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Renewed diversification following Miocene landscape turnover in a Neotropical butterfly radiation

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Abstract

Aim: The landscape of the Neotropical region has undergone dynamic evolution throughout the Miocene, with the extensive Pebas wetland occupying western Amazonia between 23 and *c*. 10 Ma and the continuous uplift of the Andes mountains. The complex interaction between the Andes and Amazonia probably influenced the trajectory of Neotropical biodiversity, but evidence from time-calibrated phylogenies of groups that diversified during this period is lacking. We investigate the role of these landscape transformations in the dynamics of diversification in the Neotropical region using a 26-Myr-old endemic butterfly radiation.

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Location: Neotropics.

Time period: Oligocene to present.

Major taxa studied: Ithomiini butterflies.

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

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

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

KEYWORDS

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

1 | INTRODUCTION

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

Notwithstanding the uncertainty about the precise timing and magnitude of surface uplift, the formation of the Andean cordilleras during the Cenozoic broadly shaped Neotropical landscapes and affected diversification in the Neotropics. As the Andes arose, they created new biotic and abiotic conditions along their slopes, modified the climate of the Neotropical region and greatly affected the formation of the Amazonian basin by introducing large amounts of sediment and modifying water drainage (Hoorn et al., 2010). There is increasing evidence that the Andes influenced the diversification of Neotropical lineages, primarily by driving increased speciation rates, perhaps most spectacularly in the high-altitude páramo habitat (e.g., Madriñán, Cortés, & Richardson, 2013). In parallel, the western part of the Amazon basin, which is connected to the Andes, experienced major turnovers of ecological conditions. During the Oligocene, western Amazonia was occupied by a fluvial system flowing northwards (the palaeo-Orinoco basin), which transformed c. 23 Ma into an aquatic system of shallow lakes and swamps episodically invaded by marine conditions, known as the Pebas system (Antonelli, Nylander, Persson, & Sanmartín, 2009; Antonelli & Sanmartín, 2011; Hoorn et al., 2010; Wesselingh et al., 2010,2001). The Pebas was connected to the north with the Caribbean Sea and probably also with the Pacific Ocean through the Western Andean Portal ("WAP"; Antonelli et al., 2009), a low-elevation gap that separated the Central Andes and the Northern Andes until 13-11 Ma. During the late Miocene and during the Andean uplift, the accumulation of sediments combined with a sea level decrease initiated the eastward drainage of the Pebas, and by 10-8 Ma the region had changed into a fluvial system, which then evolved into the modern configuration of the Amazon. More recently, climatic fluctuations during the Pleistocene (2.5-0 Ma) might have led to episodic dryness affecting Amazonian forest habitats and has been proposed as a driver of diversification of the Neotropical biota (Haffer, 1969). The extent of the influence of Pleistocene events and their effects on Neotropical diversification, and even the importance of dryness episodes, is controversial (e.g., Colinvaux, Oliveira, & Bush, 2000; Garzón-Orduña, Benetti-Longhini, & Brower, 2014; Matos-Maraví, 2016; Rull, 2008).

In this study, we focus our attention mostly on the Miocene and Pliocene and how the interaction between the rise of the Andes and coincident large landscape modifications in western Amazonia determined diversification and dispersal over 30 Myr. The Pebas ecosystem covered up to 1.1 million km² at its maximum extent (Wesselingh et al., 2001) and was probably unsuitable for terrestrial fauna dependent on *terra firme* forest habitats. Therefore, between *c*. 23 and 10 Ma, diversification of terrestrial lineages might have been impeded in western Amazonia or restricted to its edge (Wesselingh et al., 2001). In 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 Myr, the retreat of the Pebas potentially provided new opportunities for terrestrial lineages to radiate in lowland western Amazonia. The Pebas might also have constrained dispersal, acting as a barrier between the Andes and Amazonia.

Palaeontological studies have shown that the Pebas contributed greatly to the diversification of the aquatic fauna, including molluscs (Wesselingh, 2006), ostracods (Muñoz-Torres, Whatley, & Harten, 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, Salas-Gismondi, Pujos, Ganerød, & Marivaux, 2017). The hypothesis that the Pebas has shaped patterns of terrestrial diversification and dispersal in western Amazonia has grown over the years (e.g., Antoine et al., 2016; Antonelli et al., 2009; Antonelli & Sanmartín, 2011; Wesselingh et al., 2001; Wesselingh & Salo, 2006), but support from molecular phylogenies mostly stems from the observation that many western Amazonian clades have diversified during the last 10-8 Myr and not before (Antonelli & Sanmartín, 2011; Chazot, Willmott, Condamine, et al., 2016, 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 the 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 Myr). 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 suffered from either a small size or a low sampling fraction (e.g., Chazot, Panara, et al., 2016; Chazot, Willmott, Condamine, et al., 2016; Condamine, Silva-Brandão, Kergoat, & Sperling, 2012; Elias et al., 2009; Hall & Harvey, 2002; Penz, Devries, & Wahlberg, 2012; Price et al., 2014), 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 3.000 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 Heliconius butterflies (Brown & Benson, 1974). For this reason, Ithomiini were used by both Bates (Bates, 1862) and Müller (Müller, 1879) in their original descriptions of deceptive (Batesian) and mutualistic (Müllerian) mimicry, respectively.

