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Flight over the Proto-Caribbean seaway: Phylogeny and macroevolution of Neotropical Anaeini leafwing butterflies

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ABSTRACT

Our understanding of the origin and evolution of the astonishing Neotropical biodiversity remains somewhat limited. In particular, decoupling the respective impacts of biotic and abiotic factors on the macroevolution of clades is paramount to understand biodiversity assemblage in this region. We present the first comprehensive molecular phylogeny for the Neotropical Anaeini leafwing butterflies (Nymphalidae, Charaxinae) and, applying likelihood-based methods, we test the impact of major abiotic (Andean orogeny, Central American highland orogeny, Proto-Caribbean seaway closure, Quaternary glaciations) and biotic (host plant association) factors on their macroevolution. We infer a robust phylogenetic hypothesis for the tribe despite moderate support in some derived clades. Our phylogenetic inference recovers the genus *Polygrapha* Staudinger, [1887] as polyphyletic, rendering the genera *Fountainea* Rydon, 1971 and *Memphis* Hübner, [1819] paraphyletic. Consequently, we transfer *Polygrapha tyrianthina* (Salvin & Godman, 1868) **comb. nov.** to *Fountainea* and *Polygrapha xenocrates* (Westwood, 1850) **comb. nov.** to *Memphis*. We infer an origin of the group in the late Eocene *ca.* 40 million years ago in Central American lowlands which at the time were separated from South America by the Proto-Caribbean seaway. The biogeographical history of the group is very dynamic, with several oversea colonization events from Central America into the Chocó and Andean regions during intense stages of Andean orogeny. These events coincide with the emergence of an archipelagic setting between Central America and northern South America in the mid-Miocene that likely facilitated dispersal across the now-vanished Proto-Caribbean seaway. The Amazonian region also played a central role in the diversification of the Anaeini, acting both as a museum and a cradle of diversity. We recover a diversification rate shift in the Miocene within the species-rich genus *Memphis*. State speciation and extinction models recover a significant relationship between this rate shift and host plant association, indicating a positive role on speciation rates of a switch between Malpighiales and new plant orders. We find less support for a role of abiotic factors including the progressive Andean orogeny, Proto-Caribbean seaway closure and Quaternary glaciations. Miocene host plant shifts possibly acted in concert with abiotic and/or biotic factors to shape the diversification of Anaeini butterflies.

1. Introduction

The Neotropics are the most biodiverse region on Earth and have thereby captured the attention of biogeographers and macroevolutionary biologists alike (Hoorn et al., 2010; Antonelli and Sanmartín, 2011). Yet, our understanding of the origins of this biota is still fragmentary, although it is believed that abiotic events have had a profound impact on lineage diversification in the region (Hoorn et al., 2010; Antonelli and Sanmartín, 2011; Garzón-Orduña et al., 2014; Bacon et al., 2015a, 2015b; Antonelli et al., 2018a, 2018b).

Two relatively well studied abiotic factors potentially shaping the evolution of numerous Neotropical clades are the Andean and Central American highland orogenies. Geological evidence suggests that present-day surface elevation in the Andes is the result of late Miocene tectonic activity resulting from deformation initiated in the Eocene-Oligocene (see review in Mora et al. (2010)). The rise of the Andes has

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been identified by several studies as a possible driver of diversification (*e.g.*, Antonelli et al., 2009; Elias et al., 2009; Hoorn et al., 2010; Sedano and Burns, 2010; Luebert et al., 2011; Perret et al., 2013; Chazot et al., 2016, 2018; De Silva et al., 2016; Lagomarsino et al., 2016). A global pattern emerging from these studies is the role of the Andean uplift as a species pump through allopatric speciation and ecological opportunity related to elevational gradients (*e.g.*, Willmott et al., 2001; Hall, 2005; Hughes and Eastwood, 2006). Landscape reconfiguration fostered by the orogeny in the Andes but also in Amazonia might have also played an important role in fueling diversification (*e.g.*, Condamine et al., 2012; De Silva et al., 2016). In the north of the Neotropics, the Central American highlands (CAH) stretch from the north of Mexico to Panama. This region is a composite mountainous chain of various temporal and geological origins (Mastretta-Yanes et al., 2015). The oldest block within the CAH is the Sierra Madre Oriental whose origins are tied to the Laramide orogeny, with an emergence in the Cretaceous (*ca.* 70 Ma) and the final stages of the orogeny in the Eocene (*ca.* 40 Ma). Although the exact geological sequence of its elevational gradient is not fully understood, nowadays it has summits up to > 3500 m (*e.g.*, Cerro San Rafael). The Sierra Madre Occidental and Sierra Madre del Sur likely originated in the Eocene with intense tectonic activity up to the Pleistocene (Ferrari et al., 2007), while the Chiapan-Guatemalan highlands were formed in the late Miocene and Pliocene (Manea and Manea, 2006; Mora et al., 2007). The most recent and important geological stage in the emergence of the CAH is the orogeny of the Trans-Mexican volcanic belt from the Miocene to the present (Ferrari et al., 2012). The orogeny of the CAH has been shown to have fueled diversification in several lineages and is likely a key process governing biodiversity assembly in the Neotropics (*e.g.*, Gutiérrez-García and Vázquez-Domínguez, 2013).

The closing of the Proto-Caribbean seaway (*i.e.*, the formation of the Isthmus of Panama) also referred to as Central American seaway (Iturralde-Vinent, 2006; Montes et al., 2015), is another major geological remodeling of the region. It is thought to have had a significant impact on biotic evolution. The closure of the Proto-Caribbean seaway was originally proposed to have occurred some 3 Ma (Kegwin, 1978), and several early models have been proposed for the kinematics of this event (*e.g.*, Pindell et al., 1988; Iturralde-Vinent, 2006). However, a recent debate emerged relative to its timing. A recent geological model based on Eocene zircons found in Colombia and of hypothesized Panamanian origin, suggested that the formation of the Isthmus of Panama was completed in the mid-Miocene *ca.* 13–15 million years ago (Ma) (Montes et al., 2015). A meta-analysis of molecular phylogenies also supported this view of a Miocene land bridge favoring the Great American biotic interchange between Nearctic and Neotropical regions (Bacon et al., 2015a, 2015b; but see Lessios, 2015; Marko et al., 2015). Some recent molecular studies on marine lineages specifically addressing this question also found that vicariant patterns likely resulted from an earlier closure of the Proto-Caribbean seaway (*e.g.*, Stange et al., 2018). O'Dea et al. (2016), providing an extensive review of geological, paleontological, and molecular records, rejected this hypothesis and supported the older hypothesis of a Pliocene closure. Although there is still no current consensus on the exact geological sequence and timing of the Proto-Caribbean seaway closure (O'Dea et al., 2016; Jaramillo et al., 2017a; Molnar, 2017; Jaramillo, 2018), the literature suggests that dispersal between Central America and South America was increasingly facilitated as early as the mid-Miocene by the existence of island chains in the Caribbean seaway and ephemeral land-bridges (Iturralde-Vinent, 2006).

Glaciation cycles during the Quaternary (2.6 Ma–present) are hypothesized to have had a major impact on species formation, by fostering habitat fragmentation and speciation in refugia (Haffer, 1969; Brown et al., 1974; Whitmore and Prance, 1987; Garzón-Orduña et al., 2014; Smith et al., 2014; but see Rull, 2015; Matos-Maraví, 2016). Under this scenario, populations of forest-dwelling lineages would have been separated repeatedly during Pleistocene glacial maxima and would eventually become genetically isolated before secondary contact.

The study of Neotropical butterflies has largely contributed to shedding light on some of the above-mentioned evolutionary mechanisms explaining lineage diversification in the region (*e.g.*, Willmott et al., 2001; Elias et al., 2009; Condamine et al., 2012; Chazot et al., 2016, 2018; De Silva et al., 2016). The widespread subfamily Charaxinae comprises *ca.* 400 mostly tropical butterfly species organized in six tribes, the Old World Charaxini, Pallini and Prothoini, and the New World Anaeini, Anaeomorphini and Preponini, all comprising generally colorful butterflies with sometimes cryptic undersides, that as adults feed on carrion, oozing sap, rotten fruits and dung (DeVries, 1987; D'Abrera, 1988). Although these butterflies are charismatic and their taxonomy mostly well-known, the systematics of Charaxinae have only recently been the focus of comprehensive molecular phylogenetic studies (Aduse-Poku et al., 2009; Ortiz-Acevedo and Willmott, 2013; Toussaint et al., 2015; Toussaint and Balke, 2016; Ortiz-Acevedo et al., 2017). While Preponini recently received some attention (Ortiz-Acevedo and Willmott, 2013; Ortiz-Acevedo et al., 2017) and Anaeomorphini were placed with robust support as sister to Preponini (Espeland et al., 2018), the systematics and evolution of the Neotropical tribe Anaeini (*ca.* 100 described species; Fig. 1) remain to be explored. The phylogenetic relationships among and within its genera are still largely unresolved (but see Wahlberg et al., 2009), although species groups and an intuitive phylogenetic hypothesis were proposed by Comstock (1961).

The taxonomy of the tribe is complex and has changed repeatedly (Rydon 1971; D'Abrera, 1988; Salazar and Constantino, 2001; Salazar, 2008). Here we follow the latest taxonomic arrangement of Lamas (2004) in addition to species and genera described or reinstated since this catalogue (Willmott and Hall, 2004; Choimet, 2009; Dottax and Pierre, 2009; Dias et al., 2012a, 2012b, 2015, 2019; Pierre and Dottax, 2013; Costa et al., 2014). See Table 1 for details on the classification.

Following this arrangement, Anaeini comprises *ca.* 100 described species distributed across the Neotropics, from Andean plateaus (up to 3000 m) to Amazonian lowland forests. These butterflies are also found in Central America and a few species occur in the Caribbean Basin and southern Nearctic. Using anchored phylogenomics, Espeland et al. (2018) revisited the deeper-level phylogenetic relationships and dating among butterflies. In that study, Anaeini was inferred as sister to all other tribes in the subfamily, and the crown of Charaxinae was dated from the Eocene *ca.* 41.5 Ma (95% credibility interval, CI = 30.4–54.0 Ma).

Because Anaeini butterflies are comparatively old, they represent a suitable model to test the impact of the large-scale abiotic changes shaping the geography of the Neotropical region as outlined above. Moreover, this offers the opportunity to also explore other potential drivers of lineage diversification such as host plant associations. Although host plants are not known for all species of the tribe, most caterpillars seem to feed on Malpighiales and particularly on species of the genus *Croton* Linnaeus, 1753 (Malpighiales, Euphorbiaceae, Crotonoideae), whereas only species of *Consul* Cramer, 1776 and some of *Memphis* Hübner, [1819] feed on other plant orders (Laurales and Piperales) (Beccaloni et al., 2008).

Here, we present the first molecular phylogeny of Anaeini and use it to test the putative contribution of various factors to diversification. Specifically, we test the following hypotheses (H):

- H1: The Isthmus of Panama dispersal (IPD) hypothesis where the closing of the Proto-Caribbean seaway would have enhanced the connectivity between Central America, the Nearctic region and South America, with subsequent diversification in these regions. Under this hypothesis, we expect a dynamic biogeographical pattern with most dispersal events between the North Andes and the Central American/Nearctic regions occurring after the mid-Miocene, a period that coincides with the beginning of the Proto-Caribbean seaway closure. We also expect an increase in diversification rate

Fig. 1. Habitus pictures of various Anaeini leafwing butterfly lineages *in natura.* Pictures from Andrew Neild: (a) *Coenophlebia archidona*; (b) *Siderone galanthis*, (c) *Zaretis isidora*; (d) *Consul fabius*; (e) *Anaea troglodyta*; (f) *Fountainea ryphea*, (g) *Memphis verticordia*, (h) *Memphis anna*; (i) *Memphis falcata*.

