Historical biogeography of *Polyura* butterflies in the oriental Palaeotropics: trans-archipelagic routes and South Pacific island hopping

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ABSTRACT

Aim The respective contribution of vicariance and/or dispersal events to the evolution of clades dwelling in the archipelagic parts of the Oriental and Australian regions remains equivocal. Using a complete, species-level phylogeny of *Polyura* butterflies that are widespread in the oriental Palaeotropics, we aim to test predictions related to vicariance driven by past abiotic factors in the Indo-Australian archipelago (IAA) (Miocene tectonics and Pleistocene climatic shifts) *versus* repeated trans-archipelagic dispersal events.

Location The Oriental and Australian regions with a focus on the IAA.

Methods Bayesian species tree phylogenetic analyses were conducted using a matrix comprising two mitochondrial and two nuclear gene fragments. Bayesian relaxed clocks were used to produce a chronogram, which was used in ancestral area estimations to infer the spatio-temporal evolution of the genus at different geographical scales. Diversification dynamics were investigated using the package *TreePar* in R.

Results *Polyura* originated during the mid-Miocene (*c.* 13 million years ago). Ancestral area estimations inferred an origin in Indomalaya. Wallacea was colonized out-of-Indomalaya in the *P. pyrrhus* group, while the *P. athamas* and *P. eudamippus* groups diversified in Indomalaya and the east Palaearctic. We inferred three long-distance dispersal (LDD) events. The first one implies out-of-Sunda colonization of the Solomon Islands, which have three extant, endemic species. The second implies a colonization of Vanuatu out-of-Sunda that later served as a stepping stone for the colonization of other Pacific islands (Fiji and New Caledonia). A third permitted the reverse colonization of Wallacea from the Pacific islands. These LDD events were supported by our diversification analyses that suggested no diversification rate shift throughout the evolution of the genus.

Main conclusions Our results suggest unusual colonization routes with Pacific islands as a hub for late Miocene reverse colonizations back into the centre of the Indo-Australian archipelago.

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INTRODUCTION

The eastern part of the Palaeotropics encapsulates two major biogeographical entities: the Oriental and Australian regions. The Oriental region stretches from Kashmir in the west, ori-ental China in the north, Ryukyu archipelago in the east and Greater Sunda islands (Borneo, Java and Sumatra) in the south. The Australian region as defined by Alfred Russel Wallace (1876) encompasses the large islands of Australia, New Guinea and Sulawesi, the Lesser Sunda islands and a

large number of Pacific islands such as Fiji, New Caledonia, the Solomon Islands and Vanuatu. The archipelagic part of these two biogeographical regions corresponds to Alfred Wallace's Malay Archipelago (Wallace, 1860, 1869), currently referred to as Indo-Australian archipelago (IAA) (Lohman *et al.*, 2011). This region features extraordinary biodiversity and high levels of endemism, often threatened by human activity. As a result, several biodiversity hotspots have been proposed within the region. The IAA also includes New Guinea as one of the three areas of tropical wilderness on Earth. The geological history of the archipelago is extremely intricate, particularly at its core, Wallacea, where the Asian and Australian tectonic plates abut (Fig. 1). These two plates started colliding at the Oligocene–Miocene boundary roughly 25 million years ago (Ma) (Hall, 2013). Since the Miocene, New Guinea rose high above sea level, the Lesser Sunda islands and the Moluccas became subaerial, and the Philippines and Sulawesi were assembled through collision of different geological elements (Hall, 2012, 2013). In the eastern part of the IAA, the Solomon Islands, New Hebrides, Vanuatu and Fiji originated from intense volcanism fostered by tectonic activity throughout the Cenozoic (Hall, 2002). There are also Gondwanan fragments in the archipelago. In particular, the large landmasses of Australia and New Caledonia host ancient lineages, although there is some contention regarding the age and origin of the New Caledonian biota (e.g. Grandcolas *et al.*, 2008; Heads, 2008; Sharma & Giribet, 2009; Swenson *et al.*, 2014).

Its diversity of islands, geological assemblages and climatic regimes makes the IAA an ideal setting to study biogeographical patterns and processes. Indeed, Alfred R. Wallace spent much time studying the biota of the region that he considered an ideal natural laboratory for studying evolution (Wallace, 1860, 1869). More than 100 years later, the understanding of mechanisms governing biodiversity assembly in the archipelago has made great strides forward. More

sophisticated phylogenetic methods and molecular clock divergence time estimation have greatly improved our understanding of the biogeography of several taxa (Lohman *et al.*, 2011; de Bruyn *et al.*, 2014). In particular, the study of evolutionary patterns and processes in insect clades of the IAA has recently experienced remarkable progress (Braby & Pierce, 2007; Balke *et al.*, 2009; Müller & Beheregaray, 2010; Müller *et al.*, 2010, 2013; Condamine *et al.*, 2013, 2015; Toussaint *et al.*, 2013, 2014, 2015a,c,d; Tänzler *et al.*, 2014, 2016; Clouse *et al.*, 2015). Most of the clades studied are widespread in the IAA and therefore provide a window into the impact of geological and climatic factors on lineage diversification.

The genus *Polyura* Billberg, 1820 (Lepidoptera, Nymphalidae, Charaxinae) is a relatively widespread Palaeotropical clade of butterflies referred to as Nawabs. The 34 currently recognized species are distributed from Pakistan to the Ryukyu archipelago and from North Korea to Australia and Pacific islands, such as Vanuatu and Fiji (Smiles, 1982; Toussaint *et al.*, 2015b; Toussaint *et al.* in press, a,b) (Fig. 2). The diets of the caterpillars are generally poorly documented, but in the few taxa for which it is available, the larvae appear to be polyphagous within or across rosid orders including Fabales, Malpighiales, Malvales, Myrtales, Oxalidales, Rosales and Sapindales (Smiles, 1982). *Polyura sempronius*, for which the feeding habits of the caterpillars are well-known, has even been reported to feed on multiple orders of plants in magnolids (Laurales) and rosids (Common & Waterhouse, 1972). The taxonomy and systematics of the group were recently studied comprehensively (Toussaint *et al.*, 2015b; Toussaint *et al.* in press, a,b; see also Turlin & Sato, 1995; Müller & Tennent, 1998; Turlin, 2001), allowing a clarification of species boundaries and relationships, yet the biogeography of the group remains unexplored. The close affinity of the genus with the mainly Afrotropical genera *Charaxes* and *Euxanthe* from the same tribe Charaxini suggests an origin west of Wallace's Line (Aduse-Poku *et al.*, 2009; Müller *et al.*, 2010;

