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Explosive Cenozoic radiation and diversity-dependent diversification dynamics shaped the evolution of Australian skipper butterflies

EMMANUEL F.A. TOUSSAINT FLS[1,2,*], MICHAEL F. BRABY[3,4], CHRIS J. MÜLLER[5], KELLY M. DEXTER[2], CAROLINE STORER[2], DAVID J. LOHMAN[6,7,8], AKITO Y. KAWAHARA[2]

[1]Natural History Museum of Geneva, CP 6434, CH 1211 Geneva 6, Switzerland
[2]McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA
[3]Division of Ecology and Evolution, Research School of Biology, The Australian National University, Acton, ACT 2601, Australia
[4]The Australian National Insect Collection, GPO Box 1700, Canberra, ACT 2601, Australia
[5]Australian Museum, 6 College Street, Sydney, NSW 2010, Australia
[6]Biology Department, City College of New York, City University of New York, 160 Convent Avenue, New York, NY 10031, USA
[7]Ph. D. Program in Biology, Graduate Center, City University of New York, 365 Fifth Avenue, New York, NY 10016, USA
[8]Entomology Section, National Museum of Natural History, Manila 1000, Philippines

[*]Corresponding author. E-mail: Emmanuel.toussaint@ville-ge.ch

Australia was predominantly tropical for most of the Early Cenozoic, then transitioned to a cooler and drier climate in the Oligocene. In response to this increasing aridity, some lineages either adapted to xeric ecosystems, contracted to increasingly fragmented mesic refugia, or went extinct. However, the lack of macroevolutionary studies at a continental scale precludes a better understanding of Australian biodiversity patterns and processes during the Cenozoic. Here, we infer a robust, dated phylogenomic tree for a radiation of Australian endemic butterflies, the Trapezitinae skippers, to test the impact of biotic and abiotic drivers on Cenozoic diversification dynamics in Australia. These butterflies originated during the Eocene (*c.* 42 Mya) in the mesic biome of Australia. Trapezitinae exploded in diversity during a cool, dry period in the Late Oligocene and Early Miocene, then experienced a sharp deceleration in speciation. Xeric ecosystems appear to have been colonized more recently, supporting the hypothesis of arid and semi-arid biomes as evolutionary sinks. Temperature-dependent and phytophagy-dependent diversification models received little support. Instead, we find evidence for diversity-dependent processes with declining diversification in Trapezitinae likely linked to limited ecological opportunities following a rapid initial burst of diversification.

ADDITIONAL KEYWORDS: Australian biogeography; biome shifts; butterfly evolution; Hesperiidae; Lepidoptera; target-capture phylogenomics; Trapezitinae.

INTRODUCTION

Major progress has been made towards better understanding Australian biogeographic and evolutionary diversity patterns (Byrne *et al.*, 2008, 2011, 2018; Bowman *et al.*, 2010). However, studies examining biotic and abiotic drivers at the continental scale remain scarce, particularly for invertebrates. Ongoing development of model-based comparative methods and the greater availability of robust phylogenetic timetrees enable discrimination of the potential impacts of many factors on diversification dynamics. Australia was relatively geologically stable compared to other areas of the world in the Cenozoic, but has experienced dramatic climate shifts in the past 40 Myr (Weston *et al.*, 2017). From the Eocene to the Oligocene, Australia was a warm and humid tropical landmass with flourishing forests and abundant water bodies (Martin, 2006; Byrne *et al.*, 2008, 2011; Weston *et al.*, 2017). The final separation of the Australian landmass from southern Gondwana (Antarctica-South America) approximately 35–30 Mya was the first step of this progressive climate change. Following this break-up and the continuous expansion of the Tasman Seaway, the Antarctic Circumpolar Current established and contributed to decreased regional temperatures

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(Pillans, 2018). This cooling was intensified by the simultaneous global decrease in atmospheric CO_2 levels associated with the Early Oligocene glacial maximum (Zachos *et al.*, 2008). As a result, mesic forested biomes contracted and became progressively fragmented, giving rise to vast sclerophyllous and grassland ecosystems (Martin, 2006; Byrne *et al.*, 2008, 2011; Weston *et al.*, 2017). Although these more arid-adapted floral elements existed during the Eocene, they became more widespread in the Oligocene, before becoming dominant in the Miocene with the expansion of *Casuarina* and *Eucalyptus* ecosystems and the development of open woodlands and shrublands (Martin, 2006; Byrne *et al.*, 2008, 2011). A climatic pulse in the Pliocene briefly allowed more mesic ecosystems to back-colonize some regions. This was quickly followed by an increasingly drier climate starting in the Pleistocene and extended to the present with the emergence of truly arid environments (Byrne *et al.*, 2008). Because Australia harboured mostly mesic ecosystems up to the Oligocene-Miocene, it has been proposed that most ancestral lineages were likely mesic-adapted and later colonized more arid ecosystems (Byrne *et al.*, 2008, 2011). Although some lineages adapted to the new xeric ecosystems, others remained in increasingly fragmented mesic refugia. The isolation of these refugia might have led to allopatric diversification (e.g. in Southwest Australia, the Pilbara, Kimberley and Top End regions), fostered colonisation of surrounding landmasses where mesic conditions persisted (e.g. New Guinea, Solomon Islands) or caused extinction (Byrne *et al.*, 2008, 2011).

Numerous studies have investigated the drivers of diversification of Australian lineages at local or regional scales, particularly in the Australian monsoon tropics, Australian Arid Zone and Australian Wet Tropics (Bell *et al.*, 2007; Bowman *et al.*, 2010; Pepper *et al.*, 2014; Moreau *et al.*, 2015; Rix *et al.*, 2015, Boyer *et al.*, 2016; Oliver *et al.*, 2019). At the continental scale, few studies have provided insights into understanding Australian biodiversity assembly through space and time (e.g. Rix & Harvey, 2012; Marin *et al.*, 2013; Toussaint *et al.*, 2015; Braby *et al.*, 2020; Rix *et al.*, 2021). As a result, evolutionary questions pertaining to ancestral colonization and diversification in mesic vs. arid biomes, the role of climatic refugia and global responses to climate or floristic change on herbivorous lineages remain open.

Among Australian butterflies, few groups are both largely endemic and comparatively species rich, thereby being good candidates to study the above-mentioned questions (Kodandaramaiah *et al.*, 2018; Braby *et al.*, 2020). One such group is the Trapezitinae skippers, largely endemic to Australia except for a few species found in New Guinea. The 79 described species are small butterflies feeding mostly on Poaceae, although a few species feed on other monocot families including Asparagaceae, Cyperaceae and Iridaceae (Braby, 2000). A recent phylogenetic study (Toussaint *et al.*, 2022) suggested a rapid radiation near the crown of the group, but robust estimates for both the phylogeny and divergence times are lacking. Because of their continental distribution, herbivorous diets and potentially explosive diversification, these skippers are an ideal candidate to test historical biogeographic hypotheses about evolution of the Australian biota. In this study, we use a target sequencing approach to generate data for a robust, dated phylogenomic tree of Trapezitinae, estimate ancestral ranges, and test the impact of biotic and abiotic drivers on the macroevolutionary history of this lineage in Australia and New Guinea.