Table 1
Taxonomic arrangement of the Anaeini according to different authors.

C1961	R1971	SC2001	S2008	L2004
Anaea (*Coenophlebia*)	*	*Coenophlebia*	*Coenophlebia*	*Coenophlebia*
Anaea (*Zaretis*)	*	*Zaretis*	*Zaretis*	*Zaretis*
Anaea (*Siderone*)	*	*Siderone*	*Siderone*	*Siderone*
Anaea (*Anaea*)	*Anaea*	*Anaea*	*Anaea*	*Anaea*
Anaea (*Hypna*)	*Hypna*	*Hypna*	*Hypna*	*Hypna*
Anaea (*Consul*)	*Consul*	*Consul*	*Consul*	*Consul*
Anaea (*Polygrapha*)	*Polygrapha*	*Polygrapha*	*Polygrapha*	*Polygrapha*
Anaea (*Polygrapha*)	*Polygrapha*	*Zikania***	*Prozikania*	*Polygrapha*
Anaea (*Polygrapha*)	*Polygrapha*	*Muyshondtia*	*Muyshondtia*	*Polygrapha*
Anaea (*Polygrapha*)	*Polygrapha*	*Pseudocharaxes*	*Pseudocharaxes*	*Polygrapha*
Anaea (*Memphis*) I	*Memphis*	*Fountainea*	*Fountainea*	*Fountainea*
Anaea (*Memphis*) II	*Memphis*	*Rydonia*	*Rydonia*	*Memphis*
Anaea (*Memphis*) III	*Memphis*	*Annagrapha*	*Annagrapha*	*Memphis*
Anaea (*Memphis*) IVA-B	*Fountainea*	*Fountainea*	*Fountainea*	*Fountainea*
Anaea (*Memphis*) V	*Cymatogramma*	*Fountainea*	*Fountainea*	*Fountainea*
Anaea (*Memphis*) VIA-B	*Cymatogramma*	*Cymatogramma*	*Cymatogramma*	*Memphis*
Anaea (*Memphis*) VIIA-C	*Memphis*	*Cymatogramma*	*Cymatogramma*	*Memphis*
Anaea (*Memphis*) VIIIA-D	*Memphis*	*Memphis*	*Memphis*	*Memphis*

Notes: Comstock's (1961) species groups are indicated with roman numerals and capital letters. C1961 – Comstock (1961), R71 – Rydon (1971), SC01 – Salazar and Constantino (2001), L2004 – Lamas (2004), and S08 – Salazar (2008); *not included in Anaeini **preocc. *Zikania* Borgmeister, 1925 (Diptera).

following the colonization of these regions due to new potential ecological opportunities.

- H2: The Andean and Central American highland diversity pump (ACD) hypothesis, where the uplift of the Andes and subsequent landscape rearrangements would have promoted diversification through allopatric and elevational parapatric speciation in the Andes and Central American highlands. Under this hypothesis, we expect shifts in diversification rate in Andean and/or Central American highland clades contemporaneous with the major phases of the Andean and Central American highland orogeny in the Miocene, and multiple occurrences of allopatric sister species in these mountain ranges.
- H3: The Quaternary glaciation refugia (QGR) hypothesis where glaciation cycles during the Pleistocene would have fostered ecosystem fragmentation and isolation of populations in allopatry therefore fueling speciation in refugia. Under this hypothesis, we expect bursts in diversification in the Pleistocene, with potentially a significant number of Pleistocene allopatric splits across the Neotropics.
- H4: The host plant shift (HPS) hypothesis, where a shift to a different plant order during the evolution of Anaeini would have triggered a diversification rate shift (increase or decrease) as a result of an adaptive process. Under this hypothesis, we expect the clustering of species whose caterpillar feed on the same plant orders, a diversification rate shift synchronous with the estimated ancestral host plant shift, and a statistical correlation between diversification rates and host plant preferences.

2. Material and methods

2.1. Taxon sampling and molecular biology

Legs from freshly collected specimens were removed and preserved in 96% ethanol or sampled from dried museum specimens (Table S1). We gathered all known extant genera and 92 out of *ca.* 102 currently described Anaeini species (*ca.* 90% of the currently described species richness in Anaeini). We also included 10 specimens of infraspecific taxa that are likely valid species based on both molecules and morphology (see Dias, 2013), out of *ca.* 30 taxa that might represent valid species but are currently recognized as infraspecific taxa or are placed in synonymy. Most of these likely good species are located in the genera *Fountainea* Rydon, 1971 (*ca.* 13 putative species) and *Memphis* (*ca.* 8 putative species). In total, our dataset therefore included 102 species of Anaeini out of possibly *ca.* 130 valid species (*ca.* 80% of the currently estimated total species richness in Anaeini).

Although the monophyly of Anaeini is not disputed (Espeland et al., 2018), we included several representatives from the five other described tribes of Charaxinae (Anaeomorphini, Charaxini, Pallini, Preponini and Prothoini) to permit the use of Bayesian relaxed clock molecular dating (see below). The tree was rooted with *Morpho helenor* (Cramer, 1776) based on the unequivocal sister-relationship of Satyrinae with Charaxinae (Espeland et al., 2018).

DNA was extracted using the DNeasy kit (Qiagen, Hilden, Germany). We used the PCR protocols listed in Table S2 to amplify and sequence the following gene fragments: mitochondrial cytochrome *c* oxidase subunit I (CO1, 657 bp) and NADH-ubiquinone oxidoreductase chain 5 (ND5, 420 bp), and nuclear ribosomal protein S2 (Rps2, 423 bp), ribosomal protein S5 (Rps5, 573 bp) and Wingless (408 bp). The DNA sequences were edited and aligned with MUSCLE (Edgar, 2004) in Geneious R11 (Biomatters, http://www.geneious.com/). New sequences were deposited in GenBank (accession Nos. MK850566–MK850817.

2.2. Molecular phylogenetics

We used the maximum likelihood (ML) program IQ-TREE 1.6.7 (Nguyen et al., 2015) to infer phylogenetic relationships using the concatenated matrix. The dataset was initially divided by codon position for each protein coding gene fragment resulting in 15 initial partitions. The best partitioning scheme and models of substitution for each resulting partition were simultaneously selected in IQ-TREE using ModelFinder (Kalyaanamoorthy et al., 2017), with the greedy algorithm and based on the Akaike information criterion corrected (AICc). The optimal models of nucleotide substitution were determined across all available models in IQ-TREE including the FreeRate model (+R, Soubrier et al., 2012), that relaxes the assumption of gamma distributed rates. We conducted 500 tree searches starting from random parsimony topologies to avoid local optima and selected the resulting best ML tree by comparing log-likelihood scores. To assess nodal support, we performed 5000 ultrafast bootstrap replicates (UFBoot, Minh et al., 2013; Hoang et al., 2018) with the best ML tree using the command –bb, and SH-aLRT tests (Guindon et al., 2010) with 5000 replicates using the command -alrt. To reduce the risk of overestimating branch supports with UFBoot due to severe model violations, we used hill-climbing nearest neighbor interchange (NNI) to optimize each bootstrap tree. The UFBoot has been shown to be largely unbiased compared to standard or alternative bootstrap strategies, and the SH-aLRT to be as conservative as standard bootstrap (Minh et al., 2013; Hoang et al., 2018). Because initial analyses recovered inter-tribal phylogenetic relationships that differed from the most comprehensive phylogenomic study of butterflies to date (Espeland et al., 2018), we ran the final analyses constraining the inter-tribal relationships to match the ones of Espeland et al. (2018). In the latter study based on > 350 nuclear exons, Anaeini was recovered as sister to the remainder of Charaxinae, and Anaeomorphini + Preponini was recovered as sister to Prothoini +(Charaxini + Pallini) with maximal nodal support (Espeland et al., 2018). We believe this is the best approach considering the low nodal support in studies that relied on a few loci to estimate inter-tribal relationships within this subfamily (Aduse-Poku et al., 2009; Wahlberg et al., 2009; Ortiz-Acevedo and Willmott, 2013; this study). It is also noteworthy that the monophyly of tribes within Charaxinae and especially of Anaeini was never rejected in any of these studies, therefore even if the monophyly of Anaeini is not tested *per se* in this study, we wish to emphasize that it has never been and is not currently subject to controversy based on molecular and/or morphological grounds. Besides, the analysis of preliminary anchored phylogenomic data confirms the monophyly and intra-subfamilial placement of Anaeini (Toussaint unpublished data).

2.3. Divergence time estimation

Divergence times were inferred in a Bayesian framework with BEAST 1.8.4 (Drummond et al., 2012). As for the phylogenetic analyses, the dataset was initially divided by codon position for each protein coding gene fragment resulting in 15 initial partitions. The best partitioning scheme and models of substitution were then selected in PartitionFinder2 (Lanfear et al., 2017) using the greedy algorithm and the Bayesian Information Criterion across all models included in BEAST (option *models = beast*). We tested different clock partitioning schemes by assigning either (i) a unique uncorrelated lognormal relaxed clock for all partitions; (ii) two uncorrelated lognormal relaxed clocks, one for all mitochondrial partitions and one for all nuclear partitions; or (iii) eight uncorrelated lognormal relaxed clocks, one for each partition. We also tested different tree models by using a Yule (pure birth) or a birth-death model in different analyses. The rates of the uncorrelated lognormal relaxed clocks were set with an approximate continuous time Markov chain rate reference prior (Ferreira and Suchard, 2008). The analyses consisted of 20 million generations with a parameter and tree sampling every 1000 generations. We estimated marginal likelihood estimates (MLE) for each analysis using path-sampling and stepping–-stone sampling (Xie et al., 2011; Baele et al., 2012, 2013), with 1000 path steps, and chains running for one million generation with a log likelihood sampling every 1000 cycles.

Since there is no described fossil of the subfamily Charaxinae available, we relied on the large-scale fossil-based dating framework of Espeland et al. (2018) inferred for the butterfly superfamily Papilionoidea, where the crown of Charaxinae was dated to 41.5 Ma (95% CI = 30.4–54.0 Ma) and the split of Charaxinae and Satyrinae was dated to 63.8 Ma (CI = 48.5–79.5 Ma). Independent studies using different and/or overlapping fossil calibrations investigating the timeline of Nymphalidae evolution found very similar age estimates (Peña and Wahlberg, 2008; Wahlberg et al., 2009). As a result, we constrained the two nodes corresponding to the crown of the subfamily Charaxinae and to the split of Charaxinae and Satyrinae with two uniform priors encompassing the credibility intervals specified above. The tribal relationships were constrained as for the IQ-TREE analyses to avoid inferring unrealistic topologies and violating calibration priors.

2.4. Ancestral range estimation and geological rationale for model design

We inferred the biogeographical history of Anaeini using the R-package BioGeoBEARS 1.1.1 (Matzke, 2018). We conducted the analyses under the Dispersal Extinction Cladogenesis (DEC) model (Ree and Smith, 2008). We used the BEAST Maximum Clade Credibility (MCC) tree from the best analysis (see Results) with outgroups pruned. The distribution of taxa in the Neotropics was extrapolated from the labels of *ca.* 9000 specimens deposited at various institutions (listed in Table S1) and published records (*e.g.*, Comstock, 1961). The estimation of ancestral ranges in Neotropical clades is challenging due to the very dynamic geological history of the landmasses involved. This is even more apparent in clades whose temporal origin predates to some extent the orogeny of the Andes and Central American highlands (*i.e.*, Anaeini leafwing butterflies, see Results), and for which the subsequent definition of biogeographic areas is not trivial.

