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## Title

Renewed diversification following Miocene landscape turnover in a Neotropical butterfly radiation

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#### **Author contributions:**

NC and ME conceived the study, with contribution from KRW, GL and AVLF. All co-authors provided specimens and sequence data. NC, ME, FPP, CFA, DLDS performed the labwork. NC performed the analyses. NC wrote the paper with major contributions from ME, and contributions from all co-authors.

#### **Biosketch**

All authors on this paper have a longstanding interest in Ithomiini ‘clearwing’ butterflies, spanning systematics, the role of host-plants and mimetic interaction from micro- to macroevolutionary scale, community ecology, and/or historical biogeography. This paper is the result of 15 years of collaborative efforts to collect, describe, sequence and classify Ithomiini butterflies to unravel the evolutionary patterns of the diversification of this clade.

1    ***Abstract***

2    Aim: The landscape of the Neotropical region has undergone dynamic evolution throughout the  
3    Miocene, with the extensive Pebas wetland occupying western Amazonia between 23 and ~10 My ago  
4    and the continuous uplift of the Andes mountains. The complex interaction between the Andes and  
5    Amazonia likely influenced the trajectory of Neotropical biodiversity, but evidence from time-  
6    calibrated phylogenies of groups that diversified during this period are lacking. We investigate the role  
7    of these landscape transformations on the dynamics of diversification in the Neotropical region using a  
8    26 My-old endemic butterfly radiation.

9    Location: Neotropics

10    Time period: Oligocene to present

11    Major taxa studied: Ithomiini butterflies

12    Methods: We generated one of the most comprehensive time-calibrated molecular phylogenies of a  
13    large clade of Neotropical insects, the butterfly tribe Ithomiini, comprising 340 species (87% of extant  
14    species) and spanning 26 My of diversification. We applied a large array of birth-death models and  
15    historical biogeography estimations to assess the dynamics of diversification and biotic interchanges,  
16    especially at the Amazonia/Andes interface.

17    Results: Our results suggest that the Amazonian Pebas wetland system played a major role in the  
18    timing and geography of diversification of Ithomiini, by constraining dispersal and diversification in  
19    the Amazon basin until ~10 My ago. During the Pebas wetland period Ithomiini diversification mostly  
20    took place in the Andes, where terrestrial habitats were not affected. An explosion of interchanges  
21    with Amazonia and with the Northern Andes accompanied the demise of the Pebas system (11-8 My  
22    ago), and was followed by local diversification in those areas, which led to a substantial renewal of  
23    diversification.

24    Main conclusions: While many studies on Neotropical diversity have focused only on the Andes, we  
25    show that it is the waxing and waning of the Pebas mega-wetland, interacting with Andean uplift, that  
26    have determined the timing and patterns of regional interchanges and diversification in Ithomiini.

27    ***Keywords***

28    Neotropics, phylogeny, diversification, biogeography, Andes, Pebas system, Western-Andean Portal,  
29    Ithomiini, butterflies

30 **INTRODUCTION**

31 There has been a long fascination among biologists for the Neotropics and the origin of its intriguingly  
32 high biodiversity. Nevertheless, the timing of Neotropical diversification, and therefore its major  
33 driving processes, are still controversial despite the large amount of publications that have addressed  
34 these questions (e.g., Hoorn et al. 2010, Rull et al. 2008, Smith et al. 2014).

35 Notwithstanding uncertainty about the precise timing and magnitude of surface uplift, the formation of  
36 the Andean cordilleras during the Cenozoic broadly shaped Neotropical landscapes and affected  
37 diversification in the Neotropics. As the Andes arose they created new biotic and abiotic conditions  
38 along their slopes, modified the climate of the Neotropical region and deeply affected the formation of  
39 the Amazonian basin by introducing large amounts of sediment and modifying water drainage (Hoorn  
40 et al. 2010). There is increasing evidence that the Andes influenced the diversification of Neotropical  
41 lineages, primarily by driving increased speciation rates, perhaps most spectacularly in the high  
42 altitude páramo habitat (e.g., Madriñán et al. 2013). In parallel, the western part of the Amazon basin,  
43 which is connected to the Andes, experienced major turnovers of ecological conditions. During the  
44 Oligocene, western Amazonia was occupied by a fluvial system flowing northward (the paleo-Orinoco  
45 basin), which transformed ~23 million years (My) ago into an aquatic system of shallow lakes and  
46 swamps episodically invaded by marine conditions, known as the Pebas system (Wesselingh et al.  
47 2001, Antonelli et al. 2009, Wesselingh et al. 2010, Hoorn et al. 2010, Antonelli & Sanmartín 2011).  
48 The Pebas was connected northward with the Caribbean Sea and likely also with the Pacific Ocean  
49 through the Western-Andean portal (“WAP”, Antonelli et al. 2009), a low-elevation gap that separated  
50 the Central Andes and the Northern Andes until 13-11 My ago. During the late Miocene and during  
51 the Andean uplift, the accumulation of sediments combined with a sea level decrease initiated the  
52 eastward drainage of the Pebas, and by 10-8 My ago the region had changed into a fluvial system,  
53 which then evolved into the modern configuration of the Amazon. More recently, climatic fluctuations  
54 during the Peistocene (2.5-0 My ago) may have led to episodic dryness affecting Amazonian forest  
55 habitats and have been proposed as a driver of diversification of the Neotropical biota (Haffer 1969).  
56 The extent of the influence of Pleistocene events and their effects on Neotropical diversification, and  
57 even the importance of dryness episodes, are controversial (e.g., Colinvaux et al. 2000, Rull et al. 2008,  
58 Garzón-Orduña et al. 2014, Matos-Maraví 2016).

59 In this study we focus our attention mostly on the Miocene and Pliocene and how the interaction  
60 between the rise of the Andes and coincident large landscape modifications in western Amazonia  
61 determined diversification and dispersal over 30 million years. The Pebas ecosystem covered up to 1.1  
62 million km<sup>2</sup> at its maximum extent (Wesselingh et al. 2001) and was probably unsuitable for terrestrial  
63 fauna dependent on *terra firme* forest habitats. Therefore, between ca. 23 and 10 My ago,  
64 diversification of terrestrial lineages may have been impeded in western Amazonia or restricted to its

edge (Wesselingh et al. 2001). By contrast, the uplift of the Central and the Northern Andes, also occurring throughout the Miocene and the Pliocene, and the ecological gradients present along this mountain chain, probably constituted an important driver of diversification. In the last 10-8 My, the retreat of the Pebas potentially provided new opportunities for terrestrial lineages to radiate in lowland western Amazonia. The Pebas may also have constrained dispersal, acting as a barrier between the Andes and Amazonia.

Paleontological studies have shown that the Pebas greatly contributed to the diversification of the aquatic fauna, including molluscs (Wesselingh 2006), ostracods (Muñoz-Torres et al. 2006) and crocodilians (Salas-Gismondi et al. 2015). However, the fossil record also suggests a negative effect of the Pebas system on the terrestrial fauna (Antoine et al. 2016, Antoine et al. 2017). The hypothesis that the Pebas has shaped patterns of terrestrial diversification and dispersal in western Amazonia has grown over the years (e.g., Wesselingh & Salo 2006, Antonelli et al. 2009, Wesselingh et al. 2001, Antonelli & Sanmartín 2011, Antoine et al. 2016), but support from molecular phylogenies mostly stems from the observation that many western Amazonian clades have diversified during the last 10-8 My and not before (Antonelli & Sanmartín 2011, Chazot et al. 2016b, and references therein). Yet, there is very little information on what happened before, when the Pebas was occupying western Amazonia, particularly on whether the presence of Pebas constrained diversification and interchange patterns in this region. A thorough assessment of the role of the Pebas ecosystem on diversification and dispersal requires phylogenies of large Neotropical clades that originated before the formation of the Pebas, i.e. clades older than 23 My. Phylogenies of Neotropical clades meeting these conditions are surprisingly rare. In insects – which are among the most diverse terrestrial organisms – attempts to build phylogenies of Neotropical groups to test different drivers of Neotropical diversification have either suffered from a small size or a low sampling fraction (e.g. Hall & Harvey 2002, Elias et al. 2009, Penz et al. 2012, Condamine et al. 2012, Price et al. 2014, Chazot et al. 2016a,b), and therefore from low statistical power and reliability.

Among the most emblematic Neotropical insects is the butterfly tribe Ithomiini (Nymphalidae: Danainae, 393 species), also referred to as ‘clearwing’ butterflies because of the transparent wings of the majority of species. Ithomiini are forest-dwellers distributed throughout the Neotropics, from sea level up into montane cloud forests (to 3000 m), where their larvae feed on plants of the families Solanaceae, Gesneriaceae and Apocynaceae (Drummond & Brown 1987, Willmott & Freitas 2006). Species richness is primarily concentrated in the Andes, where about half the species occur (mostly on the eastern slopes), and in western Amazonia. Ithomiini are chemically-defended and engage in Müllerian mimicry, whereby co-occurring species exhibit convergent wing colour patterns that advertise their toxicity to predators (Müller 1879). Ithomiini butterflies represent a keystone group in Neotropical forests by numerically dominating mimetic butterfly communities and sharing wing colour patterns with a large number of other palatable and unpalatable Lepidoptera, such as the iconic

101 *Heliconius* butterflies (Brown & Benson 1974). For this reason, Ithomiini were used by both Bates  
102 (Bates 1862) and Müller (Müller 1879) in their original descriptions of deceptive (Batesian) and  
103 mutualistic (Müllerian) mimicry, respectively.

104 The diversity and the intriguing ecology of Ithomiini has generated a great interest among researchers  
105 and a broad and diverse literature on topics including life history (Bolaños Martinez et al. 2011, Hill et  
106 al. 2012, McClure & Elias 2016, McClure & Elias 2017), chemical ecology (Brown 1984, Trigo &  
107 Brown 1990, Schulz et al. 2004, McClure et al. accepted), systematics (Brown & Freitas 1994,  
108 Mallarino et al. 2005, Willmott & Freitas 2006, Brower et al. 2006, Elias et al. 2009, De-Silva et al.  
109 2010, Brower et al. 2014, Chazot et al. 2016b, De-Silva et al. 2017), cytogenetics (Brown et al. 2004,  
110 McClure et al. 2018), population ecology (Freitas 1993, 1996), community ecology (Beccaloni 1997,  
111 DeVries et al. 1999, Elias et al. 2008, Hill 2010, Chazot et al. 2014, Willmott et al. 2017), wing colour  
112 pattern evolution (Jiggins et al. 2006), and biogeography (Elias et al. 2009, Dasmahapatra et al. 2010,  
113 De-Silva et al. 2016, 2017, Chazot et al. 2016b, Chazot et al. 2018).

114 In this study, we generate the first species-level molecular phylogeny of the entire tribe, providing a  
115 large and densely sampled (340 species included out of 393 currently recognized) phylogenetic dataset  
116 for a Neotropical insect clade that underwent diversification during the last ~30 million years  
117 (Wahlberg et al. 2009, De-Silva et al. 2017). We investigate the dynamics of diversification and  
118 dispersal rates in Ithomiini, focusing on the turnover of ecological conditions in Western Amazonia  
119 and the concomitant Andean uplift during the Miocene. Specifically, an important role for Andean  
120 uplift and the Pebas wetland would be supported if: (1) *During the Pebas period*: (a) Andean  
121 diversification largely exceeded Amazonian diversification, due to increased diversification in the  
122 Andes driven by the evolving ecological gradient and uplift dynamics and/or a reduced diversification  
123 rate in Amazonia accompanying the loss of terrestrial habitats; (b) interchanges between the Andes  
124 and Amazonia were reduced; (c) interchanges between the Central and the Northern Andes were  
125 reduced, because of the existence of the WAP. (2) *During the retreat of the Pebas*: interchanges  
126 between the Andes and Amazonia and between the Central and the Northern Andes increased, as a  
127 result of new terrestrial habitats and the disappearance of the WAP, respectively. (3) *After the Pebas*  
128 *period*: Diversification rates in Amazonia increased and biotic interchanges became newly  
129 unconstrained. In addition, decreasing speciation rates would suggest the existence of post-Pebas  
130 radiations in Amazonia, while a scenario of increasing speciation rates would be consistent with  
131 speciation driven by climatic fluctuations during the last 2.5 My.

132

## 133 MATERIAL AND METHODS

### 134 Time-calibrated phylogeny

135 *Molecular data*

136 We compiled sequences of 1474 Ithomiini individuals (see Appendix S1.1 in Supporting Information)  
137 that included 718 sequences newly generated for this study and 3147 previously published sequences  
138 (Whinnett et al. 2005, Mallarino et al. 2005, Brower et al. 2006, Elias et al. 2009, De-Silva et al. 2010,  
139 Chazot et al. 2014, De-Silva et al. 2016, Chazot et al. 2016b, De-Silva et al. 2017, Chazot et al. 2018).  
140 We used a concatenation of nine gene fragments, a mitochondrial fragment spanning genes COI-  
141 tRNA-COII, and fragments of nuclear genes EF1 $\alpha$ , Tektin, CAD, RPS2, MDH, GAPDH, representing  
142 a total of 7083 bp (Wahlberg & Wheat 2008). Primers and PCR conditions followed Mallarino et al.  
143 (2005) and Wahlberg & Wheat (2008). We obtained at least one gene fragment for 340 species out of  
144 393 currently known in the group, which represents 87% of the known species richness of the tribe.  
145 For each species we produced the consensus sequence of all sequences belonging to individuals of that  
146 species to obtain the longest sequence possible. We added 41 outgroups, which spanned all Danainae  
147 genera as well as representatives of the main Nymphalidae clades. In total, nine concatenated genes  
148 from 381 taxa were used to generate the time-calibrated phylogeny of the Ithomiini.

149 *Tree topology and time calibrations*

150 We used PartitionFinder v.1.1 (Lanfear et al. 2012) to find the best gene partitioning and substitution  
151 models (Appendix S2.1, Table S2.1). Then we generated a phylogeny under maximum likelihood  
152 inference (ML), using IQ-Tree software as implemented in the W-ID-TREE server (Trifinopoulos et  
153 al. 2016, Nguyen et al. 2014) in order to obtain a tree topology (Appendix S2.2). Finally, this topology  
154 was time-calibrated using BEAST v1.8.2 (Drummond et al. 2012). We used four secondary  
155 calibrations from Wahlberg et al. (2009)'s phylogeny of Nymphalidae that were placed outside of  
156 Ithomiini, and six secondary calibrations consisting of maximum ages of hostplant (Solanaceae)  
157 lineages from De-Silva et al. (2017), and placed on Ithomiini lineages (Appendix S2.3, Table S2.2).  
158 We used a birth-death tree prior and we set an uncorrelated lognormal relaxed clock for each gene  
159 partition. We performed two independent runs of BEAST v.1.8.2, which were combined after applying  
160 a 15% burnin. Median branch lengths and credibility intervals were recovered using TreeAnnotator  
161 v.1.8.3 (Drummond et al. 2012) (Appendix S2.3), and outgroups were pruned to generate an Ithomiini  
162 tree with median branch lengths (hereafter, median tree). Full details are given in Appendix S2.

163

164 **Diversification rates**

165 *Time*

166 We investigated the dynamics of diversification rates through time and across the phylogeny using two  
167 different birth-death models: (1) Morlon et al.'s (2011) method allowing both speciation and

extinction rates to vary as a function of time, and allowing extinction to be higher than speciation, (2) TreePar (Stadler 2011) which accommodates models where diversification rates can vary at points in time but are constant between these points. Because none of these methods can automatically detect the number and position of different diversification processes, we first ran MEDUSA (Alfaro et al. 2009, Appendix S3.1.1) on the whole tree in order to partition the tree into different diversification processes. MEDUSA detected two shifts from the background diversification rates (see Results and Table S3.3, and additional analyses using BAMM v.2.5.0 (Rabosky 2014, Appendix S3.1.4, Figure S3.3): 1) one shift at the root of a large clade, hereafter referred to as the core-group, and 2) one shift at the root of a subclade of the genus *Melinaea* that, for simplicity, is referred to as the *Melinaea*-group. The remaining lineages are collectively referred to as the backbone.

Six models from Morlon et al. (2011) were fitted on the core-group, the *Melinaea*-group and the backbone including time-constant and time-dependent functions of speciation and extinction rates (Appendix S3.1.2). We also explored the effect of potential residual diversification rate heterogeneity in the backbone on the results from Morlon et al. (2011) method (Appendix S3.1.5). TreePar (Stadler 2011) analyses were performed on the core-group and the backbone, and diversification rate estimated in bins of 4 My (Appendix S3.1.3). We did not fit TreePar on the *Melinaea*-group since it is only 1 My old. We allowed diversification rate to be negative but we did not allow mass-extinction events. For both methods the models were fitted on 100 trees randomly sampled from BEAST's posterior distribution for each partition.

#### 187 *Diversification in the Andes*

To investigate the pattern of diversification in the Andes with respect to the rest of the Neotropical region, we classified species as Andean or non-Andean, based on a combination of published and unpublished (i.e., databases generated from museum collections and our own field collections) georeferenced distribution data and elevation ranges of species (Appendix S1.2). We used models of trait-dependent diversification implemented in ClaSSE (Goldberg & Igić 2012), to compare the rates of speciation, of extinction and of transition between the Andean area and the non-Andean regions (Chazot et al. 2016b, Beckman & Witt 2015, Appendix S3.2). Ten models were fitted on the full tree and the core-group alone to account for the major diversification shift at the root of the core-group. In both cases all models were fitted on the 100 trees randomly sampled from BEAST's posterior distribution. We also performed additional analyses with the model HiSSE (Beaulieu & O'Meara 2016) to test the hypothesis that a hidden character explained the pattern of trait-dependent diversification estimated with ClaSSE (Appendix S3.2). Finally, we also conducted ancestral state estimations based on the two best models of trait-dependent diversification, which were compared to the ancestral areas estimated by the historical biogeography analyses outlined below.

#### 202 *Diversification in Amazonia*

203 We further investigated the pattern of diversification in Amazonia during the post-Pebas period, where  
204 speciation in some taxa may have been driven by climatic fluctuations during the Quaternary (Haffer  
205 1969). To test whether speciation rates had increased in the last 2.5 My, we identified seven major  
206 Amazonian diversification events from the BioGeoBEARS ancestral state reconstruction (see below)  
207 and we fitted a model of time-dependent speciation rate (no extinction) to see whether speciation rates  
208 increased through time (supporting a recent diversification potentially caused by Pleistocene climatic  
209 fluctuations) or decreased through time (supporting radiations accompanying the post-Pebas  
210 recolonizations) (Appendix S3.3). Analyses were performed on 100 trees randomly sampled from  
211 BEAST's posterior distribution.

212

### 213 **Historical biogeography**

214 We inferred the historical biogeography of Ithomiini with BioGeoBEARS v.0.2.1 (Matzke 2014)  
215 using the model DEC. We divided the Neotropics into nine distinct biogeographic regions (Figure  
216 S4.7): 1) Central America, 2) Caribbean Islands, 3) lowlands on the Western part of the Andes,  
217 including the Magdalena valley, 4) Northern Andes that comprise the Western and eastern Ecuadorian  
218 and Colombian cordilleras and the Venezuelan cordillera, 5) Central Andes, 6) Western Amazonia, 7)  
219 eastern Amazonia, 8) Guiana Shield and 9) Atlantic Forest. We proceeded in three steps. First, we  
220 performed an estimation of ancestral ranges using a model with refined area adjacency (unrealistic  
221 distributions, such as disjunct distributions, were avoided) but uniform dispersal multipliers (null-  
222 model, Appendix S4.1). Second, we performed 1000 biogeographic stochastic mapping replicates to  
223 compute rates of dispersal between specific regions per million years (Appendix S4.2). We computed  
224 rates of dispersal in the core-group between Andean and non-Andean regions, between the Andes and  
225 Amazonia, between the Central and the Northern Andes, between the Atlantic Forest and the other  
226 regions, and between Central America and the rest of the Neotropics (Figure S4.8). This allowed us to  
227 test some biogeographic hypotheses but also to identify relevant time frames among which dispersal  
228 probabilities between areas might vary. Third, we implemented a time-stratified model designed from  
229 the previous information to refine our biogeographic reconstruction. (Appendix S4.3). We created four  
230 time frames: i) 0-4, ii) 4-8, iii) 8-13, and iv) 13-30 My ago, where dispersal multipliers between areas  
231 reflected the colonization rate variations identified previously (Table S4.6). All these analyses were  
232 performed on the median tree.

