

1 ***Title***

2 Renewed diversification following Miocene landscape turnover in a Neotropical butterfly radiation

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21

22 ***Abstract***

23 The Neotropical region has experienced a dynamic landscape evolution throughout the Miocene, with
24 the large wetland Pebas occupying western Amazonia until 11-8 my ago and continuous uplift of the
25 Andes mountains along the western edge of South America. Although the complex dynamics between
26 the Andes and Amazonia may have strongly affected the trajectory of Neotropical biodiversity, there
27 is little evidence for such an influence from time-calibrated phylogenies of groups that diversified
28 during this period. Here, we generate one of the most comprehensive time-calibrated molecular
29 phylogenies of a group of Neotropical insects: the butterfly tribe Ithomiini. Our tree includes 340
30 species (87% of extant species), spanning 26 million years of diversification in the Neotropics. We
31 investigate temporal and spatial patterns of diversification, focusing on the influence of Miocene
32 landscape transformations on the dynamics of speciation, extinction and biotic interchanges at the

1 Amazonia/Andes interface. We find that Ithomiini likely began diversifying at the interface between
2 the Andes and the Amazonia around 26.4 my ago. Five subtribes with a very low extant diversity
3 started diversifying early in western Amazonia, but a rapid decrease in diversification rate due to
4 increased extinction rate between 20 and 10 my ago suggests a negative impact of the Pebas wetland
5 system on these early lineages. By contrast, the clade containing the five most species-rich subtribes
6 (85% of extant species) was characterized by a high, positive net diversification rate. This clade
7 diversified exclusively in the Central Andes from 20 to 10 my ago. After the demise of the Pebas
8 system (11-8 my ago), we found a sudden increase of interchanges with the Northern Andes and
9 Amazonia, followed by local diversification, which led to a substantial renewal of diversification. In
10 general, ecological turnovers throughout the Miocene strongly determined the dynamics of speciation,
11 and extinction and interchanges, and appear as a key driving force shaping the current extraordinary
12 biodiversity of the Neotropical region.

13

14 ***Keywords***

15 Neotropics, phylogeny, diversification, biogeography, Andes, Pebas system, Western-Andean Portal,
16 Ithomiini, butterflies

1 INTRODUCTION

2 There has been a long fascination among biologists for the Neotropics and the origin of its intriguingly
3 high biodiversity. The timing of Neotropical diversification, and therefore its major driving processes,
4 is still controversial despite the large amount of publications that have addressed the question (e.g., ^{1, 2,}
5 ³).

6 Despite uncertainty about the precise timing and magnitude of surface uplift, the formation of the
7 Andean cordilleras during the Cenozoic greatly shaped Neotropical landscapes and affected
8 diversification in the Neotropics. As the Andes arose, they brought about new biotic and abiotic
9 conditions along their slopes, modified the climate of the Neotropical region and deeply affected the
10 formation of the Amazonian basin by bringing large amounts of sediments and modifying water
11 drainage ¹. There is increasing evidence that the Andes influenced the diversification of Neotropical
12 lineages, primarily by increasing speciation rate, perhaps most spectacularly in the high altitude
13 páramo habitat (e.g., ⁴). In parallel, the western part of the Amazon basin, which is connected to the
14 Andes, has experienced major turnovers of ecological conditions. During the Oligocene, western
15 Amazonia was occupied by a fluvial system flowing northward (paleo-Orinoco basin), which
16 transformed ~23 million years (my) ago into an aquatic system of shallow lakes and swamps
17 episodically invaded by marine conditions, known as the Pebas system ^{1, 5, 6, 7, 8}. The Pebas was
18 connected northward with the Caribbean Sea and likely also with the Pacific Ocean through the
19 Western-Andean portal (“WAP”, ⁵), a low-altitude gap that separated the Central Andes and the
20 Northern Andes until 13-11 my ago. During the late Miocene, during the Andean uplift, the
21 accumulation of sediments combined with a sea level decrease initiated the eastward drainage of the
22 Pebas, and by 10-8 my ago the region had changed into a fluvial system, which then turned into the
23 modern configuration of the Amazon. More recently, climatic fluctuations during the Peistocene (2.5-
24 0 my ago) may have led to episodic dryness affecting Amazonian forest habitats ⁹. The extent of the
25 influence of Pleistocene events and their effects on Neotropical diversification, and even the
26 importance of dryness episodes, are controversial (e.g., ^{2, 10, 11, 12}).

27 In this study we purposely focus our attention mostly on the Miocene and Pliocene and how the
28 interaction between the rise of the Andes and coincident large landscape modifications in western
29 Amazonia have determined diversification and dispersal over 30 million years. The Pebas ecosystem
30 covered up 1.1 million km² at its maximum ⁷ and was probably not suitable for a terrestrial fauna.
31 Therefore, between 23 and 10 my ago, diversification of terrestrial lineages may have been impeded in
32 western Amazonia or restricted to its edges ⁷. By contrast, the uplift of the Central and the Northern
33 Andes, also occurring throughout the Miocene and the Pliocene, and the ecological gradients present
34 along this mountain chain probably constituted an important driver of diversification. In the last 10 to
35 8 my, the retreat of the Pebas may have provided opportunities for terrestrial lineages to radiate in

1 western Amazonia. The Pebas may also have constrained dispersal, acting as a barrier between the
2 Andes, the Guiana shield and western Amazonia. Thus, whether rates of interchanges have been
3 constant throughout time since the origin of Ithomiini or instead have increased after the Pebas's
4 retreat would be an indication for the importance of this ecosystem in building the modern pattern of
5 diversity⁶.

6 Paleontological studies have shown that the Pebas greatly contributed to the diversification of aquatic
7 fauna such as molluscs¹³, ostracods¹⁴ and crocodylians¹⁵. However, the fossil record also suggests a
8 negative effect of the Pebas system on terrestrial fauna^{16,17}. The hypothesis that the Pebas has shaped
9 patterns of terrestrial diversification and dispersal in western Amazonia has grown over the years (e.g.,
10^{5, 6, 7, 16, 18}) but support from molecular phylogenies mostly stems from the observation that western
11 Amazonian clades have diversified during the last 10-8 my and not before (^{19,6} and reference therein).
12 Yet, there is very little information on what happened before, when the Pebas was occupying western
13 Amazonia, particularly on whether the presence of Pebas constrained diversification and interchange
14 patterns in this region. A thorough assessment of the role of the Pebas ecosystem on diversification
15 and dispersal requires phylogenies of large Neotropical clades that originated before the formation of
16 the Pebas, i.e. clades older than 23 my old. Phylogenies of Neotropical clades meeting these
17 conditions are surprisingly rare. In insects – which are among the most diverse terrestrial organisms -
18 attempts to build phylogenies of Neotropical groups to test different drivers of Neotropical
19 diversification have either suffered from a small size or a low sampling fraction (e.g.^{19, 20, 21, 22, 23}), and
20 therefore from low statistical power and reliability.

21 Butterflies are among the best candidates for addressing the evolution of the Neotropical biota at such
22 time scales. They are probably the best-known insects and Neotropical butterfly lineages have
23 benefited from substantial phylogenetic research compared to other insects (e. g.,^{19, 20, 21, 24, 25, 26, 27, 28, 29,}
24^{30, 31, 32, 33, 34, 35}). Among the most emblematic Neotropical butterflies is the tribe Ithomiini
25 (Nymphalidae: Danaeinae, 393 species), also referred to as the clearwing butterflies because of the
26 transparent wings of the majority of species. Ithomiini are forest-dwellers distributed throughout the
27 Neotropics, from sea level up into montane cloud forests (to 3000 m), where their larvae feed on
28 plants of the families Solanaceae, Gesneriaceae and Apocynaceae³⁶. Species richness is primarily
29 concentrated in the Andes, where about half of the species occur (mostly on the eastern slopes) and in
30 western Amazonia. Ithomiini are chemically defended and they engage in Müllerian mimicry,
31 whereby co-occurring species exhibit convergent wing colour patterns that advertise their toxicity to
32 predators³⁷. Ithomiini butterflies represent a keystone group in Neotropical forests by numerically
33 dominating mimetic butterfly communities and sharing wing colour patterns with a large number of
34 other palatable and unpalatable Lepidoptera, such as the iconic *Heliconius* butterflies³⁸. For this
35 reason, Ithomiini were used by both Bates³⁹ and Müller³⁷ in their original descriptions of deceptive
36 (Batesian) and mutualistic (Müllerian) mimicry, respectively.