MATERIAL AND METHODS

Taxon sampling and DNA sequencing

We obtained hybrid enrichment sequence data from 70 Trapezitinae species collected in the field or sampled from museum collections. This dataset is significantly more comprehensive than the one used in Toussaint *et al.* (2022) that was based on a selection of five gene fragments. We also added whole-genome data from two additional species, and added *COI* barcodes from four additional species. These 76 described species represent *c.* 96% of the species richness in the subfamily and includes all genera and species-groups. Three rare New Guinea endemics *Prada maria*, *Prada papua* and *Toxidia arfakensis* could not be sampled. Taxa representing all other described subfamilies as well as three Hedylidae and one Nymphalidae were selected as outgroups (Supporting Information, Table S1).

DNA was extracted using an OmniPrep DNA extraction kit (G-Biosciences, St Louis, MO, USA). Quantified DNA extracts were submitted to RAPiD Genomics (Gainesville, FL, USA) for library preparation, hybridization enrichment and sequencing. Random mechanical shearing of DNA was conducted with an average size of 300 bp, followed by an end-repair reaction and ligation of an adenine residue to the 3'-end of the blunt-end fragments to allow ligation of barcode adapters and PCR-amplification of the library. Following library construction, solution-based anchored hybrid enrichment (AHE) of Agilent SureSelect probes (Santa Clara, California, USA) was conducted in a pool containing 16 libraries. These libraries were enriched with the SureSelect Target Enrichment System for Illumina Paired-End Multiplexed Sequencing (Santa Clara, California, USA). Paired-end 150-bp reads were sequenced from each library on an Illumina HiSeqsequencing system.

We used the BUTTERFLY2.0 probe set (Kawahara *et al.*, 2018) to capture 13 gene regions including those often used in studies on butterfly phylogenetics, and the BUTTERFLY1.1 probe set (Toussaint *et al.*, 2018) to capture 383 gene regions including all loci captured with the BUTTERFLY2.0 probe set. The final matrix comprised 111 taxa including 76 described Trapezitinae species and up to 383 concatenated loci totalling up to 164 099 aligned nucleotides (Supporting Information, Table S2).

AHE data assembly and clean-up

The bioinformatic pipeline allowing to generate the final phylogenomic datasets from the raw Illumina reads followed Breinholt *et al.* (2018). Paired-end Illumina data were cleaned with Trim Galore! v.0.4.0 (www.bioinformatics.babraham.ac.uk), allowing a minimum read size of 30 bp, and bases were removed with a Phred score below 20. The loci were assembled with iterative baited assembly (IBA, Breinholt *et al.*, 2018) in which only reads with both forward and reverse reads that passed filtering were included. Assembled reads from the probe region were blasted against the reference genome of *Danaus plexippus* and BLAST results were used for single hit and orthology filtering. Loci were screened for orthology with a single hit threshold of 0.9 and genome mapping following Breinholt *et*

al. (2018). Orthologous sequences were screened for contamination by identifying and removing sequences that were nearly identical at the family and genus level (Breinholt *et al.*, 2018). Loci were aligned with MAFFT v.7.245 (Katoh & Standley, 2013) and concatenated with FASconCAT-G v.1.0.4 (Kück & Meusemann, 2010).

Datasets, model partitioning and phylogenetic analysis

The best partitioning scheme and substitution models were estimated using PartitionFinder2 (Lanfear *et al.*, 2017) using the *rcluster* algorithm and ModelFinder as implemented in IQ-TREE v.2.1.2 (Minh *et al.*, 2020). In IQ-TREE, 100 maximum likelihood (ML) searches were conducted with two metrics of branch support: ultrafast bootstrap (UFBoot, 1000 replicates) and SH-aLRT tests (1000 replicates).

To detect potential gene tree incongruence, we inferred a phylogenetic tree under the multispecies coalescent model (MSC). We first inferred individual locus trees using IQ-TREE 2.2.1. We estimated the best substitution model for each locus using ModelFinder and computed ultrafast bootstrap branch support. All resulting locus trees were then gathered in a single file that served as an input for species tree reconstruction in wASTRAL from the ASTER family of optimization algorithms (Zhang & Mirarab, 2022). This method is a reimplementation of ASTRAL-III (Zhang *et al.*, 2018) allowing to take into account phylogenetic uncertainty by relying on branch length and branch support across locus trees. We used the *astral-hybrid_precise* algorithm to estimate the species tree of Trapezitinae under the MSC based on unrooted IQ-TREE locus trees with attached branch length and support information.

All analyses were conducted on the University of Florida HiPerGator High Performance Computing Cluster. Raw data, datasets and appendices from this study can be found on Dryad (https://doi.org/10.5061/dryad.5tb2rbp75).

Divergence time estimation

We estimated divergence times with BEAST 1.10.4 (Suchard *et al.*, 2018). Because the full concatenated dataset of 383 loci was too large to be analysed, we used a 'gene shopping' approach implemented in SortaDate (Smith *et al.*, 2018). Single locus trees were estimated in IQ-TREE and the best-scoring ML tree from the concatenated dataset was used as the reference species tree. Loci were filtered in the order of the following three criteria: (i) clock-likeness, (ii) reasonable tree length, and (iii) least topological conflict with the species tree. We selected the 50 best loci based on this filtering to produce a new nucleotide matrix of 44 384 bp for BEAST analyses.

The best partitioning scheme and substitution models were estimated with PartitionFinder2 (Lanfear *et al.*, 2017) using the *greedy* algorithm. We implemented clock partitioning by conducting analyses with: (i) two clocks, one for the mitochondrial locus and one for all nuclear loci, and (ii) one clock for each partition recovered in PartitionFinder (14 in total). We assigned a Bayesian lognormal relaxed clock model to the different clock partitions. We also tested different tree models by using a Yule (pure birth) or a birth-death model in different analyses. Analyses consisted of 100 million generations with parameter and tree sampling every 5000 generations. We estimated marginal likelihood estimates (MLE) for each analysis using stepping-stone sampling, with 1000 path steps, and chains running for 1 million generations with a log likelihood sampling every 1000 cycles. Maximum Clade Credibility (MCC) trees were generated in TreeAnnotator v.1.10.4 (Suchard *et al.*, 2018) after removing the first 20 million generations as burn-in.

We used secondary calibrations derived from Espeland *et al.* (2018) to estimate divergence times because there are few skipper fossils. We constrained ten nodes with soft lognormal prior densities encompassing the 95% credibility intervals estimated in Espeland *et al.* (2018) (with mean and standard deviation in real space in BEAUti): root (=Nymphalidae+Hedylidae+Hesperiidae) (mean = 118.8, SD = 20.0, offset = 2.88), crown of Hedylidae+Hesperiidae (mean = 106.01, SD = 18.62, offset = 2.6), crown of *Macrosoma hyacinthina+Macrosoma rubedinaria* (mean = 30.71, SD = 7.525, offset = 0.42), crown of Hesperiidae (mean = 76.13, SD = 13.09, offset = 1.9), stem of Euschemoninae (mean = 67.12, SD = 11.44, offset = 1.71), stem of Eudaminae (mean = 59.69, SD = 10.134, offset = 1.41), stem of Heteropterinae (mean = 53.01, SD = 9.034, offset = 1.29), crown of Heteropterinae (mean = 34.12, SD = 7.412, offset = 0.41), stem of Hesperiinae (mean = 48.48, SD = 8.32, offset = 1.18) and crown of Hesperiinae (mean = 43.9, SD = 7.447, offset = 1.09).