In this study, we opted for a biogeographic regionalisation based on climatic and geological evidence following the reconstructions presented in different seminal studies focusing on the South American region (*e.g.*, Iturralde-Vinent, 2006; Wesselingh and Salo, 2006; Hoorn et al., 2010; Mora et al., 2010; Mastretta-Yanes et al., 2015; Montes et al., 2015; O'Dea et al., 2016). Therefore, the following areas were used in the analyses: N, Nearctic region corresponding to the lands north of Mexico, L, Central American lowlands from sea level to the Central American highland foothills, H, Central American highlands from their foothills (at about 500–1000 m) to their summits, this arrangement resulting in three disjunct areas, one in northern Mexico, one in southern Mexico, Guatemala, Honduras and Nicaragua, and one between Costa Rica and Panamá, R, the Caribbean archipelago, C, "Chocó" region corresponding to lands west of the Andes south of the Darién gap, from sea level to the Andean foothills, W, west Andean ranges and slopes including all of the western and central Andean mountain ranges draining to the Pacific or the Caribbean, from their foothills to their summits and the eastern Andean mountain ranges draining to the Pacific or the Caribbean, from their foothills to their watershed boundary; E, east Andean slopes (Yungas) including eastern Andean slopes draining to the Amazon and the Orinoco, from their foothills to their watershed boundary; A, the Amazon region and Guianas corresponding to lands east of the Andes draining to the Amazon or the Atlantic north coast, from sea level to the Andean foothills; and T, the Atlantic region including all remaining forested lands draining to the Atlantic east coast.

We compared a null model ignoring geological evolution of the Neotropical region with a designed model taking into account knowledge of the tectonic history of the region with six time slices and differential dispersal rate scalers between the defined areas (Fig. S1). The six time slices were designed as follows; TS1 (root age–34 Ma), corresponding to the early stages of the Andean and Central American highland orogeny and the emergence of the Pozo embayment corresponding to a large marine incursion in present-day western Amazonia connected to both the Atlantic and Pacific Oceans and located east of the low to mid-elevation proto-Andes therefore possibly limiting dispersal between the proto-Andes and eastern Amazonia (Hoorn et al., 2010; Wesselingh and Hoorn, 2011), TS2 (34–32 Ma), corresponding to the existence of the ephemeral Greater Antilles and subaerial Aves Ridge (GAARlandia land bridge) facilitating dispersal between the Caribbean and Neotropical regions (Iturralde-Vinent and McPhee, 1999; Iturralde-Vinent, 2006), TS3 (32–23 Ma), corresponding to the disappearance of the GAARlandia land bridge and Pozo embayment (Iturralde-Vinent and McPhee, 1999; Iturralde-Vinent, 2006; Hoorn et al., 2010; Wesselingh and Hoorn, 2011), associated with reduced connectivity between the Caribbean archipelago and northern South America, and renewed terrestrial connectivity between the Andean and Amazonian regions, TS4 (23–15 Ma), corresponding to the acceleration of the Andean and Central American highland orogenies with the transformation of the paleo-Orinoco drainage system into the Pebas system, a large complex of shallow swamps and wetlands stretching from the Atlantic to the Pacific coasts and submerging most of the western Amazonian region (Wesselingh et al., 2001; Wesselingh and Salo, 2006; Antonelli et al., 2009; Hoorn et al., 2010; Wesselingh and Hoorn, 2011; Jaramillo et al., 2017b), thereby separating the northern Andes and western Neotropics from the rest of South America (Hoorn et al., 2010; Wesselingh and Hoorn, 2011), TS5 (15–7 Ma), corresponding to the appearance of the Acre system dividing the Amazonian region by a large aquatic system, therefore continuing to reduce dispersal between the Andean and Amazonian regions, as well as the early stages of the Proto-Caribbean seaway closure, progressively generating the formation of the Isthmus of Panama and associated with possible strings of islands between Central America and northern South America, facilitating dispersal between these two regions (Bacon et al., 2015a,2015b; Montes et al., 2015; Jaramillo et al., 2017a; Jaramillo, 2018), TS6 (7 Ma–present), corresponding to the disappearance of the Acre system replaced by the current Amazon river drainage system, thereby restoring connectivity between the Andean and Amazonian regions, as well as the full closure of the Proto-Caribbean seaway (*e.g.*, O'Dea et al., 2016), and the formation of present-day Andean and Central American highland landscapes. The dispersal rate scaler values were selected according to terrain and water body positions throughout the timeframe of the group evolution. Dispersal between adjacent areas was not penalized while dispersal between areas separated by water barriers or another area was penalized using a dispersal rate scaler of dr = 0.75 (*i.e.*, a penalty of 0.25 was applied). Penalties were summed as areas were progressively more distant from each other or separated by other barriers. The adjacency matrices were left unconstrained.

2.5. Ancestral altitudinal range estimation

We estimated ancestral altitudinal ranges under the DEC model as implemented in Lagrange (Ree et al., 2005; Ree and Smith, 2008), to take into account the widespread distribution of some taxa (*e.g.*, from low elevation to high elevation). We compiled elevational distribution for each species included in the topology based on our records and the literature (see above). We considered three different altitudinal zones: lowland (L, < 1000 m), premontane (M, 1000–2000 m) and montane (H, > 2000 m), based on our personal observations of Anaeini butterfly altitudinal patterns and vegetation transition zones across the Andes. The adjacency and dispersal rate scaler matrices were left unconstrained.

2.6. Ancestral host plant estimation

We reconstructed ancestral host plant order preferences in a Bayesian framework using the R-package phytools 0.6 (Revell, 2012) based on the threshold model (Revell, 2014), to account for missing host plant data. We used the function *ancThresh* (Revell, 2014), to run a Bayesian Markov chain Monte Carlo (MCMC) analysis under the threshold model. We assumed a Brownian motion model for the

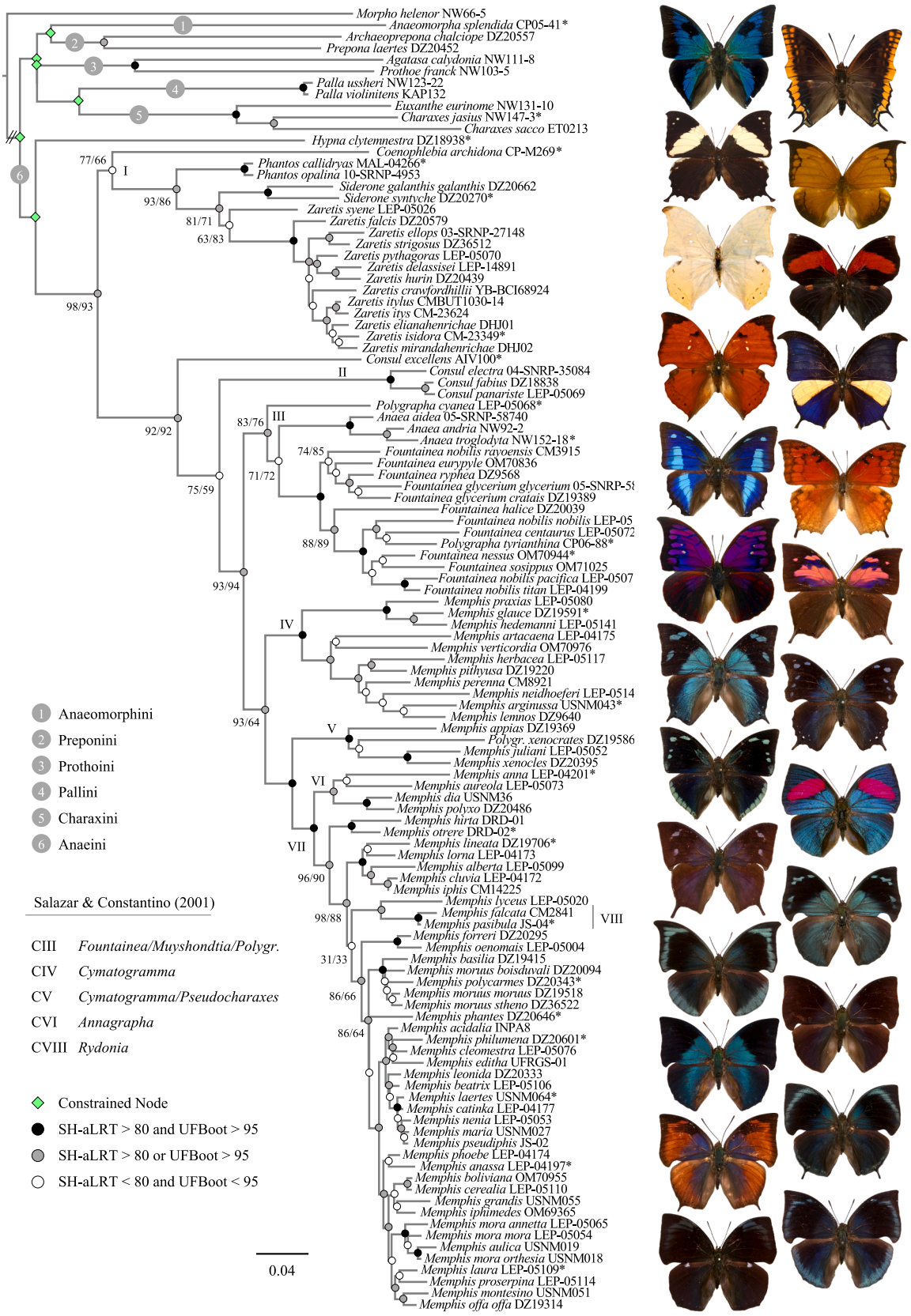

Fig. 2. Molecular phylogeny of Anaeini leafwing butterflies. Maximum likelihood topology of the best-scoring tree inferred in IQ-TREE. Nodal support expressed in SH-aLRT and ultrafast bootstrap (UFBoot) is given as indicated in the caption except for major nodes that are all labelled when SH-aLRT < 80 and/or UFBoot < 95. Habitus of some outgroups and Anaeini species marked with an asterisk are highlighted on the right of the figure and ordered from top to bottom. Picture credits: Emmanuel Toussaint.

evolution of the liability. The latter is an underlying, continuous, unobserved trait used in the threshold model to estimate discrete trait values of taxa included in the phylogeny (see Revell, 2014 for details about this model). The analysis consisted of 1 million generations sampled every 1000 generations, with a final burn-in of 25%. The host plant data was collected from our field notes as well as the literature (DeVries, 1986, 1987; Ackery, 1988; Queiroz, 2002; Beccaloni et al., 2008; Janzen and Hallwachs, 2017). To avoid having to select an ordering for the liability model, we assumed only two host plant states: Malpighiales or Laurales/Piperales (*i.e.*, non-Malpighiales) (Table S1). A prior probability of 1.0 was placed on the state of terminals for which host plant observation data was available. An uninformative prior probability of 0.5 equivalent to a flat prior probability was placed on the state of the terminals for which we did not have host plant information (Revell, 2014).

2.7. Diversification rate analyses

We used the program Bayesian Analysis of Macroevolutionary Mixtures (BAMM) to estimate putative diversification rates among and within clades (Rabosky, 2014). The analyses were conducted in BAMM 2.5.0 with four reversible jump MCMC running for 10 million generations and sampled every 1000 generations. Parameter priors were estimated through the *setBAMMpriors* command in R (*expectedNumberOfShifts* = 1.0; *lambdaInitPrior* = 2.108; *lambdaShiftPrior* = 0.0278; *muInitPrior* = 2.108). We used different values (0.1, 0.5, 1, 2, 5 and 10) for the parameter controlling the compound Poisson process that determines the prior probability of a rate shift along branches of the chronogram (*expectedNumberOfShift*, equivalent to the *poissonRatePrior* parameter in earlier versions of BAMM). Missing taxon sampling was considered by setting the sampling fraction for all major clades of the phylogeny, based on the recognized and presumed species richness of major clades in Anaeini (unpublished data and references hereinabove; see Table S3). The BAMM output files were then analyzed using the R-package BAMMtools 2.1.6 (Rabosky et al., 2014). The posterior distribution of the BAMM analysis was used to estimate the best shift configuration and the 95% credible set of distinct diversification models.