1 The diversity and the intriguing ecology of Ithomiini has generated a great interest and a broad and
2 diverse literature including life history^{40, 41, 42, 43}, chemical ecology^{44, 45, 46}, systematics^{19, 21, 26, 32, 34, 36,}
3^{47, 48}, cytogenetics⁴⁹, community ecology^{50, 51, 52, 53, 54, 55}, wing colour pattern evolution⁵⁶, and
4 biogeography^{19, 21, 26, 27}. In this study, we build on existing molecular data and provide a large amount
5 of novel DNA sequences for ithomiine species to generate the first species-level phylogeny of the
6 entire tribe, providing a large and densely sampled (340 species included out of 393 currently
7 recognized) phylogenetic dataset for a Neotropical insect clade that underwent diversification during
8 the last ~30 million years^{27, 57}. This phylogenetic framework provides an ideal opportunity for
9 investigating Neotropical diversification over a large evolutionary time-scale. Ithomiini originated
10 before the Pebas system, thus offering the opportunity to investigate diversification before, during and
11 after the environmental changes during the Miocene with a high statistical power. Importantly,
12 contrary to many other large radiations of similar age, Ithomiini are endemic to the Neotropics. Their
13 diversification therefore occurred without interaction with other biogeographic regions such as the
14 Nearctic.

15 Here, we investigated the dynamics of speciation, extinction and dispersal rates in Ithomiini through
16 space and time, using a combination of time- and trait- dependent models of diversification and
17 historical biogeography. We focused on the interaction between the turnover of ecological conditions
18 in western Amazonia and the Andean uplift during the Miocene, and we investigated whether
19 geological and ecological events in both regions affected synergistically the diversification of
20 Ithomiini. More specifically, a joint role of the Andean uplift and the Pebas would be supported if: (1)
21 *During the Pebas period*: (a) Andean diversification largely exceeds Amazonian diversification, due
22 to an increased diversification in the Andes driven by the evolving ecological gradient and uplift
23 dynamics and/or a reduced diversification rate in Amazonia accompanying the loss of terrestrial
24 habitats; (b) interchanges with western Amazonia are reduced; (c) interchanges between the Central
25 and the Northern Andes are reduced, because of the existence of the WAP. (2) *During the retreat of*
26 *the Pebas*: interchanges with western Amazonia and between the Central and the Northern Andes
27 largely increase, as a result of new terrestrial habitats and the disappearance of the WAP, respectively.
28 (3) *After the Pebas period*: Diversification rates in Amazonia globally increase and biotic interchanges
29 are not constrained anymore. After the Pebas retreat, decrease of speciation rates through time suggest
30 a post-Pebas radiations in Amazonia, while increase of speciation rates through time may suggest a
31 role of climatic fluctuations during the last 2.5 my.

32

33 **RESULTS**

34 **Time-calibrated phylogeny**

1 *Tree topology and time-calibration*

2 We generated a time-calibrated phylogeny that comprised 340 out of 393 Ithomiini species
3 (Supporting Information S1-S2-S3). The tree topology was generally well supported, including deep
4 nodes (Supporting Information S4). We found a crown age of Ithomiini of 26.4 my ago (Figure 1,
5 Supporting Information S5) and a divergence time from its sister clade Tellervini of 42.1 my ago. All
6 subtribes (10 in total) diverged in the first 10 million years, in the following order (Figure 1): (1)
7 Melinaeina (26.4 my ago), (2) Mechanitina (24.2 my ago), (3) the clade consisting of Tithoreina and
8 the Methonina (23.6 my ago), (4) Athesitina (22.1 my ago) and (5) a large clade that comprises the
9 five most species-rich subtribes (Ithomiina, Napeogenina, Oleriina, Dircennina and Godyridina),
10 hereafter called the “core-group”. The relationships between tribes were similar to those recovered in a
11 recent higher-level phylogeny of Ithomiini based on a combination of 3 gene regions and
12 morphological characters³⁴, except that Brower *et al.* (34) recovered Mechanitina as sister to
13 Tithoreina+Methonina. Lineage ages in our phylogeny were generally younger than those inferred in
14 Wahlberg *et al.* (57), but older than those inferred in Garzon-Orduña *et al.* (58) (see De-Silva *et al.* 48 for
15 further discussion of such differences).

16 **Diversification rates**

17 *Time-dependent diversification*

18 We combined three methods to assess patterns of diversification through time and across clades. We
19 first used MEDUSA⁵⁹, which automatically detects shifts of diversification processes across a
20 phylogeny. The analysis detected two significant shifts from the background process of diversification
21 (Figure 1, Supporting Information S6). One was at the root of and the core-group accounting for ~85%
22 of present-day diversity of Ithomiini. The second shift was at the root of a subgroup within the genus
23 *Melinaea*, which appears to have diversified rapidly during the last million year (hereafter “*Melinaea*-
24 group”).

25 We then used the method developed by Morlon *et al.* 60 to fit time-dependent models of speciation and
26 extinction on the different partitions based on the MEDUSA results. The results confirmed that the
27 partitioned models (either or both of the two shifts detected by MEDUSA) had a significantly better fit
28 and that the two-shift model was significantly better than the one-shift models (Table 1). For the core-
29 group, no model of time-dependent diversification had a significantly better fit than the null model of
30 constant speciation rate without extinction (0.255 lineage⁻¹my⁻¹, Figure 2, Table 1, Supporting
31 Information S6). Under the null model, the core-group diversity increased exponentially during the
32 last 20 million years and reached its current diversity (334 extant species). For the *Melinaea*-group,
33 the best fitting model was an exponentially decreasing speciation rate without extinction, with a very
34 high initial speciation rate (7.62 lineage⁻¹my⁻¹ at the root, 0.342 lineage⁻¹my⁻¹ at present, Figure 2,

1 Table 1, Supporting Information S6). The *Melinaea*-group radiated into eight species during a time
2 lapse of only 1 million year. On the remaining background lineages, the best fitting model involved a
3 time-dependency of both speciation and extinction rates. The resulting diversification rate was high
4 during the initial stages of diversification ($0.75 \text{ lineage}^{-1}\text{my}^{-1}$ at the root), but decreased rapidly and
5 became negative around 19 my ago. The background diversification rate then started a slow recovery,
6 but remained negative ($-0.038 \text{ lineage}^{-1}\text{my}^{-1}$ at present, Figure 2, Table 1, Supporting Information S6).
7 Consequently the background tree diversity reconstructed from this model shows a pattern of diversity
8 that increased during the first 10 my up to ~60 species before slowly declining toward its current
9 diversity (51 species) during the last 15 my. This signal of high relative extinction rate was not
10 affected by initial parameters for maximum likelihood search or by diversification rate heterogeneity
11 potentially remaining from the background (see discussion in Supporting Information S7). Clades
12 within the background tree showing positive net diversification rates during the last 5 my supported
13 the recovery trend described above (Supporting Information S7).

