Biological Journal of the Linnean Society, 2017, **121**, 576–591. With 6 figures.

Phylogeography of the *Delias hyparete* species group (Lepidoptera: Pieridae): complex historical dispersals into and out of Wallacea

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Received 13 November 2016; revised 6 February 2017; accepted for publication 13 February 2017

The unique and highly endemic fauna of Wallacea has been extensively examined; however, the diversification of a single animal lineage in Wallacea has not yet been studied in detail. The *Delias hyparete* (Linnaeus) species group is distributed in the Oriental and Australian regions as well as throughout Wallacea (i.e. North Maluku, South Maluku, Sulawesi and Lesser Sunda), with the highest species diversity occurring in Wallacea. The present study reconstructed the phylogeny and estimated the between- and within-species divergence of the *D. hyparete* group using two genes: mitochondrial NADH-dependent dehydrogenase subunit 5 (ND5) and nuclear elongation factor 1 alpha (EF1-α). Two out of five clades were associated with Lesser Sunda, and the remaining three clades were associated with North Maluku, South Maluku and Sulawesi, respectively. Ancestral area analyses and molecular dating suggested five colonization events into Wallacea at various times with the Australian and Oriental regions inferred as the geographical origins; Lesser Sunda may have been colonized twice. Two range expansion events from Lesser Sunda to Greater Sunda in the recent past and an older dispersal passing through Wallacea towards the Oriental region have also been inferred. The species diversity of this butterfly group in Wallacea appears to have developed due to non-sympatric speciation events. Complex historical dispersals into and out of Wallacea inferred in the *D. hyparete* group reinforce the view that animals move across Wallace's and Lydekker's Lines more frequently than classical assumptions, and these complex dispersals may have largely contributed to the high biodiversity and endemism evident in Wallacea.

ADDITIONAL KEYWORDS: biogeography – colonization – Lesser Sunda – Maluku – paraphyletic species – Sulawesi – Timor.

INTRODUCTION

Wallacea comprises a group of islands surrounding the Banda Sea, Indonesia, and is a biogeographical region between the Oriental and Australian regions (Wallace, 1867a; reviewed by Michaux, 2010; Lohman *et al.*, 2011; see also Escalante, 2016). While the definition of Wallacea has historically been complicated, we here defined it as a region delineated by Wallace's Line along the Makassar and Lombok Straits and Lydekker's Line surrounding the eastern edge of the Banda Sea (Fig. 1; Lohman *et al.*, 2011). The fauna of Wallacea is a composite of Asian and Australian/New Guinean taxa (reviewed by Michaux, 2010). The biodiversity of Wallacea islands is often high, and its fauna has a high level of endemism (Michaux, 2010; Lohman *et al.*, 2011; Ng *et al.*, 2016). Wallacea is mostly composed of oceanic