To determine the impact of host plant specialization on the diversification of Anaeini leafwing butterflies, we used the Multiple State Speciation and Extinction (MuSSE; FitzJohn et al., 2009) model implemented in a ML framework in the R-package diversitree 0.9–10 (FitzJohn, 2012). The likelihood function was generated using the function '*make.musse*', and optimized using the function '*find.mle*'. We built a series of models to test whether speciation (λ), extinction (μ), or transition rates (q) between host plant orders (Malpighiales:1, Piperales:2, Laurales:3) were dependent on trait evolution. The models were built by constraining the different parameters ($\lambda 1$, $\lambda 2$, $\lambda 3$, $\mu 1$, $\mu 2$, $\mu 3$, q12, q21, q13, q31, q23, q32) to be free or equal in different combinations. Bayesian MCMC analyses running for 10,000 steps sampled every 100 steps were conducted to estimate posterior distributions of parameters for the best model (see Results). Although MuSSE analyses can provide interesting results relative to trait diversification, caution should be exercised when studying the results of SSE models as emphasized by several recent studies (*e.g.*, Rabosky and Goldberg, 2015; Beaulieu and O'Meara, 2016).

Therefore, we complemented our diversification rate investigation with analyses relying on a hidden state speciation and extinction model (HiSSE; Beaulieu and O'Meara, 2016). The HiSSE model accounts for the presence of unmeasured factors that could impact diversification rate dynamics estimated for the states of the trait under scrutiny (*i.e.*, host plant association). The HiSSE model therefore assumes "hidden" states that are related to each observed state in the model (Malpighiales-feeding and non-Malpighiales-feeding) and exhibit potentially distinct diversification dynamics than the observed states in isolation (Beaulieu and O'Meara, 2016). We used the R-package hisse 1.9.1 (Beaulieu and O'Meara, 2016) to compare different models including the original BiSSE model (Maddison et al., 2007), as well as character-independent diversification models (CID-2 and CID-4). The states that we used were; 0 (association with Malpighiales) and 1 (association with a different host plant order). Dual transitions between both the observed trait and the hidden trait were not included and all transition rates were setup to be equal because they can be difficult to estimate (Beaulieu and O'Meara, 2016).

3. Results

3.1. Phylogenetic relationships

Original unconstrained ML searches inferred poorly supported intertribal phylogenetic relationships, but the resolution within Anaeini was very similar to the one obtained with constrained ML searches. The ML tree searches conducted in IQ-TREE and the BEAST analyses with intertribal relationships constrained, yield very similar estimates with only slight topological changes (Figs. 2 and 3). The phylogeny reconstructed in the best ML tree (LnL = −24101.930) is well resolved and generally presents moderate (UFBoot ≥ 95 or SH-aLRT ≥ 80) to robust (UFBoot ≥ 95 and SH-aLRT ≥ 80) nodal support along the backbone and for major clades within Anaeini, although some of the most derived nodes have lower nodal support (Fig. 2). The BEAST topology inferred under the preferred model (see below) recovers high posterior probabilities (PP ≥ 0.95) along the backbone and for major clades (Fig. 3). Within Anaeini, we recover the monotypic *Hypna* Hübner, [1819] as sister to the rest of the tribe. We also recover *Coenophlebia* Felder & Felder, 1862, *Phantos* Dias 2019, *Siderone* Hübner, [1823] and *Zaretis* Hübner, [1819] as a clade CI with strong support in both IQ-TREE and BEAST analyses. *Coenophlebia* is recovered as sister to *Phantos*, which is in turn recovered as sister to a clade *comprising Siderone* and *Zaretis* (Figs. 2 and 3). *Consul* is recovered as paraphyletic with moderate support in IQ-TREE due to the placement of *C. excellens* (Bates, 1864) outside of clade CII, but as monophyletic with moderate support in BEAST (clade CII, PP = 0.63). In both ML and Bayesian analyses, we recover with strong support a clade CIII comprising the genera *Anaea* Hübner, [1819], *Fountainea* as well as *Polygrapha cyanea* Salvin & Godman, 1868 and *P. tyrianthina* Salvin & Godman, 1868. In clade CIII, *P. cyanea* is found as sister to *Anaea* and *Fountainea*, the latter including *P. tyrianthina* as sister to *F. centaurus* with moderate support in both ML and BI (Figs. 2 and 3). The genus *Memphis* is recovered as monophyletic with four main clades CIV, CV, CVI and CVII inferred with high nodal support (Fig. 2). However, interspecific relationships within clade CVII are moderately to poorly supported in IQ-TREE, albeit more robust nodal support is obtained in the BEAST topology (Figs. 2 and 3).

3.2. Divergence time estimates and ancestral character state estimations

The BEAST dating analysis based on a Yule model and a unique uncorrelated lognormal relaxed clock has a better marginal likelihood than the other ones based on the birth-death model and/or additional uncorrelated relaxed clocks, although divergence time estimates are very similar (Table 2). The preferred BEAST analysis (Fig. 3) recovers an origin of the crown Anaeini in the late Eocene *ca.* 41 Ma (95% CI = 30.3–53.8 Ma) (see Fig. S2 for the full chronogram). The BioGeoBEARS ancestral range estimation analysis with a null model receives a significantly lower likelihood (LnL = −466) than the one implementing dispersal rate scalers based on paleogeographic considerations (LnL = −462.76). Therefore, we present in Fig. 3 the results of the latter analysis (see Fig. S3 for more details). Under the DEC model, the ancestor of Anaeini originated in Central American lowlands. We estimate a major colonization event from Central American lowlands to the Chocó region during the Eocene to Oligocene transition in clade CI, followed by a reverse colonization of Central America in the Miocene (Fig. 3). We also estimate a long-distance dispersal from Central America to the Amazonian region in clade CI followed by a

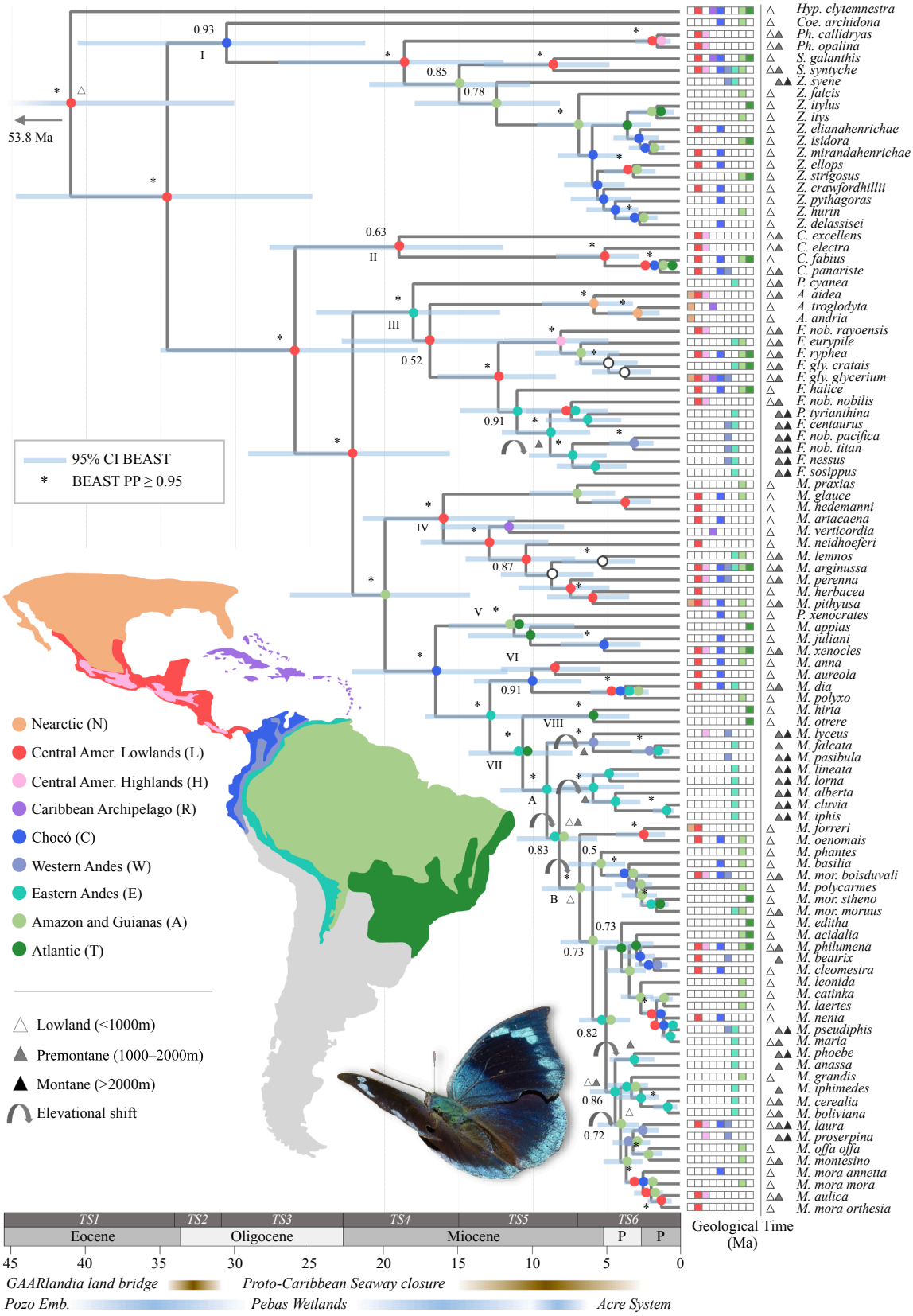

Fig. 3. Divergence time estimates and historical biogeography of Anaeini leafwing butterflies. BEAST chronogram presenting the phylogenetic relationships, median ages and 95% credibility intervals among Anaeini, as inferred under a Yule model with a unique clock and best-fit partitioning scheme. The Bayesian posterior probabilities are given, with asterisks corresponding to PP ≥ 0.95, and unlabeled nodes to PP < 0.95 (the exact PP of major nodes with PP < 0.95 are given). Current geographic and altitudinal distributions of each species are provided on the right side of the figure. The most likely ancestral range for each node is given based on the results of the DEC model as estimated in BioGeoBEARS. Results of the altitudinal DEC model are given at the root and when a shift occurred. All daughter nodes following an altitudinal shift have the same state. Shifts in elevation are highlighted with triangles of the color of the state. A picture of *Memphis anassa* is presented (credit: Andrew Neild).

Table 2
Divergence time estimates estimated in different BEAST analyses.

	Tree model	Clock model	MLE SS	MLE PS	Crown Anaeini
A1	birth-death	1 ULRC	−25233.038	−25232.540	40.613 (29.280–53.055)
A2	Yule	1 ULRC	−24972.673	−25078.023	41.408 (30.335–53.946)
A3	birth-death	2 ULRC	−25185.540	−25185.822	41.609 (30.869–53.085)
A4	Yule	2 ULRC	−25187.276	−25186.645	42.079 (31.349–53.690)
A5	birth-death	8 ULRC	−25226.000	−25224.090	41.034 (29.223–52.929)
A6	Yule	8 ULRC	−25227.394	−25227.095	41.771 (30.763–53.851)

Notes: URLC, uncorrelated lognormal relaxed clock(s); MLE, marginal likelihood estimate based on the analysis of pathLikelihood.delta using either stepping-stone sampling (SS) or path sampling (PS).