The diversity and the intriguing ecology of Ithomiini has generated a great interest among researchers and a broad and diverse literature on topics including life history (Bolaños Martinez, Zambrano Gonzalez, & Willmott, 2011; Hill et al., 2012; McClure & Elias, 2016,2017), chemical ecology (Brown, 1984; McClure et al. 2019; Schulz et al., 2004; Trigo & Brown, 1990), systematics (Brower et al., 2006; Brower, Willmott, Silva-Brandão, Garzón-Orduña, & Freitas, 2014; Brown & Freitas, 1994; Chazot, Willmott, Condamine, et al., 2016; De-Silva et al., 2010,2017; Elias et al., 2009; Mallarino, Bermingham, Willmott, Whinnett, & Jiggins, 2005; Willmott & Freitas, 2006), cytogenetics (Brown, Schoultz, & Suomalainen, 2004; McClure, Dutrillaux, Dutrillaux, Lukhtanov, & Elias, 2018), population ecology (Freitas, 1993,1996), community ecology (Beccaloni, 1997; Chazot et al., 2014; DeVries, Lande, & Murray, 1999; Elias, Gompert, Jiggins, & Willmott, 2008; Hill, 2010; Willmott, Willmott, Elias, & Jiggins, 2017), wing colour pattern evolution (Jiggins, Mallarino, Willmott, Bermingham, & Funk, 2006) and biogeography (Chazot, Willmott, Condamine, et al., 2016; Chazot et al., 2018; Dasmahapatra, Lamas, Simpson, & Mallet, 2010; De-Silva, Elias, Willmott, Mallet, & Day, 2016; De-Silva et al., 2017; Elias et al., 2009).

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

2 | MATERIALS AND METHODS

2.1 | Time-calibrated phylogeny

2.1.1 | Molecular data

We compiled sequences of 1,474 Ithomiini individuals (see Supporting Information Appendix S1.1) that included 718 sequences newly generated for this study and 3,147 previously published sequences (Brower et al., 2006; Chazot et al., 2018,2014; Chazot, Willmott, Condamine, et al., 2016; De-Silva et al., 2010, 2016, 2017; Elias et al., 2009; Mallarino et al., 2005; Whinnett, Brower, Lee, Willmott, & Mallet, 2005). We used a concatenation of nine gene fragments, a mitochondrial fragment spanning genes COI-tRNA-COII, and fragments of nuclear genes $EFI\alpha$, Tektin, CAD, RPS2, MDH and GAPDH, representing a total of 7,083 bp (Wahlberg & Wheat, 2008). Primers and PCR conditions followed Mallarino et al. (2005) and Wahlberg and Wheat (2008). We obtained at least one gene fragment for 340 species out of 393 currently known in the group, which represents 87% of the known species richness of the tribe. For each species, we produced the consensus sequence of all sequences belonging to individuals of that species to obtain the longest sequence possible. We added 41 outgroups, which spanned all Danainae genera in addition to representatives of the main Nymphalidae clades. In total, nine concatenated genes from 381 taxa were used to generate the time-calibrated phylogeny of the Ithomiini.

2.1.2 | Tree topology and time calibrations

We used PartitionFinder v.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) to find the best gene partitioning and substitution models (Supporting Information Appendix S2.1, Table S2.1). Then we generated a phylogeny under maximum likelihood (ML) inference, using IQ-Tree software as implemented in the W-ID-TREE server (Nguyen, Schmidt, Haeseler, & Minh, 2014; Trifinopoulos, Nguyen, Haeseler, & Minh, 2016) in order to obtain a tree topology (Supporting \perp Wiley-

Information Appendix S2.2). Finally, this topology was time calibrated using BEAST v.1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012). We used four secondary calibrations from the phylogeny of Nymphalidae of Wahlberg et al. (2009) that were placed outside of

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Ithomiini, and six secondary calibrations consisting of maximum ages of host-plant (Solanaceae) lineages from De-Silva et al. (2017), and placed on Ithomiini lineages (Supporting Information Appendix S2.3, Table S2.2). We used a birth-death tree prior, and we set an uncorrelated lognormal relaxed clock for each gene partition. We performed two independent runs of BEAST v.1.8.2, which were combined after applying a 15% burn-in. Median branch lengths and credibility intervals were recovered using TreeAnnotator v.1.8.3 (Drummond et al., 2012; Supporting Information Appendix S2.3), and outgroups were pruned to generate an Ithomiini tree with median branch lengths (hereafter, median tree). Full details are given in the Supporting Information (Appendix S2).

