
36.41 — Gonepteryx
32.97 — Aphrissa
12.22 — Phoebis
27.67 — Catopsilia
15.93 — Dercas
21.02 — Anteos
18.58 — Zerene
10.97 — Colias

COLIADINAE

⑨ 66.72

PIERINAE

35.08 — Pareronia
Nepheronia
36.23 — Colotis
23.13 — Eronia
41.76 — Ixias
Pinacopteryx
29 — Gideona
26.72 — Teracolus
Hebomoia
41.82 — Elphinstonia
15.99 — Anthocharis
12.85 — Euchloe
34.2 — 10.45 — Zegris
Eroessa
22.27 — Mathania
7.94 — Hesperocharis
6.64 — Cunizza

51.09

58.84

55.25

Colotini

42.45 — Elodina
Leptosia
35.32 — Aoa
27.79 — Appias
Saletara
Dixeia
28.07 — Belenois
35.93 / 29.68 — Prioneris
32.76 — Cepora
Mylothris
27.95 — Leuciacria
15.49 — Delias
25.79 — Aporia
24.24 — Melete
19.78 — Pereute
7.29 — Leodonta
18 — Neophasia
11.85 — Eucheira
14.49 — Archonias
13.65 — Catasticta
12 — Charonias

50.43

42.16

40.26

Appiadina

Pierini

19.06 — Talbotia
Pieris
Leptophobia
23.5 / 18.21 — Pieriballia
12.38 — Perrhybris
9.78 — Itaballia
22.14 — Pontia
16.78 — Baltia
21.29 — Ascia
Ganyra
6.84 — Pierphulia
14.53 — 1.44 — Phulia
2.91 — Infraphulia
5.59 — Theochila
4.88 — Hypsochila
3.89 — Tatochila

⑦ Fabaceae
(0-100 Ma)

⑧ *Mylothrites pluto*
(15.9-100 Ma)

⑨ Brassicales
(0-103 Ma)

Riodinidae - Lycaenidae - Nymphalidae

PIERIDAE

| Cretaceous | Paleogene | Neogene | |

		Curetis	CURETINAE
	36.21	Poritia	PORITIINAE
70.99	57.61	Baliochila	
	44.69	Liphyra	
	25.84	Miletus	MILETINAE
		Allotinus	
63.89		Lycaena	LYCAENINAE
	29.05	Jalmenus	
56.45	42.09	Lucia	
	38.68	Favonius	THECLINAE
	15.88	Callipsyche	
		Thecla	
54.61		Anthene	
		Pithecops	
48.03	16.97	Pseudonacaduba	
	26.6	Una	
	23.39	Azanus	
		Nacaduba	
38.43		Psychonotis	
	15.37	Caleta	
	13.31	Zintha	
	9.87	Castalius	
	26.15 17.42	Catochrysops	
		Zizeeria	
34.52	22.55 16.77	Jamides	
	13.43	Eicochrysops	
	10.04	Celastrina	
		Lycaenopsis	
	20.7	Actizera	
	16.15 6.46	Uranothauma	
	13.97	Phlyaria	
	11.51	Cacyreus	
	18.15	Lampides	
33.38		Maculinea	
	8.57	Caerulea	
	12.9	Scolitantides	
		Glaucopsyche	
	9.58	Iolana	
	7.82	Euphilotes	
	10.44	Philotes	
	9.81	Pseudophilotes	
	8.42	Otnjukovia	
	5.02	Turanana	
	27.21	Theclinesthes	
		Famegana	
31.06 20.69		Zizula	
		Oraidium	
28.93	14.84	Euchrysops	
		Lepidochrysops	
26.97		Leptotes	
		Cupido	
24.66	10.44	Tongeia	
	7.45	Talicada	
19.96		Chilades	
		Aricia	
	8.93	Lycaeides	
15.19	7.21	Albulina	
	6.51	Lysandra	
	5.56	Agrodiaetus	
13.53	3.29	Polyommatus	
		Cyclargus	
	7.59	Echinargus	
	3.69	Hemiargus	
12.01		Pseudochrysops	
		Paralycaeides	
	10.99 5.2	Madeleinea	
	4.24	Itylos	
	10.02	Pseudolucia	
	8.99	Nabokovia	
	5.26	Eldoradina	
		Riodinidae	

