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ORIGINAL ARTICLE

Delving into *Delias* Hübner (Lepidoptera: Pieridae): fine-scale biogeography, phylogenetics and systematics of the world's largest butterfly genus

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ABSTRACT

Aim Our objective was to reconstruct a species-level phylogeny of the genus *Delias*, to elucidate their finer-scale biogeography and to test boundaries between closely related taxa.

Location Indo-Australian region, with a focus on Wallacea.

Methods Sequence data from 131 taxa, representing all recognized species groups and more than half of the known species of *Delias*, were used in the analysis. Phylogenetic analyses based on molecular characters of the mitochondrial gene cytochrome *c* oxidase subunit I (*COI*) and nuclear genes *wingless* and elongation factor 1α (*EF-1α*) were carried out using maximum parsimony and Bayesian inference. Biogeographical reconstructions were undertaken using the parsimony-based method dispersal–vicariance analysis and the dispersal–extinction–cladogenesis model as implemented in RASP and LAGRANGE, respectively.

Results The phylogenetic hypothesis resolved 14 distinct clades, here designated the *nysa*, *isse*, *pasithoe*, *belladonna*, *ladas*, *geraldina*, *aroae*, *eichhorni*, *sagessa*, *aganippe*, *hyparete*, *belisama*, *albertisi* and *nigrina* species groups. *Delias blanca* Felder and *Delias chrysomelaena* Snellen van Vollenhoven were transferred to the *pasithoe* and *isse* species groups, respectively. We demonstrate that the barcode region of *COI* is useful for the delineation of closely related, more recently diverged, *Delias* species. Species diversification in *Delias*, for the most part, is shown to pre-date the Pleistocene, even in montane mainland New Guinea where numerous phenotypically similar sister species co-occur.

Main conclusions Sibling *Delias* species found in sympatry are largely restricted to those clades confined to mainland New Guinea, where most species occur in high-elevation habitats. Conversely, clades with large geographical ranges are composed of essentially allopatric taxa. Although an Australian Plate origin is plausible for the genus, *Delias* is likely to have colonized islands peripheral to Australia during the early stages of its evolution (i.e. during the Miocene), as evidenced by the presence of older lineages in Wallacea and also in islands of the south-western Pacific.

Keywords
Butterflies, DEC model, historical biogeography, Indo-Australian region, Miocene, molecular phylogeny, Müllerian mimicry, plate tectonics, Pliocene.

INTRODUCTION

Delias butterflies, commonly known as Jezebels, are popular with naturalists, artists, researchers and collectors world-wide and have been the focus of numerous taxonomic and, more recently, molecular studies (see Braby & Pierce, 2007 and references therein). *Delias* Hübner (Lepidoptera: Pieridae) are widely distributed in the Indo-Australian region, with the highest species diversity in the highlands of mainland New Guinea (NG) (Parsons, 1998; Morinaka *et al.*, 2002; Braby &

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Pierce, 2007). The first detailed taxonomic assessment of *Delias* was undertaken by Talbot (1928–1937), who classified 153 species into 20 groups. Yagishita *et al.* (1993) identified 216 species and assigned them to 22 groups. Currently, there are 255 recognized species, rendering *Delias* the largest genus of butterflies, surpassing the species diversity of *Acraea* Fabricius and *Euphaedra* Hübner (Nymphalidae), *Polyommatus* Latreille and *Arhopala* Boisduval (Lycaenidae) and *Papilio* Linnaeus (Papilionidae).

Morinaka *et al.* (2002) showed that *Delias* is monophyletic and Braby & Pierce (2007) conducted a species group-level phylogeny, which identified eight major clades. Braby *et al.* (2006) confirmed that *Delias* belonged to the Aporiina, a cosmopolitan group of genera, and showed that the genus is closely related to *Leuciacria* Rothschild & Jordan. The origins of *Delias* and their broad-scale cladogenesis have been surmised by Braby & Pierce (2007). In contrast, our study focused on estimating finer-scale relationships to clarify the processes that have influenced the extraordinary rate of diversification in the genus and to resolve questions about species-level systematics. Comprehensive species-level phylogenies have a greater capacity to resolve systematic relationships as well as biogeographical history in Indo-Pacific butterflies (Müller *et al.*, 2010; Müller & Beheregaray, 2010) and to clarify current taxonomic issues in *Delias* (e.g. Braby & Pierce, 2007).

In recent years, several studies have investigated the origins of various groups of Indo-Pacific butterflies. An 'out-of-Africa' origin is evident for two nymphalid genera, *Charaxes* (Aduse-Poku *et al.*, 2009) and *Junonia* Hübner (Kodandaramaiah & Wahlberg, 2007), while a Gondwanan origin has been inferred for *Delias* (Braby & Pierce, 2007) and troidine butterflies (Braby *et al.*, 2005), and a Palaearctic origin for Papilionidae (Condamine *et al.*, 2013). Here we attempt to disentangle the biogeographical history of the genus as a part of a larger comparative biogeographical study of butterflies in the Indo-Pacific region (Müller & Beheregaray, 2010; Müller *et al.*, 2010). The genus *Delias* is ideal for biogeographical studies within this region because it is generally widespread, yet local endemism is particularly high; for example in single mountain ranges or small islands. Indeed, there are very few wide-ranging species (Talbot, 1928–1937).

The Indo-Pacific region is geologically highly complex with a very dynamic history (Hinschberger *et al.*, 2005; Hall, 2009). During the late Oligocene, approximately 25 million years ago (Ma), fundamental changes took place as a result of the collision between the Australian continent and the Philippine Sea Plate arc in NG. The Philippine Sea Plate began to rotate clockwise and this ultimately resulted in the accretion of fragments (micro-continents) from the northern Australian margin onto the Southeast Asian margin, notably in Sulawesi (Hall, 2009). In the middle Miocene, *c.* 15 Ma, subduction at the Banda Trench was initiated and subsequently led to back-arc spreading and the inception of the Banda Basins (Banda Sea) (Réhault *et al.*, 1994; Honthaas *et al.*, 1998; Hinschberger *et al.*, 2005). That process further resulted in the fragmentation of this agglomeration of micro-continents, generating the group of islands known as Wallacea. Wallacea is of utmost importance in a biogeographical context as it is situated between the Oriental and Australian regions. While *Delias* comprises several species groups endemic to mainland NG, a number of wide-ranging groups span both zoogeographic regions. Such groups are potentially highly informative to our understanding of the biogeographical history of the genus in the region. Indeed, Braby & Pierce (2007) called for finer-scale phylogenies of the groups that are widespread across Wallacea. Here we reconstruct a species-level phylogeny of *Delias* and elucidate its biogeographical history based on the most complete molecular dataset generated so far for this genus. Our emphasis is on fine-scale biogeography and, in particular, assessments of patterns of dispersal within and across Wallacea.

MATERIALS AND METHODS

Taxon sampling

Our combined dataset consisted of 138 taxa, including seven pierid outgroups. We sampled *Delias* from all 21 species groups of Talbot (1928–1937), some entirely (e.g. Talbot's *stresemanni* and *chrysomelaena* groups) or almost entirely (e.g. the *belisama* and *hyparete* groups). In particular, we focused on sampling comprehensively within widely distributed clades. Of the 44 species recorded from Wallacea, we sampled 30 (68%). We also sampled multiples of closely related species within the *nigrina* and *albertisi* clades to assess genetic and phenotypic relationships. Several species sequenced here are known only from the types, or very few specimens (e.g. *Delias brandti* Müller, 2001, *Delias pulla* Talbot, 1937 and *Delias laknekei* Miller, Simon & Wills, 2007). The cladograms were rooted using *Eurema hecabe* (Linnaeus, 1758) and *Pareronia tritaea* (C. & R. Felder, 1859), as well as five outgroup taxa of the tribe Aporiina (see Appendix S1 in Supporting Information).