Figure 1 Biogeographical regions of the Indo-Australian archipelago examined in this study. Schematic representation of the 15 areas used in the first BioGeoBEARS analyses. The areas of the second set of analyses can be summarized as follows: Indomalaya (A+B+D+E+O), Wallacea (F+G+H), Sahul (I+K) and Pacific Islands (J+L+M+N). All regions and islands belonging to the same region are presented in the same colour and indicated in the legend.
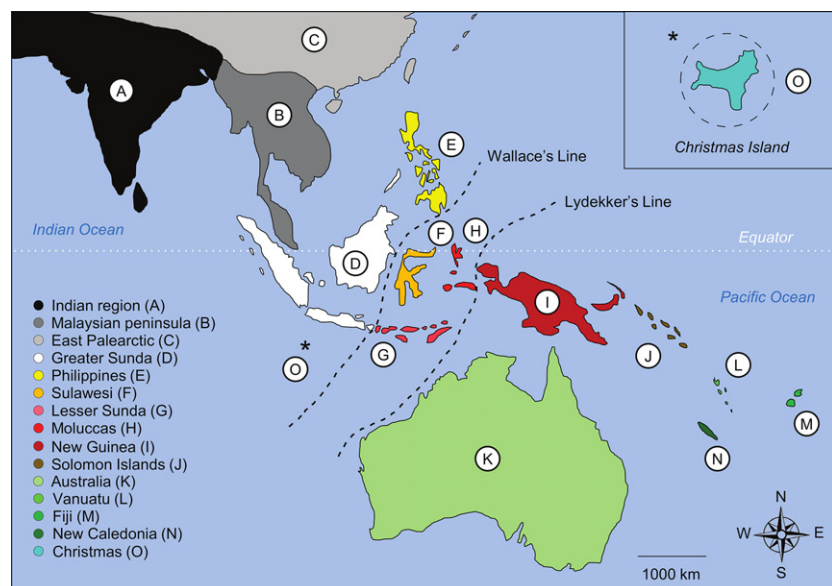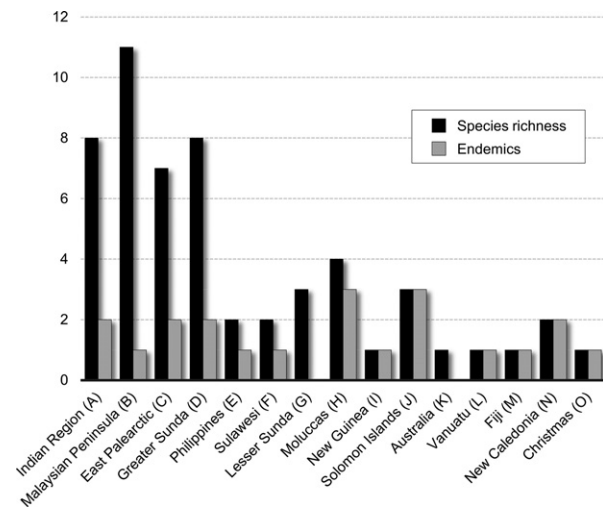
Figure 2 Distribution of species richness and endemicity in *Polyura* butterflies. Histogram presenting the number of species and endemic species found in each region as defined in Fig. 1.

Toussaint *et al.*, 2015b). However, the role of vicariant *versus* dispersal events in shaping present distributional ranges of *Polyura* species is equivocal. A null hypothesis can be drawn from the geological and climatic histories of the region. Under the hypothesis of past abiotic vicariance (PAV), the biogeographical evolution of *Polyura* is expected to reflect the geological assembly of the IAA in the Miocene (Hall, 2012, 2013) and the enhanced connectivity between IAA islands in the Pleistocene due to sea-level fluctuations (Voris, 2000; Miller *et al.*, 2005). Under the PAV hypothesis, we also expect *Polyura* ancestors to have colonized the Australian region as and when distances between landmasses permitted. Late vicariance events are expected to have occurred in the Pleistocene due to Quaternary glaciation cycles that triggered sea-level fluctuations and therefore isolation of insular populations. Pacific island endemics from Fiji, New Caledonia, the Solomon Islands and Vanuatu are expected to be derived as a consequence of late stepping stone dispersal from New Guinea towards the east. The alternative hypothesis of trans-archipelagic dispersal (TAD) accords a greater role to dispersal in driving the evolution of *Polyura* in the IAA with independent colonizations of remote islands. Under this hypothesis, the phylogeny of the genus should reflect a more dynamic biogeographical pattern with geographically distant but phylogenetically closely related species and a temporal origin of the genus and subsequent evolution not necessarily synchronous with the geological assembly of the IAA. A prediction that can be derived from the TAD hypothesis is the absence of a diversification rate shift in the evolution of the group that could provide evidence of regional extinction and better explain present-day distributions than long-distance dispersal (LDD).

To test the validity of the PAV and TAD hypotheses, we combined multimarker matrices from different molecular studies on *Polyura* (Toussaint *et al.*, 2015b; Toussaint *et al.*

in press, a,b) to produce a dataset comprising 216 specimens representing all extant species of the genus across its entire distributional range. We aimed to (1) reconstruct a species-level phylogeny of the genus using species tree inference based on multi-specimen gene alignments; (2) estimate divergence times using secondary calibrations derived from a fossil-dated phylogeny of the family Nymphalidae (Wahlberg *et al.*, 2009); (3) reconstruct the historical biogeography of the genus. These analyses allowed us to test the out-of-Asia versus out-of-Australia hypothesis and to untangle the putative role of abiotic factors (i.e. geological assembly of the IAA, Pleistocene sea-level fluctuations), as defined in the PAV hypothesis against LDD events as defined in the TAD hypothesis, in shaping the biogeography of the genus. Finally, our analyses gave us insights into the directionality of colonization routes in the IAA.