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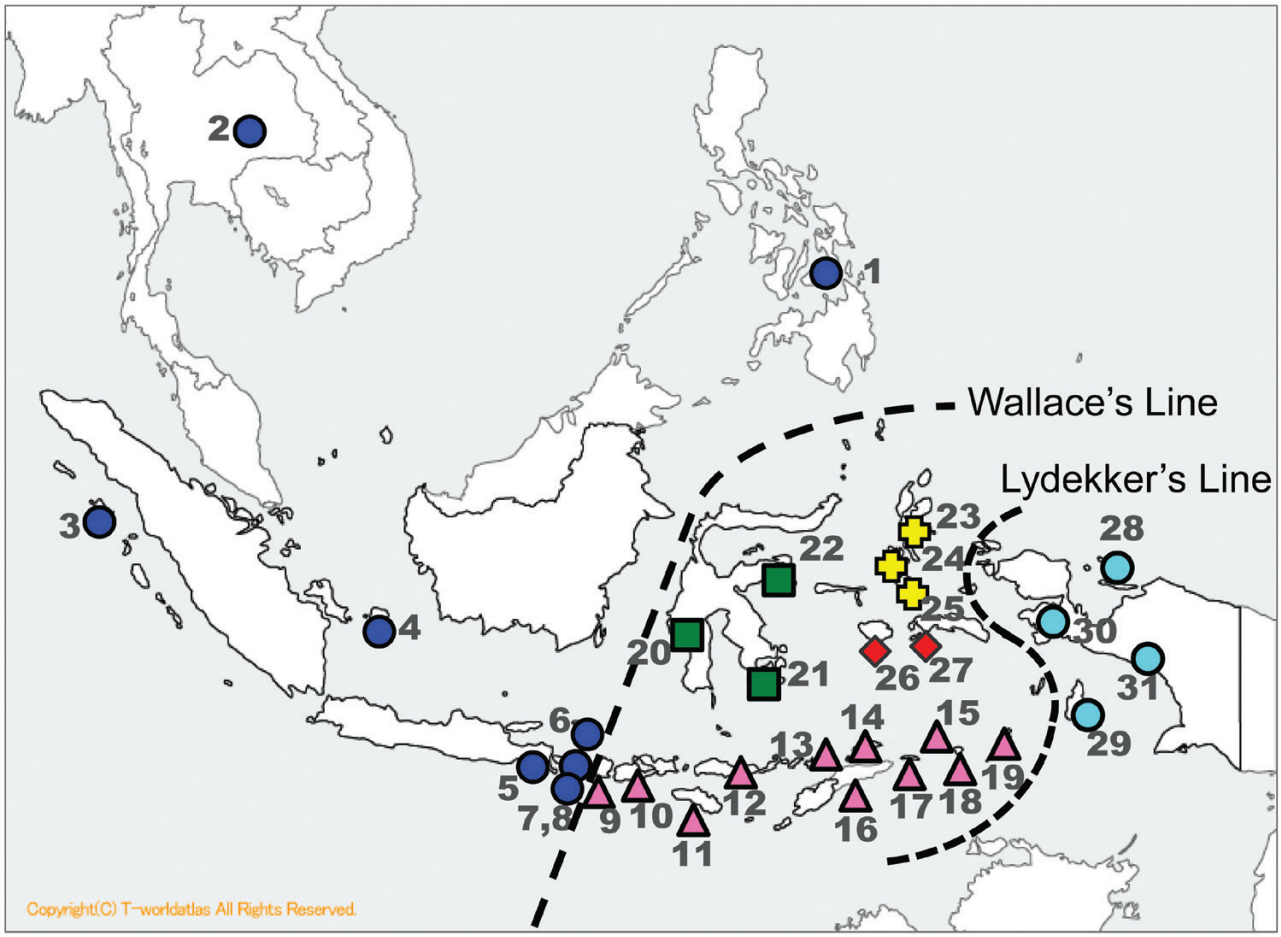