POLYOMMATINAE

LYCAENIDAE

Cretaceous | Paleogene | Neogene

Phylogenetic tree with divergence time estimates. Taxa and tribal groupings:

NYMPHALIDAE

HELICONIINAE
- Acraeini: *Cethosia*, *Acraea*, *Bematistes*, *Telchinia*, *Actinote*
- Heliconiini: *Heliconius*, *Eueides*, *Dione*, *Agraulis*, *Dryas*, *Podotricha*, *Dryadula*, *Philaethria*
- Vagrantini: *Vindula*, *Lachnoptera*, *Algia*, *Cirrochroa*, *Algiachroa*
- Argynnini: *Smerina*, *Cupha*, *Vagrans*, *Phalanta*, *Terinos*, *Euptoieta*, *Yramea*, *Pardopsis*, *Boloria*, *Issoria*, *Argynnis*, *Brenthis*

LIMENITIDINAE
- Parthenini: *Parthenos*
- Neptini: *Cymothoe*, *Harma*, *Lebadea*, *Neptis*, *Pantoporia*
- Limenitidini: *Pseudoneptis*, *Pseudacraea*, *Limenitis*, *Adelpha*, *Auzakia*, *Parasarpa*, *Moduza*, *Pandita*, *Sumalia*, *Athyma*
- Adoliadini: *Catuna*, *Bassarona*, *Lexias*, *Dophla*, *Tanaecia*, *Euthalia*, *Euptera*, *Pseudathyma*, *Bebearia*, *Euphaedra*, *Euryphura*, *Hamanumida*, *Euriphene*, *Aterica*

Pseudergolinae - Apaturinae - Biblidinae - Cyrestinae - Nymphalinae

Node values: 34.88, 23.62, 22.24, 13.84, 37.79, 15.44, 23.29, 12.01, 21.3, 15.49, 17.4, 15.14, 47.7, 36.83, 26.95, 8.19, 7.01, 40.96, 29.66, 13.67, 11.11, 34.38, 31.76, 29.2, 23.16, 20.27, 14.6, 11.81, 9.18, 61, 48.71, 13.47, 34.12, 18.06, 44.04, 30.23, 34.6, 11.44, 10.15, 16.27, 14.36, 13.83, 10.9, 9.01, 41.27, 38.32, 30.03, 21.41, 18.36, 16.51, 11.38, 32.21, 26.57, 30.45, 16.43, 26.65, 13.6, 12.4, 9.89

(18) *Doxocopa wilmattae* (33.8-140 Ma) - not used

Time scale: Cretaceous | Paleogene | Neogene

A time-calibrated phylogenetic tree of Danaidae (Libytheinae and Danainae). Taxon labels (top to bottom): *Libythea*, *Libytheana* (LIBYTHEINAE); *Lycorea*, *Anetia*, *Euploea*, *Idea*, *Tirumala*, *Danaus*, *Amauris*, *Parantica*, *Miriamica*, *Ideopsis* (Danaini); *Tellervo*, *Elzunia* (Tellervini); *Aeria*, *Thyridia*, *Sais*, *Scada*, *Mechanitis*, *Forbestra*, *Methona*, *Tithorea*, *Eutresis*, *Melinaea*, *Athyrtis*, *Olyras*, *Paititia*, *Patricia*, *Athesis*, *Hyposcada*, *Oleria*, *Ollantaya*, *Ithomia*, *Pagyris*, *Placidina*, *Hypothyris*, *Hyalyris*, *Napeogenes*, *Epityches*, *Aremfoxia*, *Hyalenna*, *Dircenna*, *Callithomia*, *Haenschia*, *Pteronymia*, *Ceratinia*, *Episcada*, *Velamysta*, *Veladyris*, *Hypomenitis*, *Greta*, *Genus2*, *Pseudoscada*, *Genus1*, *Godyris*, *Heterosais*, *Mcclungia*, *Pachacutia*, *Hypoleria*, *Brevioleria* (Ithomiini); Calinaginae - Satyrinae.