MATERIALS AND METHODS

Taxon sampling

We retrieved data from Toussaint *et al.* (2015b, in press, a,b), comprising four gene fragments from 216 ingroup specimens in addition to 14 outgroup taxa in the genera *Charaxes*, *Euxanthe* and *Palla*. The four gene fragments were the mitochondrial cytochrome *c* oxidase subunit I (COI, 471 bp), NADH dehydrogenase 5 (ND5, 417 bp), nuclear ribosomal protein subunit 5 (RPS5, 573 bp) and nuclear wingless (WGL, 396 bp). The CO1 gene fragment was amplified using the primer pair Ron (GGAGCYCCWGATATAGCTTTCCC) and Nancy (CCTGGTAAAATTAAAATATAAACTTC) with the following polymerase chain reaction (PCR) conditions; 1 min at 94 °C followed by 5 cycles of 30 s at 94 °C, 40 s at 47 °C and 1 min at 72 °C, and then 30 cycles of 30 s at 94 °C, 40 s at 52 °C and 1 min at 72 °C, with a final extension of 10 min at 72 °C. The ND5 gene fragment was amplified using the primer pair A1 (AATATDAGGTATAA ATCATAT) and C2 (ATCYTTWGAATAAAAYCCAGC) with the following PCR conditions; 5 min at 95 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C, and a final extension of 10 min at 72 °C. The RPS5 gene fragment was amplified using the primer pair RPS5F (ATGGCNGARGARAAYTGGAAYGA) and RPS5R (CGGTT RGAYTTRGCAACACG) with the following PCR conditions; 5 min at 95 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min 30 s at 72 °C, with a final extension of 10 min at 72 °C. The Wingless gene fragment was amplified using the primer pair LepWG1 (GARTGYAARTGYCAYGGY ATGTCTGG) and LepWG2 (ACTICGCARCACCARTGGA ATGTRCA) with the following PCR conditions; 5 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 47 °C and 1 min 30 s at 72 °C, with a final extension of 10 min at 72 °C. The taxon sampling and gene coverage are given in Appendix S1 in Supporting Information. The different datasets were aligned in GENEIOUS R8 (Biomatters, www.geneious.com). All gene fragment sequences can be found on

GenBank under the accession numbers KT073236–KT073670 and KT073704–KT073900.

Phylogenetic inference

We reconstructed the phylogenetic relationships of *Polyura* species using a species tree approach. We used *beast 1.8.2. (Heled & Drummond, 2010) to infer species-level relationships based on the four gene alignments from all specimens. We used the same outgroups as in Toussaint *et al.* (2015b). *Palla violinitens* was enforced at the root based on previous phylogenetic evidence (Aduse-Poku *et al.*, 2009). The best-fitting substitution model for each gene fragment was inferred using PartitionFinder 1.1.1 (Lanfear *et al.*, 2012) with the *greedy* algorithm. The likelihood of the different models implemented in the *beast* option were compared using the corrected Akaike information criterion (AICc). Each specimen in the dataset was assigned to one of the 34 extant, valid species in BEAUti 1.8.2 (Drummond *et al.*, 2012). We ran several independent analyses of *beast 1.8.2 on XSEDE as implemented in the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010). Each run consisted of 500 million generations sampled every 10,000 generations, resulting in 50,000 posterior trees. Convergence of the runs was checked in Tracer 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). Effective sample sizes of 200 for each parameter were acknowledged as a good indicator of convergence.

Divergence time estimation

We inferred divergence times using beast 1.8.2 (Drummond *et al.*, 2012) and used the topology recovered in *beast as a fixed input by manually modifying the *.xml* file and unchecking all *Tree* operators in BEAUti 1.8.2 (Drummond *et al.*, 2012). The dataset was partitioned by gene fragment with four partitions and the same substitution models used in the *beast analyses. The clock models were linked for mitochondrial genes (mtDNA) and unlinked for nuclear genes. The molecular clock test was performed in mega 6 (Tamura *et al.*, 2013) by comparing the ML value of the *beast topology with and without the molecular clock constraints under the Tamura-Nei model. The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level ($P = 0$). Therefore, we used a Bayesian relaxed clock approach as implemented in beast 1.8.2. We assigned a *lognormal relaxed clock* with uncorrelated rates to each clock model (RPS5, WGL and mtDNA). The *Tree Model* was set to *Speciation: Birth–Death Process*. The *ucld.-mean* prior of each clock model was set to an uninformative interval (0.0001–1.0) with a uniform prior distribution. The analysis consisted of 50 million generations with a tree and parameter sampling every 5000 cycles. Run convergence was ascertained as before.

To calibrate the topology, we used two secondary calibrations derived from Wahlberg *et al.* (2009). In that study, the authors sampled representatives of 400 of 540 extant genera in the family Nymphalidae. Phylogenetic relationships were reconstructed using 10 gene fragments and 235 morphological characters, allowing the recovery of a robustly supported and well-resolved phylogeny for the family. The divergence times were inferred using a comprehensive set of fossils and host plant information. Specifically, maximum age constraints were used for clades of nymphalids whose caterpillars are specialized herbivores on these families. Seven fossils from the Cenozoic were also used to enforce minimum ages. Wahlberg *et al.* (2009) recovered a median age of 22.65 Ma for the node comprising the genera *Charaxes*, *Euxanthe* and *Polyura* (95% height posterior distribution: 16.08–28.44 Ma). A median age of 40.19 Ma for the node comprising the sister genus *Palla* (95% HPD: 33.60–46.79 Ma). Therefore, we constrained these two nodes in our phylogeny with a uniform distribution encompassing the 95% HPD of the ages found in Wahlberg *et al.* (2009).