## 233 **RESULTS**

### 234 **Time-calibrated phylogeny**

235 *Tree topology and time-calibration*

236 The topology of Ithomiini phylogeny was generally well supported, including deep nodes (Figure  
237 S2.1). The inferred crown age of Ithomiini was 26.4 My ago (95% credibility interval CI=22.75-  
238 30.99) (Figure 1, Figure S2.2), and the divergence time from its sister clade Tellervini was 42.1 My  
239 ago (CI=39.50-48.44). All subtribes (10 in total) diverged during the first 10 My (Figure 1). Node ages  
240 in our phylogeny were generally slightly younger than those inferred in Wahlberg et al. (2009), but  
241 older than those inferred in Garzón-Orduña et al. (2015) (see De-Silva et al. 2017 for further  
242 discussion of such differences).

## 243 **Diversification rates**

### 244 *Time-dependent diversification*

245 We partitioned the dynamics of diversification into 1) the core-group, which accounts for ~85% of  
246 present-day diversity of Ithomiini; 2) the *Melinaea*-group (i. e., the high-diversity clade in the genus  
247 *Melinaea*) and 3) the backbone (i. e., the remaining lineages) (Figure 1, Appendix S3).

248 When fitting Morlon *et al.* (2011)'s models on the core-group, no model of time-dependent  
249 diversification had a significantly better fit than the null model of constant speciation rate without  
250 extinction (average of 0.257 lineage<sup>-1</sup>My<sup>-1</sup>, Figure 2, Table 1, Table S3.4). For the *Melinaea*-group,  
251 which radiated into eight species during a time lapse of only one million years, the best fitting model  
252 was an exponentially decreasing speciation rate without extinction, with a very high initial speciation  
253 rate (average of 14.54 lineage<sup>-1</sup>My<sup>-1</sup> at the root, average of 0.31 lineage<sup>-1</sup>My<sup>-1</sup> at present, Figure 2,  
254 Table 1, Table S3.4). On the remaining backbone, the best fitting model involved a time-dependency  
255 of both speciation and extinction rates. The resulting net diversification rate was initially high (average  
256 of 0.78 at the root), but decreased rapidly and became negative around 19 My ago, before starting a  
257 slow recovery, while remaining negative (average of -0.04 lineage<sup>-1</sup>My<sup>-1</sup> at present, Figure 2, Table 1,  
258 Table S3.4). This signal of high relative extinction rate was not affected by initial parameters for  
259 maximum likelihood search or by diversification rate heterogeneity potentially remaining in the  
260 backbone (Figure S3.4). Several clades within the backbone show positive net diversification rates  
261 during the last 5 My, which further supports the recovery trend described above.

262 We obtained congruent results with TreePar (Figure 2), with a fairly constant diversification through  
263 time for the core-group, and an initially high diversification rate for the backbone lineages, followed  
264 by a general decline during the last 15 My, leading to a negative net diversification rate between 12  
265 and 4 My ago.

### 266 *Diversification in the Andes*

267 For the whole Ithomiini, five ClasSE models were within an AIC interval of two. There was no clear  
268 consensus across those models, since three of them supported higher speciation rate in the Andes,

269 while the others had equal speciation rates. Two models found inferred different dispersal rates but in  
270 two opposite ways (Table 2, Table S3.5). This lack of consensus probably reflects the heterogeneity of  
271 diversification processes across the Ithomiini. Non-zero extinction rate was inferred in all cases. In the  
272 core-group, three out of four models within an AIC interval of two had identical speciation rates, and  
273 the fourth model had a slightly higher speciation rate in the Andes (Table 2, Table S3.5). Three of  
274 those models have identical transition rates between Andean and non-Andean regions, and the fourth  
275 one has a higher transition rate towards the Andes. All have very low extinction rate. Overall, the  
276 results for the core group converge on similar speciation and transition rates between Andean and on-  
277 Andean regions, and low extinction. This interpretation was confirmed by additional analyses in  
278 which we tested the presence of a hidden character using the model HiSSE (Box S3.1, Table S3.5,  
279 Figure S3.5).

280 The most likely state of the root of Ithomiini was non-Andean but with high uncertainty (probability of  
281 0.508 with different speciation rates [model 1] and 0.543 with different speciation and transition rates  
282 [model 2], Figure S3.6) and there was high uncertainty at the nodes leading to the core-group (Figure  
283 1, Figure S3.6). The most recent common ancestor (hereafter MRCA) of the core-group was inferred  
284 as most likely Andean (probability of 0.558 for model 1 and 0.625 for model 2). The MRCAs of  
285 backbone subtribes were always inferred to be non-Andean with a strong support, except for  
286 Athesitina (Figure 1, Figure S3.6). Within the core-group, an Andean origin for subtribes  
287 Napeogenina, Dircennina and Godyridina was inferred with model 1, whereas an Andean origin for all  
288 five subtribes was inferred with model 2 (Figure S3.6).

289 *Diversification in Amazonia*

290 Only two Amazonian diversification events in the backbone, *Mechanitis* + *Forbestra* and *Melinaea*  
291 (the whole genus), and one core-group event (*Brevioleria*-group), showed a pattern of increasing  
292 speciation rate through time (Figure 3), consistent with a potential effect of Pleistocene climatic  
293 fluctuations in driving diversification. The other four Amazonian diversification events (*onega*-group,  
294 *Hypothyris*-group, *agnosia*-group and *Methona*) showed decreasing speciation rates through time  
295 (Figure 3), suggesting an early diversification.

296

297 **Historical biogeography**

298 *Biogeographic null model and diversification of the core-group*

299 Under the null model (no dispersal multiplier) the ancestral area of the Ithomiini MRCA was unclear  
300 (Central Andes + Western Amazonia for the highest probability), and all subtribes except Melinaeina

301 and Mechanitina originated and started diversifying in the Central Andes (Figure 1, Figure S4.9).  
302 However, all estimations at the basal nodes of the backbone were highly uncertain.

303 While an absence of dispersal events out of the Central Andes characterized the initial Andean phase  
304 of diversification in the core-group (Figure 3), we found a major peak of interchanges between the  
305 Andes and Amazonia 12-8 My ago, entirely driven by colonization from the Andes towards Amazonia  
306 (Figure 3, Figure S4.8). After this peak, the rate of dispersal towards Amazonia rapidly decreased  
307 through time until around 4 My ago, when a second peak of interchanges, involving reverse  
308 colonizations towards the Andes, occurred (Figure 3). We also recorded a large increase of  
309 interchanges between the Central and the Northern Andes after around 12 My, i.e. simultaneously with  
310 the increase of dispersal rate towards Amazonia (Figure 3, Figures S4.2 and S4.3). Rates of  
311 interchanges between the Central and Northern Andes then remained relatively constant through time,  
312 although they increased during the last 3 My, due to dispersal from the Northern Andes towards the  
313 Central Andes. Colonization of Central America likely started around 8 My ago, but interchanges  
314 increased mainly during the last 4 My (Figure S4.8). Colonizations of the Atlantic Forest also started  
315 early (around 9-10 Mya), but the rate of interchanges between the Atlantic Forest and the remaining  
316 Neotropical regions remained relatively constant during the last 10 My (Figures S4.2 and S4.3).

317 Until ~10 My ago speciation events occurring in the Central Andes fully accounted for the core-group  
318 diversification (no dispersal events). During the last ~10 My, however, speciation slowed down in the  
319 Central Andes (Figure 3). At the same time, following the peaks of dispersal identified above,  
320 Northern Andean and Amazonian lineages started diversifying, although the latter diversified at a  
321 slower pace than the former. A large number of dispersal events into the Northern Andes were  
322 followed by important local diversification, for example in the genera *Hypomenitis* (17/20 species in  
323 the phylogeny occur in the Northern Andes) and *Pteronymia* (30/45 species in the phylogeny occur in  
324 the Northern Andes), or in subclades of the genera *Oleria* or *Napeogenes*. We also identified some  
325 important transitions to lowland Amazonia, for example at the origin of the *Brevioleria*-clade, during  
326 early divergence in the genus *Oleria* and in the genus *Hypotheiris*.

327 *Time-stratified biogeographic model*

328 The time-stratified model designed from rates of colonization computed above led to a significant  
329 improvement of the model (likelihoods: DECnull: -1335.802, DECstrat: -1321.805). Both ancestral  
330 state reconstructions were very congruent but the time-stratified model increased the resolution of  
331 several nodes throughout the tree (Figure S4.9). Notably, the time-stratified model greatly increased  
332 the resolution of deep nodes in the backbone, estimating that both Mechanitina and Melinaeina  
333 originated in Western Amazonia, in agreement with the ancestral state estimations performed with  
334 trait-dependent diversification models. The Andean origin for the core-group was strongly supported.

335

336 **DISCUSSION**

337 We generated one of the largest species-level phylogenies to date for a tropical insect group, the  
338 emblematic Neotropical butterfly tribe Ithomiini, to investigate the temporal and spatial patterns of  
339 diversification of this group. Here, we propose that the multiple landscape transformations during the  
340 Miocene, and more specifically the interactions between the Andes and the Pebas wetland system,  
341 strongly affected the dynamics of diversification and biotic interchanges of Ithomiini butterflies in the  
342 Neotropical region.

343 *Early diversification at the interface of the Pebas and Central Andes*

344 Ithomiini probably started diversifying along the early Andean foothills at the transition with Western  
345 Amazonia. The onset of the uplift of the eastern cordillera of the Central Andes during late Oligocene  
346 coincides with the origin of Ithomiini (Eude et al. 2015). The backbone underwent a rapid early  
347 diversification, likely following the colonization of South America during the pre-Pebas period – the  
348 sister clade of Ithomiini, Tellervini, is found in Australia and Papua New Guinea (Wahlberg et al.  
349 2009). Diversification was perhaps facilitated by an early shift to a new and diverse hostplant family,  
350 the Solanaceae, which originated in the Neotropics during the Paleogene and is particularly diverse in  
351 this region (Dupin et al. 2017).

352 From ca. 23 to 10 My ago the Pebas ecosystem replaced the previous Western Amazonian terrestrial  
353 ecosystem. Wesselingh et al. (2001) described the Pebas as an ecosystem “which was permanently  
354 aquatic with minor swamps and fluvial influence, and was connected to marine environments”, and  
355 may have reached a maximum size of 1.1 million km<sup>2</sup>. The presence of fossil marine fishes (Monsch  
356 1998) and molluscs (Wesselingh 2006) testifies to the presence of saline waters. More recently,  
357 Boonstra et al. (2015) found evidence from foraminifera and dinoflagellate cysts that marine  
358 incursions reached 2000 km inland from the Caribbean Sea during the early to middle Miocene during  
359 periods of high sea levels. The extent and duration of these marine influences is controversial (see  
360 Jaramillo et al. 2017 and references therein). Yet, it is undeniable that the Pebas system was unsuitable  
361 for the late Oligocene terrestrial fauna and flora occupying Western Amazonia, and therefore was  
362 likely to affect the timing of diversification and dispersal of Neotropical biodiversity. This scenario is  
363 congruent with recent historical biogeography analyses of vertebrates (e. g., Hutter et al. 2017) and  
364 plant lineages belonging to multiple families (e. g., Schneider and Zizka 2017, Machado et al. 2018)  
365 and by a recent evaluation of the Amazonian fossil record, which pointed at a major decline of  
366 terrestrial diversity in western Amazonia during the early and middle Miocene (Antoine et al. 2016):  
367 mammalian diversity dropped from 11 orders, 29 families and 38 species during late Oligocene down  
368 to 1 order, 2 families and 2 species during middle Miocene (see also Antoine et al. 2017). Our results

369 with Ithomiini butterflies strongly support such a scenario. Although the ancestral area of Ithomiini is  
370 ambiguous, at least the two earliest diverging Ithomiini lineages (Melinaeina and Mechanitina) were  
371 clearly endemic to Western Amazonia. We found that the diversification rate of Ithomiini rapidly  
372 decreased through time and even became negative for backbone lineages during a period  
373 corresponding to the replacement of terrestrial habitats by the Pebas system, leading to a decline of  
374 diversity in those lineages. Estimations of extinction rates from molecular phylogenies, however, are  
375 still controversial and we remain cautious in our interpretation of the role of extinction during the  
376 Pebas period. The progressive recovery of backbone lineages towards the present, including positive  
377 diversification rates in some recent lineages, also concurs with the idea that the retreat of the Pebas  
378 released constraints on diversification during the last 10 My.

379 Ithomiini are tightly linked to their larval host-plants and forest habitats. As such, early Ithomiini  
380 lineages, which are largely Amazonian, may have been strongly impacted by habitat turnover in  
381 Western Amazonia and local extinctions of larval host-plant during the Pebas period, while the core-  
382 group lineages remained unaffected and diversified fast. Yet, uncertainties surrounding the range  
383 estimations at other deep nodes and also the time of first colonization of the Andes challenges a  
384 scenario where colonization of the Andes occurred at the root of the core group. If colonization of the  
385 Andes occurred earlier than the root of the core group, the shift of diversity dynamics may be  
386 explained by other factor(s), unknown at the moment. However, biogeographic inferences in the  
387 backbone should be taken with caution. Indeed, extinction events in the backbone (potentially higher  
388 in Western Amazonian lineages, as indicated by negative net diversification rates) may bias our  
389 biogeographic ancestral state estimations. Notably, asymmetrical extinction across different  
390 geographical regions may lead to inaccurate inferences of past geographic ranges (Lieberman 2002,  
391 Sanmartín & Meseguer 2016). Such a scenario, where backbone lineages were ancestrally Western  
392 Amazonian, would be consistent with the diversification shift observed at the root of the core-group  
393 being driven by colonization of the Andes, where ecosystems were unaffected by the Pebas.

394 The strongly supported origin of the core-group in the Central-Andes (19.1-22.1 My) parallel to the  
395 events occurring in Western Amazonia during the Pebas period had profound consequences on the  
396 pattern of diversification of Ithomiini. First, this early Andean lineage diversified to produced 85% of  
397 the current Ithomiini diversity. Second, until ~10 My ago, diversification in the core-group occurred  
398 exclusively in the Central Andes, and from 19.1-22.1 to ~10 My ago we do not detect a single  
399 dispersal event out of the Central Andes. Third, the core-group corresponds to a shift of diversification  
400 dynamics, characterized by a constant and relatively high diversification rate, which greatly contrasts  
401 with the slow and even negative diversification dynamics of the backbone during the same period.

402 Drivers of diversification in the core group probably include radiation on Solanaceae (Willmott &  
403 Freitas 2006), which were already diverse in the Neotropics during the Miocene (Dupin et al. 2017), in

404 conjunction with a diversity of habitats along the slopes of the Andes. Speciation was also potentially  
405 mediated by shifts in mimicry patterns (Chazot et al. 2014, Willmott & Mallet 2004), which can  
406 generate reproductive isolation in mimetic butterflies (Jiggins et al. 2001), including Ithomiini (Jiggins  
407 et al. 2006, McClure et al. accepted). Those ecological drivers of diversification were probably at play  
408 both in the Andes and in non-Andean areas, after the Pebas retreat. Indeed, in the core-group, Andean  
409 and non-Andean lineages had similar diversification rates. The lack of support for a general increase in  
410 diversification rate in the Andes within the core-group is also supported by analyses performed  
411 independently on different core-group subtribes. For example, in both Oleriina (Chazot et al. 2018)  
412 and Godyridina (Chazot et al. 2016b), radiations occurred in both Andean and Amazonian genera.

413 *Dispersal out of the Central Andes at the demise of the Pebas*

414 Gentry (1982) pointed out a dichotomy observed in the geographic distribution of Neotropical plant  
415 diversity, showing that groups could be divided into Andean-centred *versus* Amazonian-centred  
416 patterns. Clades tend to be species-rich in one of these centres and relatively species-poor in the other.  
417 Antonelli & Sanmartín (2011) coined this observation the “Gentry-pattern”. They also suggested that  
418 in the absence of a barrier between the Andes and the Amazon basin we should observe continuous  
419 interchanges between these regions. Antonelli & Sanmartín (2011) proposed that the Pebas could be  
420 this “missing long-lasting barrier needed for creating the disjunction between Andean-centred and  
421 Amazonian-centred groups”. Therefore, in addition to the constraints on diversification discussed  
422 above, we predicted that the Pebas ecosystem should have influenced interchanges towards or across  
423 Western Amazonia.

424 Our results conform remarkably well to the scenario proposed by Antonelli & Sanmartín (2011).  
425 Ithomiini are Andean-centered with more than half of their current diversity occurring in the Andes  
426 (see also Chazot et al. 2016b,c). Here we show that interchanges have been virtually absent during the  
427 Pebas period, with a period as long as 9-12 My without interchanges. However, rates of interchanges  
428 from the Central Andes towards the Northern Andes and Amazonia suddenly peaked ~10 My ago  
429 (between 13-8 My ago) and more recently (4-0 My ago). Regarding connections between Central  
430 Andes and the Northern Andes, the closure of the Western Andean Portal (Antonelli & Sanmartín  
431 2011) may have allowed multiple colonizations of the Northern Andes facilitated by the presence of  
432 connecting higher altitude habitats. In parallel, between 10-8 My ago the Pebas system was  
433 progressively drained eastward, leading to the formation of the present-day configuration of the  
434 Amazonian basin and the expansion of terrestrial forest habitats in Western Amazonia (Leite et al.  
435 2017). This corresponds precisely to the timing at which core-group lineages colonized Western  
436 Amazonia - perhaps again because this region was concomitantly recolonized by Solanaceae - and  
437 then diversified.

438 *Diversification across the whole Neotropics following the demise of the Pebas*

439 We found a strong dampening of local speciation in the Central Andes during the last 10 My. By  
440 contrast, colonizations following the retreat of the Pebas were followed by large local bursts of  
441 diversification within the Northern Andes and Amazonia. As an illustration, from our biogeographic  
442 reconstruction, 69 divergence events occurred strictly in the Central Andes in the core-group in the last  
443 20 My. Multiple independent dispersal events followed by local diversification lead to the exact same  
444 number of divergences occurring strictly in the Northern Andes during the last 9 My only. The genera  
445 *Hypomenitis* and *Pteronymia*, for example, which comprise 23 and 53 species, respectively,  
446 diversified extensively within the Northern Andes (Chazot et al. 2016b; De-Silva et al. 2017).  
447 Lineages that dispersed into the Northern Andes and Amazonia after the demise of the Pebas probably  
448 accessed a large range of new ecological niches, including a diversity of host-plants that had already  
449 diversified or that radiated concomitantly. We detected a second peak of interchanges between the  
450 Central Andes and Amazonia or the Northern Andes. In both cases, these recent peaks of interchanges  
451 are clearly driven by reverse dispersal events. Three Amazonian radiations, the genus *Melinaea*  
452 (which comprises the rapidly-diversifying *Melinaea*-group) and the clade *Mechanitis* + *Forbestra* in  
453 the backbone and the *Brevioleria*-group in the core-group, showed increasing speciation rates towards  
454 the present. Those radiations may be interpreted as supporting an effect of recent climatic fluctuations  
455 during the Pleistocene on the diversification of the *Melinaea*-group (as well as the *Mechanitis* +  
456 *Forbestra* clade), although ecological drivers of speciation classically invoked in mimetic butterfly  
457 diversification, such as colour pattern and hostplant shifts, cannot be ruled out (Jiggins et al. 2006, Hill  
458 et al. 2012, McClure & Elias 2016, McClure & Elias 2017, McClure et al. accepted). Contrasting with  
459 those clades, four other Amazonian radiations showed diversification rates decreasing through time,  
460 meaning that diversification was highest just after the retreat of the Pebas. Recent radiations in  
461 Western Amazonia that post-date the Pebas period have repeatedly been reported. For example, in the  
462 palm genus *Astrocaryum* (Arecaceae), the upper Amazonian clade started diversifying only ~6 My ago  
463 (Roncal et al. 2013). In *Taygetis* butterflies, Amazonian lineages show rapid diversification during the  
464 last 7-8 My (Matos-Maraví et al. 2013). Such convergent timing of diversification in Western  
465 Amazonia strongly supports the scenario of a post-Pebas recovery of terrestrial habitats, which  
466 triggered dispersal followed by local diversification.

467 Taken together, our results point at a robust scenario for Neotropical diversification, which highlights  
468 the role of Miocene ecosystem turnover in determining the timing of interchanges, speciation and  
469 potentially extinction in the world's most biologically diverse region.