2.2 | Diversification rates

2.2.1 | Time

We investigated the dynamics of diversification rates through time and across the phylogeny using two different birth-death models: (a) the method of Morlon, Parsons, and Plotkin (2011), allowing both speciation and extinction rates to vary as a function of time and allowing extinction to be higher than speciation; (b) TreePar (Stadler, 2011), which accommodates models where diversification rates can vary at points in time but are constant between these points. Given that none of these methods can automatically detect the number and position of different diversification processes, we first ran MEDUSA (Alfaro et al., 2009; Supporting Information 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 additional analyses using BAMM v.2.5.0 [Rabosky, 2014], Supporting Information Appendix S3.1.4, Table S3.3, Figure S3.3): (a) one shift at the root of a large clade, hereafter referred to as the core group; and (b) 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 (Supporting Information Appendix S3.1.2). We also explored the effect of potential residual diversification rate heterogeneity in the backbone on the results from the method of Morlon et al. (2011) (Supporting Information Appendix S3.1.5). TreePar (Stadler, 2011) analyses were performed on the core group and the backbone, and diversification rate was estimated in bins of 4 Myr (Supporting Information Appendix S3.1.3). We did not fit TreePar on the *Melinaea* group because it is only 1 Myr 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 the posterior distribution of BEAST for each partition.

2.2.2 | 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 and unpublished (i.e., databases generated from museum collections and our own field collections) georeferenced distribution data and elevation ranges of species (Supporting Information 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 (Beckman & Witt, 2015; Chazot, Willmott, Condamine, et al., 2016; Supporting Information 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 the posterior distribution of BEAST. 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 (Supporting Information Appendix S3.2). Finally, we also conducted ancestral state estimations based on the two best models of trait-dependent diversification, which were compared with the ancestral areas estimated by the historical biogeography analyses outlined below.

2.2.3 | Diversification in Amazonia

We investigated the pattern of diversification in Amazonia further during the post-Pebas period, when speciation in some taxa might have been driven by climatic fluctuations during the Quaternary (Haffer, 1969). To test whether speciation rates had increased in the last 2.5 Myr, we identified seven major Amazonian diversification events from the BioGeoBEARS ancestral state reconstruction (see Historical Biogeography below), and 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) (Supporting Information Appendix S3.3). Analyses were performed on 100 trees randomly sampled from the posterior distribution of BEAST.

2.3 | Historical biogeography

We inferred the historical biogeography of Ithomiini with BioGeoBEARS v.0.2.1 (Matzke, 2014) using the model DEC. We divided the Neotropics into nine distinct biogeographical regions (Supporting Information Figure S4.7): (a) Central America; (b) Caribbean Islands; (c) Iowlands on the Western part of the Andes, including the Magdalena valley; (d) Northern Andes, which consist of the Western and eastern Ecuadorian and Colombian cordilleras and the Venezuelan cordillera: (e) Central Andes: (f) Western Amazonia: (g) eastern Amazonia; (h) Guiana Shield; and (i) Atlantic Forest. We proceeded in three steps. First, we performed an estimation of ancestral ranges using a model with refined area adjacency (unrealistic distributions, such as disjunct distributions, were avoided) but uniform dispersal multipliers (null model: Supporting Information Appendix S4.1). Second, we performed 1,000 biogeographical stochastic mapping replicates to compute rates of dispersal between specific regions per million years (Supporting Information Appendix S4.2). We computed rates of dispersal in the core group between Andean and non-Andean regions, between the Andes and 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 (Supporting Information Figure S4.8). This allowed us to test some biogeographical hypotheses, but also to

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identify relevant time frames among which dispersal probabilities between areas might vary. Third, we implemented a time-stratified model designed from the previous information to refine our biogeographical reconstruction (Supporting Information Appendix S4.3). We created four time frames: 0–4, 4–8, 8–13 and 13–30 Ma, during which dispersal multipliers between areas reflected the colonization rate variations identified previously (Supporting Information Table S4.6). All these analyses were performed on the median tree.