Ancestral range estimation

We used the R package BioGeoBEARS v.1.1.2 (Matzke, 2018) to estimate ancestral ranges. Analyses were performed under the DEC and DIVALIKE models on the BEAST chronogram. Geographic distributions of Trapezitinae species were inferred from the literature (e.g. Parsons, 1998; Braby, 2000, 2016). We used area coding largely following Ebach (2015): New Guinea (G), Northern (N), Euronotian (E), Southwest Australia (S), Northern Desert (D) and Eremaean (R).

We generated two time slices (0–15 Mya and 15–25 Mya) with different dispersal rate scalars representing the enhanced connectivity between Australia and New Guinea in the Mid-Miocene. The dispersal rate scalar values were selected according to terrain and water body positions throughout the evolutionary timeframe of the group (Supporting Information, Table S3). The maximum number of areas per ancestral state was set to four. We compared results of these analyses (M1) with null models (M0) that excluded the dispersal rate scalar and adjacency matrices, thereby relaxing all constraints derived from available geological data. We also designed a model, M2, in which colonization of New Guinea is not possible before 15 Mya to test the hypothesis of proto-New Guinea colonization [i.e. M1 allows the colonization of a proto-New Guinean archipelago *sensu* Toussaint *et al.* (2021) before 15 Mya whereas M2 disallows it].

Ancestral host plant estimation

We estimated ancestral larval host plant preferences across Trapezitinae using the function *make.simmap* (SYM model and 1000 simulations) in the R package phytools 0.7-80 (Revell, 2012). Host plant records were aggregated from the literature and **unpublished data**. All host plant observations were summarized at the family level, as follows: Poaceae (A); Cyperaceae (B); Asparagaceae (C); Arecaeae (D); Iridaceae (E); Haemodoraceae

(F); Flagellariaceae (G); and Hemerocallidaceae (H). Character states were unordered in the phytools analyses.

Diversification dynamics

We performed diversification rate analyses within Trapezitinae and within Trapezitinae+Barcinae using BAMM 2.5.0 (Rabosky, 2014) with four reversible jump Markov chains Monte Carlo running for 6 million generations and sampled every 2000 generations. Initial parameters were optimized using the function setBAMMpriors in the R package BAMMtools v.2.1.6 (Rabosky *et al.*, 2014). We also used different priors (0.1, 1, 2 and 5) for the parameter controlling the expected number of shifts. The global sampling fraction was setup to 0.96 for both chronograms (including or excluding Barcinae).

We used LS-BDS (Höhna *et al.*, 2019) implemented in RevBayes (Höhna *et al.*, 2016) to test for clade-specific shifts in diversification rates and compare results with BAMM estimates using the same two chronograms. We used three different rate categories (2, 6 and 10) and analysed the outputs of the LS-BDS runs (2500 generations with a tuning = 200) in the R package RevGadgets 1.0.0 (Tribble *et al.*, 2022).

Using RPANDA 1.8 (Morlon *et al.*, 2016) and the dd_ML function from the R package DDD 4.3 (Etienne *et al.*, 2012), we fitted different models (constant-rate, time-dependent, temperature-dependent, diversity-dependent) to the Trapezitinae chronogram [see Condamine *et al.* (2019) for more details]. Missing taxon sampling at the species level was set to 0.96 in all analyses. We tested the fit of the following models: (1) speciation is constant through time with no extinction (BCST), (2) speciation and extinction are constant through time (BCSTDCST), (3) speciation varies exponentially through time with no extinction (BtimeVarEXPO), (4) speciation varies linearly through time with no extinction (BtimeVarLIN), (5) speciation varies exponentially through time with constant extinction (BtimeVarDCSTEXPO), (6) speciation varies linearly through time with constant extinction (BtimeVarDCSTLIN), (7) extinction varies exponentially through time with constant speciation (BCSTDtimeVarEXPO), (8) extinction varies linearly through time with constant speciation (BCSTDtimeVarLIN), (9) speciation and extinction vary exponentially through time (BtimeVarDtimeVarEXPO), (10) speciation and extinction vary linearly through time (BtimeVarDtimeVarLIN), (11) speciation varies exponentially with temperature with no extinction (BtempVarEXPO), (12) speciation varies linearly with temperature with no extinction (BtempVarLIN), (13) speciation varies exponentially with temperature with constant extinction (BtempVarDCSTEXPO), (14) speciation varies linearly with temperature with constant extinction (BtempVarDCSTLIN), (15) extinction varies exponentially with temperature with constant speciation (BCSTDtempVarEXPO), (16) extinction varies linearly with temperature with constant speciation (BCSTDtempVarLIN), (17) speciation and extinction vary exponentially with temperature (BtempVarDtempVarEXPO), (18) speciation and extinction vary linearly with temperature (BtempVarDtempVarLIN), (19) speciation varies linearly with diversity without extinction (DDL), (20) speciation varies linearly with diversity with constant extinction (DDL+E), (21) speciation varies exponentially with diversity with constant extinction (DDX+E), (22) extinction varies linearly with diversity (DD+EL), (23) extinction varies exponentially with diversity (DD+EX), (24) speciation and extinction vary linearly with diversity (DDL+EL).

In these models, speciation and extinction (respectively λ and μ) were allowed to vary as a continuous (linear or exponential) function of time, past temperature or diversity. Parameters α and β measure the sign and rapidity of variation for speciation and extinction rates, respectively. Positive values of α or β can be interpreted as an indicator of speciation or extinction slowdown, whereas negative values indicate an acceleration of speciation or extinction. The parameter K measures the carrying-capacity in diversity-dependent models. These 24 models were compared with the AICc and ΔAIC to determine best-fit to the time-calibrated phylogeny.

Finally, we used the HiSSE model as implemented in the R package *hisse* (Beaulieu & O'Meara, 2016) to examine whether host plant shifts affected diversification dynamics. In total, we compared 26 different models of HiSSE and BiSSE-like implementations, accounting for hidden states to alleviate recent concerns regarding the reliability of SSE models and the high incidence of false positive results.

RESULTS AND DISCUSSION

Phylogeny and divergence times of Trapezitinae skippers

Maximum likelihood and multispecies coalescent inference approaches yield highly similar phylogenies (Supporting Information, Figs S1–S2, Files S1–S2). All inter-subfamily relationships and major nodes along the Hesperiidae backbone are identical, suggesting low conflict across locus trees and a high congruence of these alternative approaches. Only three nodes differ along the backbone of Trapezitinae, the other ones are restricted to intrageneric relationships within *Hesperilla* and *Mesodina*. Branch support for the backbone in wASTRAL analyses is low (all three conflicting nodes have a local posterior probability ≤ 0.70), and therefore these discrepancies are not statistically significantly supported. Because the IQ-TREE reconstruction offers a more robust resolution of the backbone, we base our discussion on this evolutionary tree. Our analyses recover Trapezitinae as sister to the Oriental Barcinae (two extant species endemic in Assam and Tibet/Yunnan) with robust branch support. The clade formed by these two families is sister to the Malagasy Malazinae (three extant species) (Fig. 1).