470

471

- 472 List of supporting information:
- 473 Appendix S1: List of all individuals and species used in this study (S1.1), and biogeographic  
474 distribution of species (S1.2).
- 475 Appendix S2: Detailed methods and supplementary results for phylogenetic inferences
- 476 Appendix S3: Detailed methods and supplementary results for diversification analyses
- 477 Appendix S4: Detailed methods and supplementary results for historical biogeography analyses
- 478

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734

735 **DATA ACCESSIBILITY**

736 All sequences are publicly available on Genbank (accession numbers are given in Appendix S1).  
737 Phylogenetic trees are available on TreeBase  
738 (<http://purl.org/phylo/treebase/phylows/study/TB2:S23995?x-access-code=85eb5792b9ef59452b163c39eb80ae47&format=html>).  
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741

742 Table 1. Results of time-dependent models of diversification fitted on 100 posterior trees partitioned  
 743 into the backbone, the core-group and the *Melinaea*-group. For each subclade or the backbone, only  
 744 the best fitting model is shown (Appendix 3.1.2). BCST = constant speciation, BVAR = time-  
 745 dependent speciation, DCST = constant extinction, DVAR time-dependent extinction.  $\lambda$  = speciation  
 746 rate at present,  $\alpha$  = coefficient of time variation of the speciation rate,  $\mu$  = extinction rate at present,  $\beta$   
 747 = coefficient of time variation of the extinction rate. Values given are the mean estimated for 100  
 748 posterior trees.

	model	par	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
backbone	BVARDVAR	4	-129.75	267.50	0.153	0.160	0.193	0.148
<i>Melinaea</i> -group	BVAR	2	-1.58	7.17	0.311	3.772		
core-group	BCST	1	-702.25	1406.50	0.257			
<b>Joint model</b>		<b>7</b>	<b>-835.032</b>	<b>1684.065</b>				

749

750 Table 2. Results of ClaSSE models fitted on 100 posterior trees for whole Ithomiini tree (a.) and the  
 751 core-group (b.) sorted by increasing mean AIC. Constraints of each model are indicated in the four  
 752 first columns. 1=non-Andean, 2=Andean,  $\lambda_{111}$  and  $\lambda_{222}$  are within-region speciation rates (non-  
 753 Andean and Andean, respectively),  $\lambda_{112}$  and  $\lambda_{212}$  are cladogenetic transition rates (non-Andean  
 754 towards Andean, and Andean towards non-Andean, respectively) ,  $\mu$  = extinction rates, df =number of  
 755 parameters, logL=log-likelihood, AIC= Akaike information criterion score,  $\Delta$ AIC = AIC difference  
 756 with the best fitting model. Values given are the mean estimated for 100 posterior trees (Appendix  
 757 S3.2).

758 a. Whole tree

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\Delta$ AIC	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	-1064.82	2137.63	0.00	0.154	0.186	0.052	0.052	0.003	0.003
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	-1065.89	2137.79	0.16	0.171	0.171	0.052	0.052	0.004	0.004
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	5	-1064.66	2139.31	1.68	0.151	0.188	0.046	0.058	0.002	0.002
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	4	-1065.70	2139.39	1.76	0.174	0.174	0.052	0.052	0.020	0.001
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	5	-1064.73	2139.46	1.83	0.153	0.192	0.052	0.052	0.001	0.015
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	4	-1065.88	2139.76	2.13	0.172	0.172	0.053	0.051	0.004	0.004
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-1065.65	2141.29	3.66	0.175	0.175	0.049	0.056	0.024	7E-05
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	-1064.65	2141.29	3.66	0.151	0.190	0.047	0.057	0.001	0.006
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	-1102.44	2208.88	71.25	0.111	0.111	0.111	0.111	6E-06	6E-06
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	3	-1102.42	2210.84	73.21	0.151	0.151	0.151	0.151	0.001	0.006

759

760 b. Core-group

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\Delta$ AIC	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	-877.96	1757.74	0.00	0.190	0.190	0.059	0.059	4.26E-07	4.26E-07
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	-877.00	1757.80	0.08	0.168	0.205	0.060	0.060	8.15E-07	8.15E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	4	-877.62	1759.07	1.33	0.189	0.189	0.082	0.038	4.13E-07	4.13E-07
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	4	-877.96	1759.74	2.00	0.190	0.190	0.059	0.059	8.11E-06	6.97E-06
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	5	-876.99	1759.78	2.07	0.168	0.207	0.060	0.060	1.13E-06	0.003
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	5	-874.92	1759.83	2.09	0.171	0.204	0.068	0.051	1.84E-07	1.84E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-875.52	1761.02	3.26	0.190	0.190	0.084	0.035	0.003	8.37E-07
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	-874.88	1761.75	4.01	0.170	0.205	0.064	0.054	2.65E-06	0.001
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	-905.69	1815.37	57.63	0.124	0.124	0.124	0.124	1.97E-06	1.97E-06
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	3	-905.51	1817.02	59.27	0.170	0.170	0.170	0.170	2.65E-06	0.001

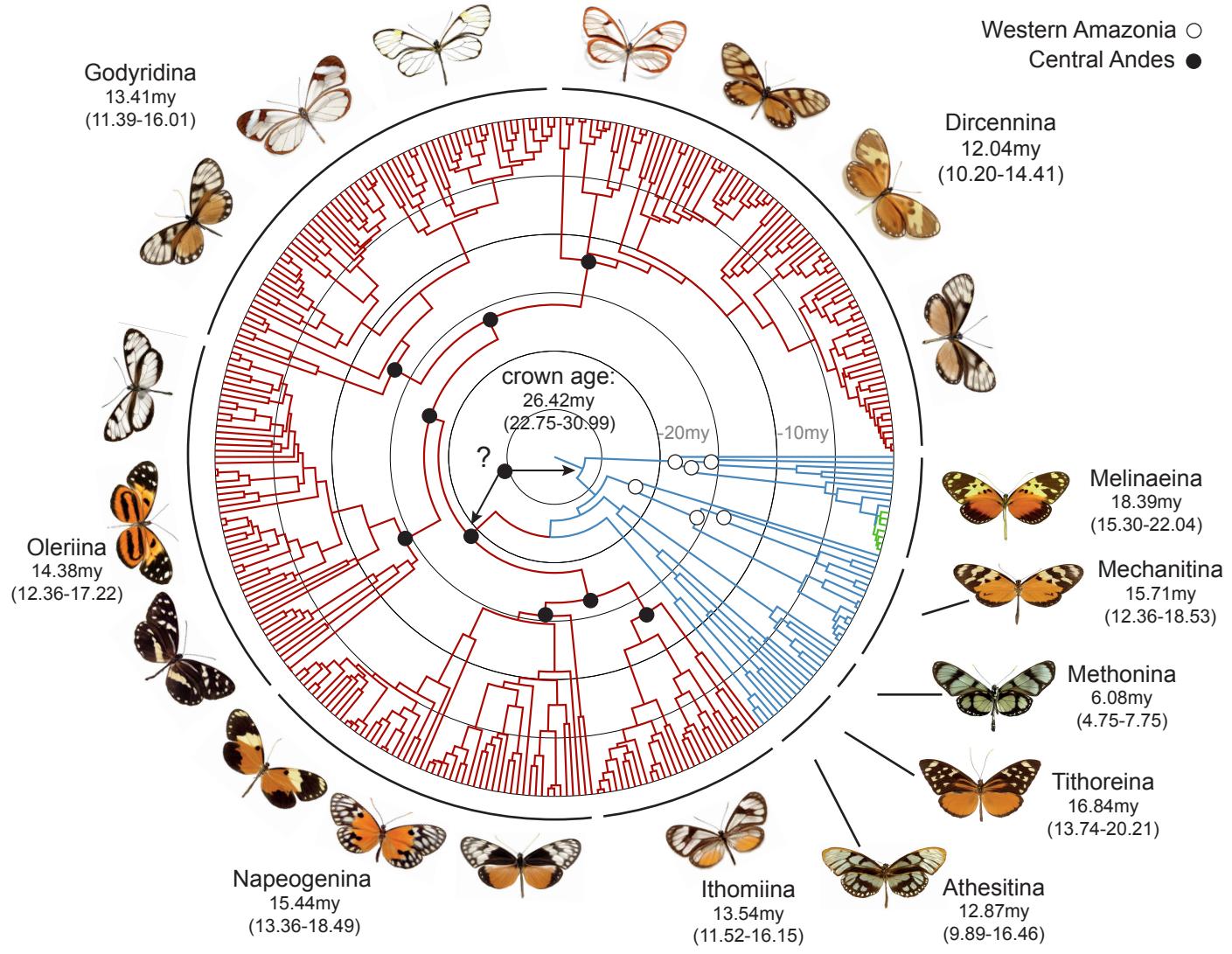
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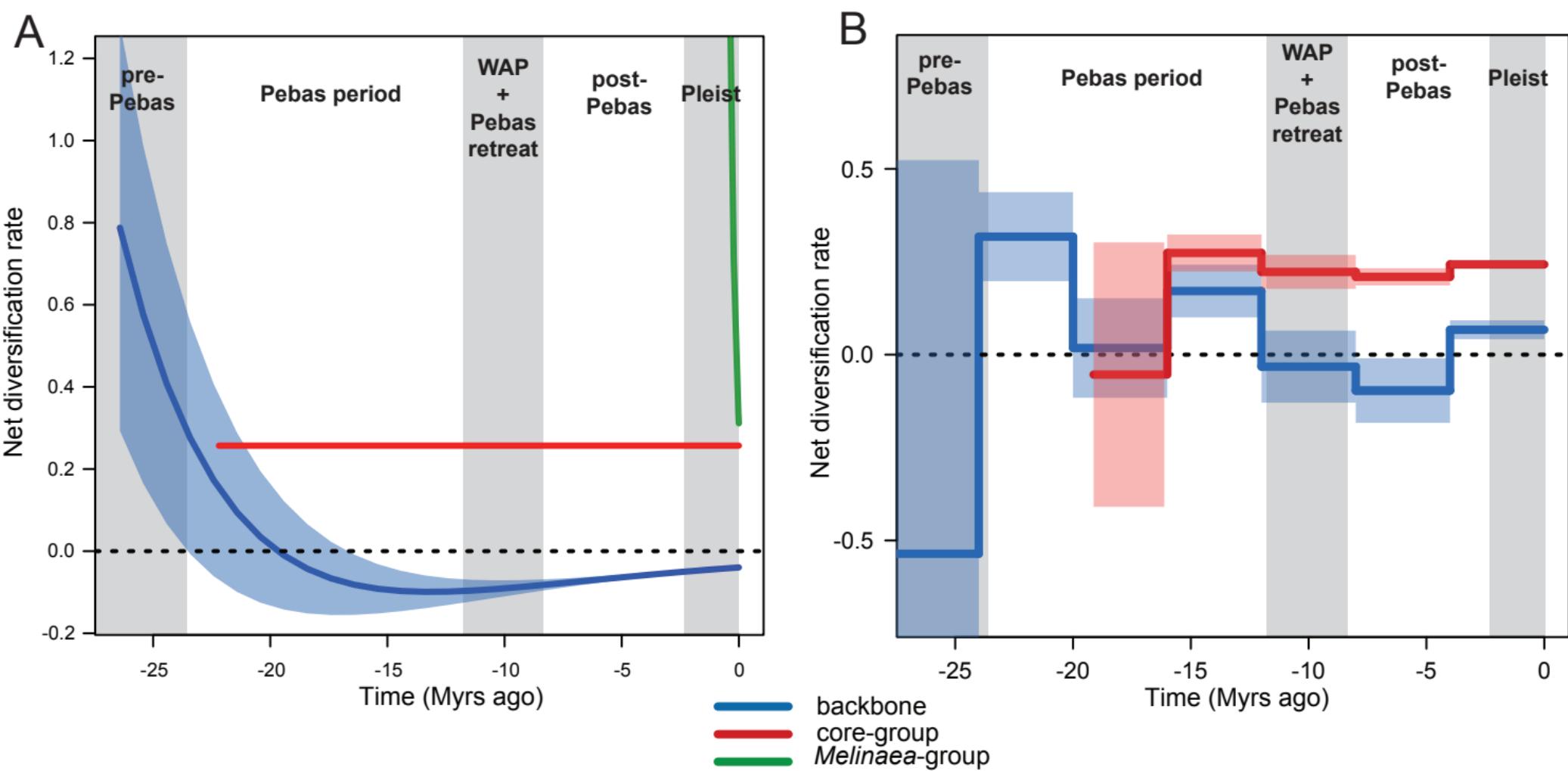
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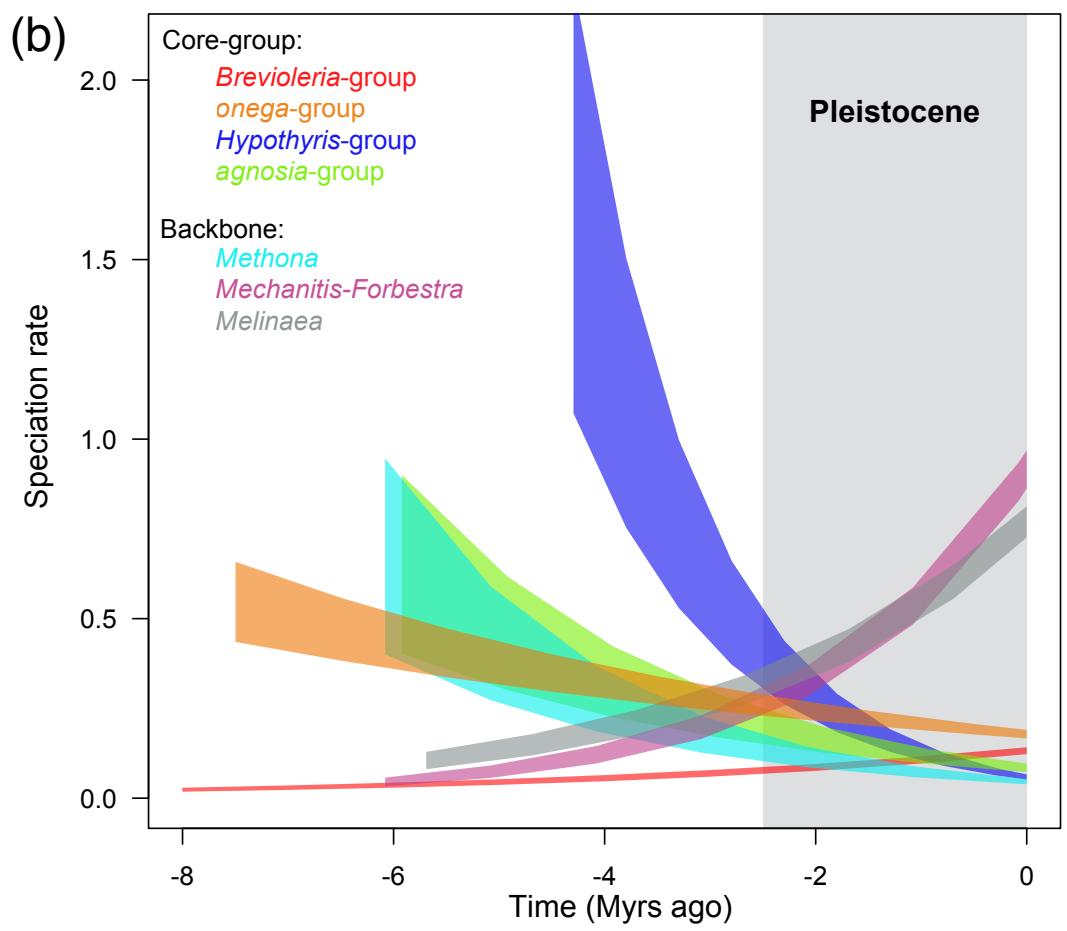
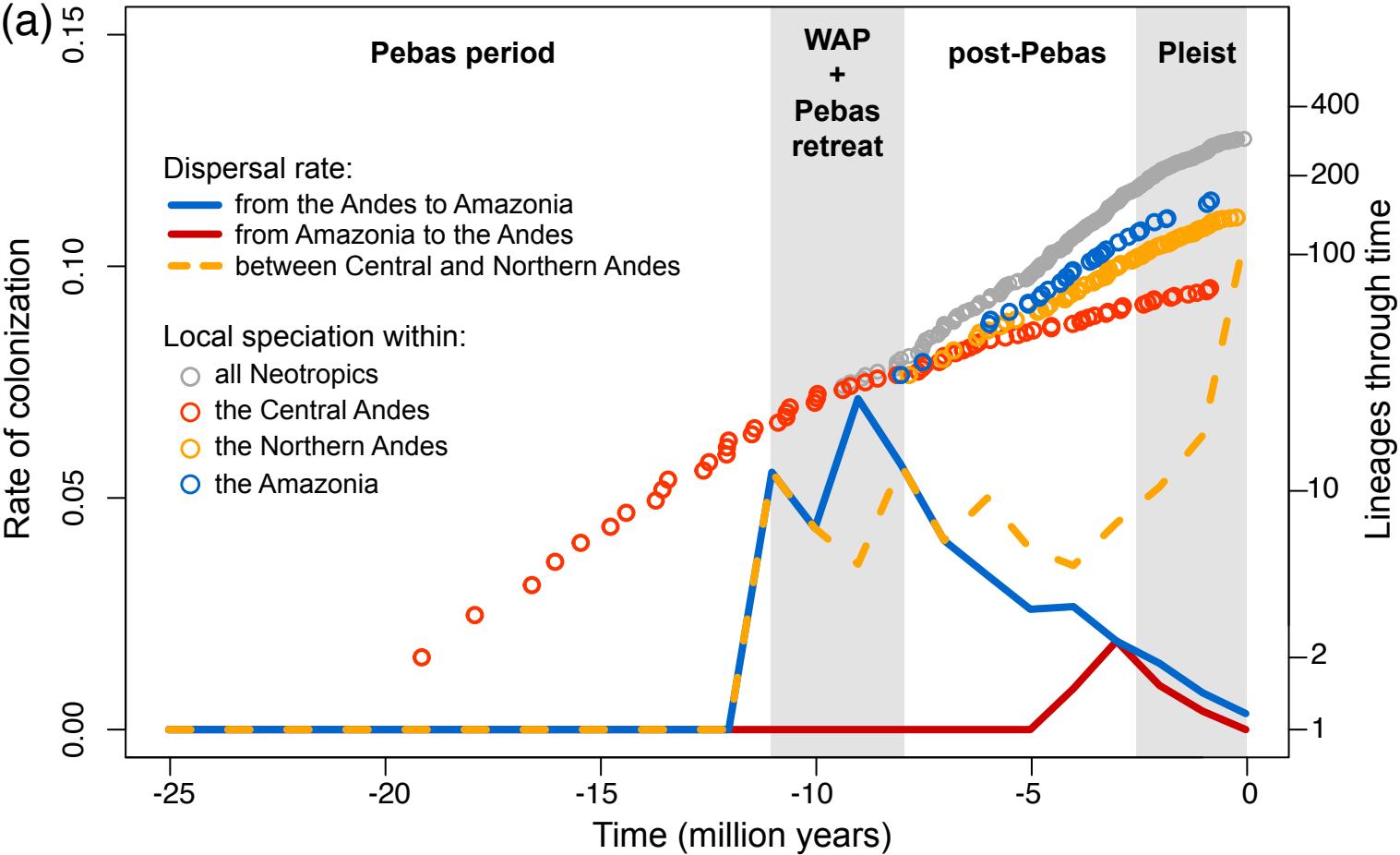
763 Figure 1. Time-calibrated phylogeny of the tribe Ithomiini. Coloured branches depict the partitions  
764 identified by MEDUSA and used for fitting diversification rate models. Red lineages constitute the  
765 core-group, green lineages the *Melinaea*-group, blue lineages the backbone. Black and white circles  
766 indicate the biogeographic ancestral states reconstructed at the basal nodes of the tree: black = Central  
767 Andes, white = Western Amazonia. Question mark and arrows indicate the position of two alternative  
768 scenarios for the first colonization of the Andes: BioGeoBEARS, at the root of the Ithomiini, and trait-  
769 dependent analyses, at the root of the core-group. Both methods are congruent for Melinaeina and  
770 Mechanitina but not for Tithoreina, Methonina and Athesitina. Names and position of the different  
771 subtribes are indicated, as well as mean crown age and 95% confidence interval.

772 Figure 2. Means and 95% confidence intervals of diversification rates through time estimated on 100  
773 posterior trees partitioned into the backbone, the core-group and the *Melinaea*-group. A.  
774 Diversification rates estimated using Morlon *et al.* (2011) for the backbone, the core-group and  
775 *Melinaea*-group (cropped). B. Diversification rates estimated using TreePar for the backbone and the  
776 core-group.

777 Figure 3. A. Colonization rates and lineage accumulation through time (speciation only) extracted  
778 from BioGeoBEARS ancestral state estimation for the core-group. The lines depicted are the median  
779 colonization rate after 1000 biogeographic stochastic mapping (Appendix S4). Dots represent the  
780 additional contribution to lineage accumulation of local diversification in three regions as  
781 reconstructed on the MCC tree. Red dots represent the cumulative number of speciation events  
782 through time in Central Andes; orange dots represent the cumulative number of speciation events  
783 through time in Central Andes and Northern Andes; blue dots represent the cumulative number of  
784 speciation events through time in Central Andes, Northern Andes and Amazonia; Grey dots represent  
785 the cumulative number of speciation events through time in the entire Neotropics. B. Confidence  
786 intervals of speciation rates through time estimated on seven Amazonian diversification events. All  
787 those clades originated during the last 8 My.