14 We also used TreePar to fit models of constant diversification rate in 5 my bins for each partition
15 identified by MEDUSA, as a second and independent assessment of diversification through time. The
16 results were congruent with those of the time-dependent models of diversification obtained with
17 Morlon *et al.* (⁶⁰)'s method (Figure 2, Supporting Information S7). The diversification rates estimated
18 for the core-group within 5 my time-bins remained relatively constant through time and the turnover
19 rate was close to 0, supporting a null or extremely low relative extinction. For the background tree, we
20 found that diversification rate was highest between 25 and 20 my ago, declining toward 0 during the
21 last 15 my, in agreement with the results obtained with the method of Morlon *et al.* (⁶⁰). Turnover
22 largely increased and reached a maximum during the last 15 million years, supporting a very high
23 relative extinction rate, similarly to our results of the time-dependent diversification models of Morlon
24 *et al.* (⁶⁰)'s method.

25 *Diversification in the Andes*

26 We compared the pattern of diversification in the Andes and in the rest of the Neotropics and we
27 assessed the rate of interchanges between these regions using character state-dependent diversification
28 models (ClaSSE, ⁶¹). Species were classified into Andean and non-Andean and we compared 10
29 models to test whether rates of speciation, extinction or transitions were different or not between
30 regions. For both, the whole Ithomiini and the core-group, we failed to identify a single model that
31 fitted the data significantly better than other models (Table 2). For the whole Ithomiini, the model with
32 the lowest AIC score had a higher speciation rate in the Andes ($\lambda_{222}=0.230$) than in non-Andean
33 regions ($\lambda_{111}=0.118$) and those speciation rates were higher than the colonization rates
34 ($\lambda_{112}=\lambda_{212}=0.079$). The second best model had again a higher speciation rate in the Andes ($\lambda_{222}=0.231$)
35 than in non-Andean regions ($\lambda_{111}=0.108$) and colonization rate out of the Andes was higher

1 ($\lambda_{212}=0.095$) than into the Andes ($\lambda_{212}=0.047$). In the core-group, there were four models within an
2 AIC difference of 2. All models had extremely low extinction rates. Only one of them inferred
3 different speciation rates and three of them inferred higher colonization rate into the Andes than out of
4 the Andes (Table 2).

5 Using BiSSE models, we additionally performed ancestral state reconstructions on the whole tree
6 according to the two best fitting models above (i.e. model1: different speciation rates, model2:
7 different speciation and transition rates). In both cases the most likely state of the root was non-
8 Andean but with high uncertainty (probability of 0.508 and 0.543 respectively) and there was
9 uncertainty at the nodes leading to the core-group (Figure 1, Supporting Information S8). The most
10 recent common ancestor (hereafter MRCA) of the core-group was inferred as most likely Andean
11 (model1: probability of 0.558, model2: probability of 0.625), which was congruent with historical
12 biogeography patterns inferred with BiogeoBEARS (see below). In both models the MRCA of all
13 background subtribes were inferred to be non-Andean with a strong support (except for Athesitina),
14 which differs from the BiogeoBEARS ancestral state reconstruction (Figure 1, Supporting Information
15 S9-S10 see Results below). In the core-group, the best model (with different speciation rates) inferred
16 an Andean origin for subtribes Napeogenina, Dircennina and Godyridina, whereas the second best
17 model (different speciation and transition rates) inferred an Andean origin for all five subtribes
18 (Supporting Information S8).

19 *Diversification in Amazonia*

20 We also investigated the pattern of Amazonian diversification during the post-Pebas period (8-0 my
21 ago). We fitted a model of time-dependent speciation rate (no extinction, based on our previous
22 results) to assess whether speciation rates decreased through time (supporting radiations
23 accompanying the post-Pebas recolonizations) or increased through time (supporting a recent
24 diversification potentially caused by Pleistocene climatic fluctuations). We identified four Amazonian
25 diversification events – clades whose nodes were inferred by BiogeoBEARS to be almost all
26 Amazonian (see results below) – in the core-group and three in the background subtribes. All core-
27 group clades showed decreasing speciation rates through time, suggesting an early diversification,
28 perhaps following the appearance of new forest habitats accompanying the Pebas retreat. Among the
29 three clades from the background lineages only the genus *Methona* followed a trend of decreasing
30 speciation rate. The other two clades, *Mechanitina* + *Forbestra* and *Melinaea* (the whole genus)
31 supported an increasing speciation rate through time, which is consistent with a potential effect of
32 Pleistocene climatic fluctuations in driving diversification.

33

34 **Historical biogeography**

1 We performed historical biogeographic reconstruction using BiogeBEARS⁶², under two models (DEC
2 and DIVALIKE). We divided the Neotropical region into 9 areas (Supporting Information S9) and we
3 assigned each ithomiine species to these areas according to their current distribution.

4 *Biogeographic null model*

5 We started with a “null” biogeographic model, which restricted the area adjacency but set all dispersal
6 probabilities to 1, and we compared the models DEC and DIVALIKE. The null DEC model had a
7 better fit than the null DIVALIKE model (likelihoods: DECnull: -1335.802, DIVALIKEnull: -
8 1347.869), hence we used the DEC model in all subsequent analyses.

9 In both models, the ancestral area of the Ithomiini MRCA was unclear (Central Andes + upper
10 Amazon for the highest probability). The areas where the two first divergences occurred, which led to
11 the Melinaeina and the Mechanitina subtribes, were also unclear (Figure 1, Supporting Information
12 S10). The ancestor of the remaining ithomiine lineages was recovered to be only occupying the
13 Central-Andes. Following this node (23.6 my ago) all the divergences occurred in the Central Andes
14 until 9.4 my ago, when the first colonization event out of the Central Andes occurred (MRCA of
15 *Oleria*, which dispersed into the western Amazonia). Hence, our null biogeographic reconstruction
16 found that all subtribes except Melinaeina and Mechanitina originated and started diversifying in the
17 Central Andes. Interchanges between regions appeared to have increased during the last 10 my. But all
18 node reconstructions at the basal nodes of the background lineages were highly uncertain.

19 *Biogeographic diversification of the core-group*

20 Using this null model, we investigated more specifically the biogeographic pattern of the core-group
21 by computing rates of dispersal among different regions. We applied the null model to 100 trees
22 randomly sampled from the BEAST posterior distribution and extracted the state with the highest
23 probability at each node. Then for each 1-my interval we computed the number of specific transitions
24 divided by the number of lineages existing during this interval and fitted a spline line on the
25 distribution of points.

26 As observed in the ancestral state reconstruction on the MCC, no dispersal event occurred during the
27 initial Central-Andean phase of diversification in the core-group (Figure 3). Between 13-8 my ago a
28 major peak of interchanges between the Andes and Amazonia occurred, followed by a second peak
29 between 4-0 my ago (Figure 3). The first peak was almost entirely driven by colonization from the
30 Andes toward the Amazonia whereas the second peak involved many reverse colonization toward the
31 Andes. We also recorded a large peak of colonization from the Central Andes toward the Northern
32 Andes between 13-8 my ago, also followed by a second peak 4-0 my ago (Figure 3, Supporting
33 Information S9-S10). Colonization of Central-America may have started 8 my ago but interchanges
34 largely increased during the last 4 my (Supporting Information S9). Colonizations of the Atlantic

1 Forest also started early (around 13 my ago) but the rate of interchanges between the Atlantic Forest
2 and the remaining Neotropical regions remained relatively constant during the last 10 my (Supporting
3 Information S9-S10).