Molecular markers

The combined dataset consisted of DNA sequences from three genes, cytochrome *c* oxidase subunit I (*COI*), *wingless* and elongation factor 1α (*EF-1α*). The great majority of sequences were from *COI* (132 taxa), whereas we used fewer samples of *wingless* (68 taxa), owing to the greater difficulty of amplifying nuclear DNA from dry-preserved material. We also incorporated the *EF-1α* sequence dataset of Braby & Pierce (2007), which comprised 33 taxa. This combination of genes was particularly successful at resolving lineages within the family Pieridae (e.g. Braby *et al.*, 2007). Mitochondrial *COI* is known to provide a better signal for resolving lower-level relationships, whereas nuclear *wingless* and *EF-1α* resolve deeper lineages (e.g. Caterino *et al.*, 2000).

Molecular techniques

Two legs and/or small amounts of body tissue were removed for subsequent extraction of DNA. Voucher specimens are deposited at the following centres: C. J. Müller reference collection, Sydney; Australian National Insect Collection (ANIC), Canberra, Australia; School of Biological Sciences Collection, Macquarie University, Sydney, Australia. A Chelex (Bio-Rad, Hercules, CA, USA) method (Walsh *et al.*, 1991), modified according to Müller & Beheregaray (2010), was used to extract genomic DNA (gDNA). Standard polymerase chain reactions (PCRs) were conducted in a PTC-100 MJ Research thermocycler (Colonel Light Gardens, Adelaide, SA, Australia), with a volume of 25 μL: 2 μL of gDNA template at various dilutions, with 11.625 μL of distilled H_2O, 2.5 μL of PCR buffer (100 mM), 2.5 μL magnesium chloride, 5 μL of each dNTP (2.5 mM), 0.5 μL of each primer and 0.375 μL of Taq polymerase (5 units μL^{-1}). Some specimens were processed at the Laboratory of Genetics, University of Turku, Finland, following the protocols described on the Nymphalidae Systematics Group webpage (http://nymphalidae.utu.fi/).

For *COI*, a 654-bp fragment was amplified using the LCO and HCO primers of Folmer *et al.* (1994). For older and/or degraded tissue samples, we used internal primers Ron (5′-GGAGCYCCWGATATAGCTTTCCC-3′) and Nancy (5′-CCTGGTAAAATTAAAATATAAACTTC-3′) from Caterino & Sperling (1999). To amplify the 433-bp *wingless* gene fragment, the primers LepWG1 and LepWG2 (Brower & DeSalle, 1998) were employed. The PCR cycling protocols for the three genes are described in Müller & Beheregaray (2010). Negative controls were included in all PCRs. The PCR products were separated by electrophoresis and purified with an UltraClean 15 DNA Purification Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). All DNA sequencing was performed on an ABI 3130 automated sequencer (Applied Biosystems, Carlsbad, CA, USA). Chromatograms were edited manually and then aligned in SEQUENCHER 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). All sequences were aligned against other published lepidopteran sequences (e.g. Brower & DeSalle, 1998; Campbell *et al.*, 2000). For *COI*, the consensus sequences were aligned against the reference sequence for *Drosophila yakuba* Burla (Clary & Wolstenholme, 1985) and/or other lepidopteran sequences in GenBank. GenBank accession numbers for all sequences are given in Appendix S1.

Phylogenetic analysis

Individual sequence properties were evaluated using MEGA 4.1 (Tamura *et al.*, 2007). Datasets for phylogenetic analyses [maximum parsimony (MP) and Bayesian inference (BI)] were generated using the software VOSEQ 1.2.4 (Peña & Malm, 2012). The MP analyses were undertaken using TNT 1.1 (Goloboff *et al.*, 2003). Heuristic searches using the New Technology Search algorithms (Goloboff, 1999; Nixon, 1999), with default settings, were executed with 100 random additions. Clade support values were assessed by bootstrap (Felsenstein, 1988) with 1000 pseudo-replicates and 10 independent replicates with tree bisection–reconnection branch swapping. Partitioned Bremer support (PBS) values (Baker & DeSalle, 1997) were calculated using the strict consensus tree recovered by the MP analysis and a script written by Carlos Peña (in Peña *et al.*, 2006; available at http://bit.ly/xfZMa3) to evaluate the contribution of and congruence between gene datasets.

Bayesian analyses with the dataset partitioned by gene fragment (*COI*, *EF-1α*, *wingless*) were conducted using MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003), after confirming the suitable model of evolution for our dataset in MODELTEST 3.06 (Posada & Crandall, 1998). All partitions were assigned with the GTR+G model. Two independent runs using four Markov chains, one cold and three heated, were run simultaneously for 10 million generations. Tree sampling was set for every 100 generations. The tree topology and posterior probabilities were summarized after confirming that the average standard deviation of split frequency estimations was below 0.05 and discarding the first 1,000,000 generations as 'burn-in'.

Age of divergence estimations

We used calibration points and a relaxed molecular clock approach, as implemented in BEAST 1.5.2 (Drummond & Rambaut, 2007), to estimate times of divergence in *Delias*. The calibration points are taken from the estimated ages of the split between *Delias* and *Leuciacria* (Braby *et al.*, 2006) and the age of the crown group of *Delias* (Braby & Pierce, 2007), which were extrapolated from fossil data included in their phylogeny of the family Pieridae. Accordingly, all resulting age estimates in this study are minimum estimates. No other calibration points are recognized as potentially reliable, because there are no known singular events that can be tied to specific lineage splits. However, the calibration points used here are supported by other estimations based on distinct methods and fossil data of the time of split between *Delias* and *Leuciacria* (Wheat *et al.*, 2007), which is concordant with that suggested by Braby & Pierce (2007). Also, various vicariance events are likely to have been overprinted to some degree.

The analysis was run within a Bayesian statistical framework. We applied the relaxed molecular clock method (uncorrelated lognormal distribution and GTR+G evolution model) independently to each gene partition and set the tree prior to the birth–death process. The calibration points were inserted as the means of normal distributions, where the split between *Delias* and *Leuciacria* was set to 31 ± 4 SD Ma and the crown age of *Delias* to 26 ± 3 SD Ma (Braby & Pierce, 2007). All other priors were left as the default values. We ran the analysis twice for 10,000,000 generations and sampled the log parameters every 1000 generations, with the initial

1,000,000 generations discarded as burn-in. The program TRACER 1.5 (Rambaut & Drummond, 2007) was used to test whether parameter estimates and tree topology had converged and reached equilibrium by verifying that the effective sample size values were higher than 100. The log and tree files from both runs were then combined using LOGCOMBINER 1.5.2 and the trees were summarized using the program TREEANNOTATOR 1.5.2 (both included in the BEAST package) into one maximum clade credibility tree where divergence times are reported as mean heights on each node.

Biogeographical analysis

The study area was subdivided into three geographical areas based on geological information and the butterfly distributions: (A) Australian region, including NG and satellite islands; (B) Wallacea; and (C) the Oriental region, including Southeast Asia (Fig. 1). The subdivision of these three major areas was intended to decipher the biogeographical patterns of radiation across Wallacea in an attempt to infer major routes of dispersal or vicariance events between Southeast Asia and Australia.