We recover a well-supported phylogeny within Trapezitinae except for a few nodes that appear to represent a rapid radiation along the backbone of the subfamily tree (Fig. 1). Although the composition of the largest clades within Trapezitinae remains largely unchanged, relationships between these lineages differ from those of Toussaint *et al.* (2022) that were based on a significantly smaller dataset and are thus less reliable. The genus *Mesodina* (clade I) is recovered with robust branch support as sister to the rest of the subfamily which is split into two large lineages. We recover a species-rich clade restricted to Australia (including Tasmania) comprising the *Anisynta*-group (clade II) as sister to the *Proeidosa*-group (clade III) and the genus *Hesperilla* (clade IV). The second diverse clade comprises two subclades: (1) *Hewitsoniella*, *Rachelia*, *Felicena, Timoconia* and

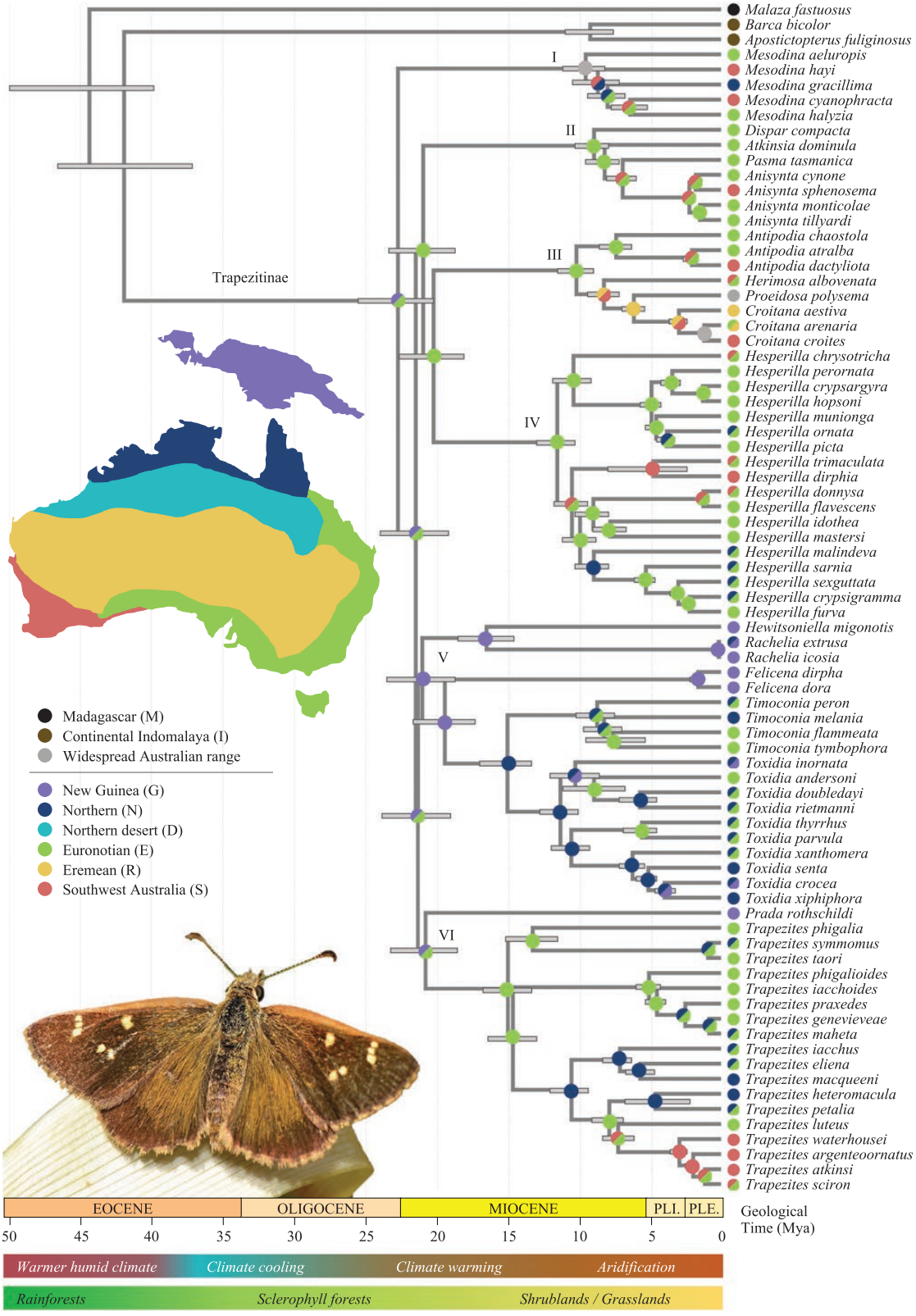

Figure 1. Phylogeny and historical biogeography of Trapezitinae skippers. BEAST maximum credibility tree with horizontal grey bars representing 95% credibility intervals. Node coloured circles indicate most likely ancestral ranges. Major clades are numbered as follows: clade I (*Mesodina*), clade II (*Anisynta*-group), clade III (*Proeidosa*-group), clade IV (*Hesperilla*), clade V and clade VI (*Trapezites*-group). Photograph of *Anisynta sphenosema* by Fred Hort. Abbreviations: PLI, Pliocene; PLE, Pleistocene.

Toxidia (clade V), and (2) *Prada* and *Trapezites* (clade VI). We infer the New Guinean *Felicena* as sister to *Timoconia* and *Toxidia* in clade V. The second subclade consists of the New Guinean genus *Prada* recovered as sister to the species-rich Australian genus *Trapezites*. Despite some regions of the tree presenting moderate branch support, our phylogenomic hypothesis is more robust than that of Toussaint *et al.* (2022) and serves as a foundation for exploring macroevolutionary trends in this group.

We infer a stem age for Trapezitinae at *c.* 42 Mya in the Late Eocene, and a crown age at *c.* 23 Mya (Fig. 1; Supporting Information, File S3, Table S4), indicating a lag of *c.* 20 Myr between the origin of this lineage and the diversification of the extant fauna. Most lineages in Trapezitinae originated 3–4 Myr after the crown age, indicating rapid diversification in the Early Miocene. Most remaining diversification began approximately in the mid-Miocene when major climatic turnovers occurred in this region.

Historical biogeography of Trapezitinae skippers

We recover a split between Barcinae and Trapezitinae *c.* 42 Mya. Palaeogeographic reconstructions of this period suggest that Wallacea, which connected the Australian and Indomalayan regions, did not yet exist, even though the two landmasses were close (Hall, 2013). Since *Apostictopterus* and *Barca* are currently confined to India and Tibet, this pattern supports the view that Barcinae diverged from Trapezitinae after dispersing over water from Australia and New Guinea, where Trapezitinae are endemic. The relatively long stem branches in both subfamilies could represent extinction signatures, and we cannot reject the hypothesis of a widespread ancestor giving rise to northern (e.g. Asian) and southern (e.g. Australian) lineages in the Eocene. However, we believe that this scenario is unlikely.