## Appendix S2: Detailed methods and supplementary results for phylogenetic inferences

We generated a phylogeny under maximum likelihood inference (ML). We then enforced this topology to generate a time-calibrated tree under Bayesian inference, using secondary calibrations.

### *S2.1 Gene partitioning and substitution models*

Prior to phylogenetic analyses, gene partitioning and substitution models were estimated using PartitionFinder v.1.1 (Lanfear et al. 2012). We divided all gene fragments into codon positions and allowed all partitions to be tested. Only substitution models available in BEAST were tested. The models of linked partitions had a better fit than unlinked partition schemes, hence the former was used. The best linked partition scheme contained 13 partitions (Table S2.1).

Table S2.1. Gene fragment partitions and substitution models associated obtained by Partition Finder v.1.1.

Gene fragment, codon position	Substitution models
COI codon 2	HKY+I+G
COII codon 3, COI codon 3	GTR+I+G
COII codon 1, COI codon 1, tRNA	GTR+I+G
COII codon 2	GTR+I+G
TKT codon 1, TKT codon 2	TrN+I+G
TKT codon 3	TrN+I+G
EF1 $\alpha$ codon 3	SYM+I+G
EF1 $\alpha$ codon 1, GAPDH codon 1	TrN+I+G
CAD codon 2, EF1 $\alpha$ codon 2, GAPDH codon 2, MDH codon 2	GTR+I+G
CAD codon 3, MDH codon 3	HKY+I+G

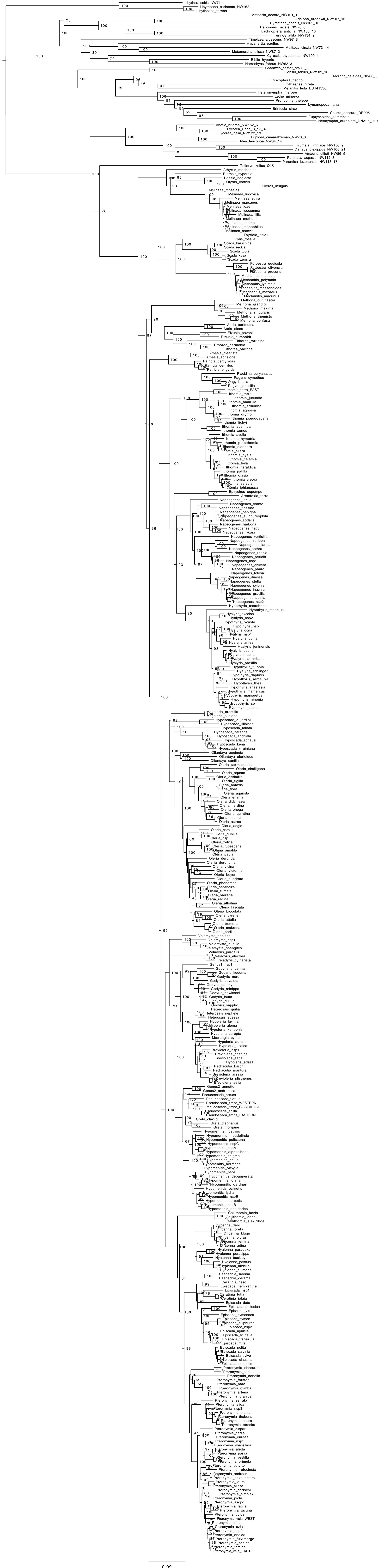
CAD codon 1, MDH codon 1, RPS2 codon 1	GTR+I+G
GAPDH codon 3, RPS2 codon 3	GTR+I+G
RPS2 codon 3	K80+I

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### *S2.2 Maximum likelihood inference (IQ-tree)*

We generated a phylogeny under maximum likelihood inference (ML), using IQ-Tree software as implemented in the W-ID-TREE server (Trifinopoulos et al. 2016, Nguyen et al. 2014) (Figure S2.1). The dataset was partitioned according to the results above (Table S2.1), and substitution rates modelled using GTR+I+G. We also assessed node support using 1000 bootstrap replicates.

Figure S2.1. Maximum likelihood tree obtained with IQ-tree, with bootstrap support indicated at nodes.



### S2.3 Bayesian inferences and time-calibration

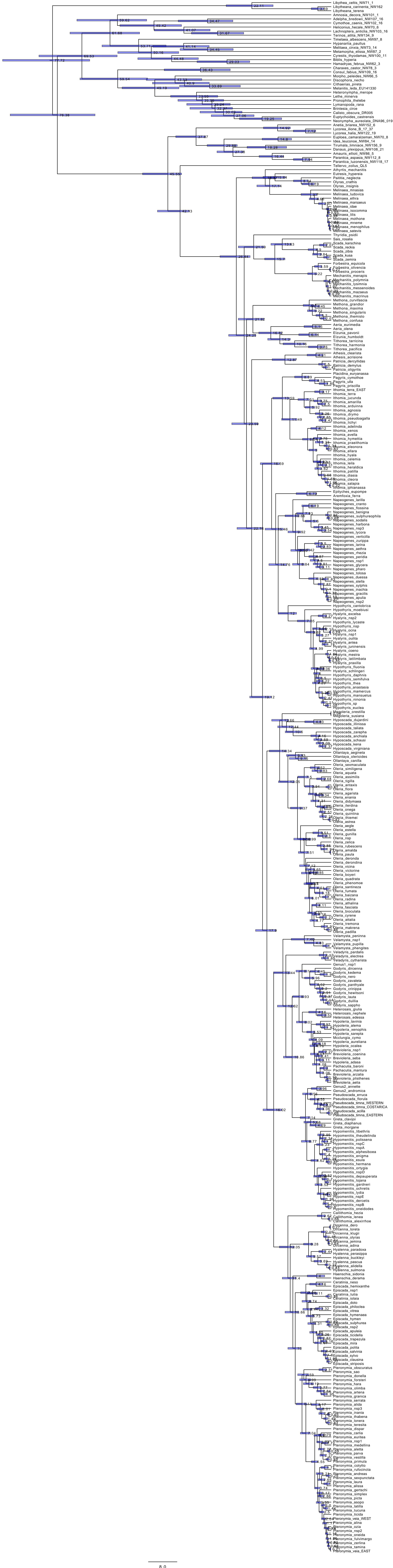
The tree was time-calibrated using BEAST v.1.8.2 (Drummond et al. 2012) with a birth-death tree prior and an uncorrelated lognormal relaxed clock for each gene partition (using gene partition and substitution models of Table S2.1), and a topology fixed to that of the ML tree. We applied a combination of secondary calibrations and host-plant calibrations (Table S2.2). Four secondary calibration points were retrieved from Wahlberg *et al.* (2009)'s phylogeny of Nymphalidae genera and were placed only outside of Ithomiini. We used uniform distribution priors, corresponding to the 95% HSPD inferred by Wahlberg *et al.* (2009). Host-plant calibrations were used as constraints only within the Ithomiini. Almost all Ithomiini species feed on Solanaceae with a relatively high specificity. A phylogeny of the Solanaceae was published by Särkinen *et al.* (2013), and recalibrated by De-Silva *et al.* (2017). We identified six relevant Solanaceae hostplant clades, which were used as maximum age constraints. Priors for host-plant calibration followed a uniform distribution. The minimum value of the uniform was 0 (present). The maximum value was the upper boundary of the 95% HSPD of the stem age of the host-plant subclade on which the calibrated Ithomiini lineage feeds as estimated in de-Silva *et al.* (2017). To get a starting tree suitable for time-calibration priors, the ML tree was made ultrametric and rescaled using PATHD8 (Britton et al. 2007) and Mesquite v.3.2 (Maddison & Maddison 2001).

We performed two independent runs of BEAST v.1.8.2 (Drummond et al. 2012) on the CIPRES server (Miller et al. 2010) of 87 million generations, sampling every 10000. Using Tracer v.1.6 we checked that the two runs had converged and the parameter's ESS values. Both runs were combined using Logcombiner v.1.8.3 (Drummond et al. 2012), applying a 15% burn-in for each run. Finally using TreeAnnotator v.1.8.3 (Drummond et al. 2012) we extracted the median branch lengths and credibility interval from the posterior distribution (Figure S2.2). The outgroups were pruned and the remaining tree was used for the following analyses (hereafter, median tree). Phylogenetic analyses with different settings (unconstrained topology in BEAST, Yule tree model or a different number of molecular clocks) produced similar topology and branch lengths (data not shown).

Table S2.2. Node constraints used for time-calibration of the tree. When node constraints were based on host-plant age the corresponding host-plant group is indicated (from de-Silva et al. 2017). The remaining constraints are secondary calibrations from Wahlberg et al. 2009.

node constrained	upper bound	lower bound	host-plant group
<i>Ollantaya + Oleria</i>	0	32	<i>Lycianthes</i>
Melinaeina	0	37.2	<i>Schultesianthus</i>
Methonina	0	41.3	<i>Brunfelsia</i>
Athesitina	0	24.8	<i>Capsicum</i>
<i>Placidina + Pagyris</i>	0	17.3	<i>Brugmansia</i>
Ithomiini	0	54.9	Solanaceae
Tellerviini + Ithomiini	39.5	52	
<i>Anetia + Lycora + Ituna</i>	11	21.5	
<i>Amauris + Danaus + Parantica</i>	24.5	35	
Danaini	35	48	

Figure S2.2. Bayesian time-calibrated tree obtained with Beast v.1.8.3 (Drummond et al. 2012) with median node ages and 95%HPD indicated at nodes.



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## Appendix S3: Detailed methods and supplementary results for diversification analyses

### Diversification rates

#### S3.1 Time

##### S3.1.1 Detection of shifts in diversification process using MEDUSA

We investigated the dynamics of speciation and extinction rates through time and across the phylogeny using two different birth-death models: (1) Morlon et al.'s (2011) method allowing both speciation and extinction rates to vary as a function of time, and allowing extinction to be higher than speciation, (2) TreePar (Stadler 2011) which accommodates models where diversification rates can vary at points in time but are constant between these points. None of these methods however, can automatically detect the number and position of different diversification processes. Hence, we first ran MEDUSA (Alfaro et al. 2009) on the whole median tree in order to partition the tree into different diversification processes. MEDUSA automatically identifies the number and the position of different diversification processes, yet making the strong assumption that speciation and extinction rates are constant through time. MEDUSA detected two shifts from the background diversification rates (Table S3.3): 1) one shift at the root of a large clade hereafter referred to as the core-group, and 2) one shift at the root of a subclade of the genus *Melinaea* that, for simplicity, is referred to as the *Melinaea*-group. The remaining lineages are collectively referred to as the backbone.

Table S3.3 Results of MEDUSA fitted on the whole tree.  $r$  represents the diversification rates inferred by MEDUSA (not considered in our study).

Steps	position	logL	AICc	Model	r
Step1	root	-863.89	1731.81	bd	0.114
Step2	Core-group	-854.99	1720.08	bd	0.236
Step3	Melinaea-group	-846.45	1709.12	bd	1.245

##### S3.1.2 Fit of Morlon et al. (2011)'s diversification models

Morlon et al. (2011)'s models were fitted on the core-group, the *Melinaea*-group and the backbone. For each partition, the models were fitted on 100 trees randomly sampled from BEAST's posterior distribution. In each case the stem branch of the shifting clade was included in the subclade, as designed in the method (Morlon et al. 2011), but we excluded the stem branch (root) of the backbone. For each partition we fitted the following models: constant

speciation (no extinction), time-dependent speciation (no extinction), constant speciation and extinction, time-dependent speciation and constant extinction, constant speciation and time-dependent extinction, time-dependent speciation and extinction. In the cases of time-dependent rates we fitted an exponential dependence to time. Sampling fraction was specified for each of the three partitions. All models were compared using AIC scores. The model with the lowest AIC score that was significantly different from the null model of constant speciation rate was used to plot the diversification rate through time. Detailed results are shown in Table S3.4.

Table S3.4 Full results of time-dependent diversification models using Morlon et al (2011)'s method fitted on 100 posterior trees partitioned into the backbone, the coregroup and the *Melinaea*-group, and ranked by increasing mean AIC score. BCST=constant speciation, BVAR=time-dependent speciation, DCST=constant extinction, DVAR time-dependent extinction.  $\lambda$  =speciation rate at present,  $\alpha$ =coefficient of time variation of the speciation rate,  $\mu$  =extinction rate at present,  $\beta$  =coefficient of time variation of the extinction rate. Models used and discussed in the manuscript are highlighted in bold.

a. Backbone excluding the core-group and the *Melinaea*-group. Mean values from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
<b>BVARDVAR</b>	<b>4</b>	<b>-129.75</b>	<b>267.50</b>	<b>0.153</b>	<b>0.160</b>	<b>0.193</b>	<b>0.148</b>
BVARDCST	3	-131.84	269.68	0.201	0.041	0.264	NA
BCST	1	-134.20	270.40	0.121	NA	NA	NA
BCSTDCT	2	-133.60	271.21	0.162	NA	0.072	NA
BCSTDVAR	3	-132.98	271.95	0.212	NA	0.245	-0.072
BVAR	2	-134.18	272.36	0.124	-0.002	NA	NA

b. Backbone excluding the core-group and the *Melinaea*-group. Standard deviations from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
<b>BVARDVAR</b>	<b>4</b>	<b>0.30</b>	<b>0.61</b>	<b>0.0017</b>	<b>0.0020</b>	<b>0.0023</b>	<b>0.0019</b>
BVARDCST	3	0.30	0.59	0.0020	0.0005	0.0037	NA
BCST	1	0.29	0.58	0.0008	NA	NA	NA
BCSTDCT	2	0.30	0.59	0.0015	NA	0.0016	NA
BCSTDVAR	3	0.30	0.59	0.0024	NA	0.0054	0.0012
BVAR	2	0.29	0.58	0.0010	0.0005	NA	NA

c. Core-group. Mean values from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
BVAR	2	-700.38	1404.77	0.229	0.030	NA	NA
<b>BCST</b>	<b>1</b>	<b>-702.25</b>	<b>1406.50</b>	<b>0.257</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>
BVARDCST	3	-700.38	1406.77	0.229	0.030	1.37E-07	NA
BCSTDCT	2	-702.25	1408.50	0.257	NA	1.80E-07	NA

BVARDVAR	4	-700.38	1408.77	0.229	0.030	8.88E-08	0.036
BCSTDVAR	3	-702.25	1410.50	0.257	NA	8.74E-08	0.015

d. Core-group. Standard deviations from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
BVAR	2	1.91	3.83	0.0016	0.0006	NA	NA
<b>BCST</b>	<b>1</b>	<b>1.92</b>	<b>3.84</b>	<b>0.0017</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>
BVARDCST	3	1.91	3.83	0.0016	0.0006	1.38E-08	NA
BCSTDCAST	2	1.92	3.84	0.0017	NA	3.01E-08	NA
BVARDVAR	4	1.91	3.83	0.0016	0.0006	7.06E-09	0.0008
BCSTDVAR	3	1.92	3.84	0.0017	NA	1.13E-08	0.0007

e. *Melinaea*-group. Mean values from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
<b>BVAR</b>	<b>2</b>	<b>-1.58</b>	<b>7.17</b>	<b>0.311</b>	<b>3.772</b>	<b>NA</b>	<b>NA</b>
BVARDCST	3	-1.55	9.10	0.375	3.838	0.488	NA
BCST	1	-4.31	10.62	1.526	NA	NA	NA
BVARDVAR	4	-1.40	10.80	0.301	4.823	1.793	-3.804
BCSTDCAST	2	-4.31	12.62	1.527	NA	1.27E-07	NA
BCSTDVAR	3	-4.29	14.58	1.633	NA	12.613	-5.569

f. *Melinaea*-group. Standard deviations from 100 posterior trees.

Model	Parameters	logL	AIC	$\lambda$	$\alpha$	$\mu$	$\beta$
<b>BVAR</b>	<b>2</b>	<b>0.18</b>	<b>0.35</b>	<b>0.023</b>	<b>0.191</b>	<b>NA</b>	<b>NA</b>
BVARDCST	3	0.17	0.35	0.037	0.185	0.117	NA
BCST	1	0.10	0.19	0.024	NA	NA	NA
BVARDVAR	4	0.17	0.34	0.030	0.229	1.505	5.366
BCSTDCAST	2	0.10	0.19	0.024	NA	2.53E-08	NA
BCSTDVAR	3	0.10	0.19	0.056	NA	12.144	5.296

### S3.1.3 Fit of TreePar diversification models

We performed TreePar (Stadler 2011) analyses on the core-group and the backbone. For each case we used 100 trees randomly sampled from BEAST's posterior distribution. For the backbone we included the times of divergence at which the core-group and the *Melinaea*-group diverged from the backbone to keep track of these cladogenetic events. We split time into time bins of four million years, for which diversification rates was estimated. We used these estimates to obtain a second estimate of diversification rate “through time”. We did not fit TreePar on the *Melinaea*-group since it is only 1 million years old. We allowed diversification

rate to be negative but we did not allow mass-extinction events. Results are shown in the manuscript (Figure 2).

#### S3.1.4 Fit of BAMM diversification models

We compared our estimates of diversification dynamics obtained with MEDUSA, Morlon et al. 2011 and TreePar with results obtained from BAMM (Rabosky et al. 2014). We ran BAMM v.2.5.0 on the whole tree of Ithomiini. We did not specify a global sampling fraction. Instead, we used a refined sampling fraction at the genus level. We ran BAMM for 30 million generations sampling every 3000 generations. The results of the MCMC were then analyzed in the R package BAMMTOOLS (Rabosky et al. 2014). First, we checked for MCMC convergence using trace plots and ESS statistics using the R package CODA, after removing 10% of the chain as burnin. Second, we investigated the number and configurations of diversification rate shifts. We report here the set of credible shifts.

The results of BAMM are congruent with our other estimates, supporting a diversification rate shift at the root of the coregroup, pointing at an increasing rate of diversification compared to the background dynamics. The configuration with two shifts of diversification rate corresponding to the partitioning analyzed in the manuscript (i.e. rate shifts at the root of the coregroup and the *Melinaea*-group) was recovered as the third most frequently sampled configuration but with the lowest support (~10%) after a configuration with no rate shift (19%) (Figure S3.3).

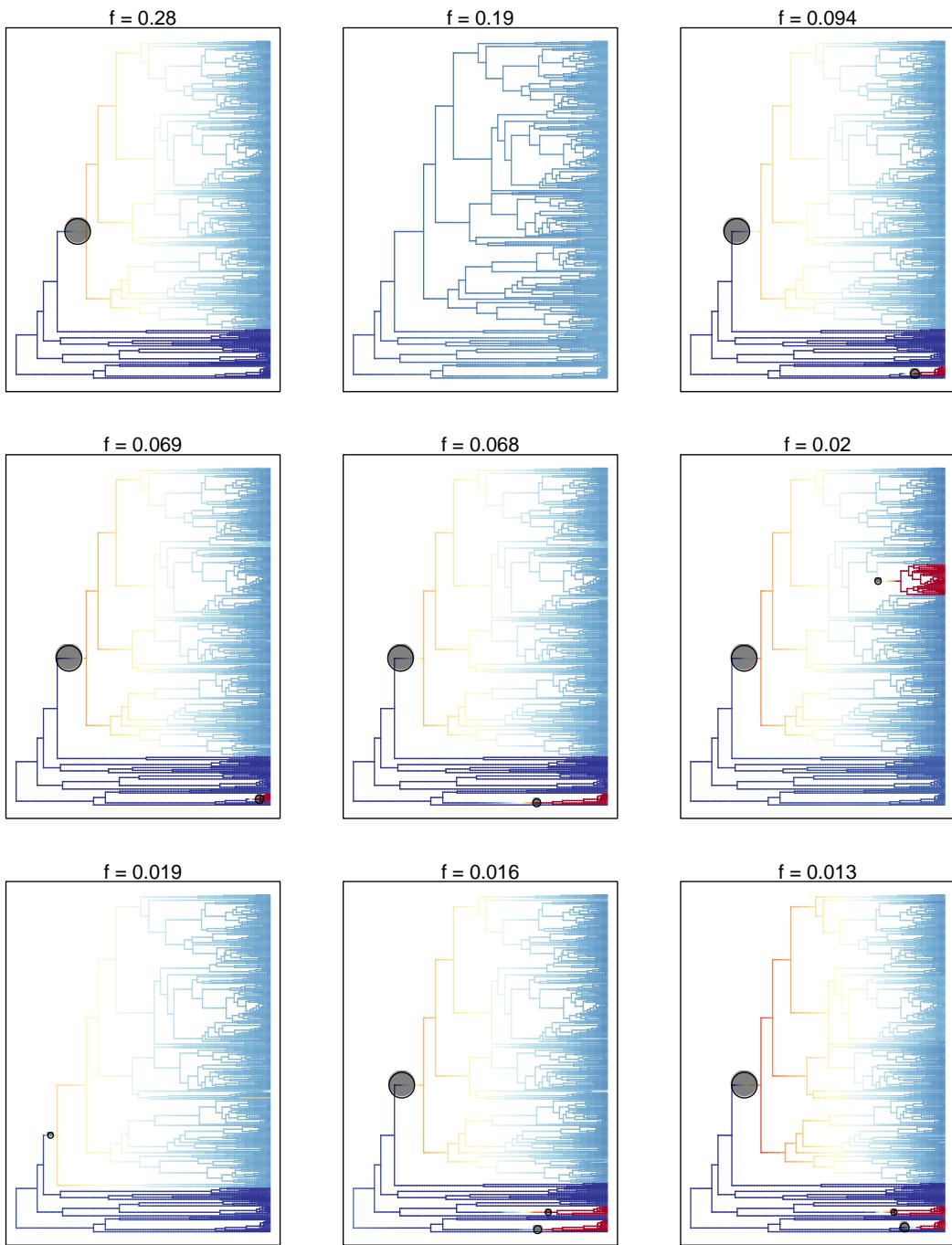


Figure S3.3 Set of credible shift configurations from the analysis performed with BAMM v.2.5.0. Branches are colored according to the diversification rate, the positions of shifts are also indicated and the frequency at which each configuration was recovered across the posterior distribution is indicated above each tree.