4 We also used the biogeographic reconstruction to estimate local diversification i.e., the cumulative
5 number of divergences inferred to have occurred exclusively in a given region. As described above,
6 until ~10 my ago speciation events occurring in the Central-Andes fully account for the core-group
7 diversification (no dispersal events). During the last ~10 my, we observed a dampening of the
8 speciation events in the Central Andes (Figure 3). At the same time, following the peaks of dispersal
9 identified above, Northern-Andean and Amazonian lineages started diversifying, although the latter
10 diversified at a slower pace than the former. This reflects the large number of dispersal events into the
11 Northern Andes that were followed by important local diversification, for example in the genera
12 *Hypomenitis* (17/20 species in the phylogeny occurring in the Northern-Andes) and *Pteronymia* (30/45
13 species in the phylogeny occurring in the Northern-Andes), or in subclades of the genera *Oleria* or
14 *Napeogenes*. We also identified some important transitions to lowland Amazonia, for example at the
15 origin of the *Breviolaria*-clade, during early divergence in the genus *Oleria*, or in the genus
16 *Hypothyris* (see results on *Diversification in Amazonia*).

17 *Time-stratified biogeographic model*

18 We used the results highlighted above to refine the biogeographic model by incorporating the
19 variations of dispersal rates identified into a model accounting for time-stratified dispersal multipliers.
20 This time-stratified model designed from rates of colonization computed above led to a significant
21 improvement of the model (likelihoods: DECnull: -1335.802, DECstrat:-1321.805). Both ancestral
22 state reconstructions were very congruent but the time-stratified model increased the resolution of
23 several nodes throughout the tree (Supporting Information S9-S10). We identified one major
24 difference in the ancestral states. From the null model the ancestral state of the subtribe Melinaeina,
25 the first lineage to diverge, was highly unclear and the first nodes within Melinaeina were identified as
26 Central-Andean, although this was not strongly supported. Likewise, in the null model, Mechanitina,
27 the second lineage to diverge, was inferred to have diversified in the Atlantic Forest but this was
28 poorly supported (Supporting Information S9-S10). The time-stratified model greatly increased the
29 resolution of all these deep nodes, inferring that both Mechanitina and Melinaeina likely occupied the
30 western Amazonia. For Melinaeina and Mechanitina this result was in agreement with the BiSSE
31 ancestral state reconstruction. The ancestral state reconstruction inferred that the Ithomiini occupied
32 the Andes from the root, however this was very weakly supported.

33

34 **DISCUSSION**

1 We generated one of the largest species-level phylogenies to date for a tropical insect group, the
2 emblematic Neotropical butterfly tribe Ithomiini. With 340 out of 393 species included and a crown
3 age of 26.4 my, this phylogeny offers a unique opportunity to investigate the dynamics of
4 diversification of an insect group throughout the Neotropical region during the major geological and
5 ecological events that have occurred since the Miocene. We discuss our findings below and we
6 propose that the dynamics of multiple landscape transformations during the Miocene, and more
7 specifically the interactions between the Andes and the Pebas system, have determined the dynamics
8 of speciation, extinction and biotic interchanges in the Neotropical region.

9 *Early diversification at the interface of the Pebas and Central Andes: has the Pebas driven extinction?*

10 The Ithomiini probably originated along the early Andean foothills at the transition with western
11 Amazonia. The onset of the uplift of the eastern cordillera of the Central Andes during late Oligocene
12 coincides with the origin of Ithomiini⁶³ and our reconstruction of the ancestral biogeographic area for
13 the MRCA of the tribe was unable to distinguish between Central Andes or western Amazonia. The
14 Pebas ecosystem replaced the previous western Amazonian terrestrial ecosystem from 23 to 10 my
15 ago. Wesselingh *et al.* (7) described the Pebas as an ecosystem “which was permanently aquatic with
16 minor swamps and fluvial influence, and was connected to marine environments”, and may have
17 reached a maximum size of 1.1 million km². The presence of fossil marine fishes⁶⁴ and molluscs¹³
18 testifies to the presence of saline waters. More recently, Boonstra *et al.*⁶⁵ found evidence from
19 foraminifera and dinoflagellate cysts that marine incursions reached 2000 km inland from the
20 Caribbean sea during the early to middle Miocene during periods of high sea levels. The extent and
21 duration of these marine influences is controversial (see⁶⁶ and references therein). Yet, it is undeniable
22 that the Pebas system was not suitable for terrestrial fauna and flora, and therefore was likely to affect
23 diversification and dispersal of the terrestrial fauna, including early Ithomiini lineages.

24 The timing of diversification of background lineages reveals a fast early diversification, perhaps
25 following the colonization of South America during the pre-Pebas period – the sister clade of
26 Ithomiini, Tellervini, is found in Australia and Papua New Guinea. Yet diversification rate rapidly
27 decreased through time, driven by an increasing relative extinction rate and at a time corresponding to
28 the replacement of the terrestrial habitats by the Pebas ecosystem, i.e., ca. 23 my ago. Although the
29 ancestral area of Ithomiini is ambiguous, the two first diverging Ithomiini lineages (Melinaeina and
30 Mechanitina) were clearly endemic to western Amazonia (time-stratified model in BiogeoBEARS and
31 BiSSE reconstruction) and therefore likely to be affected by the dramatic landscape modifications of
32 the Miocene. However, there are uncertainties surrounding the other deep nodes and also the time
33 when first colonization of the Andes occurred. Two scenarios can be envisioned: (1) The remaining
34 lineages (Tithoreina, Methonina, Athesitina and the core-group) became endemic to the Central Andes
35 (supported by the BiogeoBEARS reconstruction) and we do not know what has driven the shift of

1 diversification at the root of the core-group; and (2) All background lineages were ancestrally western
2 Amazonian (supported by BiSSE ancestral state reconstruction and the sister-clade Tellervini being a
3 group restricted to lowlands) and central Andean endemism occurred at the root of the core-group
4 only, but high extinction rate in the background lineages (potentially higher in western Amazonian
5 lineages) may have falsified the BiogeBEARS ancestral state reconstruction. Such a scenario would
6 explain the common pattern of high relative extinction rate in the background lineages and a shift of
7 diversification process at the root of the core-group.

8 The idea that the Pebas may have driven extinction is well supported by a recent evaluation of an
9 Amazonian fossil record, which pointed at a major decline of diversity in western Amazonia during
10 the early and middle Miocene ¹⁶. This study concludes that mammalian diversity dropped from 11
11 orders, 29 families and 38 species during late Oligocene down to 1 order, 2 families and 2 species
12 during middle Miocene (see also ¹⁷). These results and the pattern of extinction we found in (at least
13 some) early Amazonian Ithomiini, which occurred during the Pebas period, strongly suggest that the
14 late Oligocene fauna occupying western Amazonia suffered from extinction during the Pebas period.
15 The progressive recovery of these background lineages toward the present, including positive
16 diversification rates in some recent lineages, also concurs with the idea that the retreat of the Pebas
17 released the constraints on diversification during the last 10 my (Supporting Information S7).

18 Parallel to the events occurring in western Amazonia during the Pebas period, the core-group MRCA
19 (19.1-22.1 my old) occupied the Central Andes. This event was of major importance in shaping the
20 diversification of Ithomiini since it is the origin of 85% of the current Ithomiini diversity. Firstly, from
21 this event until ~10 my ago, all core-group lineages exclusively diversified in the Central-Andes,
22 meaning that from 19.1-22.1 to ~10 my ago not a single dispersal event occurred out of the Central-
23 Andes. Secondly, the core-group corresponds to a shift of diversification dynamics, characterized by a
24 low or absence of extinction and a constant speciation rate, which greatly contrasts with the slow and
25 even negative diversification dynamics of the background lineages during the same period.
26 Consequently, the Central Andes hosted most of the diversification during the first half of Ithomiini
27 history. A two-fold higher Andean diversification rate was found across the whole Ithomiini, which
28 may be mainly the consequence of the diversification rate shift found at the root of the core-group. By
29 contrast, when considering only the core-group, Andean and non-Andean lineages had similar
30 diversification rates. The lack of support for a general increase in diversification rate in the Andes
31 within the core-group is also supported by analyses performed independently on different core-group
32 subtribes. For example, in both Oleriina (Chazot *et al.* submitted) and Godryidina ¹⁹, radiations
33 occurred in both Andean and Amazonian genera.