3 | RESULTS

3.1 | Tree topology and time calibration

The topology of Ithomiini phylogeny was generally well supported, including deep nodes (Supporting Information Figure S2.1). The inferred crown age of Ithomiini was 26.4 Ma [95% credibility interval (CI) = 22.75–30.99; Figure 1; Supporting Information Figure S2.2],



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

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and the divergence time from its sister clade Tellervini was 42.1 Ma (CI = 39.50-48.44). All subtribes (10 in total) diverged during the first 10 Myr (Figure 1). Node ages in our phylogeny were generally slightly younger than those inferred by Wahlberg et al. (2009), but older than those inferred by Garzón-Orduña, Silva-Brandão, Willmott, Freitas, and Brower (2015) (for further discussion of such differences, see De-Silva et al., 2017).

3.2 | Diversification rates

3.2.1 | Time-dependent diversification

We partitioned the dynamics of diversification into: (a) the core group, which accounts for *c*. 85% of present-day diversity of Ithomiini; (b) the *Melinaea* group (i.e., the high-diversity clade in the genus *Melinaea*); and (c) the backbone (i.e., the remaining lineages) (Figure 1; Supporting Information Appendix S3).

When fitting the models of Morlon et al. (2011) to the core group, no model of time-dependent diversification had a significantly better fit than the null model of constant speciation rate without extinction (average of 0.257 per lineage/Myr; Figure 2; Table 1; Supporting Information Table S3.4). For the Melinaea group, which radiated into eight species during a time lapse of only 1 Myr, the best-fitting model was an exponentially decreasing speciation rate without extinction, with a very high initial speciation rate (average of 14.54 per lineage/ Myr at the root, average of 0.31 per lineage/Myr at present; Figure 2; Table 1; Supporting Information Table S3.4). On the remaining backbone, the best-fitting model involved a time dependence of both speciation and extinction rates. The resulting net diversification rate was initially high (average of 0.78 at the root), but decreased rapidly and became negative c. 19 Ma, before starting a slow recovery, while remaining negative (average of -0.04 per lineage/Myr at present; Figure 2; Table 1; Supporting Information Table S3.4). This signal of high relative extinction rate was not affected by initial parameters



FIGURE 2 Means and 95% confidence intervals of diversification rates through time estimated on 100 posterior trees partitioned into the backbone, the core group and the *Melinaea* group. (a) Diversification rates estimated using Morlon et al. (2011) for the backbone, the core group and *Melinaea* group (cropped). (b) Diversification rates estimated using TreePar for the backbone and the core group

TABLE 1	Results of time-dependent models of diversification fitted on 100 posterior trees partitioned into the backbone, the core grou
and the Meli	inaea group

	Model	Par	logL	AIC	λ	α	μ	β
Backbone	BVARDVAR	4	-129.75	267.50	0.153	0.160	0.193	0.148
Melinaea group	BVAR	2	-1.58	7.17	0.311	3.772		
Core group	BCST	1	-702.25	1,406.50	0.257			
Joint model		7	-835.032	1,684.065				

Note: Abbreviations: BCST = constant speciation; BVAR = time-dependent speciation; DCST = constant extinction; DVAR time-dependent extinction; par = number of parameters; logL = log-likelihood; α = coefficient of time variation of the speciation rate; β = coefficient of time variation of the extinction rate, λ = speciation rate at present, μ = extinction rate at present.

For each subclade or the backbone, only the best-fitting model is shown (Supporting Information Appendix 3.1.2). Values given are the mean estimated for 100 posterior trees.

for maximum likelihood search or by diversification rate heterogeneity potentially remaining in the backbone (Supporting Information Figure S3.4). Several clades within the backbone show positive net diversification rates during the last 5 Myr, which provides further support to the recovery trend described above.

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

3.2.2 | Diversification in the Andes

For the whole Ithomiini, five ClaSSE models were within an Akaike information criterion (AIC) interval of two. There was no clear consensus across those models, because three of them supported a higher speciation rate in the Andes, whereas the others had equal speciation rates. Two models inferred different dispersal rates but in two opposite ways (Table 2; Supporting Information Table S3.5). This lack of consensus probably reflects the heterogeneity of diversification processes across the Ithomiini. Non-zero extinction rate was inferred in all cases. In the core group, three out of four models within an AIC interval of two had identical speciation rates, and the fourth model had a slightly higher speciation rate in the Andes (Table 2; Supporting Information Table S3.5). Three of those models had identical transition rates between Andean and non-Andean regions, and the fourth one had a higher transition rate towards the Andes. All had very low extinction rates. Overall, the results for the core group converged on similar speciation and transition rates between Andean and non-Andean regions, and low extinction. This interpretation was confirmed by additional analyses, in which we tested the presence of a hidden character using the model HiSSE (Supporting Information Box S3.1; Table S3.5; Figure S3.5).

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

3.2.3 | Diversification in Amazonia

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

3.3 | Historical biogeography

3.3.1 | Biogeographical null model and diversification of the core group

Under the null model (no dispersal multiplier), the ancestral area of the Ithomiini MRCA was unclear (Central Andes + Western Amazonia for the highest probability), and all subtribes except Melinaeina and Mechanitina originated and started diversifying in the Central Andes (Figure 1; Supporting Information Figure S4.9). However, all estimations at the basal nodes of the backbone were highly uncertain.