Model M1 DEC was the best fit to the timetree of Trapezitinae (Table 1; Supporting Information, Figs S3–S14), suggesting that the ancestor of Trapezitinae was widespread in southern and eastern Australia (i.e. Euronotian region) as well as New Guinea in the Late Oligocene. The early geological history of New Guinea is poorly understood, but it is possible that several subaerial terranes formed a proto-Papuan archipelago in the Palaeogene until the Miocene when massive orogenies formed the current rugged, mountainous landscape (Toussaint *et al.*, 2014, 2021). Other groups of invertebrates colonized New Guinea as early as the Oligocene and Miocene [e.g. *Exocelina* diving beetles (Toussaint *et al.*, 2014, 2021); *Trigonopterus* weevils (Tänzler *et al.*, 2016; Letsch *et al.*, 2020a, b); mayflies (Cozzarolo *et al.*, 2019)]. Trapezitinae probably followed a similar evolutionary path and colonized proto-Papuan terranes around the Late Oligocene-Early Miocene (Fig. 1), likely facilitated by their capacity to fly. However, unlike most New Guinean invertebrates, they did not diversify while new habitats were forming rapidly in the Miocene (Toussaint *et al.*, 2014, 2021; Cozzarolo *et al.*, 2019). Indeed, the genera *Felicena*, *Hewitsoniella*, *Rachelia* and *Prada* are comparatively species-poor despite having originated in the Early Miocene. Interestingly, these New Guinean lineages shifted from Poaceae to other plant families (e.g. *Hewitsoniella* possibly feeding on Arecaeae, *Rachelia extrusa* feeding on Flagellariaceae) which, we hypothesize, may have resulted in reduced ecological opportunities because of the limited diversity and distributions of these plant families at the time.

The common ancestors of several early diverging clades were likely widespread in Australia and New Guinea before the appearance of Australian or New Guinean endemic ancestral lineages (Fig. 1). Our best-fit model indicates that the Euronotian region was probably the first to be colonized. Other insect groups including Hydroporini diving beetles are hypothesized to have first colonized this region within Australia too (Toussaint *et al.*, 2015). At the time, this part of Australia was likely tropical and humid, harbouring vast mesic forests (Martin, 2006; Byrne *et al.*, 2008). This biogeographic pattern is consistent with the hypothesis that arid lineages occupying xeric habitats are largely derived from mesic ancestors (Byrne *et al.*, 2008; Crisp *et al.*, 2009). The northern Australian region, largely monsoonal, appears to have been re-colonized from New Guinea by ancestors of clade V, a pattern rarely seen in butterflies (Braby *et al.*, 2020), and by *Trapezites* from the Euronotian region during the Miocene. Trapezitinae skippers mostly colonized and diversified in mesic regions of Australia between the Oligocene and Mid-Miocene, probably flourishing in the relative climatic stability of these ecosystems in an otherwise increasingly dry mainland. *Rhamphotyphlops* blind snakes (Marin *et al.*, 2013) and *Pauropsalta* cicadas (Owen *et al.*, 2017) show a similar pattern. The rest of the Australian mainland was more arid (e.g. Eremean and Southwest Australia), and was colonized more recently between the Late Miocene and Pleistocene. This colonization was synchronous with the aridification of Australia, which was associated with the establishment of dry woodlands, grasslands and shrublands, except for coastal and mountainous regions (Martin, 2006; Byrne *et al.*, 2008). The frequent pattern

Table 1. Results of the BioGeoBEARS analyses

Analysis	Time Strat.*	Proto-Papuan Arch.§	LnL	Extinction	Dispersal	Crown Trapezitinae
M0 DEC	No	Yes	-174.72	0.001	0.0103	GE (rel. prob. < 50%)
M0 DIVALIKE	No	Yes	-188.96	0.000	0.0132	GES (rel. prob. > 50%)
M1 DEC	Yes	Yes	-164.90	0.001	0.0152	GE (rel. prob. > 50%)
M1 DIVALIKE	Yes	Yes	-179.38	0.000	0.0192	GES (rel. prob. > 50%)
M2 DEC	Yes	No	-175.77	0.007	0.0160	NE (rel. prob. < 50%)
M2 DIVALIKE	Yes	No	-190.73	0.009	0.0205	NES (rel. prob. < 50%)

Crown Trapezitinae, ancestral range estimated for this node (letters correspond to region codes, see methods).
*Time Strat., time stratification.
§Proto-Papuan Arch., ancestral range comprising New Guinea allowed (or not) before 15 Mya.
GE, New Guinea + Euronotian; GES, New Guinea + Euronotian + Southwest Australia; NE, Northern + Euronotian; NES, Northern + Euronotian + Southwest Australia

of biome transition from mesic to arid ecosystems has been interpreted as evidence that the latter are evolutionary sinks (e.g. Rix *et al.*, 2021; Heimburger *et al.*, 2022), and our results are consistent with this view. The diversity of Trapezitinae skippers in Western Australia remains comparatively low, which may be due to the vagility of these butterflies and potentially low *in situ* diversification, which has been observed in other invertebrates (Rix *et al.*, 2015), or to extinction. Western Australian habitats have undergone major periods of expansion and contraction during the Pleistocene that may have caused extirpation of some species.

Interestingly, our results contrast with other lineages in which dispersal into climatic refugia fostered allopatric speciation (i.e. most extant diversity in these lineages postdates the Mid-Miocene) (e.g. Fujita *et al.*, 2010; Marin *et al.*, 2013). In Trapezitinae skippers, examples of relatively recent diversification appear decoupled from the refugium hypothesis as these lineages are mostly found in arid regions (Fig. 1).

Diversification dynamics and host plant evolution

TreePar diversification analyses supported a model with two shifts throughout the evolution of the subfamily (Supporting Information, Table S5; Fig. 2). The initial diversification rate of Trapezitinae was high ($\lambda = 0.661$) as indicated by the accumulation of short branches near the backbone of the tree (Fig. 1). Following this fast diversification in the Early Miocene, we estimate a sharp decrease in diversification rate ($\lambda = 0.0268$) at *c.* 20 Mya, followed in the Late Miocene *c.* 7 Mya by a small increase in diversification ($\lambda = 0.0619$) until the present. Our results, combined with the low endemicity of Trapezitinae skippers in focal areas, largely reject the hypothesis that refugia during the Pliocene to Quaternary fuelled diversification, as observed in other invertebrates (Bell *et al.*, 2007; Moreau *et al.*, 2015; Boyer *et al.*, 2016).

All but one of the BAMM analyses conducted on the Barcinae+Trapezitinae chronogram favoured a model with a single shift towards increased diversification at the crown of Trapezitinae, corresponding to a significant increase in speciation rates. This shift is followed by a continuous decrease in speciation rates toward the present. All BAMM analyses conducted only on Trapezitinae recovered as best configuration a model with no rate shift and a pattern of continuous declining speciation rate toward the present. The LS-BDS analyses all converged on a pattern of constant decline in net diversification toward the present, in accordance with the BAMM and TreePar analyses. These results are consistent with the similar diversity within each main clade of Trapezitinae and associated divergence time estimates (Fig. 1). These results suggest that adaptation to a particular biome (mesic, monsoonal, arid) did not affect Trapezitinae diversification rates.