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

1 Gentry (⁶⁷) pointed at a dichotomy observed in the geographic distribution of Neotropical plant
2 diversity, showing that groups could be divided into Andean-centred *versus* Amazonian-centred
3 patterns. Clades tend to be species-rich in one of these centres and relatively species-poor in the other.
4 Antonelli & Sanmartín (⁶) coined this observation the “Gentry-pattern”. They also suggested that in
5 the absence of a barrier between the Andes and the Amazon basin we should observe continuous
6 interchanges between these regions. Antonelli & Sanmartín (⁶) proposed that the Pebas could be this
7 “missing long-lasting barrier needed for creating the disjunction between Andean-centred and
8 Amazonian-centred groups”. Therefore, in addition to the constraints on diversification discussed
9 above, we predicted that the Pebas ecosystem should have influenced interchanges toward or across
10 western Amazonia.

11 Our results conform surprisingly well to the scenario proposed by Antonelli & Sanmartín (⁶). Ithomiini
12 are Andean-centered with more than a half of their current diversity occurring in the Andes (see also
13 ⁶⁸). Here we show that interchanges have been virtually absent during the Pebas period, with a period
14 as long as 9-12 my without interchanges. However, rates of interchanges from the Central Andes
15 toward the Northern Andes and Amazonia suddenly peaked ~10 my ago (between 13-8 my ago) and
16 more recently (4-0 my ago). The Western Andean Portal (WAP) is a low-altitude gap that separated
17 the Central-Andes and the Northern Andes until 13-11 my ago, and which may have connected the
18 Pebas system and the Pacific Ocean (⁶). The closure of the WAP may have allowed multiple
19 colonizations of the Northern Andes facilitated by the presence of connecting higher altitude habitats.
20 In parallel, between 10-8 my ago the Pebas system was drained eastward, leading to the formation of
21 the present-day configuration of Amazonian drainage basin. It was accompanied by the expansion of
22 terrestrial forest habitats in western Amazonia. This corresponds precisely to the timing at which the
23 core-group lineages colonized western Amazonia and then diversified.

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

25 We found a strong dampening of local speciation in the Central Andes during the last 10 my.
26 However, colonizations following the retreat of the Pebas system were followed by large local bursts
27 of diversification within the Northern Andes and Amazonia. As an illustration, from our
28 biogeographic reconstruction, 69 divergence events occurred strictly in the Central Andes in the core-
29 group from 20 my ago until present-day. However, multiple independent dispersal events followed by
30 local diversification lead to the exact same number of divergences occurring strictly in the Northern
31 Andes during the last 9 my only. The genus *Hypomenitis* and the *Pteronymia*-clade, for example,
32 diversified extensively within the Northern Andes. We also identified four Amazonian radiations in
33 the core-group and three in the background lineages. Two of these groups, the background genera
34 *Melinaea* and *Mechanitis* + *Forbestra*, showed increasing speciation rate toward the present, due in
35 the former case to the shift detected by MEDUSA (*Melinaea*-group). The recent and dramatic increase

1 in diversification rate of the *Melinaea*-group, which produced at least eight species and 50 subspecies
2 ⁴³ in just 1 my, is particularly intriguing. This may be interpreted as a support for an effect of recent
3 climatic fluctuations during the Pleistocene on the diversification of this group (as well as the
4 *Mechanitis* + *Forbestra*), although ecological drivers of speciation classically invoked in mimetic
5 butterfly diversification, such as colour pattern and hostplant shifts cannot be ruled out ^{42,43}. Five other
6 Amazonian radiations showed diversification rate decreasing through time, meaning that
7 diversification was highest just after the retreat of the Pebas. Recent radiations in western Amazonia
8 that post-date the Pebas period have been repeatedly reported. For example, in the genus *Astrocaryum*
9 (Arecaceae), the upper-Amazonian clade started diversifying only ~6 my ago ⁶⁹. In *Taygetis*
10 butterflies, Amazonian lineages show rapid diversification during the last 7-8 my ³⁵. Such convergent
11 timing of diversification in western Amazonia strongly support the scenario of a post-Pebas recovery
12 of terrestrial habitats, which triggered dispersal followed by local diversification.

13

14 *Conclusion*

15 Our research shows that the timing of diversification and biogeographic interchanges in Ithomiini
16 butterflies are tightly associated with the turnover of ecological conditions that occurred during the
17 Miocene. Our findings suggest that the ecological turnover that first accompanied the expansion of the
18 Pebas system has led to a decline of diversification, potentially driven by increasing extinction, in
19 early lineages adapted to the ecological conditions that existed during the Oligocene in the upper-
20 Amazon region. Such a decline of diversity has also been documented in the fossil record ¹⁶, which
21 calls for further investigations on the role of the Pebas in driving extinction during the Miocene. By
22 contrast, lineages that colonized the Central Andes 20 my ago rapidly diversified. However, during the
23 entire existence of the Pebas, these lineages remained trapped in the Central Andes (at least 9-12 my
24 without dispersal events out of the Andes). The closure of the West Andean Portal, connecting the
25 Central and North Andes, and the associated demise of the Pebas (10-8 my ago), apparently released
26 these long-lasting barriers, allowing interchanges with the Northern-Andes and Amazonia and opening
27 new opportunities for diversification. As a result of these multiple events, major differences appear
28 between the different faunas. Central Andean lineages started diversifying early, allowing species to
29 accumulate over a long period of time but diversification slowed down during the last 10 my. In
30 comparison, the Northern Andean fauna is recent (13-11 my old at most), driven by multiple
31 colonization events sometimes followed by important bursts of diversification. In parallel, some
32 Amazonian lineages may be old (late Oligocene) but modern diversity almost entirely arose during the
33 last 8-10 my, after the demise of the Pebas ecosystem. Taken together, all this information points to a
34 robust scenario for Neotropical diversification, which highlights the role of Miocene turnover of

1 ecosystems in determining the timing of interchanges, speciation and extinction in the world's most
2 biologically diverse region.

3

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17

18 **Author contributions:**

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

23

24 **MATERIAL AND METHODS**

25 **Time-calibrated phylogeny**

26 *Molecular data*

27 We compiled sequences of 1460 Ithomiini individuals (Supporting Information S1) that included
28 sequences newly generated for this study and previously published sequences^{19, 21, 26, 27, 32, 47, 52, 70}. We
29 used a concatenation of nine gene fragments, a mitochondrial fragment spanning genes COI-tRNA-
30 COII, and fragments of nuclear genes *EF1 α* , *Tektin*, *CAD*, *RPS2*, *MDH*, *GAPDH*, representing a total
31 of 7083 bp⁷¹. Primers and PCR conditions followed³² and⁷¹. We obtained at least one gene fragment

1 for 340 species out of 393 currently known in the group, which represents 87% of the known species
2 richness of the tribe. For each species we produced the consensus sequence of all sequences belonging
3 to individuals of that species to obtain the longest sequence possible. We added 41 outgroups, which
4 spanned all Danainae genera as well as representatives of the main Nymphalidae clades. In total, seven
5 concatenated genes from 381 taxa were used to generate the time-calibrated phylogeny of the
6 Ithomiini.

7 *Tree topology and time calibrations*

8 First, we generated a phylogeny under maximum likelihood inference (ML), using IQ-Tree software
9 as implemented in the W-ID-TREE server ^{72, 73} in order to obtain a tree topology (Supporting
10 Information S2). Then, using BEAST v1.8.2 ⁷⁴, we time calibrated this tree by enforcing the ML
11 topology and preventing BEAST from searching for a new topology in the xml file and following the
12 calibration procedure described below.