To investigate the biogeographical history of *Delias*, we used the parsimony-based method dispersal–vicariance analysis (DIVA) (Ronquist, 1997) as implemented in RASP 1.1 (Yu *et al.*, 2011) whereas to determine statistical support and account for phylogenetic uncertainty, the Bayes-DIVA approach was executed. We used the post burn-in phylogenetic trees from the analyses in BEAST and left the settings as defaults. The Markov chain Monte Carlo (MCMC) method was carried out using 10 chains (temperature 0.1) for 10,0000 cycles, sampling every 100th cycle and discarding the first 1000 samples, while the model used was F81 + G. The maximum number of ancestral areas was left unconstrained (i.e. three maxareas) and the root distribution set as null. In addition, for comparative purposes, we used the dispersal–extinction–cladogenesis (DEC) model as implemented in LAGRANGE C++ (Ree & Smith, 2008), as a second distinct

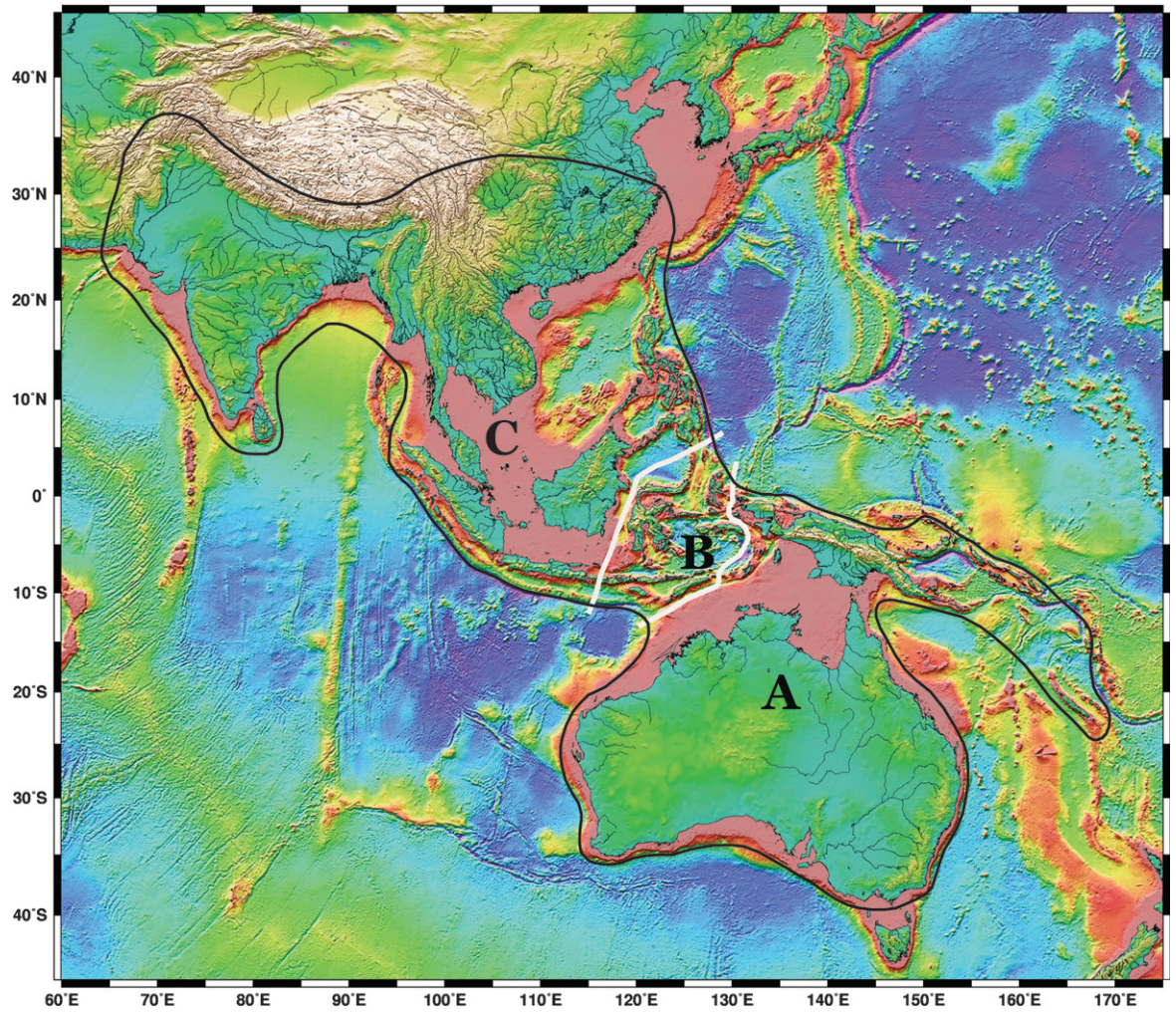

Figure 1 Map of the Indo-Australian region showing the geographical range of *Delias* (black line) and assigned biogeographical zones used in the dispersal–vicariance analysis, separated by white lines and defined as follows: Australian region (A), Wallacea (B), Southeast Asia (C).

method to compare the inferences of ancestral ranges of *Delias*. The input ultrametric tree was the maximum clade credibility inferred using BEAST and the settings were left as defaults.

RESULTS

Dataset properties

Our dataset consisted of sequences from 173 exemplars, representing 131 *Delias* species and seven outgroup taxa. The complete combined data contained 2234 bp, of which 864 (38.7%) were variable and 613 (27.4%) were parsimony informative. At the individual gene level, *wingless* had the highest proportion of parsimony informative sites, with 40.8%, while *COI* had 37.2% and *EF-1*α 17.5%. Base frequencies were near equal in *wingless* and *EF-1*α, but were A–T biased in *COI* (A = 29.2, T = 38.5, G = 14.3, C = 18.0). Appendix S2 shows the strict consensus tree of the 51 equally most parsimonious trees found for the combined dataset (length 3249, consistency index = 37, retention index = 62).

Partitioned Bremer support for the combined dataset under MP showed that there was variable conflict between the different gene partitions. *COI* contributed most to the phylogenetic signal, with only 16 nodes (13.1%) conflicting. Some conflict arose from nuclear genes *wingless* and *EF-1*α but this is probably attributable to the lack of data for many of the taxa, as well as the slower rate of evolution of these genes compared with the mitochondrial *COI*. Nonetheless, there exists congruence between the single gene and combined datasets, as well as between methods of phylogenetic reconstruction, as the species groups are recovered in every analysis.

Phylogenetic patterns

Fourteen distinct clades were recovered (Fig. 2), here designated as the *nysa*, *isse*, *pasithoe*, *belladonna*, *ladas*, *geraldina*, *aroae*, *eichhorni*, *sagessa*, *aganippe*, *hyparete*, *belisama*, *albertisi* and *nigrina* species groups. The clades were recognized by their timing of separation from sister clades, each with a split of at least 15 Ma. The monophyly of the genus *Delias* was confirmed and the genus *Leuciacria* was recovered as sister to *Delias*. The *nysa* clade was recovered as sister to all remaining *Delias*. Clade composition was nearly identical for BI and MP (Appendix S2), with the majority of clades being well supported. The relationship of *Delias aganippe* with other *Delias* taxa was not resolved under MP, nor was the position of *Delias blanca* in the phylogeny using either method. The species composition of each clade was also the same for both BI and MP, but the phylogenetic relationships among most of them were not well resolved in the MP analysis. The *nysa* and *isse* clades diverged earlier in the evolution of the genus, as clearly shown by the Bayesian analyses. The (*hyparete* (*belisama* (*albertisi* + *nigrina*))) grouping is also evident in both BI and MP.