Node dates: 20.9; 70.27; 33.37; 12.08; 10.34; 26.37; 12.46; 23.76; 42.03; 14.19; 10.39; 9.2; 37.87; 14.95; 25.64; 20.5; 14.91; 14.28; 3.88; 18.72; 23.54; 20.95; 13.7; 10.81; 12.12; 3.02; 21.97; 11.25; 10.75; 8.56; 20.42; 11.75; 6.9; 17.1; 14.73; 2.66; 13.59; 12.1; 5.38; 16.87; 4.6; 9.97; 9.09; 8.66; 7.69; 4.96; 15.03; 9.9; 7.62; 11.8; 8.69; 6.27; 9.75; 6.24; 7.47; 6.52; 1.85; 4.78; 3.47

15 *Prolibythea vagabunda* (33.8-140 Ma)

16 Apocynaceae (0-69 Ma)

17 Solanaceae (0-87 Ma)

DANAINAE

DANAIDAE

Time axis: Cretaceous | Paleogene | Neogene

A phylogenetic tree of Satyrinae (Nymphalidae) showing divergence time estimates.

Genera from top to bottom:
Satyrinae - begin, Acrophtalmia, Loxerebia, Coelites, Zipaetis, Orsotriaena, Erites, Argyronympha, Oressinoma, Sinonympha, Coenonympha, Lamprolenis, Altiapa, Hypocysta, Nesoxenica, Heteronympha, Geitoneura, Argynnina, Tisiphone, Paratisiphone, Dodonidia, Erebiola, Percnodaimon, Argyrophenga, Oreixenica, Erycinidia, Platypthima, Harsiesis, Calisto, Euptychia, Callerebia, Proterebia, Gyrocheilus, Strabena, Ypthima, Ypthimomorpha, Stygionympha, Cassionympha, Neocoenyra, Pseudonympha, Erebia, Boeberia, Hyponephele, Cercyonis, Maniola, Aphantopus, Pyronia, Faunula, Grumia, Paralasa, Melanargia, Hipparchia, Berberia, Oeneis, Neominois, Karanasa, Brintesia, Arethusana, Satyrus, Pseudochazara, Chazara, Parapedaliodes, Punapedaliodes, Praepedaliodes, Pedaliodes, Panyapedaliodes, Redonda, Steromapedaliodes, Corades, Cheimas, Eteona, Foetterleia, Junea, Pronophila, Thiemeia, Apexacuta, Pseudomaniola, Oxeoschistus, Lasiophila, Mygona, Proboscis, Steremnia, Steroma, Manerebia, Diaphanos, Ianussiusa, Lymanopoda, Idioneurula, Nelia, Chillanella, Auca, Quilaphoetosus, Elina, Pampasatyrus, Haywardella, Punargentus, Etcheverrius, Atlanteuptychia, Paramacera, Cyllopsis, Cissia, Moneuptychia, Megisto, Palaeonympha, Chloreuptychia, Zischkaia, Pharneuptychia, Amphidecta, Rareuptychia, Hermeuptychia, Pindis, Godartiana, Taygetis, Coeruleotaygetis, Taygetina, Harjesia, Forsterinaria, Taygetomorpha, Pseudodebis, Posttaygetis, Parataygetis, Capronnieria, Caeruleuptychia, Paryphthimoides, Magneuptychia, Euptychoides, Archeuptychia, Splendeuptychia, Prenda, Taydebis, Erichthodes, Pareuptychia, Megeuptychia, Satyrotaygetis, Neonympha