The best-supported models in RPANDA analyses all pointed to a decrease of speciation rates over time (Supporting Information, Table S6). The DDL model in RPANDA analyses received the highest statistical support, suggesting a diversity-dependent scenario without extinction in which diversification is a linear function of clade diversity. We estimate a clade-level carrying-capacity of 109 species, indicating that diversification is still likely occurring because the extant diversity in the clade is *c.* 80 species. However, declining diversification of Trapezitinae might indicate a near-plateau stage in which diversity becomes saturated in some biomes and/or regions as exemplified by the lack of short branches in some clades and the lack of clade-specific shifts in diversification (Figs 1–2). Three models were close in statistical support to the DDL. First, the DDL+E model was recovered as a good fit with a clade-level carrying-capacity of 104 species, largely congruent with the scenario of the DDL model. Second, we recovered the BTimeVar_LIN model as a good fit as well, suggesting a decrease in speciation rate as a linear function of time in agreement with all above-mentioned models from different methods. Third, we recovered the Btemp.Var_LIN model as a good fit, suggesting a decrease in speciation rate as a function of temperature, suggesting that the drastic changes in climatic conditions between the end of the Oligocene and the end of the Miocene might have negatively affected the diversification of these butterflies. This pattern is consistent with other studies finding temperature-dependent decreases in diversification, for instance in *Pauropsalta* cicadas (Owen *et al.*, 2017). Models including variable extinction rates were significantly less supported statistically, suggesting that extinction was probably not a fundamental force shaping the diversification of Trapezitinae since the Late Oligocene unlike other insect clades (Owen *et al.*, 2017).

Our *phytools* analyses recovered Poaceae as the ancestral host plant family on which Trapezitinae fed in the Oligocene (Fig. 2). Unfortunately, larval host plants for both Barcinae genera, *Apostictopterus* and *Barca*, are unknown, so we cannot infer Trapezitinae larval host plants in the Eocene. We hypothesize that Trapezitinae colonized Poaceae when grasslands became more common in the Oligocene but were likely feeding on other plant families before, when most ecosystems in Australia were covered with rainforest. Host plant shifts were rare and occurred mostly during the Early Miocene, primarily to Asparagaceae, Cyperaceae and Iridaceae.

We recovered a BiSSE-like model with equal extinction and speciation rates but differing transition rates as the best-fit model in our HiSSE analyses (Supporting Information, Table S7; Fig. 2). This model suggests that host plants had no effect on diversification and rejects the hypothesis of host plant-driven diversification. Less strongly supported models with a lower statistical support varied widely in their specifications, but even models implementing host plant-driven diversification found very little difference in speciation and extinction rates for the two categories (Poaceae vs. non-Poaceae). Therefore, HiSSE analyses unambiguously rejected the hypothesis that ancestral feeding on Poaceae and subsequent host plant shifts influenced diversification of Trapezitinae. These results are consistent with other studies showing that herbivory seems decoupled from macroevolutionary trends in skippers (Sahoo *et al.*, 2017).

In summary, these macroevolutionary analyses suggest an early burst of diversification in Trapezitinae skippers followed by rapid attenuation of the diversification rate. This rate heterogeneity seems decoupled from abiotic factors such as climatic change and landmass reconfiguration. Indeed, the RPANDA analyses did not support palaeoclimatic models, instead favouring models linked to diversity-dependence. Other Australian endemic groups have experienced similar diversity-dependent patterns associated with decreasing diversification rates. For instance, Toussaint *et al.*

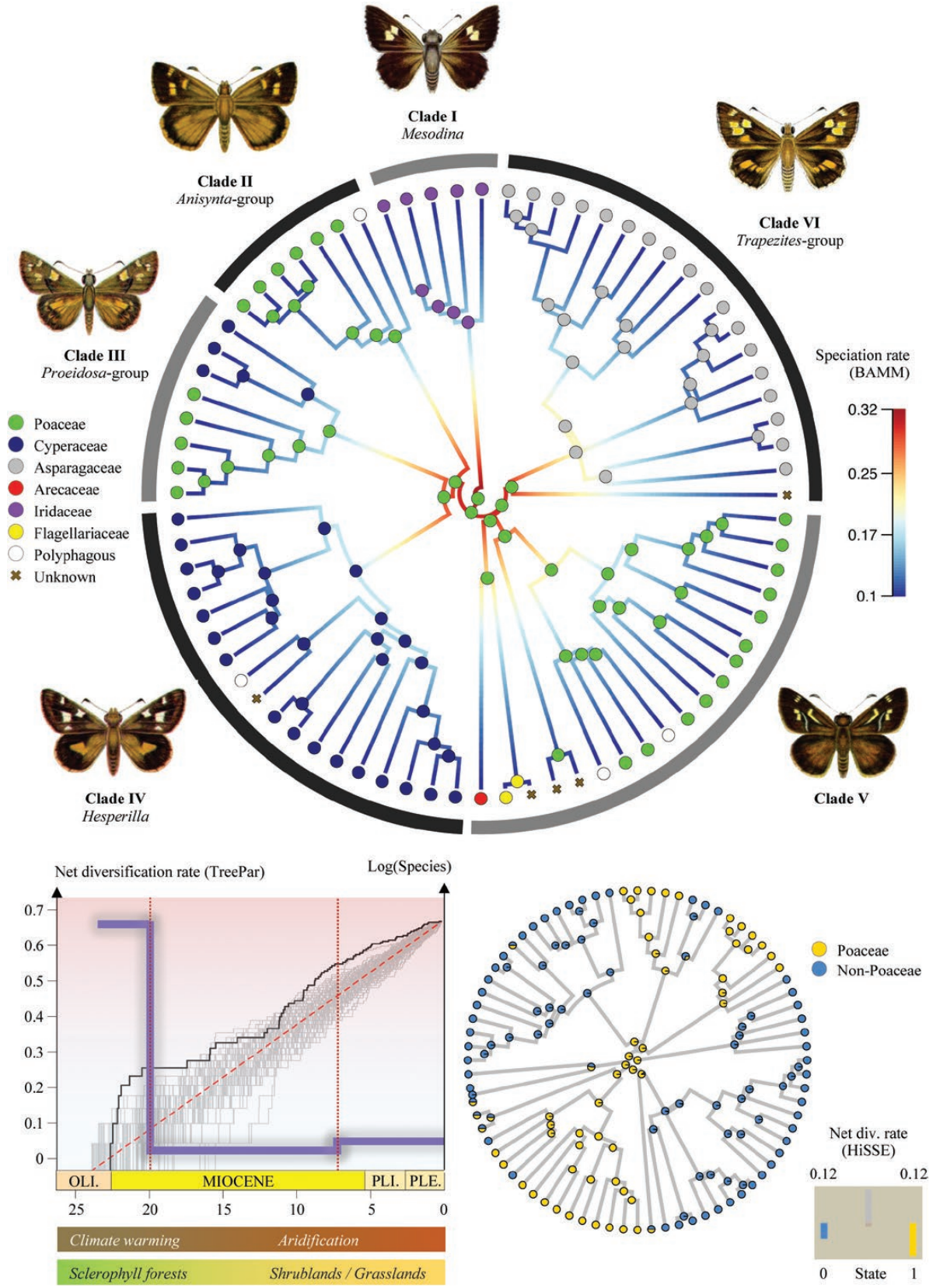

Figure 2. Summary of macroevolutionary estimations in Trapezitinae skippers. The main chronogram shows the phylorate of BAMM analysis with a prior of 0.1 for the number of shifts with most likely host plant states as optimized in *phytools*. In the bottom left corner, the net diversification rate from TreePar analyses is presented (purple line). In the bottom right corner, the most likely host plant states as optimized in HiSSE under two states (Poaceae vs. non-Poaceae) are mapped with a histogram showing the net diversification rate for each state. Abbreviations: OLI, Oligocene; PLI, Pliocene; PLE, Pleistocene.