### S3.1.5 Testing the effect of diversification rate heterogeneity among backbone lineages on diversification rate estimates.

The best fitting time-dependent model of diversification from Morlon et al. (2011) inferred a negative net diversification rate, indicating a declining diversity. We performed further tests to assess the robustness of this inference to (1) the parameters chosen for the initiating the maximum likelihood search and (2) the heterogeneity of diversification rate remaining in the backbone as defined by MEDUSA (Alfaro et al. 2009).

For the time-dependent diversification models (Morlon et al. 2011), maximum likelihood searches consistently converged toward similar parameter estimates over a range of initial parameters (results not shown). The LTT plots of the backbone tree as defined by MEDUSA (i.e excluding the *Melinaea*-group) shows a recent burst of diversification during the last 5 million years. This could be due to a global recent increase of diversification rate but also to specific subclades driven by different diversification processes from that of the remaining backbone. This may influence the selection of the best model and parameter estimations. Thus, we fitted additional models of time-dependent speciation and extinction rates on different backbones for which we removed additional subclades. Time-dependent models were also fitted on these subclades.

First, we sequentially removed the *Melinaea*-group (MEDUSA partition, results discussed in the main document), the genus *Mechanitis*, the genus *Methona* and the genus *Scada* (Figure S3.4 A). We fitted time-dependent diversification models for each of these subclades. Removing these clades reduced the final burst of diversification observed on the LTT plots but the diversification rates inferred for the backbone were only slightly affected (Figure S3.4 A & B). It slightly pushed the extinction signal backward in time and tended to infer an earlier recovery, although the differences are small. Hence, the results described in the paper are not affected by potential rate heterogeneity remaining in the backbone.

Although the timing of the divergence between shifting subclades and the backbone was recorded in the analyses performed on the backbone, the stem branches of those subclades were excluded from the backbone (this is the way the method was designed). Yet, some of those stem branches are long, especially for the *Methona* and *Scada*, and likely bear a signal of association that may have better fitted with the backbone.

Time-dependent diversification models were also fitted on the *Melinaea*-group, and the genera *Mechanitis*, *Methona* and *Scada*, following the procedure described the Method section except that we excluded the stem branches of these subclades. As explained above, some stem branches are very long and should likely be under the background process (but this cannot be

done). Results show that these four subclades all have positive net diversification rate (constant speciation rate model was always the best fitting model, Figure S3.4 C). This strengthens the observation that the lineages in the backbone suffered from high extinction, which was at its maximum around 15 my ago, but have since then started recovering, with a diversification rate generally increasing and several subclades showing positive diversification during the last 5 my.

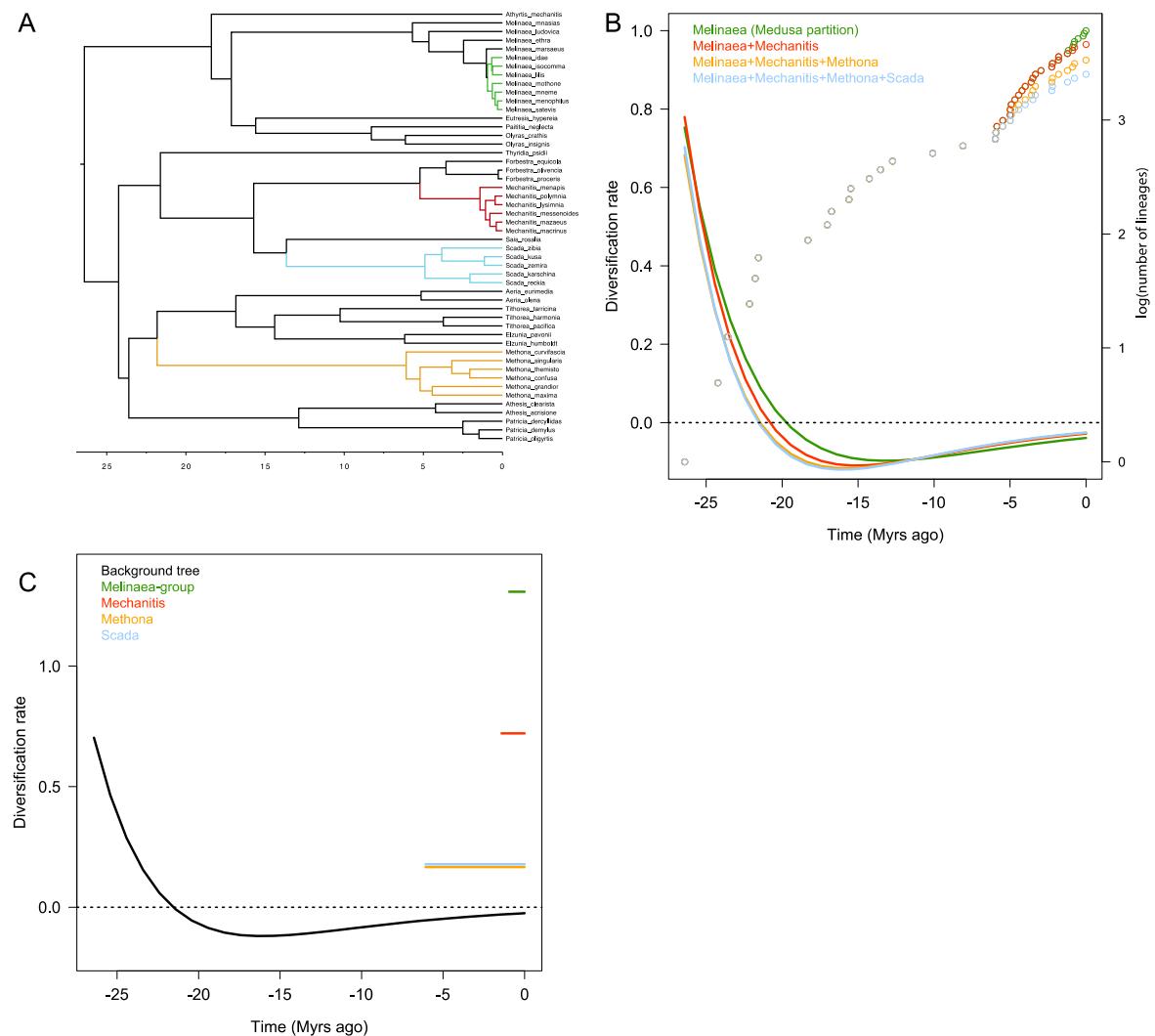


Figure S3.4 The effect of diversification rate heterogeneity among backbone lineages on diversification rate estimates using Morlon et al. (2011) on the median tree. A. the backbone, after the core-group has been removed. Colored subclades indicate the subclades that were sequentially removed from this backbone. B. Diversification rates of the different backbones estimated with Morlon et al. (2011)'s method (solid lines) and corresponding LTT plots (dotted lines). Names in the upper left hand corner indicate the subclades that were removed in each case. C. Diversification rate estimated with Morlon et al. (2011)'s method for the four subclades and the remaining backbone when these subclades were removed.

### S3.2 Diversification in the Andes

To investigate the pattern of diversification in the Andes with respect to the rest of the Neotropical region, we classified species as Andean or non-Andean, based on a combination of published (Chazot et al. 2016a, Chazot et al. 2016b, De-Silva et al. 2016, Chazot et al. 2014, De-Silva et al. 2010, Elias et al. 2009, Brower et al. 2006, Mallarino et al. 2005, Whinnett et al. 2005) and unpublished (i.e., databases generated from museum collections and our own field collections) georeferenced distribution data and elevation ranges of species (Appendix S1.2). In general species can be unambiguously classified into either of the two categories, because Andean species are never found in the lowlands, whereas species that occur in the lowlands and that sometimes also occur at the Andean foothills never occur above ca. 800m, and are therefore classified as non-Andean. We used trait-dependent models of diversification (Chazot et al. 2016a, Beckman & Witt 2015) to compare the rates of speciation, of extinction and of transition between the Andean area and non-Andean regions. We used the model ClaSSE (Goldberg & Igić 2012), which accounts for up to 10 parameters (2 speciation rates without character state change, 4 cladogenetic transition rates, 2 extinction rates and 2 anagenetic transition rates). However, we constrained parameters that were not biogeographically meaningful to zero (Chazot et al. 2016a). Those include the anagenetic transition rates, considering that transition rates from one region to the other were always accompanied by a speciation event, and the cladogenetic transition rates involving a transition in both descendant lineages, as we considered the scenario unrealistic. We therefore ended up with at most 6 parameters. We tested all models with only one parameter (speciation, extinction, transitions) free to vary, as well as all models combining two or more parameters free to vary. All models were fitted on the 100 trees randomly sampled from BEAST's posterior distribution. Models were compared using mean AIC scores (Table S3.5).

Furthermore, we considered two important sources of potential biases. (1) The major shift of diversification at the root of the core-group could affect our results of trait-dependent diversification models. We therefore repeated the analysis above on the core-group alone. (2) A hidden character may explain the pattern of trait-dependent diversification estimated with ClaSSE. We therefore performed additional analyses with the model HiSSE (Beaulieu & O'Meara 2016) (Box S3.1). All results are presented in Table S3.5.

**Box S3.1. Trait-dependent diversification using ClaSSE, BiSSE and HiSSE.**

Two papers recently pointed at a number of concerns about the character-state dependent diversification models such as ClaSSE or BiSSE, which we used for testing differences of diversification rates between Andean and non-Andean lineages (Maddison & FitzJohn 2014, Rabosky & Goldberg 2015). The main criticisms are: (1) a high rate of false positive, (2) “pseudoreplication”, in particular when diversification rates are not homogeneous across a tree (presence of diversification rate shift) and (3) the existence of “hidden” characters, not measured but influencing the results of character-state dependent models.

We “accounted” for the most important shift of diversification rate in our analysis by testing ClaSSE models both on the whole tree (which included heterogeneous diversification dynamics) and on the core-group only, which appeared as a relatively homogeneous process. While no consensual model emerged from the analyses performed on the whole tree, analyses performed on the core group, which accounts for more than 75% of the current diversity, supports the hypothesis that most of Ithomiini diversification occurred equally in both Andean and non-Andean regions. The high Andean species richness in the core-group results from an early occupation of the Andes and a late colonization of the other regions.

We complemented our ClaSSE analyses and BiSSE ancestral state estimations by testing for the presence of a hidden character that could bias our results, although this is mostly relevant for ruling out misinterpretations of strong signals, which is not our case here. We applied the recently proposed HiSSE model (Beaulieu & O’Meara 2016), which aims at fitting a four-state model, in which each observed character state (in our case Andean *versus* non-Andean) is associated with a hidden state of a character not measured. Because of the large number of parameters associated with this model and the absence of relevance of many potential nested models, we only fitted the most relevant models.

We fitted four models equivalent to BiSSE and seven HiSSE models (Table S3.5). All these models were ranked according to their AIC score and compared to identify the best fitting model. For the whole tree, the HiSSE model was always better than BiSSE. According to AIC scores, the best model corresponded to the “full” one, i.e. allowing all parameters (speciation, extinction and transitions) to be free. According to AICc scores however, the model including equal transition rates, and only differences in speciation and extinction parameters between the observed states and the hidden states was equivalent to the full models, with less parameters. Therefore, there is a significant signal of a hidden character underlying our analyses. The results were similar when performed on the core-group only.

Using the best model with the lowest number of parameters, we performed an ancestral state reconstruction, inferring both the observed states, and the hidden states. The ancestral state reconstruction (Figure S3.5) reveals the presence of a hidden state associated with high diversification rates in both Andean and non-Andean lineages and the presence of a hidden character state with low diversification rates in some poorly diversified Andean lineages. We discuss in the paper the results of previous studies (Chazot et al. 2016a,b) supporting both Andean and non-Andean radiations (and simultaneous increase of diversification rates) within the subtribes Godyridina and Oleriina. The results from the HiSSE model confirm that there is no global association, across all Ithomiini, between the Andes and diversification rate. Instead, we suggest that radiations occurred more specifically in the Northern-Andes and Amazonia following the demise of the Pebas system.

Table S3.5. Results of ClaSSE, BiSSE and HiSSE models fitted on the whole tree and the core-group. A. Full results of the models ClaSSE fitted on 100 posterior trees. B. Comparisons of the models BiSSE and HiSSE fitted on the median tree. Constraints of each model are indicated in the four first columns. 1=non-Andean, 2=Andean,  $\lambda_{111}$  and  $\lambda_{222}$  are within-region speciation rates (non-Andean and Andean, respectively),  $\lambda_{112}$  and  $\lambda_{212}$  are cladogenetic transition rates (non-Andean towards Andean, and Andean towards non-Andean, respectively),  $\mu$  = extinction rates, df =number of parameters, logL=log-likelihood, AIC= Akaike information criterion score.

A. a- Whole tree. Mean values from 100 posterior trees.

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	-1064.82	2137.63	0.154	0.186	0.052	0.052	0.003	0.003
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	-1065.89	2137.79	0.171	0.171	0.052	0.052	0.004	0.004
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	5	-1064.66	2139.31	0.151	0.188	0.046	0.058	0.002	0.002
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	4	-1065.70	2139.39	0.174	0.174	0.052	0.052	0.020	0.001
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	5	-1064.73	2139.46	0.153	0.192	0.052	0.052	0.001	0.015
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	4	-1065.88	2139.76	0.172	0.172	0.053	0.051	0.004	0.004
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-1065.65	2141.29	0.175	0.175	0.049	0.056	0.024	7E-05
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	-1064.65	2141.29	0.151	0.190	0.047	0.057	0.001	0.006
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	-1102.44	2208.88	0.111	0.111	0.111	0.111	6E-06	6E-06
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	3	-1102.42	2210.84	0.151	0.151	0.151	0.151	0.001	0.006

A. b- Whole tree. Standard deviations from 100 posterior trees.

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	23.20	46.40	0.011	0.004	0.013	0.005	0.005	
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	23.17	46.35	0.012	0.004	0.012	0.006	0.006	
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	5	23.21	46.43	0.010	0.003	0.005	0.013	0.004	
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	4	23.17	46.34	0.012	0.012	0.004	0.004	0.005	0.002
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	5	23.21	46.41	0.010	0.004	0.015	0.002	0.009	
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	4	23.17	46.34	0.012	0.004	0.012	0.006	0.006	
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	23.18	46.35	0.012	0.004	0.012	0.007	0.000	
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	23.21	46.43	0.010	0.004	0.005	0.014	0.002	0.008
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	23.17	46.34	0.008	0.008	0.008	0.008	2.15E-05	2.15E-05
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	3	23.17	46.34	0.010	0.010	0.010	0.002	0.008	

A. c- Core-group. Mean values from 100 posterior trees.

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\Delta AIC$	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	-877.96	1757.74	0.00	0.190	0.190	0.059	0.059	4.26E-07	4.26E-07
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	-877.00	1757.80	0.08	0.168	0.205	0.060	0.060	8.15E-07	8.15E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	4	-877.62	1759.07	1.33	0.189	0.189	0.082	0.038	4.13E-07	4.13E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	4	-877.96	1759.74	2.00	0.190	0.190	0.059	0.059	8.11E-06	6.97E-06
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	5	-876.99	1759.78	2.07	0.168	0.207	0.060	0.060	1.13E-06	0.003
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1=\mu_2$	5	-874.92	1759.83	2.09	0.171	0.204	0.068	0.051	1.84E-07	1.84E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-875.52	1761.02	3.26	0.190	0.190	0.084	0.035	0.003	8.37E-07
$\lambda_{111} \neq \lambda_{222}$	$\neq \lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	-874.88	1761.75	4.01	0.170	0.205	0.064	0.054	2.65E-06	0.001
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	-905.69	1815.37	57.63	0.124	0.124	0.124	0.124	1.97E-06	1.97E-06
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1 \neq \mu_2$	3	-905.51	1817.02	59.27	0.170	0.170	0.170	0.170	2.65E-06	0.001

A. d- Core-group. Standard deviations from 100 posterior trees.

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	$\mu$	df	logL	AIC	$\lambda_{111}$	$\lambda_{222}$	$\lambda_{112}$	$\lambda_{212}$	$\mu_1$	$\mu_2$
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	25.67	51.33	0.016	0.016	0.005	0.005	1.21E-06	1.21E-06
$\lambda_{111}\neq\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	25.67	51.34	0.015	0.018	0.005	0.005	2.16E-06	2.16E-06
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}\neq\lambda_{212}$	$\mu_1=\mu_2$	4	25.62	51.23	0.016	0.016	0.011	0.010	1.06E-06	1.06E-06
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	4	25.67	51.33	0.016	0.005	0.016	0.005	1.27E-05	1.79E-05
$\lambda_{111}\neq\lambda_{222}$	$\neq \lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	5	25.68	51.35	0.014	0.019	0.005	0.005	3.70E-06	0.005
$\lambda_{111}\neq\lambda_{222}$	$\neq \lambda_{112}\neq\lambda_{212}$	$\mu_1=\mu_2$	5	25.68	51.36	0.015	0.018	0.015	0.015	3.09E-07	3.09E-07
$\lambda_{111}=\lambda_{222}$	$\neq \lambda_{112}\neq\lambda_{212}$	$\mu_1\neq\mu_2$	5	25.60	51.20	0.017	0.017	0.008	0.006	0.004	2.48E-06
$\lambda_{111}\neq\lambda_{222}$	$\neq \lambda_{112}\neq\lambda_{212}$	$\mu_1\neq\mu_2$	6	25.70	51.40	0.014	0.019	0.014	0.015	7.04E-06	0.002
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	25.66	51.31	0.011	0.011	0.011	0.011	4.14E-06	4.14E-06
$\lambda_{111}=\lambda_{222}$	$= \lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	3	25.67	51.34	0.014	0.014	0.014	0.014	7.04E-06	0.002

### B. a- Whole tree

Model	- logL	AIC
Bisse: null	-1071.379	2148.759
Bisse: $\lambda$ and $\mu$ free	-1069.905	2149.811
Bisse: transitions free	-1071.405	2150.810
Bisse: allfree	-1070.148	2152.295
Hisse: all free	-1043.805	<b>2119.609</b>
Hisse: all $\lambda$ and $\mu$ free + transition fixed	-1054.777	2127.555
Hisse: observed $\lambda$ and $\mu \neq$ hidden $\lambda$ and $\mu$ + transition fixed	-1055.503	2121.007
Hisse: hidden $\lambda$ and $\mu$ free + observed $\lambda$ and $\mu$ fixed + transitions fixed	-1055.465	2124.930
Hisse: observed $\lambda$ and $\mu$ free + hidden $\lambda$ and $\mu$ fixed + transitions fixed	-1055.415	2124.829
Hisse: $\lambda$ and $\mu$ Andes (hidden/observed) free + $\lambda$ and $\mu$ non Andes fixed + transitions fixed	-1060.017	2134.033
Hisse: observed $\lambda$ and $\mu \neq$ hidden $\lambda$ and $\mu$ + transitions free	-1065.549	2155.098

### B. b- Core-group

Model	- logL	AIC
Bisse: null	-879.110	1764.220
Bisse: $\lambda$ and $\mu$ free	-876.968	1763.935

Bisse: transitions free	-877.256	1762.512
Bisse: allfree	-876.280	1764.560
Hisse: all free	-862.671	2119.609
Hisse: all $\lambda$ and $\mu$ free + transition fixed	-869.109	1756.218
Hisse: observed $\lambda$ and $\mu \neq$ hidden $\lambda$ and $\mu$ + transition fixed	-870.304	1750.608
Hisse: hidden $\lambda$ and $\mu$ free + observed $\lambda$ and $\mu$ fixed + transitions fixed	870.326	<b>1754.651</b>
Hisse: observed $\lambda$ and $\mu$ free + hidden $\lambda$ and $\mu$ fixed + transitions fixed	-870.284	<b>1754.568</b>
Hisse: $\lambda$ and $\mu$ Andes (hidden/observed) free + $\lambda$ and $\mu$ non Andes fixed + transitions fixed	-872.174	1758.349
Hisse: observed $\lambda$ and $\mu \neq$ hidden $\lambda$ and $\mu$ + transitions free	-877.021	1778.042

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Finally, we also conducted ancestral state estimation based on the models of trait-dependent diversification. Since ancestral state estimation is not available for ClaSSE models we used the model BiSSE (FitzJohn *et al.* 2009) for this purpose. We fitted the model BiSSE corresponding to the best fitting ClaSSE model, and we used these parameters to infer the ancestral states at the nodes of the phylogeny (Figure S3.6). This ancestral state estimation was compared to that obtained using the historical biogeography analyses outlined below. All these analyses were performed on both the whole phylogeny and the core-group only, to account for the diversification rate shift identified by our time-dependent diversification analyses. Ancestral state reconstruction was also performed with the HiSSE model under the best model (Figure S3.6).