13 Gene partitioning and substitution models were estimated using PartitionFinder v.1.1 ⁷⁵. We divided
14 all gene fragments into codon positions and allowed all partitions to be tested. Only substitution
15 models available in BEAST were tested. The models of linked partitions had a better fit than unlinked
16 partition schemes, hence the former was used. The best linked partition scheme contained 13 partitions
17 (Supporting Information S3).

18 Branch lengths were estimated using BEAST v.1.8.2 ⁷⁴ with a birth-death tree prior and a lognormal
19 relaxed molecular clock for each gene partition. In order to time-calibrate the tree we also used a
20 combination of secondary calibrations and host-plant calibrations (Supporting Information S4). Four
21 secondary calibration points were retrieved from Wahlberg *et al.* (⁵⁷)'s phylogeny of Nymphalidae
22 genera and were placed only outside of Ithomiini. We used uniform distribution priors, corresponding
23 to the 95% HSPD inferred by Wahlberg *et al.* (⁵⁷). Host-plant calibrations were used as constraints
24 only within the Ithomiini. Almost all Ithomiini species feed on Solanaceae with a relatively high
25 specificity. A phylogeny of the Solanaceae was published by Särkinen *et al.* (⁷⁶), and recalibrated by
26 De-Silva *et al.* (⁴⁸). We identified six relevant hostplant clades (Supporting Information S4), which
27 were used as maximum age constraints. Priors for host-plant calibration followed a uniform
28 distribution. The minimum value of the uniform was 0 (present). The maximum value was the upper
29 boundary of the 95% HSPD of the stem age of the host-plant subclade on which the calibrated
30 Ithomiini lineage feeds. To get a starting tree suitable for time-calibration priors, the ML tree was
31 ultrametrised and rescaled using PATHD8 ⁷⁷ and Mesquite v.3.2 ⁷⁸.

32 We performed two independent runs of BEAST v.1.8.2 ⁷⁴ on the CIPRES server ⁷⁹ of 87 million
33 generations, sampling every 10000. Using Tracer v.1.6 we checked that the two runs had converged
34 and the parameter's ESS values. Both runs were combined using Logcombiner v.1.8.3 ⁷⁴, applying a

1 15% burn-in for each run. Finally using TreeAnnotator v.1.8.3⁷⁴ we extracted the Maximum Clade
2 Credibility tree with median branch lengths and we computed the posterior probabilities of each node
3 (Supporting Information S5). The outgroups were pruned and the remaining tree was used for the
4 following analyses (hereafter, MCC tree).

5

6 **Diversification rates**

7 *Time*

8 We investigated the dynamics of speciation and extinction rates through time and across the
9 phylogeny. We chose a combination of three methods to infer the dynamics of diversification:
10 MEDUSA⁵⁹ followed by time-dependent models of diversification proposed by Morlon *et al.* (⁶⁰) and
11 Stadler (⁸⁰, TreePar). MEDUSA is a maximum likelihood method that uses stepwise AIC to
12 automatically identify the number and the position of different diversification processes that maximize
13 the fit of the model to the tree. However, MEDUSA makes the strong assumption that rates of
14 speciation and extinction are constant through time. To relax this assumption, we combined MEDUSA
15 with two alternative methods. (1) Morlon *et al.* (⁶⁰) developed a method that allows both speciation
16 and extinction rates to vary as a function of time and across lineages, and where extinction is allowed
17 to be higher than speciation, a situation leading to declining diversity. However, it does not
18 automatically detect rate shift points, which therefore have to be specified by the user. (2) TreePar⁸⁰
19 accommodates models where diversification rates can vary at points in time but are constant between
20 these points. Similarly to Morlon *et al.* (⁶⁰)'s method, TreePar does not automatically detect shifts
21 within a tree. We proceeded as follows: we ran MEDUSA on the whole tree (MCC tree) in order to
22 partition the tree into different diversification processes but rate estimates were ignored. Instead, we
23 used the partition inferred by MEDUSA to estimate diversification rate (speciation - extinction) and
24 turnover rate (extinction/speciation) by fitting time-dependent models of diversification using Morlon
25 *et al.* (⁶⁰)'s method and TreePar⁸⁰. MEDUSA detected two shifts from the background diversification
26 rates (see Results and Supporting Information S6-7): 1) one shift at the root of a large clade hereafter
27 referred to as the core-group, and 2) one shift at the root of a subclade of the genus *Melinaea* that, for
28 simplicity, is referred to as the *Melinaea*-group. We fitted time-dependent models using the method
29 provided in Morlon *et al.* (2011) on all four possible partitions: no shift, one shift (either the core-
30 group or the *Melinaea*-group) and both shifts. Sampling fraction was indicated for each partition.
31 Comparing the fit of all partitions allowed us to confirm that the two shifts identified by MEDUSA
32 were indeed significant. In each case the stem branch of the shifting clade was included in the
33 subclade, as designed in the method⁶⁰, but we excluded the stem branch of the background tree. For
34 each distinct part of the tree (background tree, *Melinaea*-group and core-group) we fitted the following
35 models: constant speciation (no extinction), time-dependent speciation (no extinction), constant

1 speciation and extinction, time-dependent speciation and constant extinction, constant speciation and
2 time-dependent extinction, time-dependent speciation and extinction. In the cases of time-dependent
3 rates we fitted an exponential dependence to time. Sampling fraction was specified for each of the
4 three partitions. All models were compared using AIC scores. The model with the lowest AIC score
5 that was significantly different from the null model of constant speciation rate was used to plot the
6 diversification rate through time. Finally we ran TreePar⁸⁰ in order to obtain a second, independent,
7 estimation of diversification and turnover rates across time. TreePar uses a vector of speciation times,
8 which allowed us to run it separately on the core-group and the background tree. For the background
9 tree we added the times of divergence at which the core-group and the *Melinaea*-group diverged from
10 the background to keep track of these cladogenetic events. We split time into time bins of five million
11 years, for which diversification and turnover rates were estimated. We used these estimates to obtain a
12 second estimate of diversification rate “through time” that could be compared with Morlon *et al.* (⁶⁰)’s
13 method. We did not fit TreePar on the *Melinaea*-group since it is only 1 million year old, and therefore
14 does not even span an entire time bin. We allowed diversification rate to be negative but we did not
15 allow mass-extinction events.

16 *Diversification in the Andes*

17 To investigate the pattern of diversification in the Andes with respect to rest of the Neotropical
18 regions, we classified species as Andean or non-Andean, based on a combination of GPS coordinates
19 and elevation point distribution of species. Species biogeographic distribution was used as character
20 states. Then we used trait-dependent models of diversification^{19, 81} to compare the rates of speciation,
21 of extinction and of transition between the Andean area and the non-Andean regions. We used the
22 ClaSSE model⁶¹, which accounts for up to 10 parameters (2 speciation rates without character state
23 change, 4 cladogenetic transition rates, 2 extinction rates and 2 anagenetic transition rates). However,
24 we constrained parameters that were not biogeographically meaningful to zero¹⁹. Those include the
25 anagenetic transition rates, considering that transition rates from one region to the other were always
26 accompanied by a speciation event, and the cladogenetic transition rates involving a transition in both
27 descendant lineages since we considered the scenario as unrealistic. We therefore ended up with at
28 most 6 parameters. We tested all models with only one parameter (speciation, extinction, transitions)
29 free to vary as well as all models combining two or more parameters free to vary. This allows
30 comparing the relative contribution of one biogeographic model to the others, as well as their
31 combination since they are not mutually exclusive. Models were compared using AIC scores. All
32 models were fitted on the MCC tree. Finally, we also conducted ancestral state reconstructions based
33 on the models of trait-dependent diversification. Since ancestral state reconstruction is not available
34 for ClaSSE models we used the BiSSE model⁸² for this purpose. BiSSE also includes 2 speciation
35 rates and two extinction rates, but it allows the transitions to occur only along branches (anagenetic
36 transition rates). Therefore, we fitted the BiSSE model corresponding to the best fitting ClaSSE

1 model, and we used these parameters to infer the ancestral states at the nodes of the phylogeny. This
2 ancestral state reconstruction was compared to that obtained using the historical biogeography
3 analyses outlined below. All these analyses were performed on both the whole phylogeny and the
4 core-group only, to account for the diversification rate shift identified by our time-dependent
5 diversification analyses.