Although an absence of dispersal events out of the Central Andes characterized the initial Andean phase of diversification in the core group (Figure 3), we found a major peak of interchanges between the Andes and Amazonia 12-8 Ma, entirely driven by colonization from the Andes towards Amazonia (Figure 3; Supporting Information Figure S4.8). After this peak, the rate of dispersal towards Amazonia rapidly decreased through time until c. 4 Ma, when a second peak of interchanges, involving reverse colonizations towards the Andes, occurred (Figure 3). We also recorded a large increase of interchanges between the Central and the Northern Andes after c. 12 Myr (i.e., simultaneously with the increase of dispersal rate towards Amazonia; Figure 3; Supporting Information Figures S4.2 and S4.3). Rates of interchanges between the Central and Northern Andes then remained relatively constant through time, although they increased during the last 3 Myr, owing to dispersal from the Northern Andes towards the Central Andes. Colonization of Central America is likely to have started c. 8 Ma, but interchanges increased mainly during the last 4 Myr (Supporting Information Figure S4.8). Colonizations of the Atlantic Forest also started early (c. 9-10 Ma), but the rate of interchanges between the Atlantic Forest and the remaining Neotropical regions remained relatively constant during the last 10 Myr (Supporting Information Figures S4.2 and S4.3).

Until c. 10 Ma, speciation events occurring in the Central Andes fully accounted for the core group diversification (no dispersal events). During the last c. 10 Myr, however, speciation slowed down in the Central Andes (Figure 3). At the same time, after the peaks of dispersal identified above, Northern Andean and Amazonian lineages started to diversify, although the latter diversified at a slower pace than the former. A large number of dispersal events into the Northern Andes were followed by important local diversification, for example in the genera *Hypomenitis* (17 of 20 species in the phylogeny occur in the Northern Andes) and *Pteronymia* (30 of 45 species in the phylogeny occur in the Northern Andes), or in subclades of the genera *Oleria* or *Napeogenes*. We also identified some

FABLE 2 Results	s of ClaS	SE models fitted	on 100 poste	rior tree	s for whole Ithor	niini tree (a) an	d the core gi	roup (b) sort	ed by increa	asing mean A	AIC		
111/л222	~	112/2212	ㅋ	df	logL	AIC	AIC	1111	1,222	۵112	1212	μ1	μ2
(a) Whole tree													
λ111 ≠ λ222 ×	*	$112 = \lambda 212$	μ1 = μ2	4	-1,064.82	2,137.63	0.00	0.154	0.186	0.052	0.052	0.003	0.003
λ111 = λ222	*	$112 = \lambda 212$	μ1 = μ2	ę	-1,065.89	2,137.79	0.16	0.171	0.171	0.052	0.052	0.004	0.004
λ111 ≠ λ222 ×	*	<pre>112 ≠ \\212</pre>	μ1 = μ2	5	-1,064.66	2,139.31	1.68	0.151	0.188	0.046	0.058	0.002	0.002
λ 111 = λ222 3	*	$112 = \lambda 212$	µ1 ≠ µ2	4	-1,065.70	2,139.39	1.76	0.174	0.174	0.052	0.052	0.020	0.001
λ111 ≠ λ222 ×	*	$112 = \lambda 212$	µ1 ≠ µ2	5	-1,064.73	2,139.46	1.83	0.153	0.192	0.052	0.052	0.001	0.015
$\lambda 111 = \lambda 222$	*	√ 112 ≠ λ212	μ1 = μ2	4	-1,065.88	2,139.76	2.13	0.172	0.172	0.053	0.051	0.004	0.004
$\lambda 111 = \lambda 222$	*	(112 ≠ λ212)	μ1 ≠ μ2	5	-1,065.65	2,141.29	3.66	0.175	0.175	0.049	0.056	0.024	7×10^{-5}
λ 111 ≠ λ222 ÷	*	√ 112 ≠ λ212	μ1 ≠ μ2	9	-1,064.65	2,141.29	3.66	0.151	0.190	0.047	0.057	0.001	0.006
$\lambda 111 = \lambda 222$	=	$112 = \lambda 212$	μ1 = μ2	2	-1,102.44	2,208.88	71.25	0.111	0.111	0.111	0.111	6×10^{-6}	6×10^{-6}
λ 111 = λ222 =		.112 = λ212	μ1 ≠ μ2	ю	-1,102.42	2,210.84	73.21	0.151	0.151	0.151	0.151	0.001	0.006
(b) Core group													
λ111 = λ222	*	$112 = \lambda 212$	μ1 = μ2	с	-877.96	1,757.74	0.00	0.190	0.190	0.059	0.059	4.26×10^{-7}	4.26×10^{-7}
λ111 ≠ λ222 ×	*	$(112 = \lambda 212)$	μ1 = μ2	4	-877.00	1,757.80	0.08	0.168	0.205	0.060	090.0	8.15×10^{-7}	8.15×10^{-7}
λ111 = λ222	*	112 ≠ λ212	μ1 = μ2	4	-877.62	1,759.07	1.33	0.189	0.189	0.082	0.038	4.13×10^{-7}	4.13×10^{-7}
λ111 = λ222	*	112 = 1212	µ1 ≠ µ2	4	-877.96	1,759.74	2.00	0.190	0.190	0.059	0.059	8.11×10^{-6}	6.97×10^{-6}
λ 111 ≠ λ222 š	*	112 = λ212	µ1 ≠ µ2	5	-876.99	1,759.78	2.07	0.168	0.207	090.0	090.0	1.13×10^{-6}	0.003
λ 111 ≠ λ222 ÷	*	(112 ≠ λ212)	μ1 = μ2	5	-874.92	1,759.83	2.09	0.171	0.204	0.068	0.051	1.84×10^{-7}	1.84×10^{-7}
$\lambda 111 = \lambda 222$	*	112 ≠ λ212	µ1 ≠ µ2	5	-875.52	1,761.02	3.26	0.190	0.190	0.084	0.035	0.003	8.37×10^{-7}
λ 111 ≠ λ222 ÷	*	(112 ≠ λ212)	µ1 ≠ µ2	9	-874.88	1,761.75	4.01	0.170	0.205	0.064	0.054	$2.65 imes 10^{-6}$	0.001
λ 111 = λ222 :	~	112 = λ212	μ1 = μ2	2	-905.69	1,815.37	57.63	0.124	0.124	0.124	0.124	1.97×10^{-6}	1.97×10^{-6}
λ111 = λ222	=	112 = $\lambda 212$	µ1 ≠ µ2	ო	-905.51	1,817.02	59.27	0.170	0.170	0.170	0.170	2.65×10^{-6}	0.001
Vote: Abbreviations: /	AIC = Ak	aike information c	criterion score;	ΔAIC=	AIC difference wi	th the best-fittir	ıg model; df ∶	= number of	parameters;	logL = log-lik	elihood; 1 = r	ion-Andean; 2 = Ar	idean; λ111 and