Age of divergence

The Bayesian tree inferred in BEAST is highly congruent with the BI and MP trees, recovering the major groupings of species as well as the timings of the origins and diversification of the main clades found in previous studies of *Delias* (Braby & Pierce, 2007; Wheat *et al.*, 2007). Following the split between *Leuciacria* and *Delias* at approximately 31 Ma (37–25 Ma), the majority of species-groups originated in the early to mid Miocene (*c.* 24–15 Ma). There is no apparent difference in the overall timing of speciation between the 'insular clades' and the mainland NG clades. Throughout the late Miocene to Pliocene (*c.* 10–3 Ma), there was a remarkable increase in speciation events, contrasting with the fewer sister pairings dating from the Pleistocene. During the past 2.5 Ma, five splits are implied for the Oriental Region (C) and four for the Australian Region (A), whereas only the recent divergence between *Delias periboea* and *Delias timorensis* is reported for Wallacea (B).

Biogeographical analysis

Both the Bayes-DIVA and LAGRANGE analyses suggest that very early in the evolution of the group, *Delias* was present as several clades in Wallacea (Fig. 3a,b). Despite the differences in rationale between the two methods, general biogeographical patterns are recovered. Due to the occurrence of the sister group *Leuciacria* in Australia, and given the extant distribution of *Delias* species, both analyses suggest an Australian origin of *Delias*, as suggested by Braby & Pierce (2007). However, Wallacea was very important for the diversification of the group, as the butterflies have apparently crossed this region towards both Southeast Asia and Australia several times (Fig. 3). For instance, the analyses suggest that there were early dispersal events from Wallacea towards the Oriental region in the *nysa*, *isse*, *hyparete* and *belisama* clades during the late Miocene and Pliocene. In the case of the *belisama* clade, dispersal events back into Wallacea from the Oriental region were inferred about 5 Ma, and dispersal back into the Australian region at around 7.5 Ma was inferred for the *nysa* clade.

The main difference between the LAGRANGE and Bayes-DIVA outputs is the widespread ancestral distributions (involving more than one geographical area) closer to the crown node of *Delias* in the former, thus implying ancient dispersal events earlier in the evolution of the genus than in Bayes-DIVA. Furthermore, there is evidence of conflict between the methods because certain nodes show different inferred ancestral ranges or different relative probabilities for the same reconstructed ancestral ranges.

DISCUSSION

Historical biogeography and diversification

As *Delias* is highly species rich and many species have restricted ranges, their fine-scale biogeographical patterns

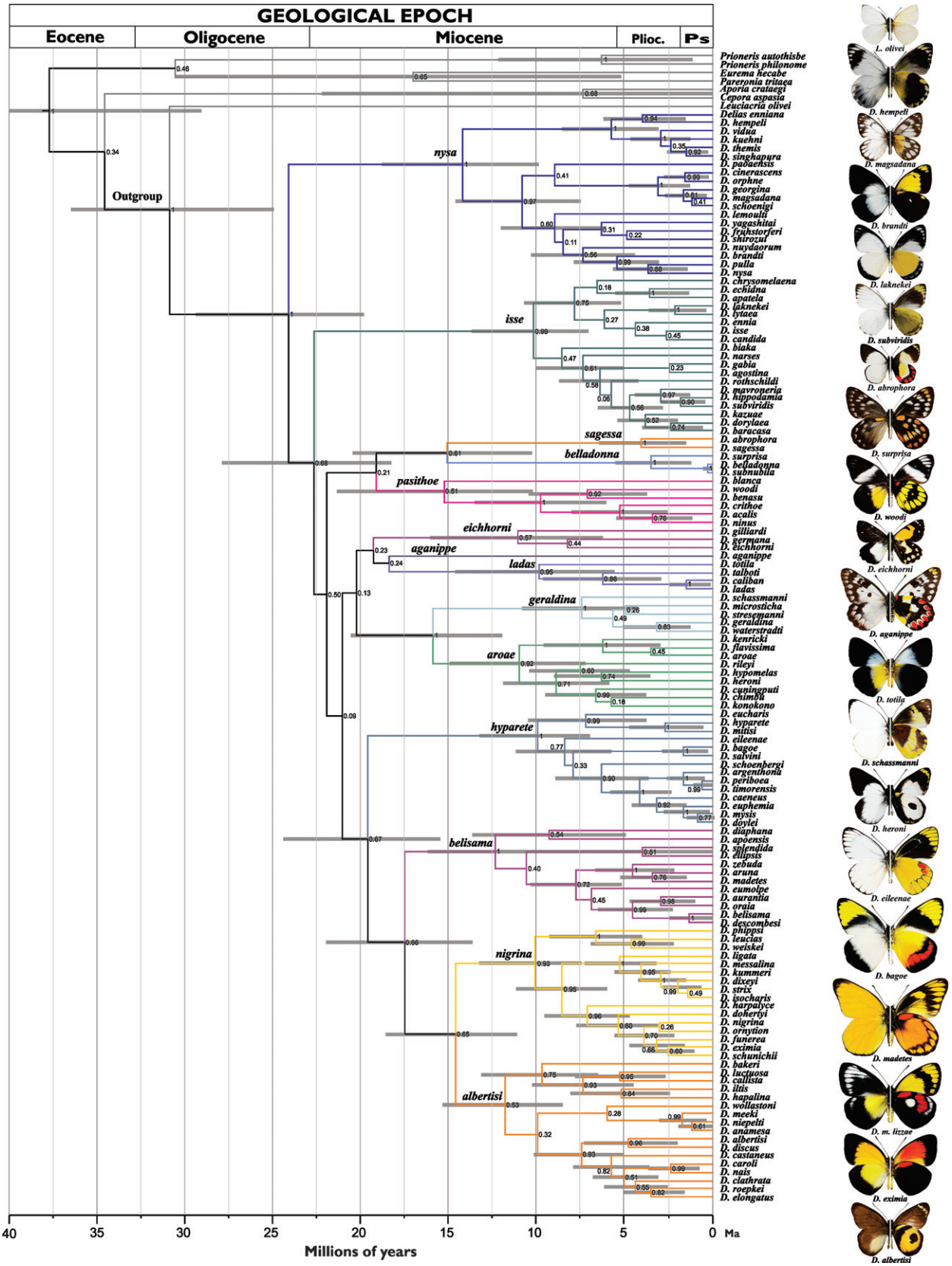

Figure 2 Calibrated tree showing phylogenetic relationships of *Delias* and estimates of divergence times for the combined cytochrome *c* oxidase subunit I (*COI*), elongation factor 1α (*EF-1α*) and *wingless* datasets. Clades are coloured according to their species group taxonomy. Numbers on the right of each node represent posterior probabilities and the horizontal bars are the 95% credibility interval estimated in BEAST. The scale of the ultrametric tree is million years ago (Ma). Plioc is Pliocene and Ps is Pleistocene.