Figure 1. Map of Wallacea showing some of the collection localities of the *D. hyparete* group used in the present study. Solid dots, open dots, rectangles, triangles, crosses and rhombi represent the Oriental region, Australian region, Sulawesi, the Lesser Sunda Islands, North Maluku and South Maluku, respectively. 1, Leyte [*D. hyparete*]; 2, Thailand [*D. hyparete*]; 3, Nias [*D. hyparete*]; 4, Belitung [*D. hyparete*]; 5, East Java [*D. periboea*]; 6, Kangean [*D. periboea*]; 7, Bali [*D. sambawana*, *D. hyparete* and *D. periboea*]; 8, Nusa Penida [*D. hyparete*]; 9, Lombok [*D. sambawana* and *D. periboea*]; 10, Sumbawa [*D. sambawana*]; 11, Sumba [*D. fasciata*]; 12, Flores [*D. sambawana*]; 13, Alor [*D. sambawana* and *D. periboea*]; 14, Wetar [*D. timorensis*]; 15, Damar [*D. timorensis*]; 16, Timor [*D. eileenae* and *D. timorensis*]; 17, Moa [*D. timorensis*]; 18, Babar [*D. timorensis*]; 19, Tanimbar [*D. timorensis*]; 20, Centre Sulawesi [*D. rosenbergi*]; 21, Muna [*D. rosenbergi*]; 22, Peleng [*D. mitisi*]; 23, Halmahera [*D. poecilea*]; 24, Bacan [*D. poecilea*]; 25, Obi [*D. edela*]; 26, Buru [*D. ceneus*]; 27, Ambon [*D. ceneus*]; 28, Biak [*D. euphemia*]; 29, Aru [*D. mysis*]; 30, New Guinea (Fakfak) [*D. lara*]; 31, New Guinea (Timika) [*D. lara*]. The following localities of samples are not shown. New Guinea [*D. doylei*], Australia [*D. argenthona* and *D. mysis*], India [*D. hyparete* and *D. eucharis*], Nepal [*D. hyparete*] and Andaman [*D. hyparete*].

islands which have not been connected to continents (Lohman *et al.*, 2011). Therefore, ancestors of animal lineages now endemic to Wallacea immigrated via oversea dispersal from the Oriental and/or Australian regions. Beck *et al.* (2006) biogeographically examined the diversity of hawkmoths in Wallacea, and suggested that dispersal was a relatively important determinant of fauna similarities among the islands there.

Until recently, it was often implicitly assumed that historical dispersal into Wallacea was rare (Bellemain & Ricklefs, 2008; Balke *et al.*, 2009). However, several studies demonstrated more frequent and complex historical dispersals across Wallace's and Lydekker's Lines. Birds of the genus *Pericrocotus* dispersed out of the Australian region into Greater Sunda and other Asian regions through Wallacea (Jønsson *et al.*, 2008). A group of diving beetles historically jumped across Wallace's and Lydekker's Lines during a single main event (Balke *et al.*, 2009). Lohman *et al.* (2011) reviewed molecular biogeographical studies for Wallacea, and indicated that Wallace's and Lydekker's Lines are permeable boundaries for fauna.

A few groups of flightless weevils were suggested to have transgressed Wallace's and/or Lydekker's Line(s) repeatedly (Tänzler *et al.*, 2014, 2015; Toussaint *et al.*, 2015). The ancestral area reconstruction analysis on other insects suggested repeated colonization into Wallacea (carpenter ants *Camponotus maculatus* and its allies: Clouse *et al.*, 2015; diving beetles of the genus *Platynecetes*: Toussaint *et al.*, 2016; burying beetles of the genus *Nicrophorus*: Toussaint & Condamine, 2016). Thus, Wallacean islands may have been colonized by animals more frequently than classically assumed. Wallacean islands may have served not only as the recipients of colonizers from continents but also as a source of emigrants to continents (reverse colonization, Bellemain & Ricklefs, 2008; Grismer *et al.*, 2016). However, empirical studies critically examining these hypotheses remain limited both in number and in taxonomic range. The historical processes responsible for the formation of endemic species in Wallacea have not yet been sufficiently examined (Braby & Pierce, 2007). The diversification histories of more groups of animals diversifying within Wallacea warrant further study.