Ancestral area estimation

We used the R implementation of BioGeoBEARS (Matzke, 2013a,b) to infer the biogeographical history of *Polyura* butterflies across the IAA and test the support for either the PAV or TAD hypothesis to explain the present-day distribution of *Polyura* species in the Palaeotropics. This program allows the estimation of ancestral areas under different biogeographical models. It implements a parameter describing founder-event speciation (+j) likely to be important in oceanic settings (Matzke, 2014). The comparison of the different models is made in a statistical framework allowing the selection of the best-fitting model. We used the DEC (Ree *et al.*, 2005; Ree & Smith, 2008) and DIVA (Ronquist, 1997) models to estimate ancestral areas. The analyses were conducted with the beast maximum clade credibility (MCC) tree from which we pruned outgroups. We conducted two biogeographical analyses to reconstruct the history of *Polyura* colonization in the IAA at different scales. The geographical distribution of each species was gathered from the review of Smiles (1982), our previous work (Toussaint *et al.*, 2015b; Toussaint *et al.* in press, a,b), and collection voucher information from the first author's collection and the Bavarian State Collection of Zoology (ZSM).

The first set of analyses comprised the 15 following areas (Fig. 1): Indian region (A), Malay Peninsula (B), East Palaearctic (C), Greater Sunda (D), Philippines (E), Sulawesi (F), Lesser Sunda (G), Moluccas (H), New Guinea (I), Solomon Islands (J), Australia (K), Vanuatu (L), Fiji (M), New Caledonia (N) and Christmas Island (O).

The second set of analyses comprised the five following areas: East Palaearctic (C), Wallacea (Lesser Sunda, Moluccas, Sulawesi) (P), Sahul (Australia/New Guinea) (Q), Indomalaya (Indian region/Malay Peninsula/Greater Sunda/Philippines/Christmas Island) (R), and Pacific Islands (Solomon Islands/Vanuatu/Fiji/New Caledonia) (S).

We did not use time slices, as most of the geological assemblage of the archipelago was probably concluded by 13 Ma

(Hall, 2012, 2013). In order to improve the resolution and exclude implausible areas, only combinations of adjacent areas were considered (e.g. in the five area analyses, Indomalaya + Wallacea was allowed, whereas Indomalaya + Sahul was not allowed; in the 15 area analyses, New Guinea + Solomon Islands was allowed but New Guinea + Vanuatu was not allowed). The maximum number of ancestral areas was set to four in both sets of analyses. We specified dispersal rates between the different regions based on palaeogeographical (Hall, 2012, 2013) and palaeoclimatic (Miller *et al.*, 2005) evidence (Tables 1 & 2).

Diversification analyses

We used the package *TreePar* (Stadler, 2011) in R with the BEAST MCC tree from which we pruned all outgroups to test for potential shifts in diversification rate throughout the evolution of *Polyura* that could suggest regional extinction and therefore support the PAV over the TAD hypothesis.

To estimate the potential shifts in speciation and extinction rates in the whole phylogeny, we used the function '*bd.shifts.optim*'. This function uses the empirical branching times from the MCC tree as an input and fits several birth–death models including 0 (constant-rate model) to several diversification rate shifts during the lineage evolution. We tested different models ranging from 0 to 3 rate shifts. All the analyses were carried out with the following non-default settings: taxon sampling was set to 34/34, start = 0, end = 13 and grid = 0.1 Ma for a fine-scale estimation of rate shifts. We calculated AICc scores and computed likelihood ratio tests (LRT) to select the best-fit between the different models allowing incrementally more shifts during the evolution of the clade.

RESULTS

Phylogenetic relationships

Although nodal support was only moderate in some parts of the *BEAST tree (Fig. 3), *Polyura* was recovered as monophyletic (PP = 1.0), and as the sister taxon to the African species *Charaxes paphianus* (PP = 1.0). The monophyly of the *P. athamas* and *P. pyrrhus* species groups was strongly supported (PP = 1.0), while the monophyly of the *P. eudamippus* group had weaker support (PP = 0.93).

Divergence time estimates

The BEAST analyses converged very rapidly and all parameters had ESS values >500. The median ages derived from the

Table 2 Matrix of dispersal rates used in the five area BIOGEOBEARS estimation of ancestral ranges in Palaeotropical *Polyura* butterflies.

	R	C	P	Q	S
R	1	1	0.5	0.1	0.01
C	1	1	0.1	0.01	0.01
P	0.5	0.1	1	0.5	0.1
Q	0.1	0.01	0.5	1	0.5
S	0.01	0.01	0.1	0.5	1

Letters correspond to the following biogeographical regions: Indomalaya (Indian region/Malay Peninsula/Greater Sunda/Philippines/Christmas Island) (R), East Palaearctic (C), Wallacea (Lesser Sunda, Moluccas, Sulawesi) (P), Sahul (Australia/New Guinea) (Q) and Pacific Islands (Solomon Islands/Vanuatu/Fiji/New Caledonia) (S). Numbers correspond to the dispersal rate multipliers (0–1) between pairs of areas.

Table 1 Matrix of dispersal rates used in the 15 area BIOGEOBEARS estimation of ancestral ranges in Palaeotropical *Polyura* butterflies.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
A	1	1	1	0.5	0.25	0.1	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1
B	1	1	1	1	0.5	0.25	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.25
C	1	1	1	0.5	0.5	0.1	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.1
D	0.5	1	0.5	1	0.5	0.5	0.5	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.5
E	0.25	0.5	0.5	0.5	1	0.5	0.1	0.5	0.1	0.01	0.01	0.01	0.01	0.01	0.1
F	0.1	0.25	0.1	0.5	0.5	1	0.5	0.5	0.1	0.01	0.01	0.01	0.01	0.01	0.1
G	0.1	0.25	0.01	0.5	0.1	0.5	1	0.5	0.1	0.1	0.5	0.01	0.01	0.1	0.5
H	0.01	0.01	0.1	0.1	0.5	0.5	0.5	1	0.5	0.1	0.5	0.01	0.01	0.1	0.1
I	0.01	0.01	0.01	0.01	0.1	0.1	0.1	0.5	1	0.5	1	0.1	0.01	0.01	0.25
J	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.1	0.5	1	0.5	0.5	0.1	0.1	0.1
K	0.01	0.01	0.01	0.01	0.01	0.01	0.5	0.5	1	0.5	1	0.1	0.01	0.5	0.5
L	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.5	0.1	1	0.5	0.5	0.01
M	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.01	0.5	1	0.1	0.01
N	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.1	0.01	0.1	0.5	0.5	0.1	1	0.1
O	0.1	0.25	0.1	0.5	0.1	0.1	0.5	0.1	0.25	0.1	0.5	0.01	0.01	0.1	1