 λ 222 = within-region speciation rates (non-Andean and Andean, respectively); λ 112 and λ 212 = cladogenetic transition rates (non-Andean towards Andean towards non-Andean, respectively); μ = extinction rates. Note: /

Constraints of each model are indicated in the four first columns. Values given are the means, each estimated for 100 posterior trees (Supporting Information Appendix S3.2). The best models (i.e., the model with the lowest AIC and all models with an AIC value within an interval of 2 from that of the best model) are highlighted in bold. FIGURE 3 (a) Colonization rates and lineage accumulation through time (speciation only) extracted from **BioGeoBEARS** ancestral state estimation for the core group. The lines depicted are the median colonization rate after 1,000 biogeographical stochastic mapping (Supporting Information Appendix S4). Dots represent the additional contribution to lineage accumulation of local diversification in three regions as reconstructed on the tree with median node ages. Red open circles represent the cumulative number of speciation events through time in the Central Andes; orange open circles represent the cumulative number of speciation events through time in the Central Andes and Northern Andes; blue open circles represent the cumulative number of speciation events through time in the Central Andes, Northern Andes and Amazonia; grey open circles represent the cumulative number of speciation events through time in the entire Neotropics. (b) Confidence intervals of speciation rates through time estimated on seven Amazonian diversification events. All those clades originated during the last 8 Myr



important transitions to lowland Amazonia, for example at the origin of the *Brevioleria* clade, during early divergence in the genus *Oleria* and in the genus *Hypothyris*.

3.3.2 | Time-stratified biogeographical model

The time-stratified model designed from rates of colonization computed above led to a significant improvement of the model (likelihoods: DECnull: -1,335.802; DECstrat: -1,321.805). Both ancestral state reconstructions were very congruent, but the time-stratified model increased the resolution of several nodes throughout the tree (Supporting Information Figure S4.9). Notably, the time-stratified model greatly increased the resolution of deep nodes in the backbone, estimating that both Mechanitina and Melinaeina originated in Western Amazonia, in agreement with the ancestral state estimations performed with trait-dependent diversification models. The Andean origin for the core group was strongly supported.