(2015) inferred that diversification of Hydroporini diving beetles was diversity-dependent and possibly linked to the aridification of Australia in the Miocene, which would have triggered extinction in this clade. The diversity-dependent pattern in Trapezitinae appears to be decoupled from Miocene climatic shifts, and there is no signal of rampant extinction. Although the saturation of ecological opportunities suggested by the DDL model could have been explained as a dearth of host plant taxa, HiSSE analyses rejected a link between diversification dynamics and host plants. We therefore hypothesize that Trapezitinae skippers experienced diversity-dependent processes after shifting to new host plant families (i.e. Asparagaceae, Cyperaceae and Iridaceae) in the Early Miocene. Following a potential radiation of Trapezitinae skippers linked to early host plant shifts, diversification dynamics in the different clades of Trapezitinae were almost synchronous and homogeneous regardless of their newly evolved diets. This indicates that other biotic and/or abiotic factors such as competition and/or lack of ecological opportunities acted in concert to foster diversity-dependent processes in these butterflies, resulting in declining diversification dynamics since the Miocene.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Maximum likelihood phylogeny.
Figure S2. Multispecies coalescent phylogeny.
Figures S3–S14. BioGeoBEARS analysis.
File S1. IQ-TREE best scoring ML tree.
File S2. wASTRAL MSC tree.
File S3. BEAST MCC 14ULRC BDeath.
Table S1. Taxon sampling.
Table S2. Genomic matrix composition.
Table S3. Biogeography data.
Table S4. BEAST results.
Table S5. TreePar results.
Table S6. RPANDA results.
Table S7. HiSSE results.

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DATA AVAILABILITY

The data underlying this article is available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.5tb2rbp75).

REFERENCES

Beaulieu JM, O'Meara BC. 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic Biology* 65: 583–601.
Bell KL, Moritz C, Moussalli A, Yeates DK. 2007. Comparative phylogeography and speciation of dung beetles from the Australian Wet Tropics rainforest. *Molecular Ecology* 16: 4984–4998.
Bowman DM, Brown GK, Braby MF, Brown JR, Cook LG, Crisp MD, Ford F, Haberle S, Hughes J, Isagi Y, Joseph L. 2010. Biogeography of the Australian monsoon tropics. *Journal of Biogeography* 37: 201–216.
Boyer SL, Markle TM, Baker CM, Luxbacher AM, Kozak KH. 2016. Historical refugia have shaped biogeographical patterns of species richness and phylogenetic diversity in mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) endemic to the Australian Wet Tropics. *Journal of Biogeography* 43: 1400–1411.
Braby MF. 2000. *Butterflies of Australia: their identification, biology and distribution*. Collingwood: CSIRO Publishing.
Braby MF. 2016. *The complete field guide to butterflies of Australia, 2nd ed*. Collingwood: CSIRO Publishing.
Braby MF, Espeland M, Müller CJ, Eastwood R, Lohman DJ, Kawahara AY, Maunsell SC, Pierce NE. 2020. Molecular phylogeny of the tribe Candalidini (Lepidoptera: Lycaenidae): systematics, diversification and evolutionary history. *Systematic Entomology* 45: 703–722.
Breinholt JW, Earl C, Lemmon AR, Lemmon EM, Xiao L, Kawahara AY. 2018. Resolving relationships among the megadiverse butterflies and moths with a novel pipeline for anchored phylogenomics. *Systematic Biology* 67: 78–93.
Byrne M, Joseph L, Yeates DK, Roberts JD, Edwards D. 2018. Evolutionary history. In: Lambers H, ed. *On the ecology of Australia's arid zone*. Cham: Springer, 45–75.
Byrne M, Steane DA, Joseph L, Yeates DK, Jordan GJ, Crayn D, Aplin K, Cantrill DJ, Cook LG, Crisp MD, Keogh JS, Melville J, Moritz C, Porch N, Sniderman JMK, Sunnucks P, Weston PH. 2011. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* 38: 1635–1656.
Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J. 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* 17: 4398–4417.
Condamine FL, Rolland J, Morlon, H. 2019. Assessing the causes of diversification slowdowns: temperature-dependent and diversity-dependent models receive equivalent support. *Ecology Letters* 22: 1900–1912.
Cozzarolo CS, Balke M, Buerki S, Arrigo N, Pitteloud C, Gueuning M, Salamin N, Sartori M, Alvarez N. 2019. Biogeography and ecological diversification of a mayfly clade in New Guinea. *Frontiers in Ecology and Evolution* 7: 233.
Crisp MD, Arroyo MT, Cook LG, Gandolfo MA, Jordan GJ, McGlone MS, Weston PH, Westoby M, Wilf P, Linder HP. 2009. Phylogenetic biome conservatism on a global scale. *Nature* 458: 754–756.
Ebach MC, Gonzalez-Orozco CE, Miller JT, Murphy DJ. 2015. A revised area taxonomy of phytogeographical regions within the Australian Bioregionalisation Atlas. *Phytotaxa* 208: 261–277.
Espeland M, Breinholt J, Willmott KR, Warren AD, Vila R, Toussaint EFA, Maunsell SC, Aduse-Poku K, Talavera G, Eastwood R, Jarzyna MA. 2018. A comprehensive and dated phylogenomic analysis of butterflies. *Current Biology* 28: 770–778.
Etienne RS, Haegeman B, Stadler T, Aze T, Pearson PN, Purvis A, Phillimore AB. 2012. Diversity-dependence brings molecular phylogenies closer to agreement with the fossil record. *Proceedings of the Royal Society B: Biological Sciences* 279: 1300–1309.
Fujita MK, McGuire JA, Donnellan SC, Moritz C. 2010. Diversification and persistence at the arid-monsoonal interface: Australia-wide biogeography of the Bynoe's gecko (*Heteronotia binoei*; Gekkonidae). *Evolution* 64: 2293–2314.
Hall R. 2013. The palaeogeography of Sundaland and Wallacea since the Late Jurassic. *Journal of Limnology* 72: 1–17.

Heimburger B, Schardt L, Brandt A, Scheu S, Hartke TR. 2022. Rapid diversification of the Australian Amitermes group during late Cenozoic climate change. *Ecography* 2022(9): e05944.

Höhna S, Freyman WA, Nolen Z, Huelsenbeck JP, May MR, Moore BR. 2019. A Bayesian approach for estimating branch-specific speciation and extinction rates. *bioRxiv*, doi: 10.1101/555805, 20 February 2019, preprint: not peer reviewed.

Höhna S, Landis MJ, Heath TA, Boussau B, Lartillot N, Moore BR, Huelsenbeck JP, Ronquist F. 2016. RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Systematic Biology* 65: 726–736.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.