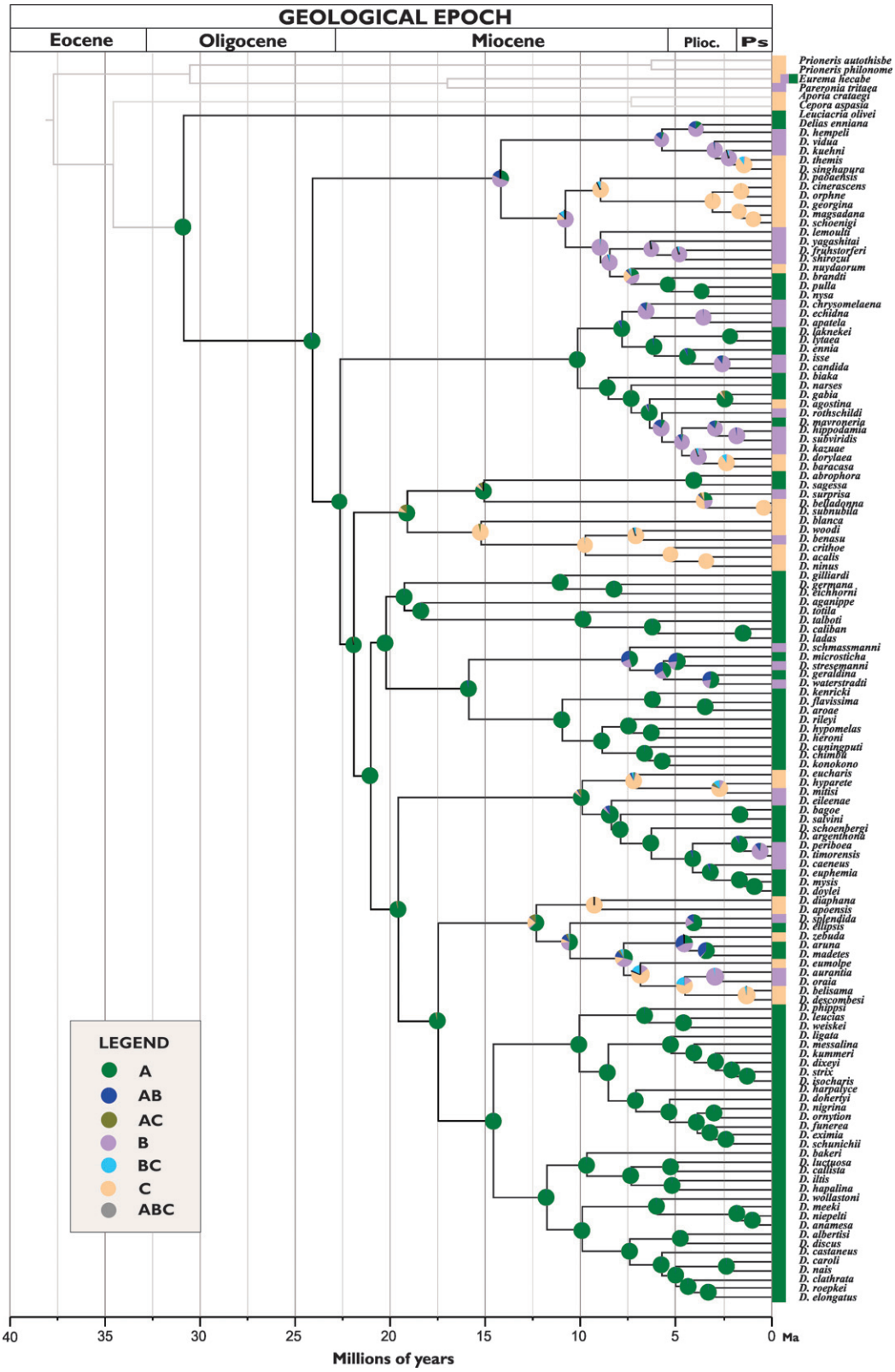

Figure 3 Ancestral areas of distribution reconstructions for *Delias* as inferred by (a) Bayes-DIVA and (b) LAGRANGE, where A is the Australian region, B is Wallacea and C is Southeast Asia. Pie charts on each node represent marginal probabilities (Bayes-DIVA) and relative probabilities (LAGRANGE) of ancestral ranges (states). Ancestral area probabilities lower than 0.1 were combined in the category 'junk'. The scale of the ultrametric tree is million years ago (Ma). Plioc is Pliocene and Ps is Pleistocene.