4 | DISCUSSION

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

4.1 | Early diversification at the interface of the Pebas and Central Andes

Ithomiini probably started to diversify along the early Andean foothills at the transition with Western Amazonia. The onset of the uplift of the eastern cordillera of the Central Andes during late Oligocene coincides with the origin of Ithomiini (Eude et al., 2015). The backbone Global Ecology

underwent a rapid early diversification, probably after the colonization of South America during the pre-Pebas period; the sister clade of Ithomiini, Tellervini, is found in Australia and Papua New Guinea (Wahlberg et al., 2009). Diversification was perhaps facilitated by an early shift to a new and diverse host-plant family, the Solanaceae, which originated in the Neotropics during the Palaeogene and is particularly diverse in this region (Dupin et al., 2017).

From c. 23 to 10 Ma, the Pebas ecosystem replaced the previous Western Amazonian terrestrial ecosystem. Wesselingh et al. (2001) described the Pebas as an ecosystem "which was permanently aquatic with minor swamps and fluvial influence, and was connected to marine environments", and might have reached a maximum size of 1.1 million km^2 . The presence of fossil marine fishes (Monsch, 1998) and molluscs (Wesselingh, 2006) testifies to the presence of saline waters. More recently, Boonstra, Ramos, Lammertsma, Antoine, and Hoorn (2015) found evidence from foraminifera and dinoflagellate cysts that marine incursions reached 2,000 km inland from the Caribbean Sea during the early to middle Miocene during periods of high sea levels. The extent and duration of these marine influences is controversial (see Jaramillo et al., 2017 and references therein). Yet, it is undeniable that the Pebas system was unsuitable for the late Oligocene terrestrial fauna and flora occupying Western Amazonia, and therefore was likely to affect the timing of diversification and dispersal of Neotropical biodiversity. This scenario is congruent with recent historical-biogeographical analyses of vertebrates (e.g., Hutter, Lambert, & Wiens, 2017) and plant lineages belonging to multiple families (e.g., Machado, Rønsted, Bruun-Lund, Pereira, & Paganucci de Queiroz, 2018; Schneider & Zizka, 2017) and by a recent evaluation of the Amazonian fossil record, which indicated a major decline of terrestrial diversity in western Amazonia during the early and middle Miocene (Antoine et al., 2016): mammalian diversity dropped from 11 orders, 29 families and 38 species during late Oligocene down to one order, two families and two species during middle Miocene (see also Antoine et al., 2017).

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

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

The strongly supported origin of the core group in the Central Andes (19.1–22.1 Myr), parallel to the events occurring in Western Amazonia during the Pebas period, had profound consequences on the pattern of diversification of Ithomiini. First, this early Andean lineage diversified to produce 85% of the total extant diversity of Ithomiini. Second, until *c*. 10 Ma, diversification in the core group occurred exclusively in the Central Andes, and from 19.1–22.1 to *c*. 10 Ma we do not detect a single dispersal event out of the Central Andes. Third, the core group corresponds to a shift of diversification dynamics, characterized by a constant and relatively high diversification rate, which greatly contrasts with the slow and even negative diversification dynamics of the backbone during the same period.

Drivers of diversification in the core group probably include radiation on Solanaceae (Willmott & Freitas, 2006), which were already diverse in the Neotropics during the Miocene (Dupin et al., 2017), in conjunction with a diversity of habitats along the slopes of the Andes. Speciation was also potentially mediated by shifts in mimicry patterns (Chazot et al., 2014; Willmott & Mallet, 2004), which can generate reproductive isolation in mimetic butterflies (Jiggins, Naisbit, Coe, & Mallet, 2001), including Ithomiini (Jiggins et al., 2006; McClure et al. 2019). Those ecological drivers of diversification were probably at play both in the Andes and in non-Andean areas, after the Pebas retreat. Indeed, in the core group, Andean and non-Andean lineages had similar diversification rates. The lack of support for a general increase in diversification rate in the Andes within the core group is also supported by analyses performed independently on different core group subtribes. For example, in both Oleriina (Chazot et al., 2018) and Godyridina (Chazot, Willmott, Condamine, et al., 2016), radiations occurred in both Andean and Amazonian genera.

4.2 | Dispersal out of the Central Andes at the demise of the Pebas

Gentry (1982) pointed out a dichotomy observed in the geographical distribution of Neotropical plant diversity, showing that groups

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could be divided into Andean-centred versus Amazonian-centred patterns. Clades tend to be species rich in one of these centres and relatively species poor in the other. Antonelli and Sanmartín (2011) coined this observation the "Gentry-pattern". They also suggested that in the absence of a barrier between the Andes and the Amazon basin, we should observe continuous interchanges between these regions. Antonelli and Sanmartín (2011) proposed that the Pebas could be this "missing long-lasting barrier needed for creating the disjunction between Andean-centred and Amazonian-centred groups". Therefore, in addition to the constraints on diversification discussed above, we predicted that the Pebas ecosystem should have influenced interchanges towards or across Western Amazonia.