Table 3
Results of the BAMM analyses.

Prior nb. Shifts	ESS nb. shifts	ESS LnL	Nb. Config.	Best Config.	Shift Location
0.1	1162.694	813.2825	9 (7, 1 shift/1, 0 shift/1, 2 shifts	1 shift (f = 0.30)	*Memphis*
0.5	2931.252	2120.345	9 (6, 1 shift/1, 0 shift/2, 2 shifts	1 shift (f = 0.29)	*Memphis*
1	3856.962	2695.392	8 (6, 1 shift/1, 0 shift/1, 2 shifts	1 shift (f = 0.35)	*Memphis*
2	3220.482	3518.893	9 (6, 1 shift/1, 0 shift/2, 2 shifts	1 shift (f = 0.33)	*Memphis*
5	2828.662	4129.17	5 (4, 1 shift/1, 0 shift)	1 shift (f = 0.36)	*Memphis*
10	3128.869	3911.6	5 (4, 1 shift/1, 0 shift)	1 shift (f = 0.37)	*Memphis*

Notes: Prior nb. Shifts, prior on the *expectedNumberOfShift* parameter; ESS, effective sample size; LnL, log-likelihood; Nb. config., number of shift configurations in the credibility set; Best config., highest probability shift configuration in the credibility set.

reverse colonization to the Chocó region in the Miocene. In clade CIII, the colonization of Eastern Andes from Central America is followed by a reverse colonization back to Central America in the Miocene (Fig. 3). Further dispersal events allowed the colonization of Central American highlands, Nearctic region and of Eastern and Western Andes in the late Miocene. In clade CIV, the Caribbean archipelago was colonized out of Central America in the Miocene. We estimate a colonization of the Amazonian region from Central America followed by a reverse colonization of the Chocó region in the early Miocene between clades CIV and CV. The colonization of the Chocó region is followed by dispersal into the Andes and back to the Amazonian region in clade CVII. The Atlantic region is mostly colonized in the Pliocene and Pleistocene from the Amazonian region. Multiple reverse colonization events from South America to Central America are estimated in clade CVII throughout the Miocene, Pliocene and Pleistocene (Fig. 3).

Our DEC analyses significantly support lowland elevation (LnL = −170.3) as the most likely ancestral state for the altitudinal preference of the Anaeini ancestor, with premontane (LnL = −177.1) and montane (LnL = −178.0) elevations receiving lower likelihood scores. We recover several late Miocene shifts towards premontane elevation in clades CIII and CVII (Fig. 3). The distribution of species at montane elevation is inferred as the result of very recent range expansions.

The ordering Malpighiales- Piperales-Laurales receives a DIC = 1050.1329 (mean deviance across samples Dbar = 871.6413, mean parameter values from the posterior sample Dhat = 693.1496, effective number of parameters of the model pD = 178.4917), and the ordering Malpighiales-Laurales-Piperales receives a DIC = 1050.4235 (Dbar = 869.9298, Dhat = 689.4360, pD = 180.4938). The results of the ancestral host plant preference optimization using the ordering with the best DIC are given in Fig. 5. We recover Malpighiales as the ancestral host plant of the tribe, with a shift to Piperales in *Consul* (clade CII*). We also recover a shift from Malpighiales to Piperales in clade CVII (Fig. 5). Three late Neogene shifts from Piperales to Laurales are also inferred within *Memphis* (clade CVII).

3.3. Diversification analyses

Regardless of the prior number of rate shift prior specified in the BAMM analyses, all rate shift configurations among the best credible configuration set for all analyses include a shift located on the node following the crown of clade CVII in *Memphis* in the late Miocene, corresponding to a net increase in speciation rate compared to the rest of the tribe (Table 3, Fig. 5). Overall, the net increase in speciation rate in *Memphis* is recovered in all BAMM best shift configurations, as well as in all alternative shift configurations. Other rate shifts are not recovered systematically across analyses. Apart from the speciation rate increase inferred in *Memphis*, we recover a somewhat constant background speciation rate through time, both for this specific clade and other

Table 4
Results of the MuSSE analyses of diversification linked to host plant preference.

	Df	lnLik	AIC	ChiSq	Pr(> |Chi|)	
minimal	3	−337.99	681.97	–	–	
all.lambda.free	5	−326.86	663.72	22.253	0.000	***
all.mu.free	5	−336.01	682.02	3.949	0.139	
all.q.free	8	−331.22	678.44	13.535	0.019	*
all.lambda.mu.free	7	−326.70	667.39	22.58	0.000	***
all.lambda.q.free	10	−319.56	659.11	36.861	0.000	***
all.mu.q.free	10	−327.66	675.33	20.647	0.004	**
all.lambda.mu.q.free	12	−318.94	661.87	38.102	0.000	***
lambda1.free	4	−326.97	661.93	22.041	0.000	***
mu1.free	4	−336.05	680.10	3.872	0.049	*
q1.free	7	−331.55	677.09	12.879	0.012	*
lambda1mu1.free	5	−326.97	663.93	22.041	0.000	***
lambda1q1.free	8	−319.59	655.17	36.803	0.000	***
mu1q1.free	8	−327.73	671.46	20.508	0.001	**
lambda1mu1q1.free	9	−319.58	657.17	36.804	0.000	***
lambda2.free	4	−335.40	678.80	5.169	0.023	*
mu2.free	4	−337.96	683.93	0.043	0.836	
q2.free	7	−334.38	682.77	7.207	0.125	
lambda2mu2.free	5	−334.78	679.57	6.403	0.041	*
lambda2q2.free	8	−320.23	656.46	35.513	0.000	***
mu2q2.free	8	−333.70	683.40	8.569	0.128	
lambda2mu2q2.free	9	−320.23	658.46	35.512	0.000	***
lambda3.free	4	−331.00	670.00	13.975	0.000	***
mu3.free	4	−336.10	680.19	3.778	0.052	.
q3.free	7	−331.22	676.44	13.535	0.009	**
lambda3mu3.free	5	−331.00	672.00	13.977	0.001	***
lambda3q3.free	8	−319.73	655.47	36.503	0.000	***
mu3q3.free	8	−329.31	674.61	17.359	0.004	**
lambda3mu3q3.free	9	−319.73	657.47	36.503	0.000	***

Notes: lambda, speciation rate; mu, extinction rate; q, transition rate; Df, degrees of freedom; lnLik, log-likelihood.

Table 5
Results of the HiSSE analyses of diversification linked to host plant preference.

Model	lnLik	AIC
BiSSE null	−323.2091	654.4182
HiSSE null	−320.2077	650.4154
Host plant dependent	−314.0644	640.1287
Host plant dependent with hidden state	−312.5028	645.0057

Notes: lnLik, log-likelihood; AIC, Akaike Information Criterion.

lineages across the topology (Fig. 5).

The MuSSE analyses support a model in which the speciation rates of and transitions rates between Malpighiales (λ1 and q1) on one hand and Piperales and Laurales (λ2/λ3, q2/q3) on the other hand vary but where extinction rates (μ1/μ2/μ3) are similar (Table 4). In this model (lambda1q1.free, see Table 4), the speciation rate of lineages associated with Laurales and Piperales is faster than the one for linages associated with Malpighiales (λ2 = λ3 > λ1, Fig. 5), confirming the pattern suggested by the *phytools* and BAMM analyses. The HiSSE analyses also confirm this pattern, with the best overall model supporting host plant dependent diversification dynamics (Table 5).

4. Discussion

4.1. Systematics of Anaeini

We present the first comprehensive molecular phylogenetic hypothesis for the tribe Anaeini, with moderate to strong nodal support for its major clades (Figs. 2 and 3). The results are generally in accordance with previous systematic treatments of the tribe, albeit with significant differences in some clades (Table 1, Fig. 2). We recover the monotypic genus *Hypna* as sister to the rest of Anaeini, a relationship previously suggested (Peña and Wahlberg, 2008; Wahlberg et al., 2009; Ortiz-Acevedo and Willmott, 2013). This unique species is widespread in Central and South America, from Mexico to Brazil and into the Caribbean (Fig. 3) and likely represents a cryptic complex in need of an integrative taxonomic revision (see also Röber, 1916; Rydon, 1971). For instance, populations from Mexico likely represent a different species.

In clade CI, we recover the monotypic genus *Coenophlebia* as sister to the genera *Phantos*, *Siderone* and *Zaretis* (Figs. 2 and 3). This placement is at odds with Peña and Wahlberg (2008) and Ortiz-Acevedo and Willmott (2013), who found this genus more closely related to *Anaea*, *Fountainea*, *Memphis* and *Polygrapha*, but in line with Wahlberg et al. (2009) and inferred with robust nodal support in both our ML and BI reconstructions (Figs. 2 and 3). This morphologically conspicuous species is widespread from Colombia to Bolivia and more data is needed to understand the possible genetic differentiation along this latitudinal gradient. The recently erected genus *Phantos* comprising species previously belonging to *Zaretis* (Dias et al., 2019) is recovered as sister to a clade comprising the respectively monophyletic *Siderone* and *Zaretis*.

The genus *Phantos* is now comprised of two species referred to as ghost leafwings and restricted to Central America. These species are morphologically highly distinctive compared to the rest of *Zaretis*, whose species are relatively similar and complex to separate on a morphological basis (Dias et al., 2019). Nevertheless, our dataset only comprises fragments from the mitochondrial gene CO1 (DNA barcodes) for the two species of *Phantos*. Additional data is needed to understand the placement of this genus within Anaeini.

The morphologically highly distinctive genus *Siderone*, with round shaped wings and large red patches on the upper wings, has been the focus of several in depth studies investigating its morphological variability and taxonomy (Dottax and Pierre, 2009; Dias et al., 2015; Pierre and Dottax, 2013). Although morphological variation exists between populations from the Caribbean archipelago, Central America and South America, *Siderone* currently comprises two distinct species and a preliminary molecular study based on DNA barcodes suggests that the taxonomy in place is likely adequate (Dias et al., 2015).

Finally, the genus *Zaretis* was recently the focus of a large-scale DNA barcoding and morphological study that revised its taxonomy in depth (Dias et al., 2019), in continuation of an earlier more traditional morphology-based study (Willmott and Hall, 2004). Our molecular dataset expands the one of Dias et al. (2019), with nuclear loci added for four species, *Zaretis falcis* Dias, Casagrande & Mielke, 2012, *Z. hurin* Dias, 2019, *Z. pythagoras* Willmott and Hall, 2004 and *Z. strigosus* (Gmelin, [1790]). In Dias et al. (2019), topologies inferred with either ML or BI were conflicting with low nodal support across the topologies. Here, we recover very similar topologies between ML and BI inferences despite low nodal support for internal relationships (Figs. 2 and 3). *Zaretis falcis* is recovered as sister to all other species except *Z. syene*. These two species are the most conspicuous taxa in this morphologically homogeneous genus, along with *Z. delassisei* Choimet, 2009 and *Z. pythagoras*. We recover three subclades within *Zaretis*, the first one comprising the sister pair *Z. ellops* and *Z. strigosus*, the second comprising *Z. delassisei*, *Z. hurin* and *Z. pythagoras*, and the last one comprising the rest of the species (Figs. 2 and 3). Phylogenomic data will be needed to address the highly complex taxonomy within *Zaretis* and test species hypotheses considering the moderate genetic distances between some species.

The polyphyly of *Consul*, although moderately supported in the IQ-TREE ML best scoring tree, may also be a methodological artefact caused by *C. excellens* (Bates, 1864), represented in the analysis by a single mitochondrial DNA fragment. Besides, *Consul* is recovered as monophyletic in BEAST albeit with low nodal support (Fig. 3). Despite their superficial morphological dissimilarity, the four species currently recognized in *Consul* are clearly united by genitalic morphological features (Rydon, 1971; Miller and Miller, 1976), but additional studies are needed to understand the true diversity in this genus and the evolutionary mechanisms that lead to such a remarkable wing pattern and shape diversity.