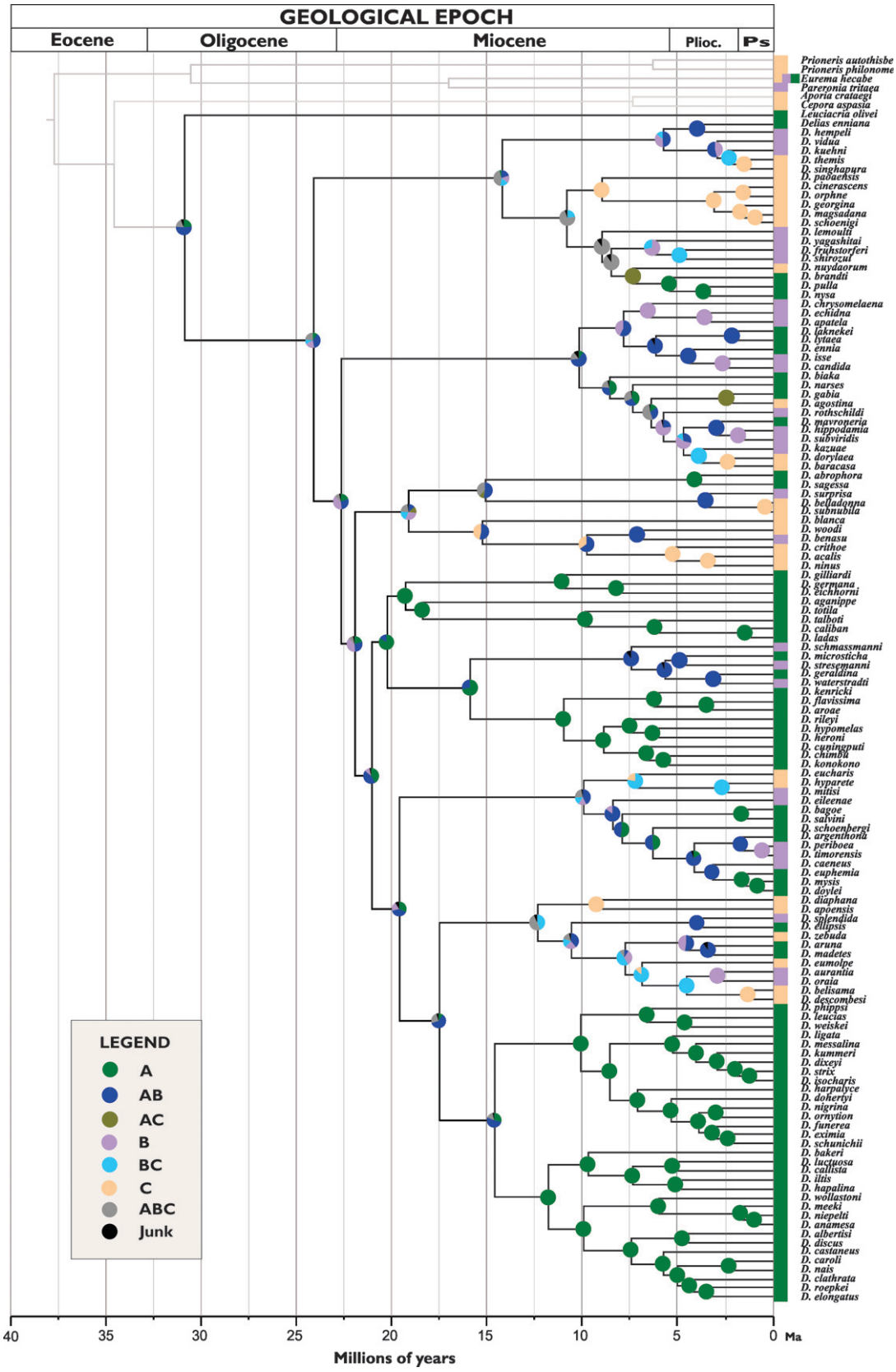

Figure 3 Continued

provide insights into the processes that led to their diversification. Broadly, two major biogeographical groups are evident within *Delias:* those that can be regarded as belonging to 'insular' clades and those that are 'NG mainland' clades. The first are relatively widespread clades, composed essentially of allopatric species that inhabit mostly low to moderate elevations below 2000 m. Such wide-ranging clades (*nysa*, *isse*, *hyparete* and *belisama*) have speciated largely on islands outside of the NG mainland. Where they occur on mainland NG, they are represented by only one or two species. Of the less widespread 'insular' clades, the *ladas* species group has diverged into highly distinctive taxa in the NG archipelagos but *Delias ladas*, distributed throughout mid-montane NG mainland, is remarkably consistent in phenotype across its wide range. Conversely, the second group of clades comprise numerous sympatric species that are confined to high elevations in mainland NG but which have not crossed even very narrow seas to satellite islands (e.g. *aroae*, *eichhorni*, *sagessa* and *albertisi* clades).

Braby & Pierce (2007) proposed an Australian Plate origin for *Delias*, based on a sister relationship with *Leuciacria* and other evidence, in contrast to several previous studies that hypothesized an Oriental origin for *Delias* (Dixey, 1894; Talbot, 1928–1937; Klots, 1933; Roepke, 1955; Holloway, 1986; Mani, 1986). However, the close relationship of *Leuciacria* was not then appreciated and Klots (1933) placed the genus as sister to *Elodina*. Both Dixey (1894) and Talbot (1928–1937) considered that the Oriental *pasithoe* and *belladonna* were the most 'primitive' groups in *Delias* but actually the *nysa* clade split off first in the evolutionary history of the genus. Our biogeographical analyses suggest that the genus spread rapidly, with early lineages crossing and, to some extent, diversifying in Wallacea. Talbot (1928–1937) noted that, with the exception of species belonging to the mainland NG clades, all other groups are represented by species occurring in Wallacea, which Talbot referred to as the 'Central Area' (also comprising Gebe and Aru). He proposed that many species originated in this area and spread in all possible directions. Our phylogenies are not indifferent to this theory, in that the older *Delias* lineage represented by the *nysa* clade is composed largely of Wallacean endemics. Indeed, Müller *et al.* (2010) and Müller & Beheregaray (2010), respectively, recorded an ancient Wallacean element in the evolutionary histories of the nymphalid genera *Charaxes* and *Cethosia* in the Indo-Pacific, and attributed such early diversifications to the fragmentation of Wallacea during the mid to late Miocene, a theory equally plausible for *Delias*. Lohman *et al.* (2011) surmised that taxa in Sulawesi, the largest island in Wallacea, are frequently basal groups with an essentially Asian origin, as revealed by molecular data.

Older lineages in islands peripheral to NG are not only restricted to Wallacea but are also apparent in islands of the south-western Pacific. The presence of *Delias ellipsis* Joannis in New Caledonia, which is an old member of the *belisama* group, as well as the early diverged *Charaxes (Polyura) gamma* Lathy (Nymphalidae) (see Smiles, 1982) attest to this theory. In this study, all 11 known Bismarckian *Delias* species were sampled, enabling an assessment of their phylogenetic relationships in detail. A possible link existed between Australia and the Bismarck Archipelago, through island arc (and ophiolite) stepping-stones in the south-western Pacific (such as Vanuatu and New Caledonia). The closer relationship between *Delias brandti* (New Ireland), *Delias nysa* (Australia, New Caledonia and Vanuatu) and *Delias pulla* (mainland NG), whose origins are dated back at around 5 Ma, is one example. Past connections between the Bismarck Archipelago and mainland NG facilitated the dispersal of *Delias* as exemplified by the splitting of *Delias messalina* (Bismarcks) and *Delias ligata* (mainland NG), *Delias madetes* (Bismarcks) and *Delias aruna* (mainland NG), and (*Delias laknekei* + *Delias lytaea*) (Bismarcks) and *Delias ennia* (mainland NG), dated at approximately 5 and 6.5 Ma, respectively. Similarly, connections between the Bismarcks and the Solomons are evidenced by the inferred splitting of (*Delias salvini* + *Delias bagoe*) from the Bismarcks and *Delias schoenbergi* from the Solomons at approximately 7.5 Ma. The modal inferred age of divergence for Bismarckian taxa is approximately 5 Ma. Talbot (1928–1937) noted a potential link between Bismarckian and Schouten Island taxa through the relationship of *Delias totila* and *Delias talboti* Joicey & Noakes, respectively, which is corroborated in this study (inferred split dated at around 9 Ma). This is also supported by the inferred split of *Delias narses* (Bismarcks) and *Delias biaka* (Schouten Island) at about 7.5 Ma. Such a link was also hypothesized for the danaines *Euploea tripunctata* (Biak) and *Euploea lacon* (Bismarcks) by Ackery & Vane-Wright (1984).

Within the Bismarck Archipelago, further splitting is evident. Three sister pairs of *Delias* species ((*Delias lytaea–Delias laknekei*, *Delias salvini–Delias bagoe*, *Delias mayrhoferi* (= *schunichii–Delias eximia*)) occur allopatrically respectively on New Britain (NB) and New Ireland (NI). The inferred ages of divergence of these pairs are *c.* 2.0, 2.0 and 2.5 Ma, respectively. There is also diversification at the subspecies level on New Ireland in the case of *Delias messalina* Arora. Two distinctive subspecies, *Delias messalina lizzae* and *Delias messalina gerrittsi*, endemic to the highest parts of the Hans Meyer and Schleinitz ranges, respectively, are separated by a narrow strip of land, which was probably submerged until relatively recently (Müller *et al.*, 2008).

Diversification of *Delias* on the NG mainland has been documented as being 'explosive', based on the high number of similar, co-occurring species. This has been largely attributed to vast climatic changes during the Pliocene–Pleistocene (e.g. Parsons, 1998; Braby & Pierce, 2007), whereby species became isolated on mountain ranges during interglacials. Here, we found that it was during the Pliocene that the main diversification events for species in mainland NG occurred, similar to what is observed for *Delias* outside of NG. Only a few examples of Pleistocene speciation are evident. Even those taxa that are phenotypically similar (e.g. members of the *albertisi* and *nigrina* clades) apparently diverged in the Pliocene. While the glaciation events of the Pleistocene and

the resultant sea-level fluctuations are well documented, those pre-dating this period are far from well understood, although there are several examples of significant Pliocene sea-level fluctuations (Hutchison, 1992; Monk *et al.*, 1997; Roy *et al.*, 2000; Roy & Whitehouse, 2003). On mainland NG, orogenesis intensified during the Pliocene, particularly in southern NG. Indeed, geological and palaeontological evidence suggests that many fossils, now located above 1000 m, represent lowland species such as crocodiles (Natus, 2005). Much of southern NG was submerged until at least the mid Pliocene, *c.* 3 Ma (Natus, 2005). The generation of mountain fold belts, in conjunction with sea-level fluctuations, would have altered the distribution of *Delias* and may have facilitated diversification of the genus.