Letters correspond to the following biogeographical regions: Indian region (A), Malay Peninsula (B), East Palaearctic (C), Greater Sunda (D), Philippines (E), Sulawesi (F), Lesser Sunda (G), Moluccas (H), New Guinea (I), Solomon Islands (J), Australia (K), Vanuatu (L), Fiji (M), New Caledonia (N) and Christmas Island (O). Numbers correspond to the dispersal rate multipliers (0–1) between pairs of areas.
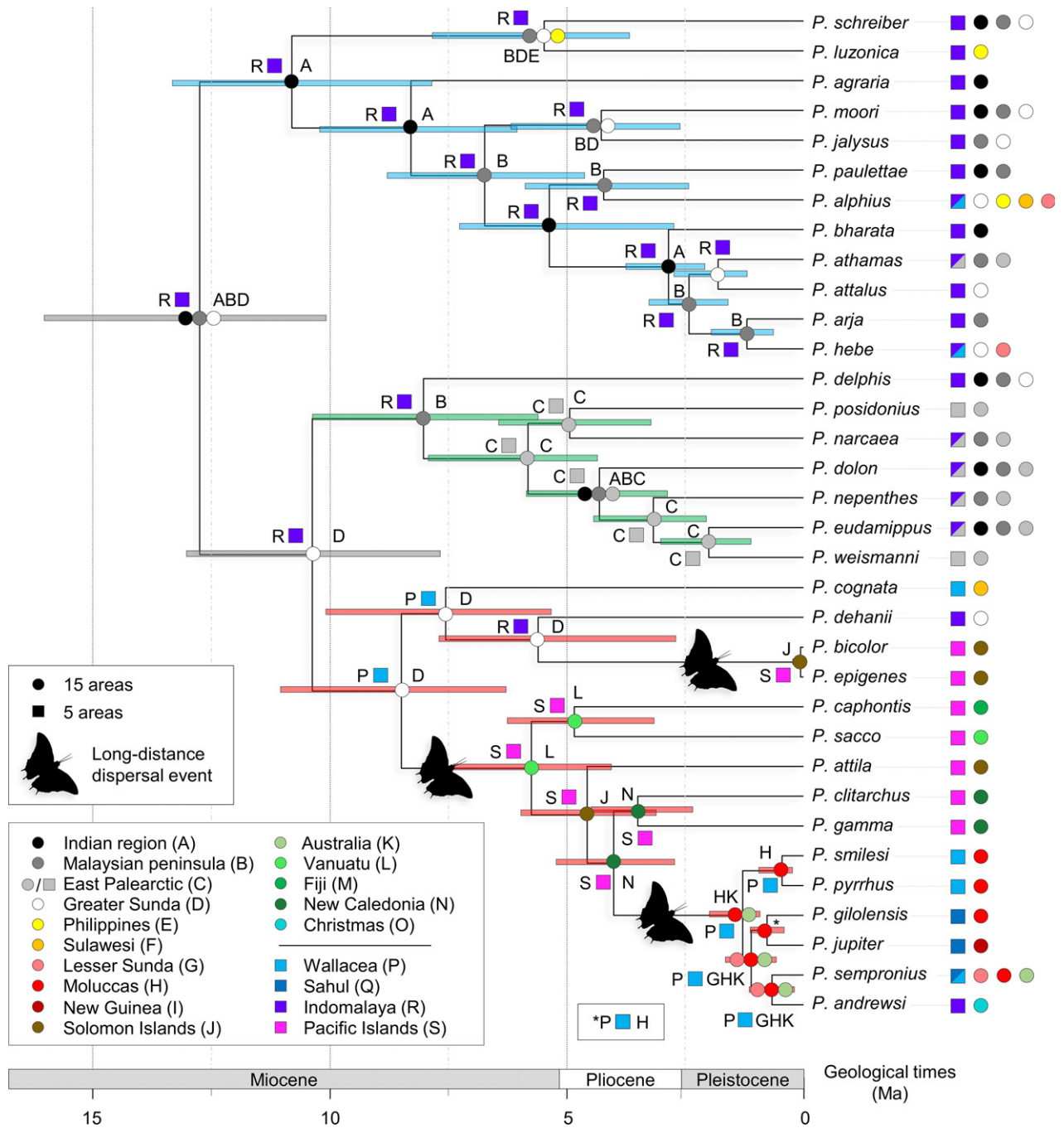
Figure 3 Bayesian phylogenetic species tree of the genus *Polyura*. Species tree recovered from the combined analysis of the four gene alignments (CO1, ND5, RPS5 and Wingless) in *beast. Branch lengths have been transformed to obtain a cladogram. The three species groups of the genus *Polyura* are highlighted.

beast divergence time estimation are given in Fig. 4. We recovered an origin of the genus *Polyura* in the mid-Miocene, 12.7 Ma (95% HPD: 10.0–16.1 Ma). The origin of the *P. athamas* group was recovered at 10.8 Ma (95% HPD: 7.8–13.3 Ma), whereas the crown of the *P. eudamippus* and *P. pyrrhus* groups were dated respectively at 7.9 Ma (95% HPD: 5.7–10.3 Ma) and 10.3 Ma (95% HPD: 7.6–13.0 Ma). Most of the branching events in the phylogeny were dated from the late Miocene to the late Pleistocene.

Ancestral area estimation

The results of the best biogeographical model for each set of areas are summarized in Fig. 4. The two analyses support similar scenarios although not at the same geographical scale.