In clade CIII, we recover *Polygrapha cyanea* as sister to the genera *Anaea* and *Fountainea* (Figs. 2 and 3). This unique species found east of the Andes and into the Amazonian region at low to medium elevation is morphologically very different from other species of *Polygrapha*, including *P. tyrianthina* that is recovered nested within *Fountainea* (Figs. 2 and 3). The polyphyly of *Polygrapha* is not surprising and in line with previous studies detailing the different wing-venation, male genitalia, and basal sensory patch of the palps in the four species placed in the genus (Rydon, 1971; Salazar and Constantino, 2001; Salazar, 2008; Salazar and Villalobos, 2016). The genus *Anaea* is inferred as sister to *Fountainea* in our phylogeny, although with moderate support (Figs. 2 and 3). This genus restricted to Central America, the Nearctic region and Caribbean islands, is a known species complex sometimes considered monotypic but within which nine subspecies have already been described, their status varying among authors (Johnson and Comstock, 1941; Lamas, 2004). Based on available barcode evidence (*e.g.*, Miller and Lutman, 2010), the three species we included in our dataset are likely to represent the true species-level richness in the genus, but additional fine-scale studies are needed to investigate species boundaries in *Anaea*, with a dense sampling across the Caribbean islands.

Clade CIII also comprises the comparatively diverse genus *Fountainea* erected by Rydon (1971) to accommodate several species of the genus *Memphis*. We recover two main clades within the genus, including one comprising all the species found at higher altitude, although the relationships among species in each of these clades are moderately supported (Figs. 2 and 3). Interestingly, *Polygrapha tyrianthina* is recovered within this clade of premontane to montane adapted species. This remarkable species was already suggested as being close to the genera *Anaea* and *Fountainea* although its placement was uncertain (Peña and Wahlberg, 2008; Aduse-Poku et al., 2009). In this study, *Polygrapha tyrianthina* is recovered as sister to *Fountainea centaurus* in both ML and BI, albeit with moderate support. This

relationship was hypothesized by Salazar (2014) and Salazar and Villalobos (2016) based on the examination of genitalia and wing patterns of specimens from both of these Andean species. Several subspecies of the widespread species *Fountainea glycerium* and *F. nobilis* have been sampled in this study because they likely represent distinct species, as evidenced for instance by the non-monophyly of *F. nobilis* subspecies (Figs. 2 and 3). Based on the branch lengths and divergence times estimated in this study, we argue that these subspecies are likely valid species and should be the focus of a taxonomic study. Additional future work in *Fountainea* should be based on a comprehensive geographic sampling of all populations within the genus to allow a clarification of its systematics and taxonomy.

Clades CIV, CV, CVI, CVII and CVIII comprise species belonging to the genus *Memphis*, the most diverse lineage within Anaeini, and yet the one for which phylogenetic relationships are virtually unknown, certainly so at the molecular level and perhaps also at the morphological one too (but see *e.g.*, Dias, 2013). Within *Memphis*, we recover several clades that have been recognized as valid genera in other studies (*e.g.*, Salazar and Constantino, 2001) but placed in synonymy by Lamas (2004). For instance, we recover the genus *Cymatogramma* Doubleday, 1849, in two clades, CIV and CV. In clade CIV, we recover *M. echemus* (Doubleday, [1849]), the type species of *Cymmatogramma*, which is treated in our study as a subspecies of *M. verticordia* (Hübner, 1824) following Lamas (2004). In clade CV, we recover *Polygrapha xenocrates* (Westwood, 1850), which was erected by Salazar and Constantino (2001) as a distinct monotypic genus *Pseudocharaxes* Salazar and Constantino, 2001. This species has a large and presumably disjunct distribution in South America, from the Magdalena river valley (type locality of the subspecies *P. xenocrates ernestoi* Salazar and Constantino, 2001) to the Amazonian region (Fig. 3). These two clades CIV and CV include most of the *Croton*-feeding species of *Memphis*, with quite similar immature stages (Dias et al., 2010). The genus *Annagrapha* Salazar and Constantino, 2001, is recovered nested within *Memphis* in clade CVI, including its type species *M. aureola* (Bates, 1866). The small genus *Rydonia* Salazar and Constantino, 2001, comprising only the morphologically remarkable *M. falcata* (Hopffer, 1874) and *M. pasibula* (Doubleday, [1849]) (type species), is recovered nested within *Memphis* and forming a clade CVIII with *M. lyceus* (Druce, 1877), although this relationship needs more scrutiny (Fig. 2).

Among the species that could not be sampled for this study, the recently described *Memphis paulus* Orellana & Costa, 2014 from Venezuela (Costa et al., 2014) is likely related to *M. arginussa* (Geyer, 1832) and *M. perenna* (Godman & Salvin, 1884), which are recovered in clade CIV in our analyses (Figs. 2 and 3). *Memphis xenippa* (A. Hall, 1935) from Colombia, likely belongs to clade CV and is related to *M. juliani* Constantino, 1999 and *M. xenocles* (Westwood, 1850) based on morphology (Constantino, 1999; Dias, 2013). *Memphis wellingi* L. Miller & J. Y. Miller, 1976 from Mexico is possibly related to *M. falcata* and *M. pasibula* (Dias, 2013) despite its superficial resemblance with the other tailless species *M. gudrun* (Niepelt, 1924) (considered a subspecies of *M. offa* H. Druce, 1877 but likely a valid species) as noted by Miller and Miller (1976). *Memphis viloriae* Pyrcz & Neild (1966), from Venezuela and only known from the holotype is possibly related to *M. phantes* (Pyrcz and Neild, 1996). *Memphis salinasi* Pyrcz, 1993 from Venezuela, likely belongs to the *Memphis iphis*-group, a clade of cloud forest-dwelling species comprising *M. alberta* (H. Druce, 1876), *M. cluvia* (Hopffer, 1874), *M. iphis* (Latreille, [1813]), *M. lineata* (Salvin, 1869) and *M. lorna* (H. Druce, 1877) (Figs. 2 and 3). This clade also likely comprises *Memphis moeris* (C. Felder & R. Felder, 1867) from Colombia and Venezuela that could not be sampled in this study (Pyrcz, 1993, Dias, 2013). *Memphis ambrosia* (H. Druce, 1874) as noted by Pyrcz (1995) and Dias (2013), is not particularly close morphologically to *M. phoebe* (H. Druce, 1877) but is possibly related to *M. lyceus*. *Memphis acaudata* (Röber, 1916) is likely closely related to *M. praxias* (Hopffer, 1874), the two species being elevationally disjunct (*M. acaudata* being found at premontane elevation and *M. praxias* in lowlands) but sharing similar morphologies. *Memphis marylena* Choimet, 2009, from Costa Rica, might be a subspecies of *M. aulica* (Röber, 1916) with which it shares a similar habitus and geographic range (Dias, 2013), but molecular data would be needed to test this hypothesis. Finally, the recently described *M. elisa* de la Maza & de la Maza, 2019 from the Sierra Madre de Chiapas in Mexico is closely related to *M. chaeronea* (Felder & Felder, 1861) and *M. xenica* (Bates, 1864), considered by Lamas (2004) and in this study as subspecies of *M. philumena* (Doubleday,[1849]). However, these subspecies were raised as valid species by de la Maza et al., (2019) based on comparative morphology. Preliminary population-level molecular data indicates that the "*M. philumena*-group" likely represents a cryptic complex, but additional data will be needed to test species boundaries in this clade.

Resolving the taxonomy of the genus *Polygrapha* allows to stabilize the classification within Anaeini and allows to reconcile the taxonomy and systematics of the tribe. Therefore, *Polygrapha xenocrates* is here assigned the new combination: *Memphis xenocrates* **comb. nov.**, and *Polygrapha tyrianthina* is here assigned the new combination: *Fountainea tyrianthina* **comb. nov.**

As a result, the genus *Muyshondtia* Salazar and Constantino, 2001 **syn. nov.** is a synonym of *Fountainea* instead of *Polygrapha*. Based on our results, *Polygrapha* is now only comprised of *P. cyanea* and *P. suprema* (Schaus, 1920). The latter is the only species of the genus *Polygrapha* that was not sampled in this study. It was placed in a different monotypic genus; *Zikania* Salazar and Constantino, 2001 but this name is not available because it is preoccupied by a dipteran genus and the replacement name *Prozikania* Salazar, 2008 was proposed instead (Salazar, 2008) (Table 1). Based on a morphological phylogeny, Marconato (2008) placed *P. suprema* close to *P. tyrianthina*, although in this reconstruction *Polygrapha* was recovered as monophyletic, which is clearly not correct (Fig. 1). In Dias (2013), *P. suprema* is found as sister to *P. xenocrates* in some reconstructions based on morphology. Morphological characters do not appear to be phylogenetically informative enough and even the study of genitalic structures does not allow a precise placement of this species within Anaeini (Bonfantti et al., 2013). Additional data is needed to understand the placement of this Brazilian endemic species within Anaeini. Considering the low nodal support of some taxonomically relevant nodes in *Memphis* (Fig. 2) and the lack of clear morphological diagnostic characters, we refrain from making further nomenclatural changes until a more stable phylogeny and a morphological revision of the different clades are available. For example, the validity of *Annagrapha*, *Cymatogramma*, *Rydonia* and the potential description of a new genus for the clade comprised of the distinctive *Memphis hirta* (Weymer, 1907) and *M. otrere* (Hübner, [1825]) will be addressed when more data is available.

4.2. Biogeography and diversification of Anaeini

Anaeini butterflies likely originated in Central American lowlands (Fig. 3), while the Central American highland orogeny was already well advanced (Ferrari et al., 2007; Mastretta-Yanes et al., 2015). This pattern is highly unusual among Neotropical butterflies that consistently present an origin in South America (*e.g.*, Elias et al., 2009; Condamine et al., 2012; Matos-Maraví et al., 2013; Chazot et al., 2016, 2018; DeSilva, 2016, 2017), but could be explained by the greater age of Anaeini compared to clades that have been studied so far. Anaeini colonized other Neotropical regions out of Central America, between the Eocene and Oligocene, when this region and South America were separated by the Proto-Caribbean seaway (Pindell et al., 1988, 2005; Iturralde-Vinent, 2006). The earliest colonization events out of Central America occurred long before the closing of that seaway, regardless of the preferred hypothesis for the timing of this event (O'Dea et al., 2016; Jaramillo et al., 2017a; Molnar, 2017; Jaramillo, 2018).