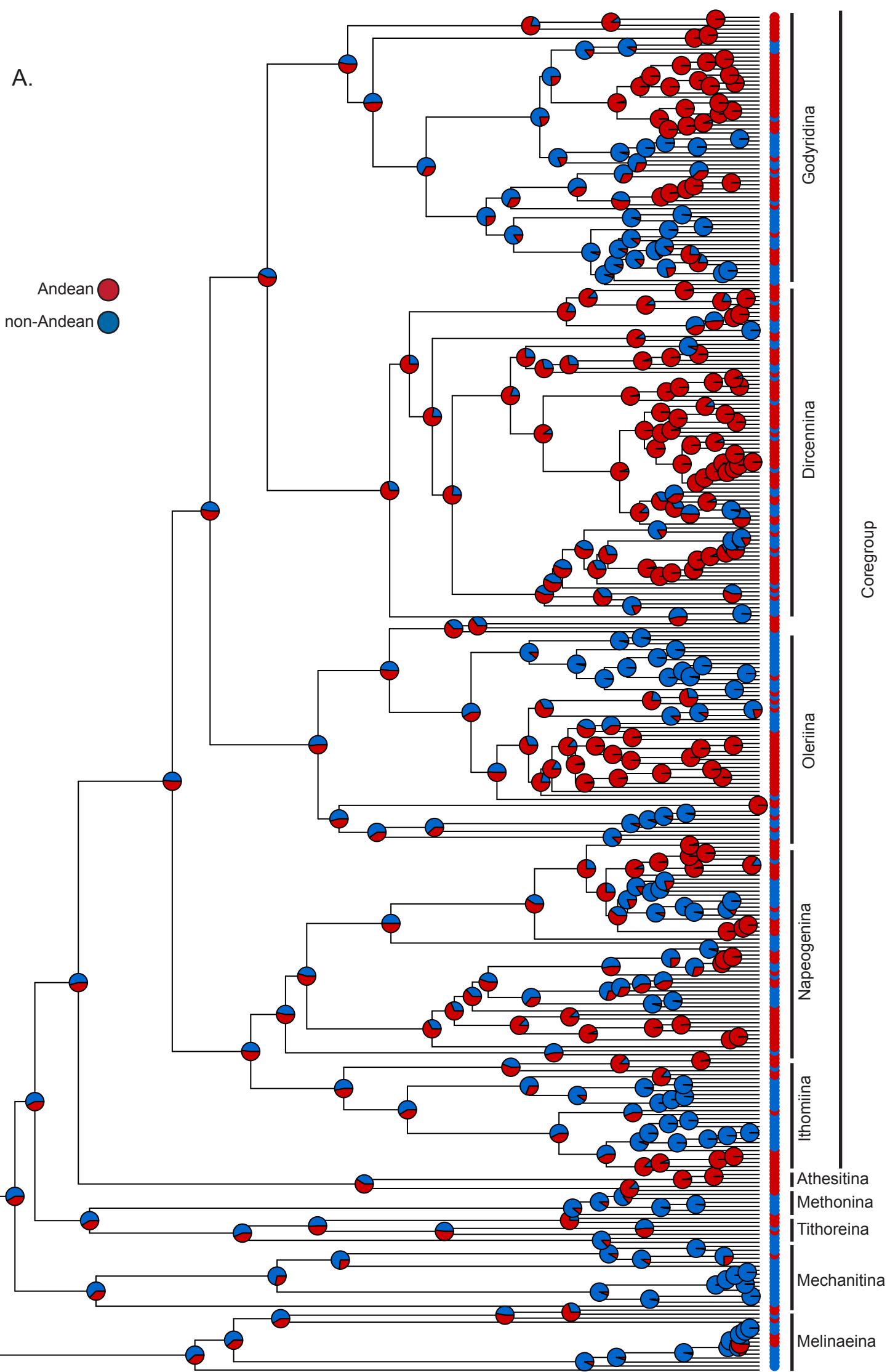
Figure S3.5. A. (Next page). Ancestral state estimation performed on the whole tree, using the best HiSSE model. White branches indicate non-Andean lineages, black branches indicate Andean lineages, blue and red branches correspond to the estimations of the hidden character states.

Athyris mechanitis  
Eutresis hyperia  
Paitita neglecta  
Olyras insignis  
Olyras crathis  
Melinaea aglaia  
Melinaea ludovicia  
Melinaea ethra  
Melinaea marsaeus  
Melinaea idaea  
Melinaea illis  
Melinaea isocomma  
Melinaea ethone  
Melinaea mneme  
Melinaea satevis  
Melinaea menophilus  
Thyridia psidii  
Sais rosalia  
Scada agatha  
Scada karschina  
Scada zibia  
Scada zemira  
Scada kusa  
Forbesia equicola  
Forbesia taurica  
Forbesia olivencia  
Mechanitis menapis  
Mechanitis lysimnia  
Mechanitis polytmia  
Mechanitis messenoides  
Mechanitis machaon  
Mechanitis mormon  
Methona curvifascia  
Methona maxima  
Methona grandior  
Methona singularis  
Methona fulfusa  
Methona himmisto  
Aeria olena  
Aeria eurimedia  
Elizunia humboldti  
Elizunia pavonia  
Tithorea tariciana  
Tithorea taurica  
Tithorea harmonia  
Athesia acrisiose  
Athesia clearista  
Patricia deryllidas  
Patricia eugyrnis  
Patricia deetus  
Placienda eurytanassa  
Pagryis cymothoe  
Pagryis priscilla  
Pagryis ulla  
Ithomia terra  
Ithomia tenebris EAST  
Ithomia lucunda  
Ithomia arduinna  
Ithomia amarilla  
Ithomia agnoscia  
Ithomia drymo  
Ithomia cyanea  
Ithomia pseudodagala  
Ithomia xenos  
Ithomia adelinda  
Ithomia avella  
Ithomia hymetta  
Ithomia aglaja  
Ithomia ellara  
Ithomia eleonora  
Ithomia hyala  
Ithomia celemia  
Ithomia heraidica  
Ithomia lycia  
Ithomia patilla  
Ithomia diaisia  
Ithomia cleora  
Ithomia iphianassa  
Ithomia salapia  
Arenaria alpina  
Lycauges eupompe  
Hypothyris cantabrica  
Hypothyris moebiusi  
Hyalynis nsp2  
Hyalynis excelsa  
Hyalynis lycaste  
Hyalynis oustaleti  
Hyalynis iunoniensis  
Hyalynis anteia  
Hyalynis nsp1  
Hyalynis nsp2  
Hyalynis coeno  
Hyalynis mestra  
Hyalynis praxilla  
Hyalynis latilimbata  
Hyalynis schlingeri  
Hyalynis euonia  
Hyalynis dana  
Hyalynis thea  
Hyalynis semifulva  
Hypothrysis anastasia  
Hypothrysis mansuetus  
Hypothrysis mamercus  
Hypothrysis leucania  
Hypothrysis euclea  
Hypothrysis sp  
Napeogenes larilla  
Napeogenes flosina  
Napeogenes cranto  
Napeogenes amathina  
Napeogenes lycora  
Napeogenes nsp3  
Napeogenes benigna  
Napeogenes sodalis  
Napeogenes sulphureophila  
Napeogenes lycioides  
Napeogenes tolosa  
Napeogenes stella  
Napeogenes duessa  
Napeogenes sylphia  
Napeogenes inachia  
Napeogenes tracis  
Napeogenes nsp2  
Napeogenes apulia  
Napeogenes aethria  
Napeogenes larina  
Napeogenes nezia  
Napeogenes parva  
Napeogenes nsp1  
Napeogenes pharo  
Napeogenes glycera  
Megoleria susiana  
Megoleria orestra  
Megoleria nivosa  
Hyposcada dujardini  
Hyposcada zarapha  
Hyposcada anchiala  
Hyposcada schausi  
Hyposcada tenuis  
Hyposcada keha  
Olanṭaya aequineta  
Olanṭaya cañilla  
Olanṭaya olieroides  
Oleria sexmaculata  
Oleria tenuis  
Oleria similiigena  
Oleria tigilla  
Oleria assimilis  
Oleria flora  
Oleria antaxis  
Oleria enhana  
Oleria victorina  
Oleria didymaea  
Oleria onega  
Oleria ilerdina  
Oleria quintina  
Oleria tenebra  
Oleria thienmei  
Oleria aegea  
Oleria victorina  
Oleria estella  
Oleria nsp  
Oleria gunilla  
Oleria orea  
Oleria rubescens  
Oleria paula  
Oleria amalda  
Oleria deronda  
Oleria derondina  
Oleria cincta  
Oleria bereri  
Oleria victorina  
Oleria quadrata  
Oleria radina  
Oleria balzana  
Oleria cincta  
Oleria santeza  
Oleria fasciata  
Oleria athalina  
Oleria tremona  
Oleria padilla  
Oleria cincta  
Oleria bioculata  
Oleria affatia  
Oleria cyrene  
Velamysta pennina  
Velamysta nsp  
Velamysta velamystes  
Velamysta pupilla  
Veladryas pardalis  
Veladryas cytharista  
Veladryas electrea  
Genus1 nsp1  
Godysia nana  
Godysia nero  
Godysia kedema  
Godysia zavaleta  
Godysia panthale  
Godysia crinippa  
Godysia gaudronsoni  
Godysia lauta  
Godysia sappho  
Godysia dulilla  
Heterosais giulia  
Heterosais edessa  
Hypoleucis epiphile  
Hypoleucis lava  
Hypoleucis xenophis  
Hypoleucis alema  
Hypoleucis saretta  
Mclouelia cymo  
Hypoleucis ocellata  
Hypoleucis carolina  
Hypoleucis adasa  
Brevoileria seba  
Brevoileria coenina  
Brevoileria nsp1  
Bachacutia mantura  
Pachacutia nana  
Brevoileria arzalia  
Brevoileria aelia  
Brevoileria pilisthenes  
Genus2 andromica  
Pseudoscada erruca  
Pseudoscada florula  
Pseudoscada limna eastern  
Pseudoscada acilla  
Pseudoscada limna costarica  
Pseudoscada limna western  
Greta morgane  
Greta libethra  
Hypomenitis theudelinda  
Hypomenitis nspC  
Hypomenitis nana  
Hypomenitis alchesboea  
Hypomenitis nspA  
Hypomenitis enigma  
Hypomenitis hermania  
Hypomenitis esula  
Hypomenitis nana  
Hypomenitis nspD  
Hypomenitis depauperata  
Hypomenitis gardneri  
Hypomenitis fojana  
Hypomenitis ochreata  
Hypomenitis nana  
Hypomenitis lycia  
Hypomenitis dercetis  
Hypomenitis oneiodes  
Hypomenitis nspB  
Calithomia albertae  
Calithomia albertae  
Calithomia seneca  
Haleenia perasipa  
Haleenia paradox  
Haleenia buckleyi  
Haleenia pascua  
Haleenia galathea  
Haleenia galathea  
Haleenia deroma  
Haenischia deroma  
Haenischia sidonia  
Episcada hemixanthae  
Ceratina neso  
Episcada nsp1  
Ceratina tenuis  
Ceratina tutia  
Episcada doto  
Episcada vitrea  
Episcada philoclea  
Episcada hymenaea  
Episcada nana  
Episcada nsp2  
Episcada sulphurea  
Episcada apuleia  
Episcada ticiella  
Episcada mira  
Episcada parvula  
Episcada pulita  
Episcada salvina  
Episcada sylvo  
Episcada striposis  
Episcada clausina  
Pteronymia sphaerula  
Pteronymia obsoletus  
Pteronymia donella  
Pteronymia forsteri  
Pteronymia hara  
Pteronymia olinda  
Pteronymia gronica  
Pteronymia nana  
Pteronymia serrata  
Pteronymia alida  
Pteronymia nsp3  
Pteronymia teresita  
Pteronymia tonia  
Pteronymia hannah  
Pteronymia mania  
Pteronymia dispar  
Pteronymia euritea  
Pteronymia carlia  
Pteronymia melbellina  
Pteronymia nana  
Pteronymia primula  
Pteronymia vestilla  
Pteronymia parva  
Pteronymia alecta  
Pteronymia rufocincta  
Pteronymia albertae  
Pteronymia alissa  
Pteronymia sexpunctata  
Pteronymia gertschi  
Pteronymia andreas  
Pteronymia simplex  
Pteronymia ascpo  
Pteronymia tucuna  
Pteronymia latilla  
Pteronymia ticiella  
Pteronymia nana  
Pteronymia WEST  
Pteronymia alpina  
Pteronymia nsp2  
Pteronymia ozia  
Pteronymia onela  
Pteronymia vulvaro  
Pteronymia vela  
Pteronymia vela EAST  
Pteronymia tamina

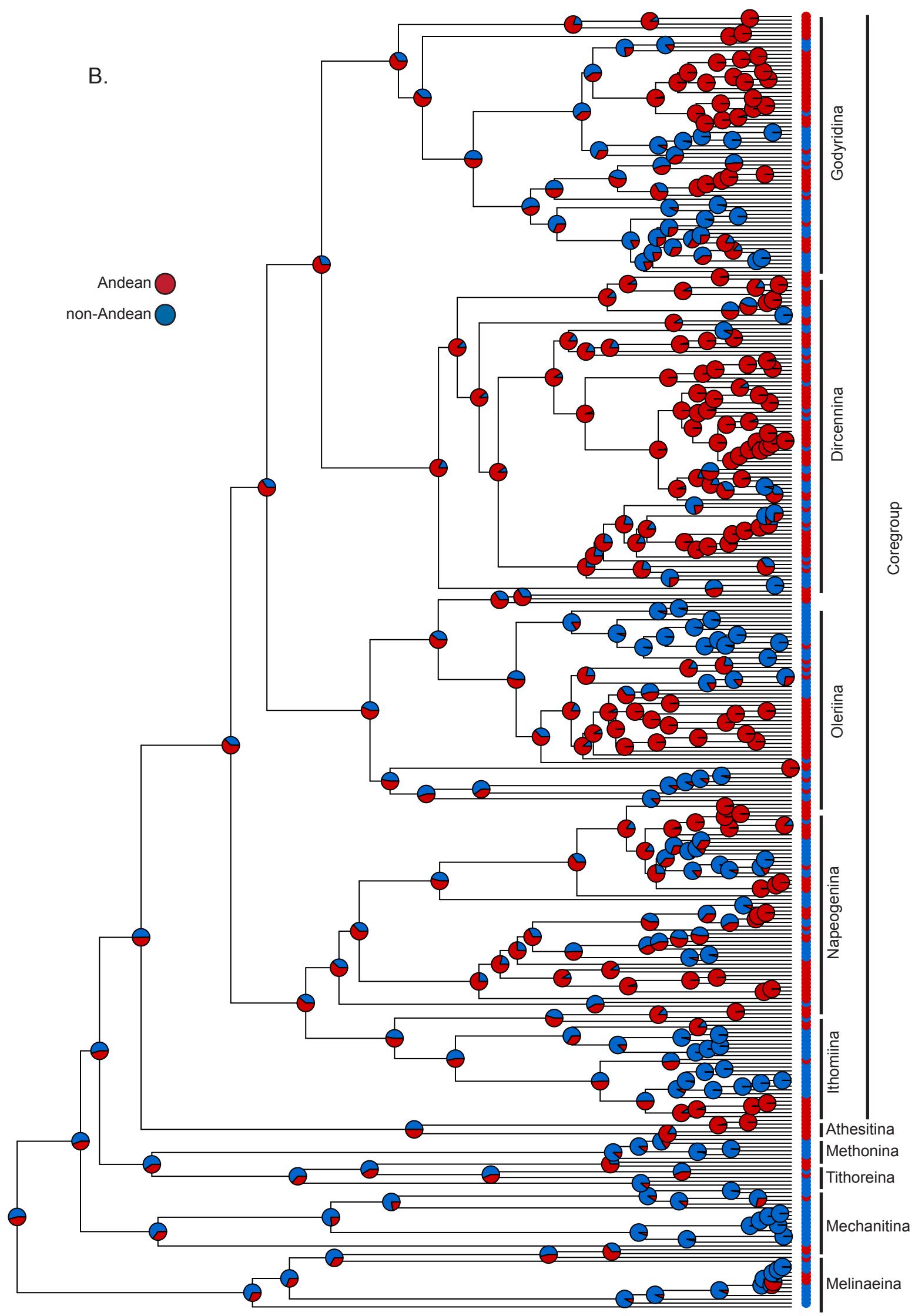


Figure S3.6. B. Ancestral state reconstruction performed using BiSSE diversification models:  
A. different speciation rates, B. different speciation and colonization rates. Red indicate Andean  
lineages, blue indicates non-Andean lineages. State probability at each node is represented with  
pie charts

A.



B.



### S3.3 Diversification in Amazonia

We further investigated the pattern of diversification in Amazonia during the post-Pebas period. The Amazon basin appears to be the second most important place for diversification after the Andes and there is a longstanding hypothesis that speciation in this region has been driven by climatic fluctuations during the Quaternary (Haffer 1969). An interpretation of this scenario is that speciation rate should increase during the last 2.5 million years (Matos-Maraví 2016). To test this hypothesis we identified seven major Amazonian diversification events, i.e. clades which nodes were inferred to be almost all Amazonian from the BioGeoBEARS ancestral state reconstruction. We fitted a model of time-dependent speciation rate (no extinction) to see whether speciation rates increased through time (supporting a recent diversification potentially caused by Pleistocene climatic fluctuations) or decreased through time (supporting radiations accompanying the post-Pebas recolonizations), using Morlon et al.'s (2011) method. Analyses were performed on 100 trees randomly sampled from BEAST's posterior distribution. Results are shown in the manuscript (Figure 3).

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## Appendix S4: Detailed methods and supplementary results for historical biogeography analyses

We proceeded in three steps to reconstruct the historical biogeography of Ithomiini. First, we performed an ancestral state reconstruction using a model with refined area adjacency but uniform dispersal multipliers (null-model). Second, we used the results of this model to compute rates of dispersal between specific regions per million years. This allowed us to test some biogeographic hypotheses but also to identify relevant time frames for which dispersal probabilities might vary. Third, we implemented a time-stratified model designed from the previous information to refine our biogeographic reconstruction. All these models were performed on the median node ages tree from BEAST.

### *S4.1 Biogeographic null model*

We inferred the historical biogeography of Ithomiini using BioGeoBEARS v.0.2.1 (Matzke 2014). We divided the Neotropics into nine distinct biogeographic regions: 1) Central America, 2) Caribbean Islands, 3) lowlands on the Western part of the Andes, including the Magdalena valley, 4) Northern Andes that comprise the Western and eastern Ecuadorian and Colombian cordilleras and the Venezuelan cordillera, 5) Central Andes, 6) Western Amazonia, 7) eastern Amazonia, 8) Guiana Shield and 9) Atlantic Forest (Figure S4.7). In this model, we constrained the combinations of areas to avoid unrealistic distributions (e.g., disjunct distributions) but all dispersal multipliers were set to 1 to avoid biasing the ancestral state reconstruction. We used the best fitting model (DEC) for the following analyses.