Among the analyses based on the 15 areas, those conducted under the DEC+j and DIVALIKE+j models were significantly better supported compared to the rest of the models (Table 3). These two models gave similar results (see

Figure 4 Divergence times and historical biogeography of *Polyura* butterflies at different geographical scales. The median ages are derived from the BEAST analysis based on secondary calibrations from Wahlberg *et al.* (2009). Horizontal bars indicate the 95% HPD at each node and is colour coded depending on the species group. The geographical distribution is presented to the right of each species' name in the five area setting with squares and in the 15 area setting with circles. The results of the best BIoGEoBEARS model (DEC+j) of each analytical set (with 5 or 15 areas respectively) are also presented. Only the most likely ancestral area is shown at each node. Refer to Appendix S3 for the relative probabilities at each node.

Appendix S2), but as the DEC+j analysis received the highest relative probability (53.1%), these results are presented. The relative probability of the most likely ancestral area was rather low across the deep nodes of the topology (see Appendix S3). An origin in a combined ancestral area including the Indian region/Malaysian Peninsula/Greater

Sunda was recovered at the root. We recovered a signal of vicariance with range contraction with the ancestor of the *P. athamas* group being restricted to the Indian region and one of the two other groups, to Greater Sunda.

In the *P. athamas* group, we infer a late range expansion for the ancestor of the clade *P. luzonica*+*P. schreiber* in the

Table 3 Results of the BioGeoBEARS model comparison for the analyses with 15 areas.

	LnL	param.	*d*	*e*	*j*	Akaike weights	Rel. prob.
DEC	−134.988	2	0.198	0.159	0.000	0.000	0.000
DEC+j	−111.488	3	0.053	0.000	0.482	0.531	53.100
DIVALIKE	−134.209	2	0.131	0.075	0.000	0.000	0.000
DIVALIKE+j	−111.613	3	0.058	0.000	0.367	0.469	46.900

LnL, log-likelihood of the model; param., number of parameters in the model; *d*, dispersal; *e*, extinction; *j*, founder-event; Rel. prob., relative probability of the model in comparison to the other ones.

Philippines and Malaysian Peninsula. The latter was repeatedly recolonized from the Indian region between the late Miocene and mid-Pleistocene. Most of the distributions in Eastern Palaearctic, Sulawesi and Lesser Sunda Islands are very recent.

In the *P. eudamippus* group, the ancestor first recolonized the Malaysian peninsula from Greater Sunda in the late Miocene before rapidly dispersing towards the eastern Palaearctic. Range expansions in this group are also recent, although we recovered an early Pliocene range expansion towards the Indian region and Malay Peninsula in the clade comprising *P. dolon*, *P. nepenthes* and the species pair *P. eudamippus* + *P. weismanni*.

The ancestor of the *P. pyrrhus* group remained in Greater Sunda until the late Miocene when some populations dispersed to Vanuatu by LDD. The remaining populations remained in Greater Sunda until a late LDD event towards the Solomon Islands archipelago that resulted in the formation of the extant sister species *P. bicolor* + *P. epigenes*. The colonizers of Vanuatu then dispersed towards the Solomon Islands archipelago at the Miocene–Pliocene boundary which led to the formation of the endemic *P. attila*. From there, they colonized

New Caledonia where some populations gave rise to the endemic species pair *P. gamma* + *P. clitarchus*, while others dispersed to Australia and islands in the Moluccas. Colonization of Christmas Island (*P. andrewsi*), New Guinea (*P. jupiter*) and the Lesser Sunda Islands (some populations of the widespread *P. sempronius*) took place in the Pleistocene.

The analyses based on five areas gave a congruent pattern to that described above, although at a larger scale. The DEC+j and DIVALIKE+j models were also significantly supported compared to the models that did not include the founder-event speciation parameter +j (Table 4). The two models gave very similar results (presented in Appendix S2). As the DEC+j analysis received the highest relative probability (58.9%) these results are presented. The relative probabilities across the chronogram were higher compared to the analyses with 15 areas (see Appendix S3), but the overall biogeographical pattern is the same as in the latter. We recovered an origin in Indomalaya west of Wallace's Line with subsequent colonization of the eastern Palaearctic and LDD events to reach the Pacific Islands.

The colonization of the eastern Palaearctic by *P. athamas* and Wallacea by *P. alphius* and *P. hebe* was inferred to be

Table 4 Results of the BioGeoBEARS model comparison for the analyses with five areas.

	LnL	param.	*d*	*e*	*j*	Akaike weights	Rel. prob.
DEC	−77.755	2	0.149	0.100	0.000	0.000	0.000
DEC+j	−60.579	3	0.046	0.000	0.151	0.589	58.900
DIVALIKE	−75.429	2	0.122	0.032	0.000	0.000	0.000
DIVALIKE+j	−60.939	3	0.050	0.000	0.132	0.411	41.100

LnL, log-likelihood of the model; param., number of parameters in the model; *d*, dispersal; *e*, extinction; *j*, founder-event; Rel. prob., relative probability of the model in comparison to the other ones.

Table 5 Results of the TreePar analyses of diversification dynamics.

	LnL	P-value	dr1	tr1	ts1	dr2	tr2	ts2	dr3	tr3	ts3	dr4	tr4
Yule	−84.517	–	0.194	0.000	–	–	–	–	–	–	–	–	–
BD 1 shift	−81.081	0.076	0.000	1.000	3.900	0.213			–	–	–	–	–
BD 2 shifts	−79.946	0.166	−0.046	1.284	3.900	0.630	0.000	5.800	0.247	0.000	–	–	–
BD 3 shifts	−78.159	0.176	−0.034	1.193	3.900	0.522	0.292	5.800	0.104	0.014	7.500	0.000	1.000

LnL, log-likelihood of the model; P-value, result of the LRT between the Yule model and competing models; dr, diversification (speciation–extinction) rate; tr, turnover rate; ts, time shift; BD, birth–death model.

recent, probably during the late Pleistocene. We found an origin of the *P. eudamippus* group in Indomalaya followed by the colonization of the eastern Palaearctic in the late Miocene. Here again, the recolonization of Indomalaya by several species of this group is inferred to be recent. We found a colonization of Wallacea out-of-Indomalaya in the *P. pyrrhus* group followed by a LDD event towards the Pacific. The colonization of Pacific Islands by the ancestor of the species pair *P. bicolor* + *P. epigenes* was inferred from Indomalaya after a reverse colonization event from Wallacea. Wallacea was finally recolonized by dispersers from Pacific islands in the Plio-Pleistocene with further colonization of Sahul and Indomalaya in the Pleistocene.