Mainland clades within *Delias* were clearly restricted by sea barriers. For example, of the mainland clades (*aroae*, *eichhorni*, *sagessa* and *albertisi*), none has been able to cross to nearby islands, e.g. the Schouten Islands, Waigeo and the d'Entrecasteaux, the sole exceptions being *Delias messalina* and *Delias ligata*. Elevation cannot be considered the limiting factor, for there are many peaks of suitable elevation. Nearly all mainland NG *Delias* clades are widespread throughout the island, including the Arfak Mountains, and a phylogeny incorporating most mainland species would shed light on the origins of each of these taxa.

Evolution of morphological traits

Wing pattern diversification in *Delias* is as intriguing as their biogeographical patterns. Red pigmentation is particularly conspicuous in the genus but its evolution has been conjectural and considered homoplasious (e.g. Braby & Pierce, 2007). In most clades such a trait is either present or absent in all included species, and this has implications for the development of red pigment in the context of a phylogeny. The few exceptions, which contain both red and non-red taxa, are the *pasithoe*, *aroae* and *albertisi* groups. Red pigment is not present in the earliest *Delias* lineages (*nysa* and *isse* clades) and is more evident in younger clades. Earlier phylogenetic hypotheses (Dixey, 1894; Talbot, 1928–1937) presumed a sister relationship of *Delias* with *Cepora* (*Huphina*), some of which have the red pigment. However, because the sister of *Delias* is *Leuciacria* (Braby *et al.*, 2006, 2007; Braby & Pierce, 2007), which has no red pigment, it is plausible that the ancestor of *Delias* did not possess red pigment. Indeed, *Aporia* Hübner, another genus with no red pigment, is part of an unresolved trichotomy with the *Catasticta* group and (*Delias* + *Leuciacria*) (Braby *et al.*, 2007).

Convergent evolution of wing pattern and coloration (Müllerian mimicry) is widespread in *Delias* and has been noted by previous authors (e.g. Dixey, 1918, 1920; Talbot, 1928–1937). Such mimicry is apparent at the fine scale within *Delias* clades and extends to different family groups, such as the nymphalid genera *Mynes* Boisduval and *Elymnias* Hübner, and even diurnal moths. For example, various zygaenid and agaristine moths are mimics of members of the *nysa* and *pasithoe* groups, respectively, in Asia. Within *Delias*, the *isse* clade is largely composed of species that are mimics of congeners from other groups, mainly within the *nysa* and *hyparete* clades. As these clades represent older lineages within *Delias* there is a suggestion that it takes considerable time for such mimicry rings to evolve. Interestingly, while several of the *hyparete* models possess red pigment, the *isse* mimics have only been able to attain an orange colour (e.g. *Delias sacha* and *Delias alberti*) suggesting an inability to replicate the coloration in its entirety.

Delias systematics

The comprehensive taxon sampling in this study resolved the 14 distinct clades. The number of clades and their fine-scale composition recognized here differs from that of Talbot (1928–1937), Yagishita *et al.* (1993) and Braby & Pierce (2007). Nonetheless, the broad-scale phylogeny of the latter authors does not differ markedly from our own. Clade relationships and composition within the new phylogenetic hypothesis was markedly constant irrespective of the analysis (i.e. MP or BI). A detailed assessment of the systematics and taxonomy of the genus is presented in Appendix S3.

CONCLUSIONS

The fine-scale species-level phylogeny of *Delias* presented here showed that early lineages mostly radiated during the Miocene and quickly spread throughout much of the Indo-Pacific region, which involved several crossings of Wallacea during the late Miocene to Pliocene. The early splitting clades contain a number of Wallacean endemics, and this may, in part, be attributed to the complex fragmentation history of the region. Younger clades are composed of species that are essentially restricted to mainland NG because their ability to disperse into surrounding satellite islands has been very low. The theory of recent 'explosive' evolution of *Delias* (e.g. Roepke, 1955; Parsons, 1998) is rejected here and the Pliocene is shown to be of much greater temporal importance in the evolution of the genus. Timing of diversification for both the widespread and the mainland NG endemic clades is similar. Numbers of cryptic *Delias* taxa, exhibiting only slight phenotypic differences, display significant genetic distance for *COI*, substantiating the prolonged separation of taxa. Although dispersal was apparent in the diversification of early clades, sea barriers, even narrow straits, appear to have been impediments to colonization within the mainland clades. Future biogeographical studies of *Delias* should focus on the origin of the various recent mainland clades within NG itself, although this would entail an analysis of a complete dataset with respect to the evolution of the numerous geological terranes that comprise the island, as well as Pliocene climate data.

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REFERENCES

Ackery, P.R. & Vane-Wright, R.I. (1984) *Milkweed butterflies*. Cornell University Press, New York.

Aduse-Poku, K., Vingerhoedt, E. & Wahlberg, N. (2009) Out-of-Africa again: a phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on five gene regions. *Molecular Phylogenetics and Evolution*, **53**, 463–478.

Baker, R.H. & DeSalle, R. (1997) Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Systematic Biology*, **46**, 674–698.

Braby, M.F. & Pierce, N.E. (2007) Systematics, biogeography and diversification of the Indo-Australian genus *Delias* Hübner (Lepidoptera: Pieridae): phylogenetic evidence supports an 'out-of-Australia' origin. *Systematic Entomology*, **32**, 2–25.

Braby, M.F., Trueman, J.W.H. & Eastwood, R. (2005) When and where did troidine butterflies (Lepidoptera: Papilionidae) evolve? Phylogenetic and biogeographic evidence suggests an origin in remnant Gondwana in the Late Cretaceous. *Invertebrate Systematics*, **19**, 113–143.

Braby, M.F., Vila, R. & Pierce, N.E. (2006) Molecular phylogeny and systematics of the Pieridae (Lepidoptera: Papilionoidea): higher classification and biogeography. *Zoological Journal of the Linnean Society*, **147**, 239–275.

Braby, M.F., Pierce, N.E. & Vila, R. (2007) Phylogeny and historical biogeography of the subtribe Aporiina (Lepidoptera: Pieridae): implications for the origin of Australian butterflies. *Biological Journal of the Linnean Society*, **90**, 413–440.

Brower, A.V.Z. & DeSalle, R. (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. *Insect Molecular Biology*, **7**, 73–82.

Campbell, D.L., Brower, A.V.Z. & Pierce, N.E. (2000) Molecular evolution of the *wingless* gene and its implications for the phylogenetic placement of the butterfly family Riodinidae (Lepidoptera: Papilionoidea). *Molecular Biology and Evolution*, **17**, 684–696.

Caterino, M.S. & Sperling, F.A.H. (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. *Molecular Phylogenetics and Evolution*, **11**, 122–137.

Caterino, M.S., Cho, S. & Sperling, F.A.H. (2000) The current state of insect molecular systematics: a thriving tower of Babel. *Annual Review of Entomology*, **45**, 1–54.

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *Journal of Molecular Evolution*, **22**, 252–271.

Condamine, F.L., Sperling, F.A.H. & Kergoat, G.J. (2013) Global biogeographical pattern of swallowtail diversification demonstrates alternative colonization routes in the Northern and Southern hemispheres. *Journal of Biogeography*, **40**, 9–23.

Dixey, F.A. (1894) On the phylogeny of the Pierinae, as illustrated by their wing markings and geographical distribution. *Transactions of the Entomological Society of London*.

Dixey, F.A. (1918) On mimicry on certain butterflies of New Guinea. *Transactions of the Entomological Society of London*, 118–129.

Dixey, F.A. (1920) The geographical factor in mimicry. *Transactions of the Entomological Society of London*, 208–211, pl. vi.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Felsenstein, J. (1988) Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics*, **22**, 521–565.