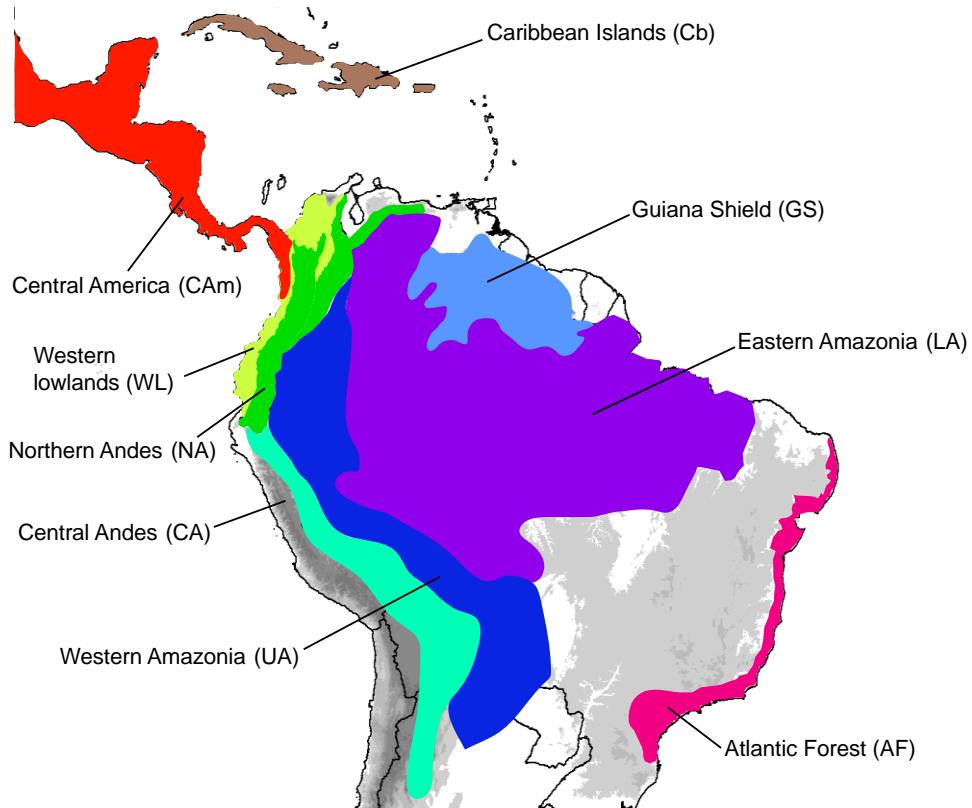


Figure S4.7. Biogeographic areas used in the BioGeoBears analyses

#### *S4.2 Biogeographic interchanges within the core-group*

One important hypothesis is that the Pebas influenced the interchanges among biogeographic regions, especially towards or across Western Amazonia. To test this hypothesis, we computed “rates” of colonization from BioGeoBEARS ancestral-state reconstructions performed with the null-model. We ran 1000 biogeographical stochastic mappings as implemented in BioGeoBEARS and extracted the times of dispersal events. Then, for each million-year interval, we computed the proportion of specific dispersal events compared to the maximum number of lineages existing during this interval, which we refer to as “rates” of colonization. We computed rates of dispersal between Andean and non-Andean regions, between the Andes and the Amazonia, between the Central and the Northern Andes, between the Atlantic Forest and the other regions, and between Central America and the rest of the Neotropics. These rates were computed only on the core-group, because (1) this group contains 85% of Ithomiini species, (2) the diversification process is homogeneous throughout the clade and (3) we found high extinction in the backbone, which may falsify the ancestral state reconstruction in these lineages, which were therefore excluded. In the paper we showed the median rates of interchanges between the Andes and Amazonia and between Central and Northern Andes (Figure 3). Below (Figure S4.8) we show the boxplots of rate values per million years for the interchanges between the Andes and Amazonia, Central and Northern Andes, Central America

and the other Neotropical regions, Atlantic Forest and the other Neotropical regions, not only for the total amount of interchanges but also the results when distinguishing one way from the other (*e.g.* from the Northern towards the Central Andes *versus* from the Central towards the Northern Andes).

Figure S4.8. Rates of colonization between different regions computed on the core-group and then used to design a time-stratified biogeographic model (next pages).

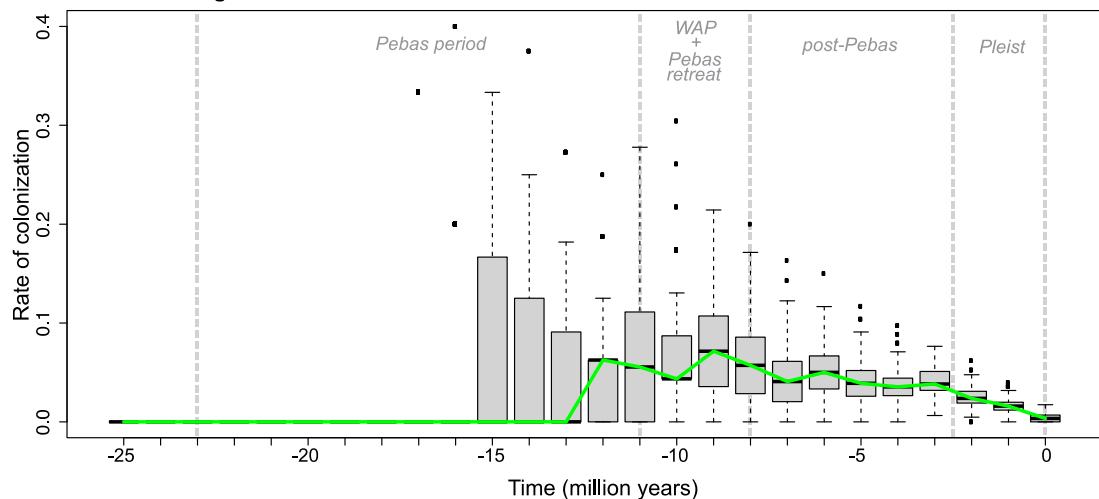
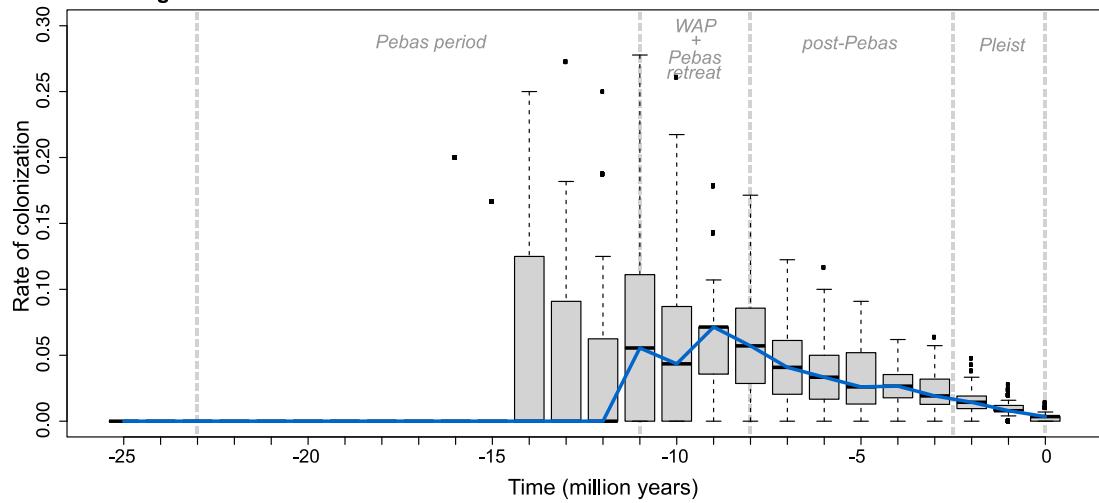
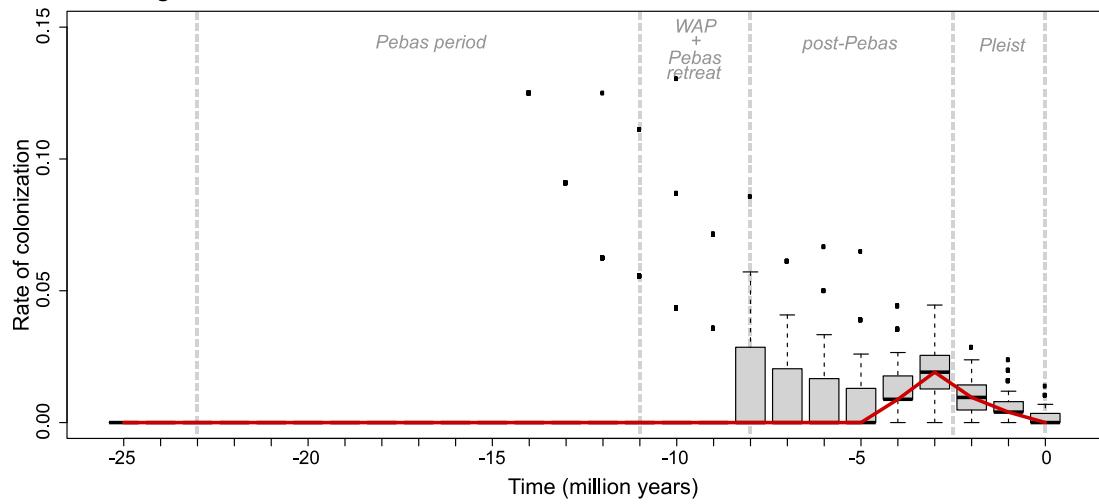
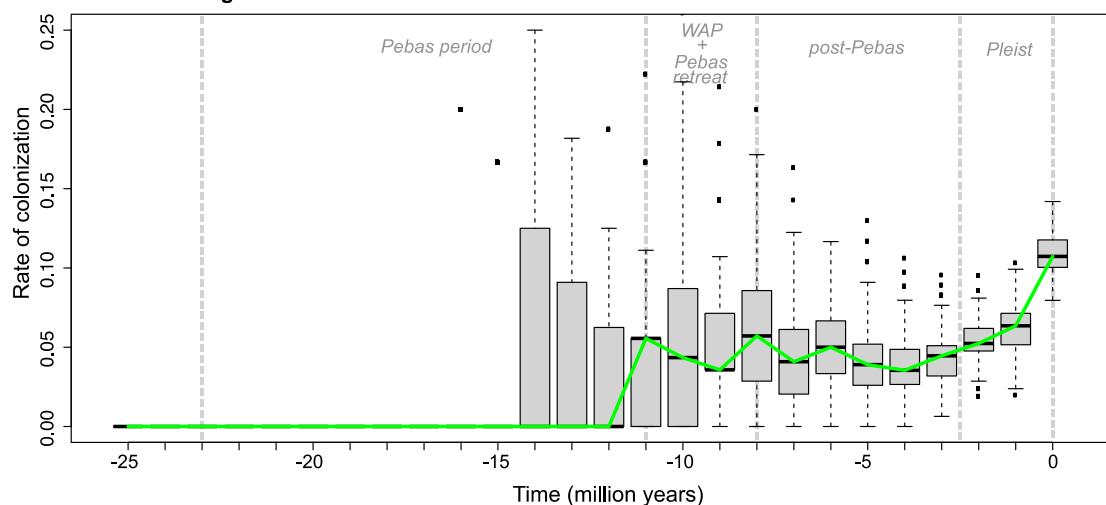
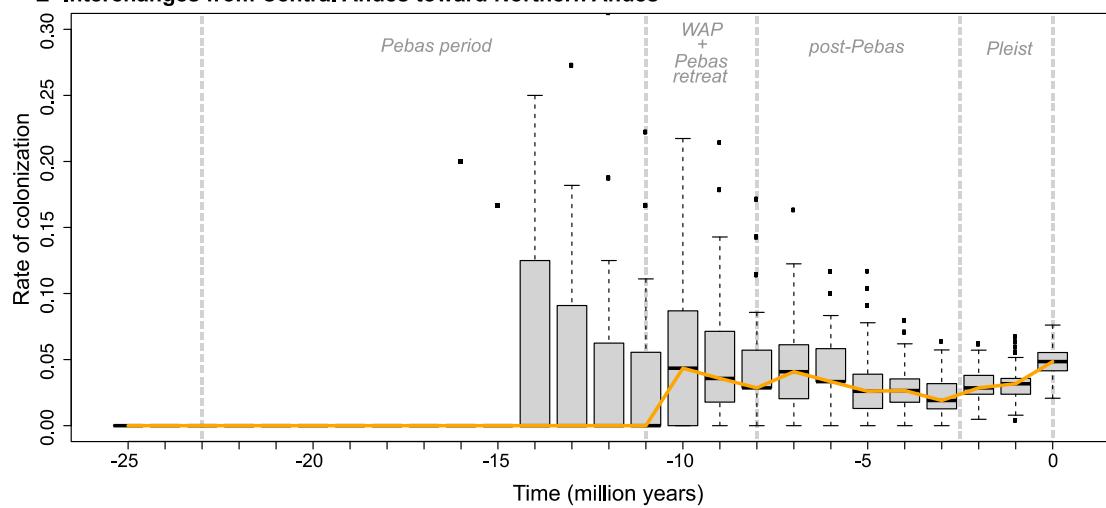
**A- Total interchanges between the Andes and Amazonia****B- Interchanges from the Andes toward Amazonia****C- Interchanges from Amazonia toward the Andes**

Figure S4.8 A, B & C. Rates of interchanges between all Andes and Amazonia. A: Total number of interchanges. B: Rates of colonization from the Andes toward Amazonia. C: Rates of colonization from Amazonia toward the Andes.

**D- Total interchanges between Central and Northern Andes**



**E- Interchanges from Central Andes toward Northern Andes**



**F- Interchanges from Northern Andes toward Central Andes**

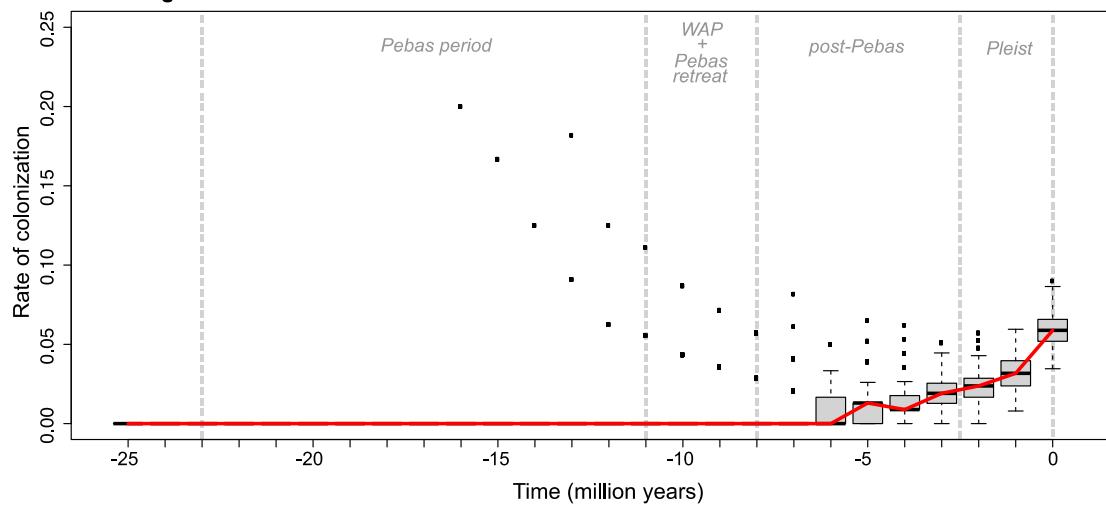
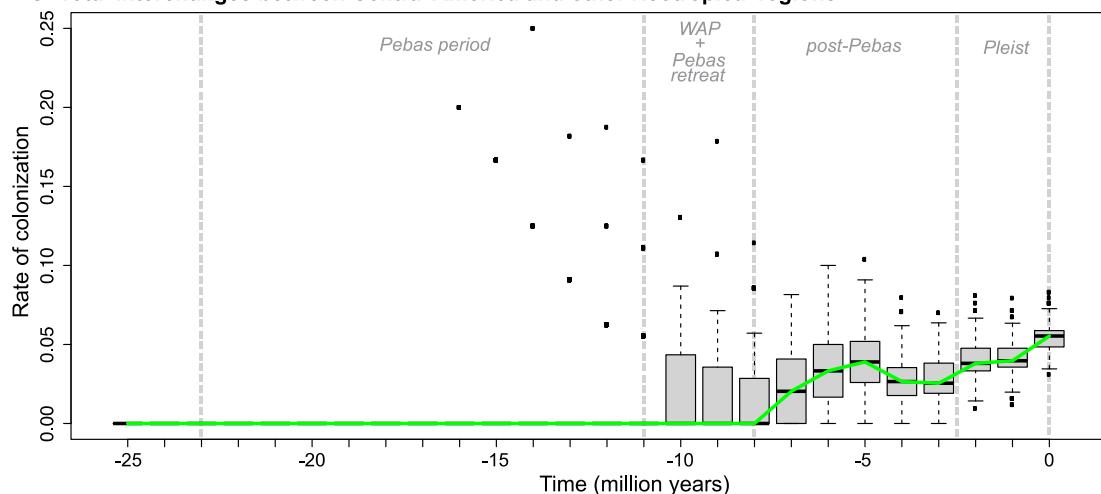
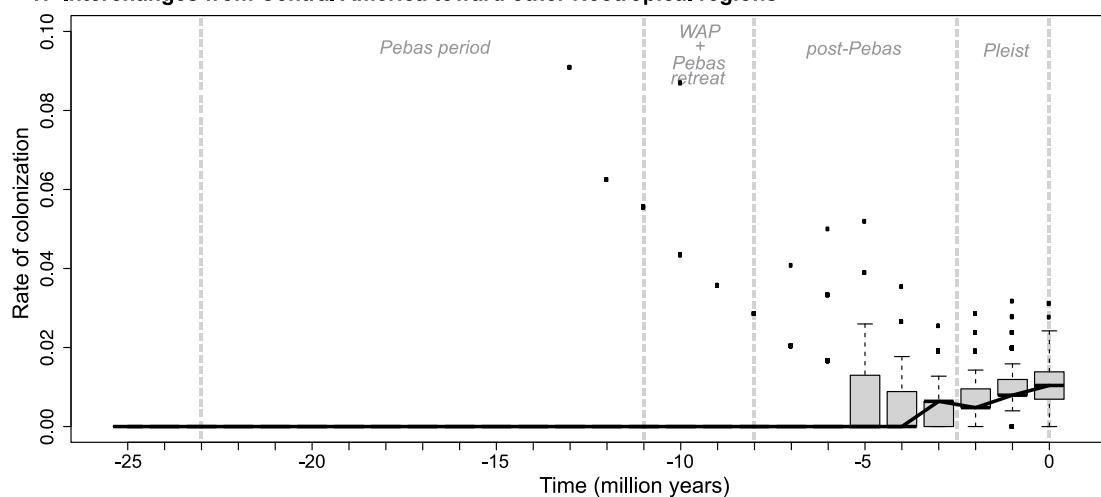


Figure S4.8 D, E & F. Rates of interchanges between Central and Northern Andes. A: Total number of interchanges. B: Rates of colonization from Central toward Northern Andes. C: Rates of colonization from Northern toward Central Andes.