6 *Diversification in Amazonia*

7 We further investigated the pattern of diversification in Amazonia during the post-Pebas period. The
8 Amazonian basin appears to be the second most important place for diversification after the Andes and
9 there is a longstanding hypothesis that speciation in this region has been driven by climatic
10 fluctuations during the Quaternary⁹. An interpretation of this scenario is that speciation rate should
11 increase during the last 2.5 million years¹². To test this hypothesis we identified the major Amazonian
12 diversification events, i.e. clades which nodes were inferred to be almost all Amazonian from the
13 BiogeoBEARS ancestral state reconstruction. We fitted a model of time-dependent speciation rate (no
14 extinction) to see whether speciation rates increased through time (supporting a recent diversification
15 potentially caused by Pleistocene climatic fluctuations) or decreased through time (supporting
16 radiations accompanying the post-Pebas recolonizations).

17

18 **Historical biogeography**

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

26 *Biogeographic null model*

27 We inferred the historical biogeography of Ithomiini using BiogeoBEARS v.0.2.1⁶². We divided the
28 Neotropics into nine distinct biogeographic regions (Supporting Information S9): 1) Central America,
29 2) Caribbean Islands, 3) lowlands on the western part of the Andes, including the Magdalena valley, 4)
30 Northern Andes that comprise the western and eastern Ecuadorian and Colombian cordilleras and the
31 Venezuelan cordillera, 5) Central Andes, 6) western Amazonia, 7) eastern Amazonia, 8) Guiana
32 Shield and 9) Atlantic Forest (Supporting Information S9). In this model, we constrained the
33 combinations of areas to avoid unrealistic distributions (e.g., disjunct distributions) but all dispersal

1 multipliers were set to 1 to avoid biasing the ancestral state reconstruction. We compared the models
2 DIVALIKE and DEC as implemented in BiogeoBEARS v.0.2.1⁶² using log likelihoods. We used the
3 best fitting model (DEC) for the following analyses.

4 *Biogeographic interchanges within the coregroup*

5 One important hypothesis is that the Pebas influenced the interchanges among biogeographic regions,
6 especially toward or across western Amazonia. To test this hypothesis, we computed “rates” of
7 colonization from BiogeoBEARS ancestral-state reconstructions performed with the null-model. For
8 100 trees randomly sampled from the BEAST posterior distribution we applied the null-model with
9 BiogeoBEARS and reconstructed the ancestral states. For each tree, we extracted the state with the
10 highest probability at each node, then for each million-year interval, we computed the proportion of
11 specific state transitions compared to the maximum number of lineages existing during this interval.
12 Hence, for each million-year interval we obtained a proportion of lineages that dispersed for example
13 from the Central-Andes toward the Northern-Andes or from the Andes toward the Amazonia, which
14 we refer to as “rates” of colonization. Therefore, for each million-year interval and each state
15 transition we obtained a distribution of values. We fitted a spline line on this distribution against time
16 to assess the pattern of variation of colonization rates through time. That way we computed rates of
17 colonization between Andean and non-Andean regions, between the Andes and the Amazonia,
18 between the Central and the Northern Andes, between the Atlantic forest and the other regions, and
19 between Central America and the rest of the Neotropics. These rates were computed only on the core-
20 group, because (1) this group contains 85% of Ithomiini species, (2) the diversification process is
21 homogeneous throughout the clade and (3) we found high extinction in background lineages, which
22 may falsify the ancestral state reconstruction in these lineages, which were therefore excluded. We
23 focused more specifically on the timing of transitions between the Andes and the Amazonian basin
24 and the timing of transitions from the Central Andes (ancestral Andean region of the core-group) to
25 the Northern Andes, because a strong influence of the Pebas and the WAP should have affected these
26 rates of interchanges (see Supporting Information S9 for the complete results).

27 Additionally, we recorded the divergence times of nodes inferred to be strictly in either the Central-
28 Andes, the Northern Andes or Amazonia (the region comprising the upper and lower Amazon and the
29 Guiana shield). We excluded all nodes inferred to be in more than one of these areas (for Amazonia,
30 only all combinations of the three areas above were considered) so that we obtained times of
31 divergences inferred to have occurred strictly within the region considered (which we call local
32 diversification). These divergence times were obtained from the unconstrained BiogeoBEARS
33 reconstruction (DEC) on the MCC tree and we used them to represent lineage accumulation through
34 time (exclusively due to speciation) in each region.

35 *Time-stratified biogeographic model*

1 We used our estimations of colonization rates among the major biogeographic regions to design a
2 time-stratified biogeographic model and improve the resolution of our biogeographic reconstruction
3 (Supporting Information S9). We created four time frames: i) 0-4, ii) 4-8, iii) 8-13, and iv) 13-30 my
4 ago, where dispersal multipliers between areas reflected the colonization rate variations identified
5 previously. This ancestral state reconstruction was compared to the null model as well as to the
6 ancestral state reconstruction obtained from trait-dependent diversification models (Supporting
7 Information S8).

1 Table 1. Results of time-dependent models of diversification fitted on the different partitions: 0 shift, 1
 2 shift (core-group or *Melinaea*-group), 2 shifts. For each subclade or the background tree, only the best
 3 fitting model is shown (see Supporting Information S6 for more details). BCST=constant speciation,
 4 BVAR=time-dependent speciation, DCST=constant extinction, DVAR time-dependent extinction. λ
 5 =speciation rate at present, α =coefficient of time variation of the speciation rate, μ =extinction rate at
 6 present, β =coefficient of time variation of the extinction rate.

		model	par	logL	AIC	λ	α	μ	β
0 shift	whole tree	BCST	1	-861.800	1725.613	0.227			
	background	BCSTDVAR	3	-144.886	296.294	0.311		0.397	-0.055
1 shift	core-group	BCST	1	-703.033	1408.080	0.255			
	Joint model		4	-847.920	1704.375				
	background	BCST	1	-850.489	1702.990	0.223			
1 shift	<i>Melinaea</i> -group	BVAR	2	-2.135	8.270	0.342	3.044		
	Joint model		3	-852.624	1711.261				
	background	BVARDVAR	4	-129.863	267.727	0.151	0.159	0.190	0.147
	<i>Melinaea</i> -group	BVAR	2	-2.135	8.270	0.342	3.044		
2 shifts	core-group	BCST	1	-703.033	1408.080	0.255			
	Joint model		7	-835.032	1684.065				

7

8 Table 2. Results of ClaSSE models fitted on the whole Ithomiini tree (a.) and the core-group (b.)
 9 sorted by increasing AIC. Constraints of each model are indicated in the four first columns. 1=non-
 10 Andean, 2=Andean, λ_{111} and λ_{222} represent within-region speciation rates, λ_{112} and λ_{212} represent
 11 cladogenetic transition rates, μ = extinction rates, df = degree of freedom (number of parameters),
 12 logL=log-likelihood, AIC= Akaike information criterion score, Δ AIC = AIC difference with the best
 13 fitting model.