Diversification analyses

Our diversification rate analyses conducted with the R package *TreePar* supported a Yule model with no rate shift as the best explanation of the diversification dynamics of *Polyura*. As indicated in Table 5, this model was a significantly better fit than all variable-rate models according to our LRT. The second best model with a unique shift was rejected with a *P*-value = 0.076. We found a diversification rate of 0.194 with a null turnover rate during the evolution of the genus.

DISCUSSION

The species tree presented here (Fig. 3) differs slightly from the one presented in Toussaint *et al.* (2015b). The three species groups were reconstructed as monophyletic and most of their internal organization is unchanged except for a few inconsistencies that received moderate support. In the *P. athamas* and *P. eudamippus* species groups, we generally recover phylogenetic relationships between geographically close lineages supporting the hypothesis of PAV exacerbated by geological and climatic events (Fig. 3). In the *P. pyrrhus* species group, however, we recovered intricate phylogenetic relationships between Pacific island species and Indo-Australian species providing some support for the alternative hypothesis of TAD.

We reconstructed an origin of the genus in the mid-Miocene (Fig. 4). It is worth mentioning that our age estimates are derived from secondary calibrations, and therefore conditioned by the accuracy of absolute ages inferred in Wahlberg *et al.* (2009). As recently hypothesized (Garzón-Orduña *et al.*, 2015), the ages determined by Wahlberg *et al.* (2009) may be overestimates although the analyses conducted by Garzón-Orduña *et al.* (2015) are equally debatable as the authors used host plants as maximum ages to infer absolute ages in Ithominii (Nymphalidae, Danainae) butterflies. Such practice (also used by Wahlberg *et al.*, 2009 in combination with fossil calibrations) assumes that present-day host plant specialization did not evolve with the clade's diversification, but is an ancestral trait. There is therefore a need for a new time-calibrated tree of nymphalids to cross-validate or invalidate the estimates of Wahlberg *et al.* (2009), although the age estimates for the tribe Charaxini inferred in Wahlberg *et al.* (2009) are largely congruent with the ones obtained in a slightly older study (Peña & Wahlberg, 2008) that implemented a fossil calibration without enforcing maximum ages
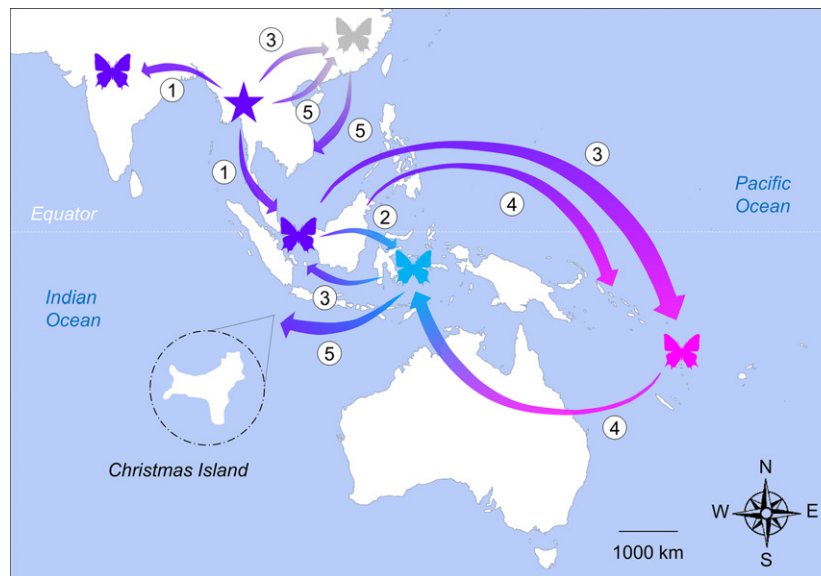

Figure 5 Biogeographical scenario of *Polyura* butterflies based on the BioGeoBEARS results. Schematic representation of the putative biogeographical history of the genus *Polyura* as derived from the results of both BioGeoBEARS analyses (with 5 and 15 areas respectively). The purple star indicates the origin of the genus in Indomalaya. The butterflies indicate newly colonized regions in the IAA. Coloured arrows indicate the potential routes of colonization and the colours refer to the areas defined in Figs 1 and 3. The arrows figuring the three long-distance dispersal events are not representative of actual routes. The numbers are proportional to time, with 1 being the oldest colonization event and 5 the last with reference to the dating framework presented in Fig. 3.

based on host plant information. Using our dated phylogeny, we inferred an origin of *Polyura* in Indomalaya with a possible ancestral area in the Indian region (Fig. 4) which unambiguously supports the out-of-Asia hypothesis. This is in line with the African origin of the genus *Charaxes* in which *Polyura* appears to be included (Aduse-Poku *et al.*, 2009; Müller *et al.*, 2010; Toussaint *et al.*, 2015b; this study).

In Fig. 5, we summarized the two biogeographical reconstructions conducted at different scales to illustrate the likely scenario that led to the ranges of extant *Polyura* species in the IAA. Most of the colonization in the oceanic part of the IAA occurred after the assemblage of the archipelago *c.* 15–20 Ma (Hall, 2002, 2012, 2013). Biological events thus reflect to some extent the geological evolution of the IAA as expected under the PAV hypothesis. The early evolution of *Polyura* broadly agrees with the dating of several other clades of IAA butterflies that colonized the archipelago out-of-Asia (Müller & Beheregaray, 2010; Müller *et al.*, 2010; Condamine *et al.*, 2013, 2015). This is also in line with the origin of several other insect groups whose evolution was likely shaped by the assemblage of the IAA in the Miocene (e.g. Balke *et al.*, 2009; Tänzler *et al.*, 2014, 2016; Toussaint *et al.*, 2014, 2015c; Clouse *et al.*, 2015).