Folmer, O., Black, M.B., Hoch, W., Lutz, R.A. & Vrijehock, R.C. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Goloboff, P.A. (1999) Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics*, **15**, 415–428.

Goloboff, P.A., Farris, J.S. & Nixon, K.C. (2003) *TNT: tree analysis using new technology, version 1.1*. Program and documentation, available from the authors and at: http://www.zmuc.dk/public/phylogeny/TNT/.

Hall, R. (2009) Southeast Asia's changing palaeogeography. *Blumea – Biodiversity, Evolution and Biogeography of Plants*, **54**, 148–161.

Hinschberger, F., Malod, J.A., Réhault, J.P., Villeneuve, M., Royer, J.Y. & Burhanuddin, S. (2005) Late Cenozoic geodynamic evolution of eastern Indonesia. *Tectonophysics*, **404**, 91–118.

Holloway, J.D. (1986) Origins of lepidopteran faunas in high mountains of the Indo-Australian tropics. *High altitude tropical biogeography* (ed. by F. Vuilleumier and M. Monasterio), pp. 533–556. Oxford University Press, New York.

Honthaas, C., Réhault, J.P., Maury, R.C., Bellon, H., Hémond, C., Malod, J.A., Cornée, J.J., Villeneuve, M., Cotton, J., Burhanuddin, S., Guillou, H. & Arnaud, N. (1998) A Neogene back-arc origin for the Banda Sea

basins: geochemical and geochronological constraints from the Banda ridges (east Indonesia). *Tectonophysics*, **298**, 297–317.

Hutchison, C.S. (1992) The Eocene unconformity on Southeast and East Sundaland. *Geological Society of Malaysia Bulletin*, **32**, 69–88.

Klots, A.B. (1933) A generic classification of the Pieridae (Lepidoptera) together with a study of the male genitalia. *Entomologica Americana*, **12**, 139–242.

Kodandaramaiah, U. & Wahlberg, N. (2007) Out-of-Africa origin and dispersal mediated diversification of the butterfly genus *Junonia* (Nymphalidae: Nymphalinae). *Journal of Evolutionary Biology*, **20**, 2181–2191.

Lohman, D.J., de Bruyn, M., Page, T., von Rintelen, K., Hall, R., Ng, P.K.L., Shih, H.T., Carvalho, G.R. & von Rintelen, T. (2011) Biogeography of the Indo-Australian Archipelago. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 205–226.

Mani, M.S. (1986) *Butterflies of the Himalaya*. Dr W. Junk bv Publishers, Dordrecht.

Monk, K.A., De Fretes, Y. & Reksodiharjo-Lilley, G. (1997) *The ecology of Nusa Tenggara and Maluku*. Oxford University Press, Oxford.

Morinaka, S., Miyata, T. & Tanaka, K. (2002) Molecular phylogeny of the *eichhorni* group of *Delias* Hübner, 1819 (Lepidoptera: Pieridae). *Molecular Phylogenetics and Evolution*, **23**, 276–287.

Müller, C.J. & Beheregaray, L.B. (2010) Palaeo island-affinities revisited – biogeography and systematics of the Indo-Pacific genus *Cethosia* Fabricius (Lepidoptera: Nymphalidae). *Molecular Phylogenetics and Evolution*, **57**, 314–326.

Müller, C.J., Jakusch, J. & Wills, L. (2008) A further new subspecies of *Delias messalina* Arora (Lepidoptera: Pieridae) from New Ireland, Papua New Guinea. *Australian Entomologist*, **35**, 73–77.

Müller, C.J., Wahlberg, N. & Beheregaray, L.B. (2010) 'After Africa': the evolutionary history and systematics of the genus *Charaxes* Ochsenheimer (Lepidoptera: Nymphalidae) in the Indo-Pacific Region. *Biological Journal of the Linnean Society*, **100**, 457–481.

Natus, I.R. (2005) *Biodiversity and endemic centres of Indonesian terrestrial vertebrates*. PhD Dissertation, Fachbereich VI (Geographic/Geowissenschaften), Trier University, Trier. Available at: http://ubttest.opus.hbz-nrw.de/volltexte/2005/349/pdf/diss_inar_r_natus.

Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, **15**, 407–414.

Parsons, M.J. (1998) *The butterflies of Papua New Guinea: their systematics and biology*. Academic Press, London.

Peña, C. & Malm, T. (2012) VoSeq: a voucher and DNA sequence web application. *PLoS ONE*, **7**, e39071.

Peña, C., Wahlberg, N., Weingartner, E., Kodandaramaiah, U., Nylin, S., Freitas, A.V.L. & Brower, A.V.Z. (2006) Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. *Molecular Phylogenetics and Evolution*, **40**, 29–49.

Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.5*. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14.

Réhault, J.P., Maury, R.C., Bellon, H., Sarmili, L., Burhanuddin, S., Joron, J.L., Cotton, J. & Malod, J.A. (1994) La Mer de Banda Nord (Indonésie): un bassin arrière-arc du Miocéne supérieur. *Comptes Rendus de l'Académie des Sciences*, **318**, 969–976.

Roepke, W. (1955) The butterflies of the genus *Delias* Hübner (Lepidoptera) in Netherlands New Guinea. *Nova Guinea (n.s.)*, **6**, 185–260.

Ronquist, F. (1997) Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, **46**, 195–203.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Roy, P.S. & Whitehouse, J. (2003) Changing Pliocene sea levels and the formation of heavy minerals beach placers in the Murray Basin, southeastern Australia. *Economic Geology*, **98**, 975–983.

Roy, P.S., Whitehouse, J., Cowell, P.J. & Oakes, G. (2000) Mineral sands occurrences in the Murray Basin, southeastern Australia. *Economic Geology*, **95**, 1107–1128.

Smiles, R.L. (1982) The taxonomy and phylogeny of the genus *Polyura* Billberg (Lepidoptera: Nymphalidae). *Bulletin of the Natural History Museum, Entomology Series*, **44**, 115–237.

Talbot, G. (1928–1937) *A monograph of the pierine genus* Delias, *Parts I–VI*. British Museum (Natural History), London.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.

Walsh, P.S., Metzger, D.A. & Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.

Wheat, C.W., Vogel, H., Wittstock, U., Braby, M.F., Underwood, D. & Mitchell-Olds, T. (2007) The genetic basis of a plant-insect coevolutionary key innovation. *Proceedings of the National Academy of Sciences USA*, **104**, 20427–20431.

Yagishita, A., Nakano, S. & Morita, S. (1993) *An illustrated list of the genus* Delias *Hübner of the world*. Khepera Publishers, Tokyo.

Yu, Y., Harris, A.J. & He, X.J. (2011) *RASP (reconstruct ancestral state in phylogenies) 1.1*. Available at: http://mnh.scu.edu.cn/soft/blog/RASP.

SUPPORTING INFORMATION

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

Appendix S1 Exemplar taxa used in this study, with collection data and GenBank accession numbers.
Appendix S2 Maximum parsimony and Bayesian trees for the combined cytochrome *c* oxidase subunit I (*COI*), elongation factor 1α (*EF-1α*) and *wingless* dataset.
Appendix S3 *Delias* systematics.

BIOSKETCH

Chris J. Müller is an economic geologist with a lifelong interest in the taxonomy, ecology and biogeography of Indo-Pacific butterflies.

Pável Matos-Maraví is interested in molecular systematics, historical biogeography and diversification of insects.

This study formed part of Chris Müller's PhD research supervised by **Luciano B. Beheregaray**, a molecular ecologist with interests in conservation and evolution of biodiversity.

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