**G- Total interchanges between Central America and other Neotropical regions**



**H- Interchanges from Central America toward other Neotropical regions**



**I- Interchanges from other Neotropical regions toward Central America**

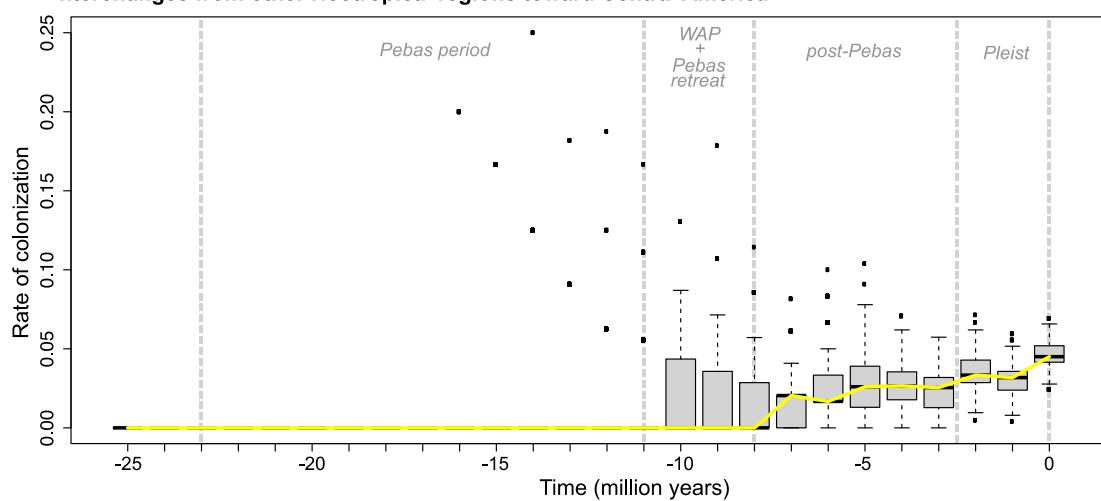
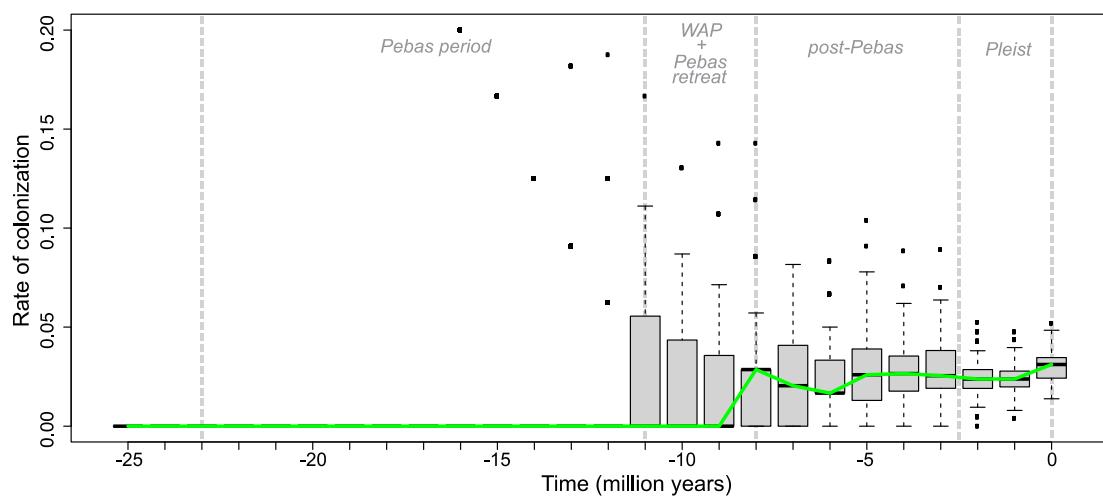
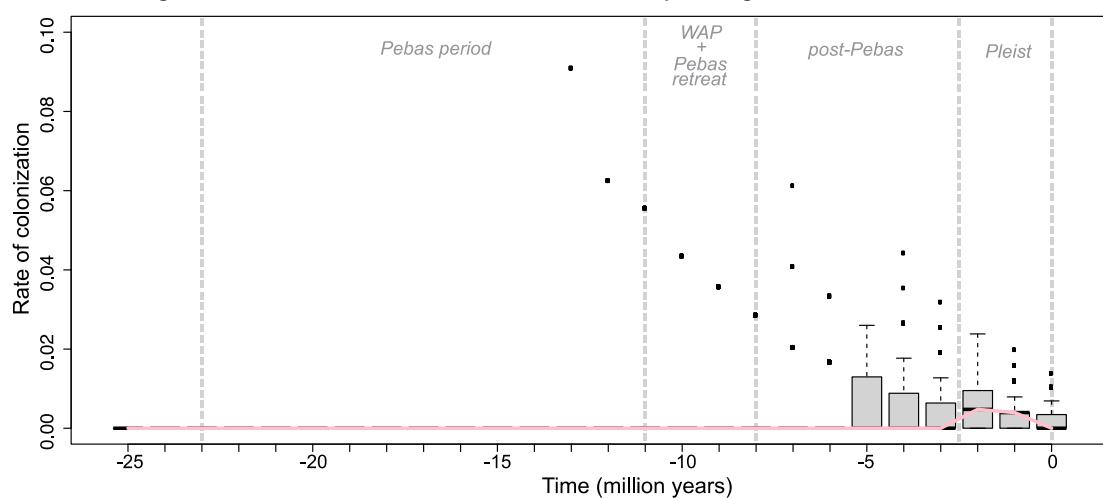


Figure S4.8 G, H & I. Rates of interchanges between Central America and the other Neotropical regions. A: Total number of interchanges. B: Rates of colonization from Central America toward the other regions. C: Rates of colonization from other regions toward Central America.

**J- Total interchanges between Atlantic Forest and other Neotropical regions**



**K- Interchanges from the Atlantic Forest toward other Neotropical regions**



**L- Interchanges from other Neotropical regions toward the Atlantic Forest**

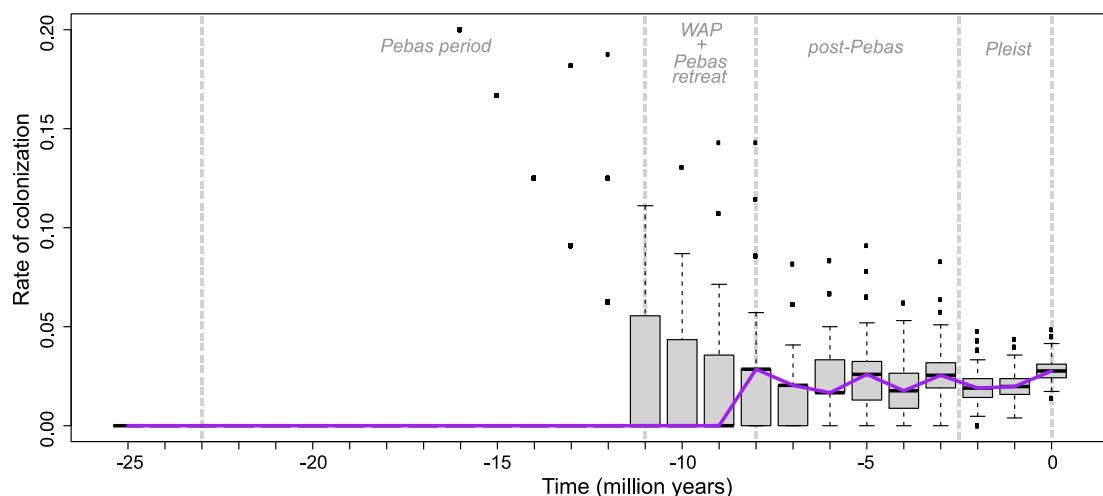


Figure S4.8 J, K & L. Rates of interchanges between Atlantic Forest and the other Neotropical regions. A: Total number of interchanges. B: Rates of colonization from Atlantic Forest toward the other regions. C: Rates of colonization from other regions toward Atlantic Forest.

We used these results of dispersal rates estimated with the null model to design a time-stratified model and improve the resolution of our biogeographic reconstruction. We interpreted the variations of rates of interchanges (including also the interchanges with the Atlantic forest) as indications for time-varying dispersal probabilities to design a time-stratified model (Table S4.6).

Table S4.6. A. Matrix of adjacency and B. stratified dispersal multipliers estimated from the transition rates among regions, and implemented in the time-stratified biogeographic analysis

**A. MATRIX OF ADJACENCY**

	Cam	Cb	WL	NA	CA	GS	UA	LA	AF
Cam	1	1	1	1	1	0	0	0	0
Cb	1	1	1	0	0	0	1	1	0
WL	1	1	1	1	1	0	1	1	0
NA	1	0	1	1	1	1	1	1	0
CA	1	0	1	1	1	0	1	1	1
GS	0	0	0	1	0	1	1	1	0
UA	0	1	1	1	1	1	1	1	1
LA	0	1	1	1	1	1	1	1	1
AF	0	0	0	0	1	0	1	1	1

**B. DISPERSAL MULTIPLIERS**

<b>0-4 mya</b>	Cam	Cb	WL	NA	CA	GS	UA	LA	AF
Cam	1.00	0.40	0.30	0.30	0.18	0.18	0.18	0.18	0.00
Cb	0.40	1.00	0.10	0.06	0.00	0.06	0.01	0.10	0.00
WL	0.60	0.10	1.00	0.60	0.60	0.18	0.30	0.30	0.00
NA	0.60	0.06	0.60	1.00	0.60	0.6	0.60	0.60	0.00
CA	0.36	0.00	0.60	0.60	1.00	0.36	0.60	0.60	0.36
GS	0.36	0.06	0.18	0.6	0.36	1	0.6	0.6	0.36
UA	0.18	0.01	0.30	0.60	0.60	0.6	1.00	0.60	0.36

LA	0.18	0.10	0.30	0.60	0.60	0.6	0.60	1.00	0.60
AF	0.00	0.00	0.00	0.00	0.36	0.36	0.36	0.60	1.00

<b>4-8 mya</b>	Cam	Cb	WL	NA	CA	GS	UA	LA	AF
Cam	1.00	0.40	0.30	0.30	0.18	0.18	0.00	0.00	0.00
Cb	0.40	1.00	0.10	0.06	0.00	0.06	0.01	0.10	0.00
WL	0.30	0.10	1.00	0.60	0.60	0.18	0.30	0.30	0.00
NA	0.30	0.06	0.60	1.00	0.40	0.6	0.20	0.20	0.00
CA	0.12	0.00	0.60	0.40	1.00	0.24	0.20	0.20	0.12
GS	0.18	0.06	0.18	0.6	0.24	1	0.6	0.6	0.36
UA	0.00	0.01	0.30	0.20	0.20	0.6	1.00	0.60	0.36
LA	0.00	0.10	0.30	0.20	0.20	0.6	0.60	1.00	0.60
AF	0.00	0.00	0.00	0.00	0.12	0.36	0.36	0.60	1.00

<b>8-13 mya</b>	Cam	Cb	WL	NA	CA	GS	UA	LA	AF
Cam	1.00	0.40	0.30	0.30	0.18	0.18	0.00	0.00	0.00
Cb	0.40	1.00	0.10	0.06	0.00	0.06	0.01	0.10	0.00
WL	0.30	0.10	1.00	0.60	0.60	0.18	0.30	0.30	0.00
NA	0.30	0.06	0.60	1.00	0.40	0.6	0.60	0.60	0.00
CA	0.12	0.00	0.60	0.40	1.00	0.24	0.60	0.60	0.36
GS	0.18	0.06	0.18	0.6	0.24	1	0.6	0.6	0.36
UA	0.00	0.01	0.30	0.60	0.60	0.6	1.00	0.60	0.36
LA	0.00	0.10	0.30	0.60	0.60	0.6	0.60	1.00	0.60
AF	0.00	0.00	0.00	0.00	0.36	0.36	0.36	0.60	1.00

<b>13-30 mya</b>	Cam	Cb	WL	NA	CA	GS	UA	LA	AF
Cam	1.00	0.40	0.00	0.00	0.00	0	0.00	0.00	0.00
Cb	0.40	1.00	0.10	0.06	0.00	0.06	0.01	0.10	0.00

WL	0.00	0.10	1.00	0.60	0.01	0.01	0.01	0.01	0.00
NA	0.00	0.06	0.60	1.00	0.10	0.1	0.10	0.10	0.00
CA	0.00	0.00	0.01	0.10	1.00	0.01	0.10	0.20	0.02
GS	0	0.06	0.01	0.1	0.01	1	0.1	0.6	0.06
UA	0.00	0.01	0.01	0.10	0.10	0.1	1.00	0.10	0.01
LA	0.00	0.10	0.01	0.10	0.20	0.6	0.10	1.00	0.10
AF	0.00	0.00	0.00	0.00	0.02	0.06	0.01	0.10	1.00

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Cam: Central-America

Cb: Caribbean Islands

WL: Lowlands west of the Andes, including the Magdalena Valley

NA: Northern Andes

CA: Central Andes

GS: Guiana Shild

UA: Upper Amazon

LA: Lower Amazon

AF: Atlantic Forest

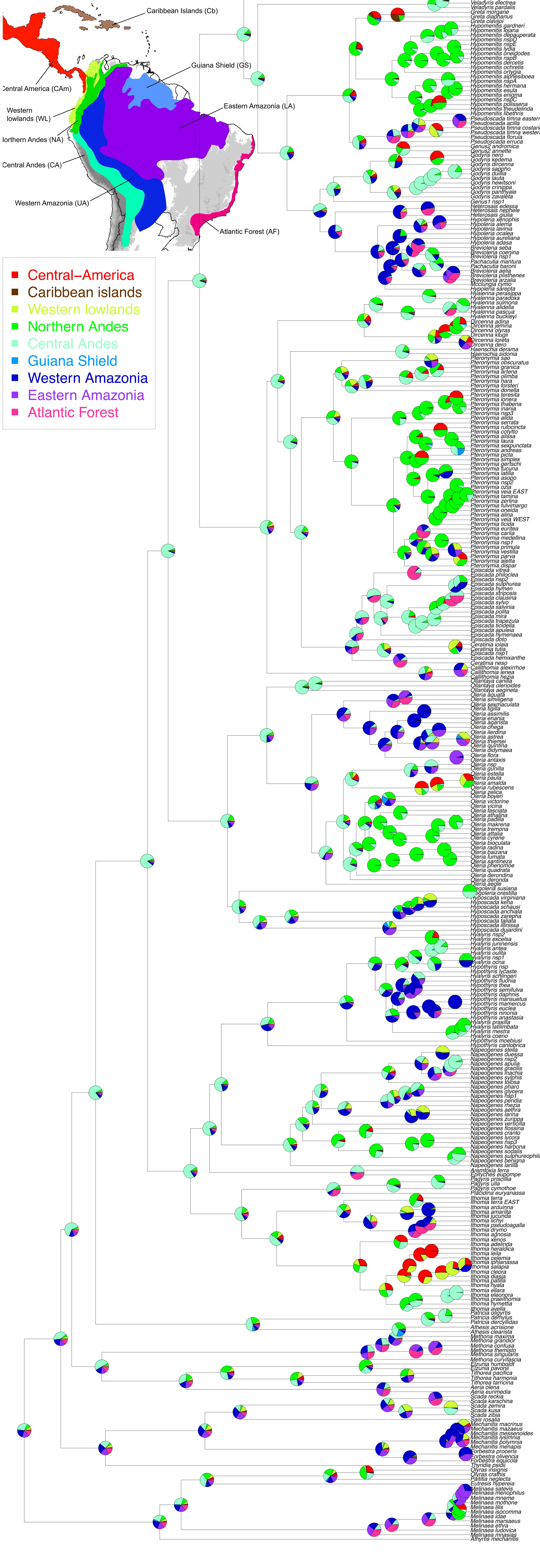
#### *S4.3 Time-stratified biogeographic model*

We used our estimations of colonization rates among the major biogeographic regions to design a time-stratified biogeographic model and improve the resolution of our biogeographic reconstruction. We created four time frames: i) 0-4, ii) 4-8, iii) 8-13, and iv) 13-30 My ago, where dispersal multipliers between areas reflected the colonization rate variations identified previously (Table S4.6). This ancestral state reconstruction was compared to the null model as well as to the ancestral state reconstruction obtained from trait-dependent diversification models (Figure S3.6). Ancestral state reconstructions for the null and the time-stratified models are shown in Figure S4.9.

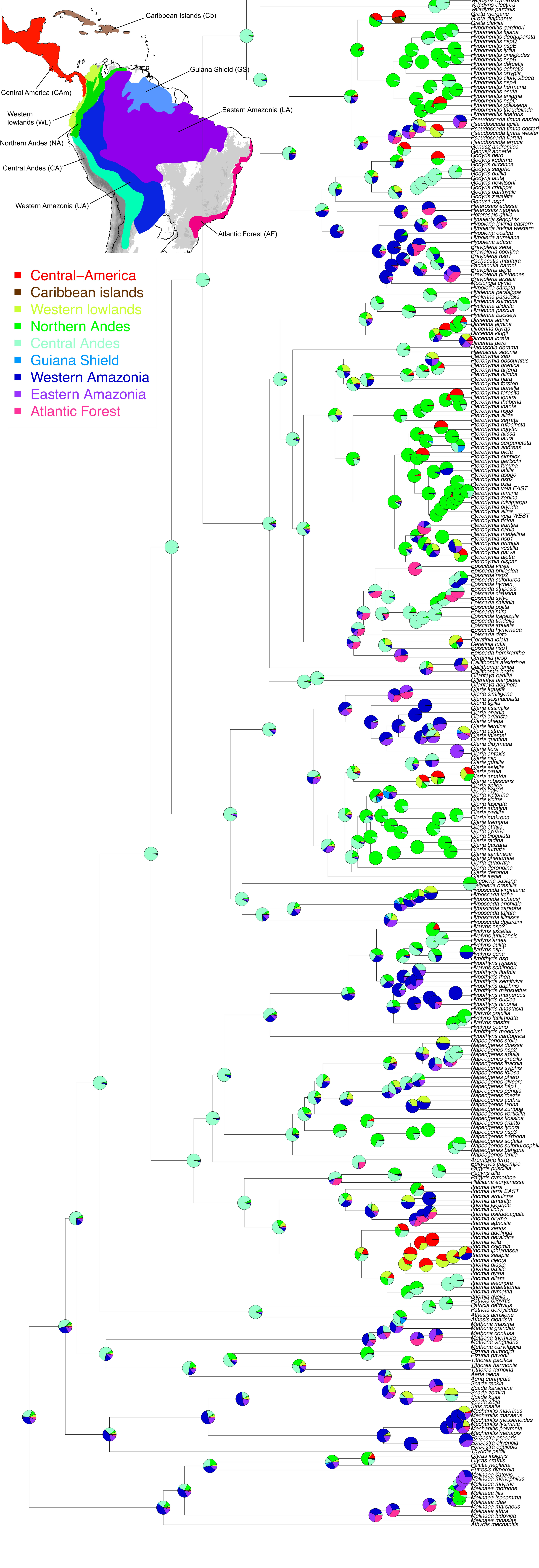
Figure S4.9. Results of biogeographic ancestral state estimation obtained with Biogeobears and using the “null” or time-stratified model.

- A. Biogeographic ancestral state estimation obtained from the “null” model using Biogeobears. Pie charts are the probability of each area summed across all possible ranges and standardized.
- B. Biogeographic ancestral state estimation obtained from the time-stratified model using Biogeobears. Pie charts are the probability of each area summed across all possible ranges and standardized.
- C. Biogeographic ancestral state estimation obtained from the time-stratified model using Biogeobears. Ranges with the highest probability at the nodes are shown. The figure also shows the different sections of the tree that we refer to throughout the paper, including MEDUSA partition (yellow names), the Amazonian radiations (red names) and the North Andean radiation (orange names).

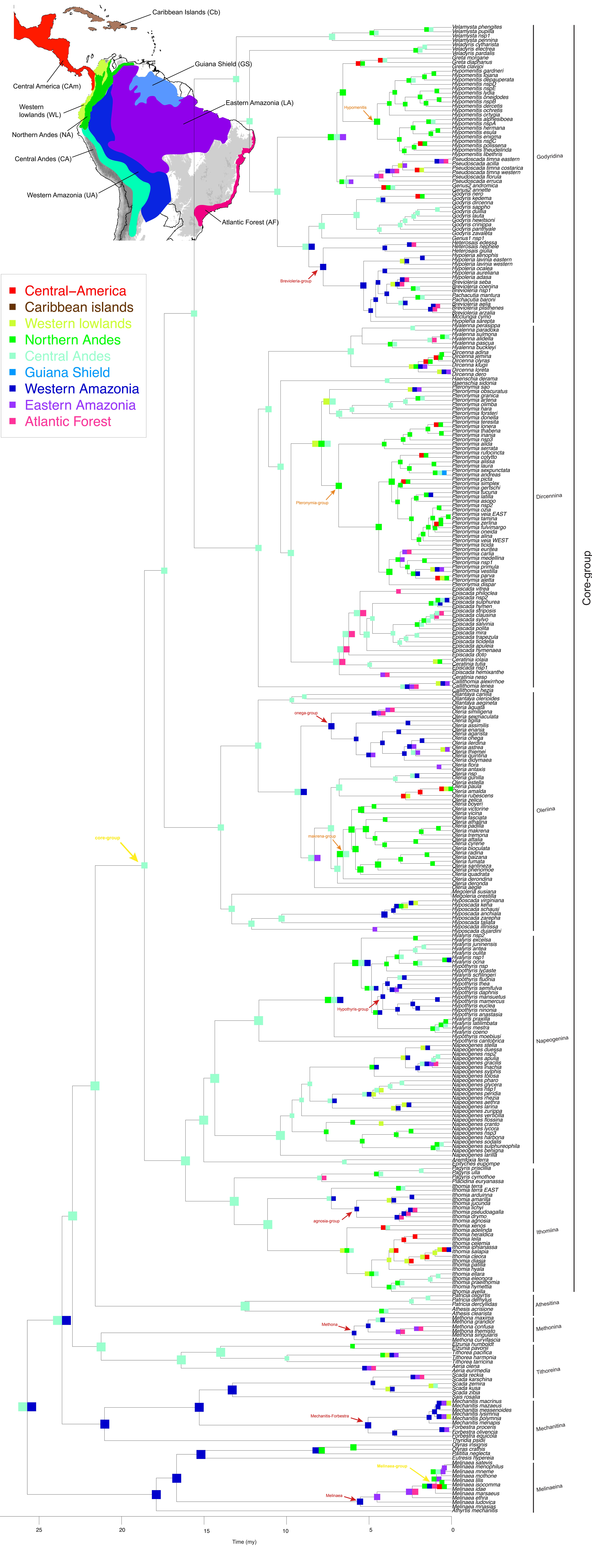
A.



B.



C.



## **References**

Matzke NJ. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, 63(6), 951-970.