Kawahara AY, Breinholt JW, Espeland M, Storer C, Plotkin D, Dexter KM, Toussaint EFA, St Laurent RA, Brehm G, Vargas S, Forero D, Pierce NE, Lohman DJ. 2018. Phylogenetics of moth-like butterflies (Papilionoidea: Hedylidae) based on a new 13-locus target capture probe set. *Molecular Phylogenetics and Evolution* 127: 600–605.

Kodandaramaiah U, Braby MF, Grund R, Müller CJ, Wahlberg N. 2018. Phylogenetic relationships, biogeography and diversification of Coenonymphina butterflies (Nymphalidae: Satyrinae): intercontinental dispersal of a southern Gondwanan group? *Systematic Entomology* 43: 798–809.

Kück P, Meusemann K. 2010. FASconCAT: convenient handling of data matrices. *Molecular Phylogenetics and Evolution* 56: 1115–1118.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.

Letsch H, Balke M, Toussaint EFA, Narakusumo RP, Fiedler K, Riedel A. 2020a. Transgressing Wallace's Line brings hyperdiverse weevils down to earth. *Ecography* 43:1329–1340.

Letsch H, Balke M, Toussaint EFA, Riedel A. 2020b. Historical biogeography of the hyperdiverse hidden snout weevils (Coleoptera, Curculionidae, Cryptorhynchinae). *Systematic Entomology* 45: 312–326.

Marin J, Donnellan SC, Blair Hedges S, Doughty P, Hutchinson MN, Cruaud C, Vidal N. 2013. Tracing the history and biogeography of the Australian blindsnake radiation. *Journal of Biogeography* 40: 928–937.

Martin HA. 2006. Cenozoic climatic change and the development of the arid vegetation in Australia. *Journal of Arid Environments* 66: 533–563.

Matzke NJ. 2018. *BioGeoBEARS: BioGeography with Bayesian (and likelihood) Evolutionary Analysis with R Scripts, version 1.1.1*, published on GitHub on November 6, 2018. https://zenodo.org/record/1478250.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534.

Moreau CS, Hugall AF, McDonald KR, Jamieson BG, Moritz C. 2015. An ancient divide in a contiguous rainforest: endemic earthworms in the Australian Wet Tropics. *PLoS One* 10: e0136943.

Morlon H, Lewitus E, Condamine FL, Manceau M, Clavel J, Drury J. 2016. RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. *Methods in Ecology and Evolution* 7: 589–597.

Oliver PM, Ashman LG, Bank S, Laver RJ, Pratt RC, Tedeschi LG, Moritz CC. 2019. On and off the rocks: persistence and ecological diversification in a tropical Australian lizard radiation. *BMC Evolutionary Biology* 19: 1–15.

Owen CL, Marshall DC, Hill KB, Simon C. 2017. How the aridification of Australia structured the biogeography and influenced the diversification of a large lineage of Australian cicadas. *Systematic Biology* 66: 569–589.

Parsons M. 1998. *The butterflies of Papua New Guinea: their systematics and biology*. Princeton: Princeton University Press

Pepper M, Scott Keogh J. 2014. Biogeography of the Kimberley, Western Australia: a review of landscape evolution and biotic response in an ancient refugium. *Journal of Biogeography* 41: 1443–1455.

Pillans BJ. 2018. Seeing red: some aspects of the geological and climatic history of the Australian arid zone. In: Lambers H, ed. *On the ecology of Australia's arid zone*. Cham: Springer, 5–43.

Rabosky DL. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS One* 9: e89543.

Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huang H, Larson JG. 2014. BAMM tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution* 5: 701–707.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217–223.

Rix MG, Edwards DL, Byrne M, Harvey MS, Joseph L, Roberts JD. 2015. Biogeography and speciation of terrestrial fauna in the south-western Australian biodiversity hotspot. *Biological Reviews* 90: 762–793.

Rix MG, Harvey MS. 2012. Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: evidence for Miocene speciation within Tertiary refugia. *Molecular Phylogenetics and Evolution* 62: 375–396.

Rix MG, Wilson JD, Huey JA, Hillyer MJ, Gruber K, Harvey MS. 2021. Diversification of the mygalomorph spider genus *Aname* (Araneae: Anamidae) across the Australian arid zone: tracing the evolution and biogeography of a continent-wide radiation. *Molecular Phylogenetics and Evolution* 160: 107127.

Smith SA, Brown JW, Walker JF. 2018. So many genes, so little time: a practical approach to divergence-time estimation in the genomic era. *PLoS One* 13: e0197433.

Sahoo RK, Warren AD, Collins SC, Kodandaramaiah U. 2017. Hostplant change and paleoclimatic events explain diversification shifts in skipper butterflies (Family: Hesperiidae). *BMC Evolutionary Biology*, 17: 1–9.

Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4: vey016.

Tänzler R, Van Dam MH, Toussaint EFA, Suhardjono YR, Balke M, Riedel A. 2016. Macroevolution of hyperdiverse flightless beetles reflects the complex geological history of the Sunda Arc. *Scientific Reports* 6:1–12.

Toussaint EFA, Braby MF, Müller CJ, Petrie EA, Kawahara AY. 2022. Molecular phylogeny, systematics and generic classification of the butterfly subfamily Trapezitinae (Lepidoptera: Papilionoidea: Hesperiidae). *Zoological Journal of the Linnean Society* 195: 1407–1421.

Toussaint EFA, Breinholt JW, Earl C, Warren AD, Brower AV, Yago M, Dexter KM, Espeland M, Pierce NE, Lohman DJ, Kawahara AY. 2018. Anchored phylogenomics illuminates the skipper butterfly tree of life. *BMC Evolutionary Biology* 18: 1–11.

Toussaint EFA, Condamine FL, Hawlitschek O, Watts CH, Porch N, Hendrich L, Balke M. 2015. Unveiling the diversification dynamics of Australasian predaceous diving beetles in the Cenozoic. *Systematic Biology* 64: 3–24.

Toussaint EFA, Hall R, Monaghan MT, Sagata K, Ibalim S, Shaverdo HV, Vogler AP, Pons J, Balke M. 2014. The towering orogeny of New Guinea as a trigger for arthropod megadiversity. *Nature Communications* 5: 1–10.

Toussaint EFA, White LT, Shaverdo H, Lam A, Surbakti S, Panjaitan R, Sumoked B, von Rintelen T, Sagata K, Balke M. 2021. New Guinean orogenic dynamics and biota evolution revealed using a custom geospatial analysis pipeline. *BMC Ecology and Evolution* 21: 1–28.

Tribble CM, Freyman WA, Landis MJ, Lim JY, Barido-Sottani J, Kopperud BT, Höhna S, May MR. 2022. RevGadgets: an R Package for visualizing Bayesian phylogenetic analyses from RevBayes. *Methods in Ecology and Evolution* 13: 314–323.

Weston P, Jordan G, Keith DA. 2017. Evolutionary biogeography of the Australian flora in the Cenozoic Era. In: Keith DA, ed. *Australian vegetation, 3rd ed*. Cambridge: Cambridge University Press, 40–62.

Zachos JC, Dickens GR, Zeebe RE. 2008. An Early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* 451: 279–283.

Zhang C, Mirarab S. 2022. Weighting by gene tree uncertainty improves accuracy of quartet-based species trees. *Molecular Biology and Evolution* 39: msac215.

Zhang C, Rabiee M, Sayyari E, Mirarab S. 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* 19: 15–30.