14 a. Whole tree

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	μ	df	logL	AIC	Δ AIC	λ_{111}	λ_{222}	λ_{112}	λ_{212}	μ_1	μ_2
$\lambda_{111} \neq \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 = \mu_2$	4	-250.061	508.122	0	0.118	0.230	0.079	0.079	6.10E-06	6.10E-06
$\lambda_{111} \neq \lambda_{222}$	$\lambda_{112} \neq \lambda_{212}$	$\mu_1 = \mu_2$	5	-249.801	509.602	1.48	0.108	0.231	0.047	0.094	5.20E-08	5.20E-08
$\lambda_{111} \neq \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-250.061	510.122	2	0.118	0.230	0.079	0.079	3.75E-06	9.11E-06
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 = \mu_2$	3	-252.359	510.718	2.596	0.187	0.187	0.077	0.077	1.00E-09	1.00E-09
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 \neq \mu_2$	4	-251.94	511.88	3.758	0.198	0.198	0.080	0.080	0.061	1.26E-06
$\lambda_{111} \neq \lambda_{222}$	$\lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	6	-249.801	511.602	3.48	0.108	0.231	0.047	0.094	9.95E-07	2.79E-07
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} \neq \lambda_{212}$	$\mu_1 = \mu_2$	4	-252.335	512.67	4.548	0.189	0.189	0.066	0.081	5.26E-07	5.26E-07
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} \neq \lambda_{212}$	$\mu_1 \neq \mu_2$	5	-251.726	513.452	5.33	0.204	0.204	0.052	0.101	0.089	9.00E-09
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 = \mu_2$	2	-256.181	516.362	8.24	0.133	0.133	0.133	0.133	1.87E-07	1.87E-07
$\lambda_{111} = \lambda_{222}$	$\lambda_{112} = \lambda_{212}$	$\mu_1 \neq \mu_2$	3	-255.657	517.314	9.192	0.108	0.108	0.047	0.094	9.95E-07	2.79E-07

15

16 b. Core-group

$\lambda_{111}/\lambda_{222}$	$\lambda_{112}/\lambda_{212}$	μ	df	logL	AIC	Δ AIC	λ_{111}	λ_{222}	λ_{112}	λ_{212}	μ_1	μ_2	
$\lambda_{111}=\lambda_{222}$	\neq	$\lambda_{112}\neq\lambda_{212}$	$\mu_1=\mu_2$	4	-169.942	347.884	0	0.156	0.156	0.068	3.48E-07	7.12E-05	7.12E-05
$\lambda_{111}=\lambda_{222}$	\neq	$\lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	3	-171.109	348.218	0.334	0.156	0.156	0.037	0.037	5.00E-09	5.00E-09
$\lambda_{111}\neq\lambda_{222}$	\neq	$\lambda_{112}\neq\lambda_{212}$	$\mu_1=\mu_2$	5	-169.399	348.798	0.914	0.177	0.129	0.07	1.71E-07	2.96E-07	2.96E-07
$\lambda_{111}=\lambda_{222}$	\neq	$\lambda_{112}\neq\lambda_{212}$	$\mu_1\neq\mu_2$	5	-169.94	349.88	1.996	0.156	0.156	0.068	1.63E-07	3.95E-06	7.70E-08
$\lambda_{111}\neq\lambda_{222}$	\neq	$\lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	4	-171.075	350.15	2.266	0.163	0.15	0.037	0.037	1.90E-07	1.90E-07
$\lambda_{111}=\lambda_{222}$	\neq	$\lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	4	-171.109	350.218	2.334	0.156	0.156	0.037	0.037	7.89E-06	3.14E-06
$\lambda_{111}\neq\lambda_{222}$	\neq	$\lambda_{112}\neq\lambda_{212}$	$\mu_1\neq\mu_2$	6	-169.399	350.798	2.914	0.177	0.129	0.07	4.03E-07	1.43E-06	5.06E-06
$\lambda_{111}\neq\lambda_{222}$	\neq	$\lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	5	-171.075	352.15	4.266	0.163	0.15	0.037	0.037	4.11E-07	2.76E-05
$\lambda_{111}=\lambda_{222}$	=	$\lambda_{112}=\lambda_{212}$	$\mu_1=\mu_2$	2	-179.29	362.58	14.696	0.097	0.097	0.097	0.097	4.00E-09	4.00E-09
$\lambda_{111}=\lambda_{222}$	=	$\lambda_{112}=\lambda_{212}$	$\mu_1\neq\mu_2$	3	-179.291	364.582	16.698	0.177	0.177	0.07	4.00E-06	1.43E-06	5.06E-06

1 Figure 1. Time-calibrated phylogeny of the Ithomiini tribe. Coloured branches depict the partitions
2 identified by MEDUSA and used for fitting diversification rate models. Red lineages constitute the
3 core-group, green lineages the *Melinaea*-group, blue lineages the background tree. Black and white
4 circles indicate the biogeographic ancestral states reconstructed at the basal nodes of the tree:
5 black=Central Andes, white=western Amazonia. Question mark and arrows indicate the position of
6 two alternative scenarios for the first colonization of the Andes: BiogeoBEARS at the root of the
7 Ithomiini, BiSSE at the root of the core-group. Both methods agree for Melinaeina and Mechanitina
8 but diverge for Tithoreina, Methonina and Athesitina. The names and position of the different
9 subtribes are indicated.

10

11 Figure 2. Results of diversification rate estimates through time using MEDUSA's partition. A.
12 Diversification rate estimated using the method designed by Morlon *et al.* ⁽⁶⁰⁾ for the background and
13 the core-group. Results for the *Melinaea*-group are not plotted because of its very high diversification
14 rate. B. Diversity trajectories inferred from the best fitting models of diversification obtained using
15 Morlon *et al.* ⁽⁶⁰⁾'s method. C. and D. Diversification rate and turnover of the background and core-
16 group respectively, estimated using 5 million-year time bins with TreePar ⁸⁰.

17

18 Figure 3. A. Colonization rates and lineage accumulation through time (by speciation) extracted from
19 BiogeoBEARS ancestral state reconstruction for the core-group. Colonization rates correspond to the
20 proportion of transitions compared to the number of lineages existing during each 1-million year
21 interval. The lines depicted are spline lines fitted over 100 reconstructions on the posterior
22 distribution. The blue line is the rate of total interchanges between the Andes and Amazonia, the
23 orange line the rate of colonization from the Central Andes toward the Northern Andes (see
24 Supporting Information S9 for details). Dots represent the additional contribution to lineage
25 accumulation of local diversification in three regions. Red dots represent the cumulative number of
26 divergence events through time in Central Andes; orange dots represent the cumulative number of
27 divergence events through time in Central Andes and Northern Andes; blue dots represent the
28 cumulative number of divergence events through time in Central Andes, Northern Andes and
29 Amazonia; Grey dots represent the cumulative number of divergence events through time in the entire
30 Neotropics. B. Time-dependent speciation rates estimated on seven Amazonian clades (four in the
31 core-group and three in the background lineages). All those clades originated during the last 8 my.

- 1 List of supporting information:
- 2 S1. List of all individuals and species used in this study, and biogeographic distribution of species.
- 3 S2. Gene fragment partitions and substitution models associated obtained by Partition Finder v.1.1
- 4 S3. Node constraints used for time-calibration of the tree.
- 5 S4. Maximum likelihood tree obtained with IQ-tree, with bootstrap support indicated at the nodes.
- 6 S5. Bayesian time-calibrated tree obtained with BEAST v.1.8 with median node ages and 95%HPD
- 7 indicated at the nodes.
- 8 S6. Full results of diversification analyses.
- 9 S7. Testing the effect of diversification rate heterogeneity within the background on diversification
- 10 rate estimates
- 11 S8. Ancestral state reconstruction performed using trait-dependent diversification models
- 12 S9. Rates of colonization between different region, computed on the core-group and used to design a
- 13 time-stratified biogeographic model.
- 14 S10. Results of biogeographic ancestral state reconstruction obtained with BiogeoBEARS and using
- 15 the “null” or time-stratified model.
- 16

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