SATYRINAE
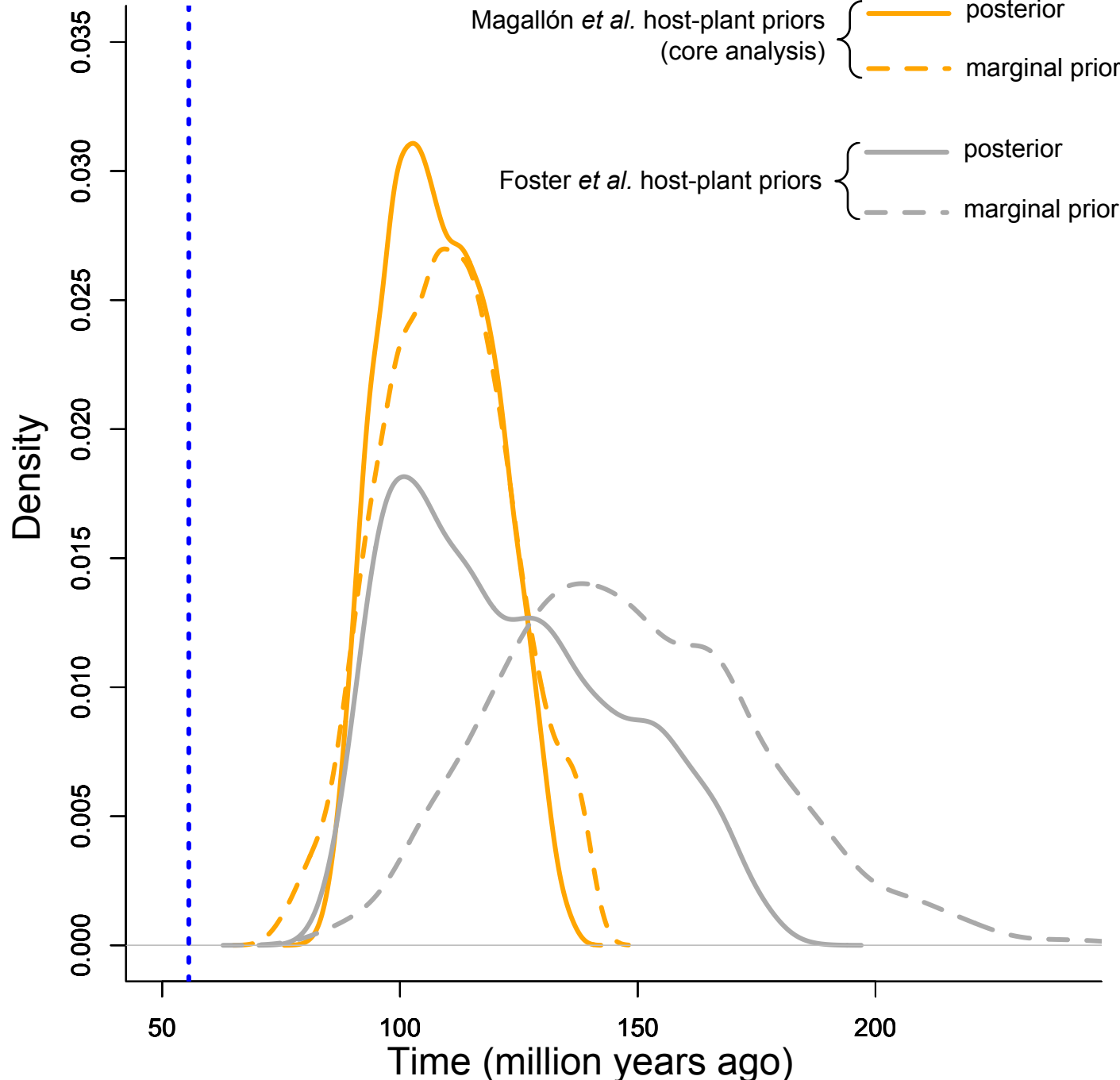
Satyrini

NYMPHALIDAE

Paleogene | Neogene

Figure showing density distributions over time (million years ago) for various taxa grouped as higher-level fossils, lower-level fossils, and host-plants. Panels: Protocoeliades, Prolibythea, Vanessa, Pamphilites, Lethe, Mylothrites, Neorinella, Dynamine, Doritites, Thaites, Theope, Voltinia, Apocynaceae, Brassicales, Fabaceae, Poaceae, Rubiaceae, Sapindaceae, Solanaceae, Euphorbiaceae.

Legend: posterior distribution (orange), marginal prior distribution (gray), maximum constraint (green dashed), minimum constraint (blue dashed).

a)

b)