These results are not surprising for charaxine butterflies, which are able to cross sea straits (*e.g.*, Toussaint and Balke, 2016). Similar cases of crosses before and after the formation of an isthmus have also been

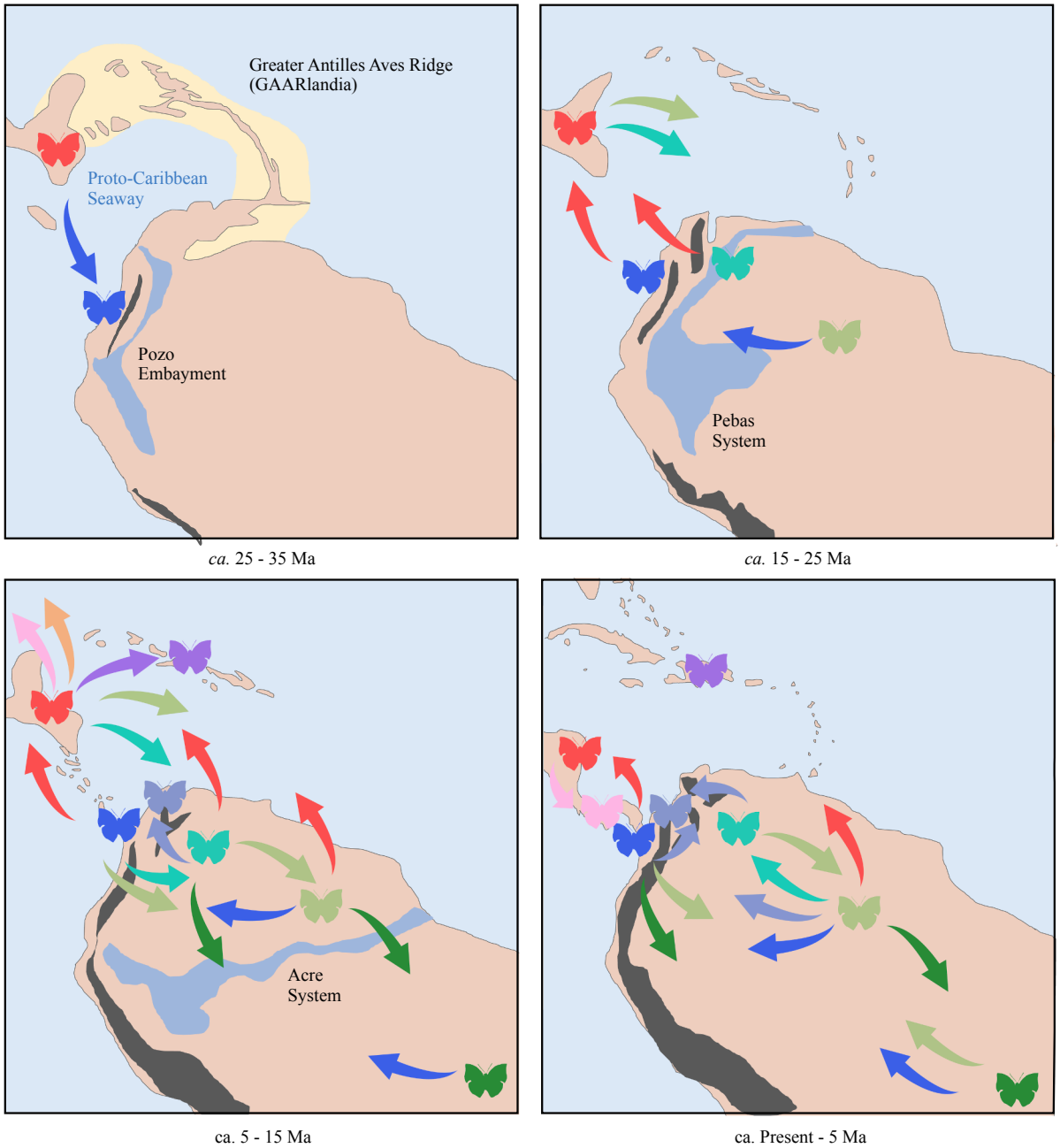

Fig. 4. Proposed scenario for the historical biogeography of Anaeini leafwing butterflies in the Neotropics. Paleomaps drawn based on the combined information summarized in Iturralde-Vinent and MacPhee (1999), Iturralde-Vinent 2006 and Hoorn et al. (2010). The four paleomaps represent landmass and water body positions at the time indicated below each drawing. Dispersal events corresponding to the colonization of new biogeographic regions throughout the evolution of Anaeini are indicated with colored arrows and butterfly shapes. The color of butterfly shapes corresponds to the biogeographic region where their lineages occur, following the coding illustrated in Fig. 3. The color of the arrows corresponds to the color of the biogeographic region that was colonized.

reported for other butterfly groups, both for Central America and other regions (*e.g.*, Vila et al., 2011). We estimate multiple colonization events out of and into Central America in the past 20 Ma, highlighting a very dynamic pattern that seems largely unrelated to the formation of the Isthmus of Panama since we do not recover a major increase in colonization events *ca.* 15 Ma or 4 Ma, but rather multiple dispersal and reverse colonization events in the Miocene and Plio-Pleistocene (Figs. 3 and 4). Our diversification rate analyses do not lend support to the Isthmus of Panama dispersal (IPD) hypothesis, with a unique rate shift found in BAMM in a derived *Memphis* clade (Fig. 5). This part of the tree is comparably poor in dispersal events from or toward Central America and the few events we estimate are more recent than the hypothesized closure of the Proto-Caribbean seaway. Our results suggest that Anaeini butterflies dispersed over the Proto-Caribbean seaway repeatedly during the Cenozoic (Fig. 4), when the progressive closure of this water corridor was accompanied by the continuous existence of a Panamanian archipelago (Iturralde-Vinent, 2006), likely facilitating transcontinental dispersal events (*e.g.*, Bacon et al., 2015a, 2015b; Winston et al., 2017).

The Amazonian region seems to have played an important role in the evolution of the Anaeini, acting both as a region where diversity continuously thrived (museum of diversity), and as a major hub for diversification and dispersal (cradle of diversity) (Figs. 3 and 4). This is not surprising, as the region was highly modified during the Cenozoic by water systems (Pozo embayment, Acre and Pebas systems;

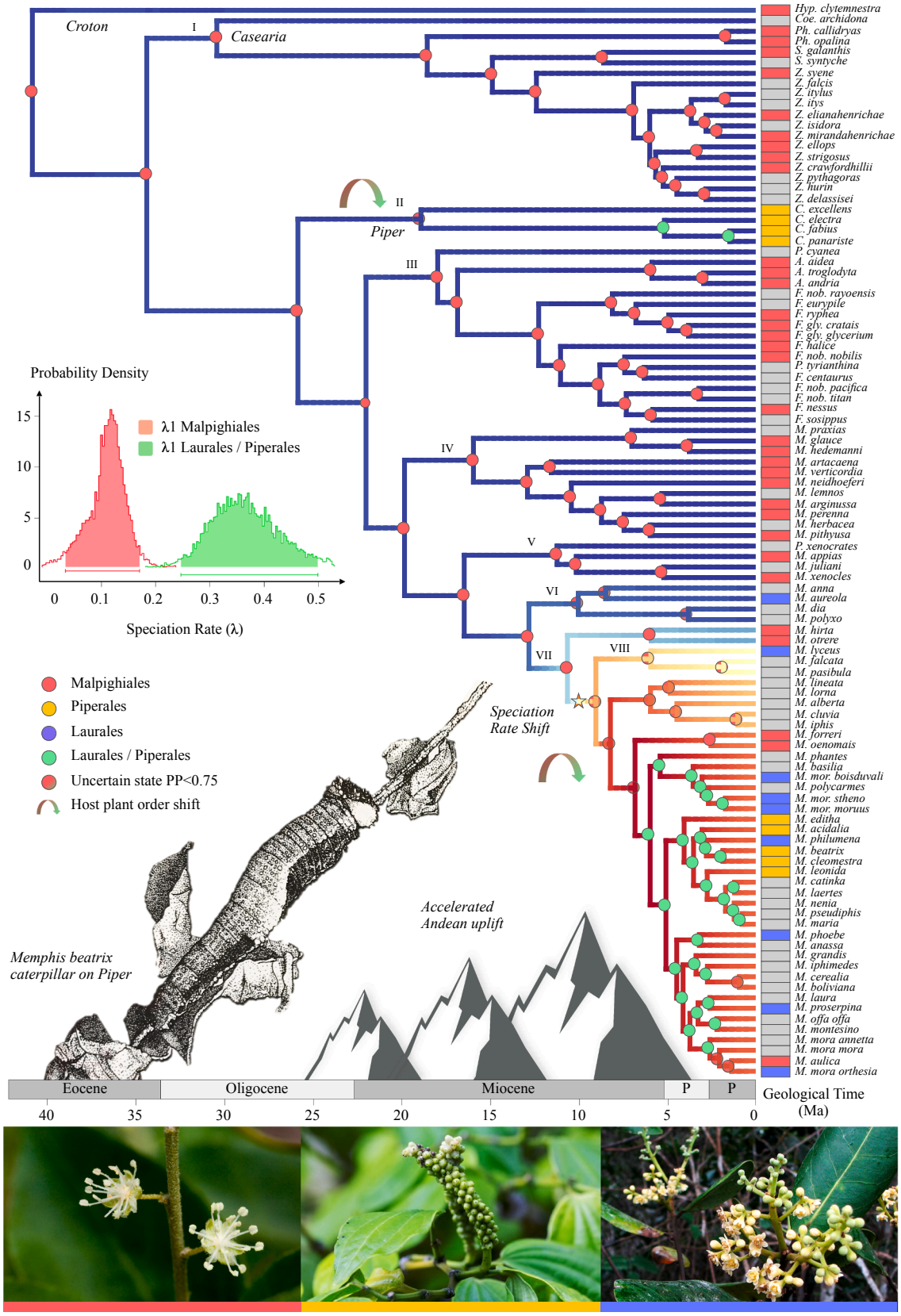

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Fig. 5. Ancestral host plant preference and diversification dynamics of Anaeini butterflies. BAMM phylorate from the analysis with a prior of 1 shift, with branches colored according to speciation rates. The shift recovered in the best BAMM rate shift configuration (1 shift prior, Table 3) is indicated with an orange star. A graphic indicates the speciation rates (λ) of the Anaeini lineages whose caterpillars feed on either Malpighiales or Laurales/Piperales as estimated in the best-fit MuSSE model. The posterior distributions are derived from a MCMC analysis conducted in the R-package diversitree using the best MuSSE model of trait diversification (see Table 4). Ancestral host plant states are mapped onto the tree using colored circles following the inserted caption. The ancestral host plant state with the highest posterior probability is only given when PP > 0.75. Photographs of host plants are given at the bottom of the figure, from left to right; *Croton fruticulosus* (Malpighiales, credit: Patrick Alexander), *Piper amalago* (Piperales, credit: Brent Miller), and *Ocotea lancifolia* (Laurales, credit: Alex Popovkin). A drawing of a 3rd instar caterpillar of the Beatrix leafwing *Memphis beatrix* (H. Druce, 1874) from the book of DeVries (1987) is also presented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Wesselingh and Salo, 2006; Hoorn et al., 2010; Jaramillo et al., 2017b) and by the Andean orogeny, therefore creating opportunities for speciation and extinction to shape the evolution of the group (Fig. 4).

Most dispersal events into the Andean regions (East and West) occurred during the orogeny of the Andes in the Miocene (Fig. 3). This orogeny seems to have been an important process in the biogeographic evolution of Anaeini in particular in clades CIII and CVII. The BAMM analyses recover a unique supported shift in clade CVII of *Memphis*, the latter presenting multiple range shifts from and toward the Andes, however, this clade also comprises several cladogenetic events that appear to be unrelated to the Andean orogeny (Fig. 3). Similarly, we do not recover a diversification rate shift in Clade CIII, where the genus *Fountainea* presents a small Andean radiation. Some widespread species of Anaeini have well-differentiated subspecies not included in this study that could potentially represent valid cryptic species (*e.g., F. halice* (Godart, [1824]), *M. anna* (Staudinger, 1897), *M. xenocles* (Westwood, 1850)), some of them possibly originating from the Andean orogeny. However, other polytypic species represented in the study by only one terminal (*e.g.*, *M. perenna* (Godman & Salvin, [1884]), *M. verticordia*) likely comprise valid closely related species of recent origin, but not related to the Andean orogeny. As a result, we do not recover a pattern supporting the Andean and Central American highland diversity pump (ACD) hypothesis. These orogenies do not appear to have beena crucial mechanism driving Anaeini diversification, unlike in other butterfly lineages (*e.g.*, Elias et al., 2009; Chazot et al., 2016, 2018; De Silva et al., 2016). This is also evidenced by the fact that most Anaeini species are in fact found in lowlands or at medium elevation, and were apparently already lowland-dwelling lineages in the early stages of the diversification (Fig. 3). Surprisingly, our biogeographic estimation does not indicate that the Andes played a major role as a robust barrier either (Figs. 3 and 4). We recover multiple instances of dispersal from regions separated at the time by the Andean orogeny, indicating that Anaeini butterflies were able to find other colonization routes, as they likely did not disperse over the Andes. The widespread distribution of many species resulting from this unique biogeographical pattern might therefore be the result of dispersal over large distances. Anaeini leafwings have been proposed as good dispersers, which could explain this surprising pattern. For instance, a study investigating dispersal capacity in multiple genera of brush-footed butterflies from the Serra da Canastra National Park in Brazil, found that *Fountainea ryphea* could disperse over almost a kilometer over a few days in a fragmented landscape, the record among the species included in the experimental design (Marini-Filho and Martins, 2010).