Our results conform remarkably well to the scenario proposed by Antonelli and Sanmartín (2011). Ithomiini are Andean centred, with more than half of their current diversity occurring in the Andes (see also Chazot, Willmott, Condamine, et al., 2016; Chazot, Willmott, Freitas, et al., 2016). Here, we show that interchanges have been virtually absent during the Pebas period, with a period as long as 9-12 Myr without interchanges. However, rates of interchanges from the Central Andes towards the Northern Andes and Amazonia suddenly peaked c. 10 Ma (between 13 and 8 Ma) and more recently (4-0 Ma). Regarding connections between Central Andes and the Northern Andes, the closure of the Western Andean Portal (Antonelli & Sanmartín, 2011) might have allowed multiple colonizations of the Northern Andes facilitated by the presence of connecting higher-elevation habitats. In parallel, between 10 and 8 Ma the Pebas system was progressively drained eastwards, leading to the formation of the present-day configuration of the Amazonian basin and the expansion of terrestrial forest habitats in Western Amazonia (Leite, Paz, do Carmo, & Silva-Caminha, 2017). This corresponds precisely to the timing at which core group lineages colonized Western Amazonia, perhaps again because this region was concomitantly recolonized by Solanaceae, and then diversified.

4.3 | Diversification across the whole Neotropics after the demise of the Pebas

We found a strong dampening of local speciation in the Central Andes during the last 10 Myr. In contrast, colonizations after the retreat of the Pebas were followed by large local bursts of diversification within the Northern Andes and Amazonia. As an illustration, from our biogeographical reconstruction, 69 divergence events occurred strictly in the Central Andes in the core group in the last 20 Myr. Multiple independent dispersal events followed by local diversification also led to 69 divergences occurring strictly in the Northern Andes during the last 9 Myr only. The genera Hypomenitis and Pteronymia, for example, which comprise 23 and 53 species, respectively, diversified extensively within the Northern Andes (Chazot, Willmott, Condamine, et al., 2016; De-Silva et al., 2017). Lineages that dispersed into the Northern Andes and Amazonia after the demise of the Pebas probably accessed a large range of new ecological niches, including a diversity of host plants that had already diversified or that radiated

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concomitantly. We detected a second peak of interchanges between the Central Andes and Amazonia or the Northern Andes. In both cases, these recent peaks of interchanges are clearly driven by reverse dispersal events. Three Amazonian radiations, the genus Melinaea (which comprises the rapidly diversifying Melingea group) and the clade Mechanitis + Forbestra in the backbone and the Brevioleria group in the core group showed increasing speciation rates towards the present. Those radiations may be interpreted as supporting an effect of recent climatic fluctuations during the Pleistocene on the diversification of the Melinaea group (and the Mechanitis + Forbestra clade), although ecological drivers of speciation classically invoked in mimetic butterfly diversification, such as colour pattern and host-plant shifts, cannot be ruled out (Hill et al., 2012; Jiggins et al., 2006; McClure & Elias, 2016, 2017; McClure et al. 2019). Contrasting with those clades, four other Amazonian radiations showed diversification rates decreasing through time, meaning that diversification was highest immediately after the retreat of the Pebas. Recent radiations in Western Amazonia that post-date the Pebas period have repeatedly been reported. For example, in the palm genus Astrocaryum (Arecaceae), the upper Amazonian clade started to diversify only c. 6 Ma (Roncal, Kahn, Millán, Couvreur, & Pintaud, 2013). In Taygetis butterflies, Amazonian lineages show rapid diversification during the last 7-8 Myr (Matos-Maraví, Peña, Willmott, Freitas, & Wahlberg, 2013). Such convergent timing of diversification in Western Amazonia strongly supports the scenario of a post-Pebas recovery of terrestrial habitats, which triggered dispersal followed by local diversification.

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

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AUTHOR CONTRIBUTIONS

N.C. and M.E. conceived the study, with contributions from K.R.W., G.L. and A.V.L.F. All co-authors provided specimens and sequence data. N.C., M.E., F.P.-P., C.F.A. and D.L.D.-S. performed the laboratory work. N.C. performed the analyses. N.C. wrote the paper, with major contributions from M.E. and contributions from all co-authors.

DATA ACCESSIBILITY

All sequences are publicly available on GenBank (accession numbers are given in Supporting Information Appendix S1). Phylogenetic trees are available on TreeBase (http://purl.org/phylo/treebase/phylows/ study/TB2:S23995?x-access-code=85eb5792b9ef59452b163c39e b80ae47&format=html).

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BIOSKETCH

All authors of 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.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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