The PAV hypothesis is also supported by our biogeographical reconstruction where we find the signature of past geological and/or climatic events. In particular, several allopatric relationships in the *athamas* species group might result from sea-level fluctuations in the Pleistocene (Voris, 2000) that may have allowed some populations from the Malaysian Peninsula to colonize the Greater Sunda islands. The vicariant pattern found in the species pair *P. luzonica* (endemic to the Philippines) and *P. schreiber* (widespread in the Oriental region but absent from the Philippines) is remarkably congruent with the separation of the Philippine arc from the Sunda shelf in the past 5 Ma (Hall, 2013). Likewise, the split between the Okinawa Islands endemic *P. weismanni* and its widespread closest relative *P. eudamippus* coincides with possible Pleistocene glacial land bridges between continental China and the Ryukyu archipelago (Kimura, 2000). These examples clearly substantiate the PAV hypothesis over the TAD hypothesis.

However, the *P. pyrrhus* species group provides a rather different biogeographical history (Fig. 4). The early colonization of Wallacea by the ancestor of this clade is congruent with the dating of Sulawesi's assemblage (Hall, 2013) and therefore supports the PAV hypothesis. However, we infer a stunning LDD event from Indomalaya to Solomon Islands where the ancestor of the pair *P. bicolor*–*P. epigenes* settled in the Pleistocene (Toussaint *et al.* in press b). In the second major branch of the *P. pyrrhus* group, we infer another LDD event from Wallacea to the Pacific islands, most likely to Vanuatu where only the endemic *P. sacco* currently occurs. We recover a third LDD event in a more derived part of the phylogeny from New Caledonia to a joint area including Australia and the Moluccas. Under the scenario of early presence in Vanuatu, the other Pacific

Ocean archipelagos could have been colonized by island hopping before a last reverse colonization towards Sahul and Wallacea where it led to the evolution of multiple endemics including the remarkable Christmas emperor *P. andrewsi*. These LDD events, along with the diversification rate analysis results, substantiate the TAD hypothesis over the PAV hypothesis and therefore shed light on the intricate biogeographical history of *Polyura* resulting from a combination of both mechanisms (LDD and abiotic vicariance).

Such patterns of LDD have rarely been suggested in the hyperdiverse arthropods of the Indo-Australian archipelago. Peacock swallowtail butterflies [*Papilio* (*Achillides*)] show a potential case of LDD from Wallacea towards New Caledonia, for instance, although island hopping in the Melanesian archipelago was not ruled out (Condamine *et al.*, 2013). *Camponotus* ants might also represent a clade with multiple LDD events (Clouse *et al.*, 2015), but the lack of proper divergence time estimates hampers the testing of this hypothesis. *Polyura* butterflies represent to our knowledge only the second example of colonization from Pacific islands back to the centre of the IAA. Grudinski *et al.* (2014) inferred such a pattern in the woody genus *Aglaia* (Sapindales), where Pacific lineages probably colonized Sundaland and the Philippines in the Pleistocene by bird dispersal. Although LDD events are difficult to substantiate and presumably extremely rare (Gillespie *et al.*, 2012), an alternative scenario to explain the present distribution of *Polyura* species would imply repeated and massive extinctions. Such an evolutionary trajectory, although not entirely impossible, is not supported by our diversification analyses (Table 5). Therefore, we suggest that our biogeographical reconstruction is the most parsimonious to explain the present-day geographical ranges observed in this charismatic group of tropical butterflies. This is consonant with the multiple transgressions of Wallace's and Lydekker's Line inferred during the evolution of the genus that support the view of *Polyura* butterflies as good dispersers (but see Tänzler *et al.*, 2014, 2016 and Toussaint *et al.*, 2015c for flightless clades able to cross these boundaries). The fact that most *Polyura* species are probably polyphagous also supports the idea that these butterflies are good dispersers, able to feed on a wide variety of host plants once settled on a newly colonized island. Our findings of fine-scale allopatric speciation and LDD events substantiating both PAV and TAD hypotheses suggest that the triggers of speciation and biogeographical structure in this group are diverse and intricate.

CONCLUSION

We highlight the remarkable historical biogeography of Nawab butterflies in the Palaeotropics and particularly in the Indo-Australian archipelago. We show that different parts of the phylogenetic tree support either past abiotic vicariance (PAV) or trans-archipelagic dispersal (TAD) hypotheses. The genus' biogeographical history was mainly driven by geologi-

cal and climatic vicariance west of Lydekker's line and by trans-archipelagic dispersal events to the east of that line. Pacific islands, in particular Vanuatu and New Caledonia, apparently served as hubs for the reverse colonization of Wallacea from the Pacific and the recent settlement of populations on Christmas Island and New Guinea. Our results provide a better understanding of biogeographical processes in the IAA, and also illustrate and support a view of islands as potential source areas for the evolution of regional diversity, rather than evolutionary sinks (Balke *et al.*, 2009; Jønsson *et al.*, 2011; de Bruyn *et al.*, 2014).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Details of all *Polyura* specimens used in this study.
Appendix S2. Results of the BioGeoBEARS analyses showing the most likely ancestral areas.
Appendix S3. Results of the BioGeoBEARS analyses showing the relative probabilities of ancestral areas of the two best analyses.

BIOSKETCHES

Emmanuel F.A. Toussaint is a post-doctoral researcher at the University of Kansas in Lawrence, USA. His main research interest focuses on the origin and evolution of biodiversity at global and regional scales. He combines ecological and molecular data to study lineage diversification and biogeography of insects, mainly aquatic beetles and tropical butterflies.

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Author contributions: E.F.A.T. conceived the study, contributed the data, analysed them, designed the figures and wrote the article; M.B. made significant comments on and improvements to the manuscript.

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