In our DEC analyses, we recover an ancestral altitudinal preference for Anaeini at lowland elevation. This result agrees with our estimates of an origin for the tribe in Central American lowlands (Fig. 3). The range expansion events toward higher elevations seem to have be very recent and are probably linked to the formation of suitable habitat during the Andean orogeny (Fig. 3). Most species of *Memphis* are currently distributed in lowlands with only some species being more generalist with distributions at mid- and high elevations. Similarly, De Silva et al. (2016) recovered that species of Oleriina (Nymphalidae, Ithomiini) occurring at higher elevations are more recently evolved. This pattern lends some support to the ACD where the orogeny of the Andes would have promoted speciation through the emergence of geographical barriers between populations, as well as by the formation of new habitats and subsequent dispersal and adaptation to them. However, this orogeny-driven mechanism of lineage diversification is limited to few lineages within Anaeini, among higher altitude *Fountainea* and *Memphis* (*i.e.*, *Memphis lineata*-group).

The diversification of Anaeini is not comparatively high in the Pleistocene with respect to older periods, as shown in the diversification analysis (Fig. 5). Most species recognized in the tribe have an origin predating the Pleistocene glaciation cycles and, although a few have speciated recently, our results disprove the Quaternary glaciation refugia (QGR) hypothesis (H3), as a main factor involved in the macroevolution of Anaeini. However, ecosystem fragmentation and isolation of populations in allopatry due to Pleistocene glaciations (Brown, 1974), or more complex ecological and intrinsic biological factors (*e.g.*, Dasmahapatra et al., 2010), might have fostered diversification at the intraspecific level.

Our assessment of the potential influence of three main abiotic factors in the diversification of the Anaeini resulted in rejecting IPD (H1) and QGR (H3), but accepting to some extent ACD (H2) as a potential mechanism explaining a fraction of the diversification dynamics in this tribe. Our diversification rate analyses relative to the association of Anaeini leafwings to their host plants show that Malpighiales were most likely the ancestral host plants of the tribe, with subsequent shifts to Piperales and Laurales. We remain cautious with the ancestral reconstruction performed here, because the host plant of many species remains unknown (Fig. 5). The shift to Laurales and Piperales in *Memphis* is somewhat contemporaneous with the diversification shift recovered in BAMM (Fig. 5), therefore substantiating the HPS (H4). This result is reinforced by the MuSSE and HiSSE diversification analyses, which clearly support a model where lineages specialized on Laurales or Piperales have faster speciation rates than the ones specialized on Malpighiales (Table 4, Fig. 5).

Most species of Malpighiales-feeding Anaeini are associated with the genus *Croton* including *Hypna* (Fig. 5). Notable exceptions are species belonging to the genera *Phantos*, *Siderone* and *Zaretis* which all seem to feed on *Casearia* Jacquin (1760) (Malpighiales, Salicaceae, Samydeae). To this regard, discovering the host plant of *Coenophlebia archidona* (Hewitson, 1860) would be important to understand the transition from *Croton*-feeding to *Casearia*-feeding in clade II (Fig. 5). The genus *Croton*, on which many species of Anaeini feed, is thought to have originated in the Neotropics possibly in the Eocene (*ca.* 55 Ma), therefore slightly predating the origin of Anaeini in the region and potentially allowing them to diversify simultaneously (Van Ee et al., 2008; Haber et al., 2017). The genus *Casearia* is not closely related to *Croton* (Tokuoka and Tobe, 2006), and is likely not monophyletic, rendering the study of its evolution complicated (Samarakoon, 2015; Sun et al., 2016). The origin of its Neotropical lineages possibly dates back to Oligocene (Samarakoon, 2015), which is compatible with the likely shift from *Croton*-feeding to *Casearia*-feeding in clade II (Fig. 5). Species from the genus *Consul* feed on *Piper* L, 1753 (Piperales, Piperaceae, Piperoideae), although the host plant of *C. excellens* is not yet known with certainty (Glassberg, 2018: Glassberg pers. comm.). *Consul* butterflies use different species of *Piper* that are not closely related and belong to different clades recognized by Jaramillo et al., (2008). The genus *Piper* is comparatively old, with a possible split from its sister genus *Peperomia* Ruiz & Pav. in the Cretaceous and a crown age in the late Cretaceous suggesting an ongoing diversification in the Neotropics throughout the Cenozoic

(Smith et al., 2008; Martínez et al., 2015). Unlike with *Croton* and *Casearia*, host plants of the genus *Piper* were therefore available long before the cladogenesis of *Consul*. This shift from Malpighiales to Piperales requires additional scrutiny, especially since our results suggest that a similar shift in *Memphis* possibly fostered higher speciation rates (Fig. 5). Why two synchronous shifts in two different but geographically co-occurring clades did not result in similar diversification dynamics is unclear and possibly linked to inherent ecological, behavioral or developmental features of these two genera. The shift from Malpighiales to Laurales and Piperales recovered in *Memphis* (Fig. 5) is possibly associated with an increase in speciation rates as suggested by our BAMM, MuSSE and HiSSE analyses (Fig. 5). Within *Memphis*, the major genera on which species feed are *Piper* and members of the *Ocotea* complex (Laurales, Lauraceae, Chanderbali et al., 2001), among which *Aniba* Fusée-Aublet, 1775, *Endlicheria* Nees von Esenbeck, 1833, *Nectandra* Rolander, 1778 and *Ocotea* Fusée-Aublet, 1775. As mentioned above, *Piper* host plants were present long-before *Memphis* species shifted from Malpighiales to these plants. However, the *Ocotea*-complex might have an origin *ca.* 20 Ma in the early Miocene (Chanderbali et al., 2001; Nie et al., 2007) or even in the mid-Miocene (Massoni et al., 2015). The emergence of the *Ocotea*-complex in South America might therefore have been synchronous with the diversification of *Memphis* leafwings (Fig. 5). The shift from Malpighiales to other host plant orders in clade CVII may have been the result of climatic conditions intermittently changing the floristic composition of biogeographic regions, the result of adaptation from the butterflies to feed on and process different host plants, or a combination of both these mechanisms. Several species of *Croton*, *Piper* and *Ocotea* are known to host toxic chemical compounds, and some have even been used as insecticides (Rafael et al., 2008; Mossi et al., 2014). Perhaps the different levels of toxicity of these plants and the evolutionary pathways involved in the establishment of this toxicity might have allowed the evolution of a key innovation in *Memphis* that seem to be more generalists than other members of Anaeini and could have developed novel physiological innovations to cope with different plant defenses (Ehrlich and Raven, 1964; Janz, 2011; Jousselin & Elias, 2019). Teasing apart whether this host plant driven diversification is linked to classical hypotheses such as the escape and radiate hypothesis (Ehrlich and Raven, 1964), oscillation hypothesis (Janz and Nylin, 2008) or musical chairs hypothesis (Hardy and Otto, 2014) will require a more robust phylogeny and additional host plant records as well as a better understanding of toxicity among the various host plants these butterflies feed upon.

We also propose that this host plant shift might have triggered speciation to new adaptive zones and promoted an adaptive radiation in *Memphis*, such as the one observed in butterflies in the genus *Adelpha* Hübner, 1819 (Ebel et al., 2015). Species of *Croton*, *Casearia*, *Piper* and, to a lesser degree, *Ocotea* (the main host plant genera for the tribe), are ubiquitous in most Neotropical forests. The fact that these host plants are widely distributed, abundant and species-rich, may facilitate the dispersal of taxa to new areas, where they may or may not compete with other related Anaeini species. Specialization in one species or group of species of host plants might be a mechanism to avoid competition. This seems to occur for instance in species of *Zaretis* specializing in different *Casearia* species in Central America (Janzen and Hallwachs, 2017). A similar process describing the diversification of moths as a function of available *Piper* species in the Neotropics was proposed for moths in the genus *Eois* Hübner, 1818 (Geometridae) (Strutzenberger and Fiedler, 2011; Jahner et al., 2017).

In addition to the impact of host plant preferences, we suggest that the turnover in biome composition across the Cenozoic likely affected the distribution and diversification of Anaeini butterflies. Although there is strong evidence that closed-canopy tropical rainforests were widespread in the Neotropics as early as the late Paleocene to early Eocene (Burnham and Johnson, 2004; Jaramillo et al., 2006), the extent of landscape reconfiguration throughout the Cenozoic is unclear. It is possible that changes in the composition of rainforests, their productivity and geographic extent from the Eocene until today shaped the geographic ranges of Anaeini and governed their evolutionary trajectory. Unfortunately, it is not trivial to infer diversification patterns in a paleoenvironmental framework to test for instance the time-integrated area hypothesis (*e.g.*, Fine and Ree, 2006; Jetz and Fine, 2012), that is certainly relevant to a majority of lineages associated with particular biomes across the planet. New models are emerging, that allow integrating past climatic or bathymetric data into diversification inferences (*e.g.*, Condamine et al., 2013). Integrating the evolution of biomes into historical biogeography and other macroevolutionary analyses represents the future of the field and efforts should be made to broaden our understanding of landscape reconfigurations throughout the Cenozoic in overwhelmingly diverse regions such as the Neotropics.

4.3. Conclusions

We infer a new and well-resolved phylogenetic hypothesis for the tribe Anaeini that represents the foundation for future systematic and ecological studies. Although the evolution of this clade is obscured by its comparatively ancient origin, we propose that this clade of leafwing butterflies originated in Central America and later colonized neighboring biogeographic regions with a very dynamic and highly complex biogeographic scenario (Fig. 4). Our results indicate that this evolution was only moderately affected by the different abiotic factors that governed the assemblage of the Neotropics since the Eocene (Figs. 3, 4 and 5). We present evidence for a role of both one biotic (host plant shifts) and several abiotic factors (the Andean orogeny and Amazonian cradle/museum of diversity) on the diversification of Anaeini. This combination of both types of factors is likely at work in most lineages, although their respective contribution certainly varies depending on the dispersal capacity of the organisms. Species of Anaeini are usually good and fast fliers (DeVries, 1987; Pyrcz and Neild, 1996; Marini-Filho and Martins, 2010). Their high dispersal ability plays an important role in shaping distributions, as demonstrated by the existence of quite recently evolved species with pan-Neotropical distribution, but also possibly on the modes of speciation. Since good dispersers, like most butterflies, are less affected by physical barriers, ecological speciation may be over-represented in such lineages, as they might expand their ranges more frequently and thus establish populations in areas with different ecological properties, thus fostering the evolution of new ecological niches. Decoupling the relative contributions of all the factors involved in diversification requires comparative macroevolutionary studies that shed light on the interplay between environment and organisms. Special attention needs to be directed to the fact that the ecological features of a lineage are both cause and consequence of diversification.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.04.020.

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