Butterflies have long been a model group to understand the pattern and history of faunal diversification in the Indo-Pacific Archipelago including Wallacea (reviewed by Braby & Pierce, 2007; Condamine *et al.*, 2015; Toussaint & Balke, 2016). Recently, molecular phylogeographical studies have been shedding light on the detailed diversification history of Wallacean butterflies such as birdwing butterflies (Condamine *et al.*, 2015), nymphalids (Müller *et al.*, 2010; Toussaint & Balke, 2016) and pierids (Braby & Pierce, 2007; Müller *et al.*, 2012). Müller *et al.* (2010, 2012) reported that Wallacea was not only a transitional zone between the Oriental and Australian regions, but also by itself important in the evolution of animals, such as butterflies. The genus *Delias* (Hübner) has the most species in the family Pieridae. *Delias* butterflies are distributed in South and Southeastern Asia, Wallacea, New Guinea, Australia and Melanesia, and are most diversified in New Guinea (Yagishita *et al.*, 1993). The occurrence of many *Delias* species in the Oriental region, the Australian region and Wallacea makes the genus particularly attractive to investigate the historical biogeography in and around Wallacea (Braby & Pierce, 2007). The number of species in *Delias* has continued to increase since Wallace (1867b) first listed 57 species: *c*. 250 species in 22 groups of species are currently recognized (Yagishita *et al.*, 1993; Braby & Pierce, 2007; Müller *et al.*, 2012). Morinaka *et al.* (2002) and Braby & Pierce (2007) examined phylogenetic relationships among the species groups of *Delias*. Braby & Pierce (2007) suggested that *Delias* originated on the Australian plate. This was later supported by the findings of a phylogenetic analysis of 138 *Delias* species (Müller *et al.*, 2012). Braby & Pierce (2007) suggested at least seven dispersal events from the Australian region to the Oriental region across Wallacea. Müller *et al.* (2012) inferred that several species groups of *Delias* colonized Wallacea during the late Miocene and Pliocene, the early stages of the evolutionary history of the genus.

The present study focused on the *Delias hyparete* (Linnaeus) species group (Supporting Information, Fig. S1), which was suggested by Braby & Pierce (2007) to be a good model to look at historical dispersal across Wallacea. In the *D. hyparete* species group, 21 species have been identified to date, ranging from India in the west to the Solomon Islands in the east, and from China in the north to Australia in the south (Yagishita *et al.*, 1993; Parsons, 1998; Braby, 2000; Davenport & van Mastrigt, 2008). *Delias hyparete* and *Delias eucharis* (Drury) are distributed in mainland Asia, while the others are distributed in Australia, New Guinea, Melanesia and/or Wallacea. Ten out of the 21 species are endemic to or centred in Wallacea, covering the entirety of Wallacea (North Maluku, South Maluku, Sulawesi and Lesser Sunda; Fig. 1). Thus, the species diversity of the *D. hyparete* group is higher in Wallacea than in the Oriental or Australian region. In addition, *Delias sambawana* Rothschild and *Delias periboea* (Godart) attracted interest because they are distributed in Lesser and Greater Sundas, i.e. both sides of Wallace's Line (Fig. 1). These latter two species provide us with the opportunity to phylogenetically examine the time and direction of their historical dispersal across Wallace's Line. Therefore, this group is considered suitable for studying the diversification process of animals in Wallacea. Braby & Pierce (2007) assumed that the *D. hyparete* group dispersed only once across Wallacea to infer the minimum number of historical dispersals among the Oriental region, the Australian region and Wallacea; however, they also pointed that fine-scale phylogeny is required to infer the number and timing of historical dispersals within Wallacea and among Wallacea, the Oriental region and the Australian region for geographically widespread species-groups such as the *D. hyparete* group. Müller *et al.* (2012) used 14 species of this group in their molecular phylogenetic analysis of 138 *Delias* species, suggesting early dispersal events from Wallacea towards the Oriental region in the *D. hyparete* group. However, the diversification of the Wallacea-endemic species in the *D. hyparete* group has not been investigated in detail. Some Wallacean species were not sampled, and sequence variations within species were not examined by Müller *et al.* (2012).

The aim of the present study was to examine the history of colonization into/out of and diversification within Wallacea of the *D. hyparete* group using most known Wallacean species and many subspecies in the *D. hyparete* group. Molecular phylogenetic, molecular

dating and ancestral area analyses were combined to infer the route and timing of the historical dispersal of the *D. hyparete* group into, out of and/or within Wallacea. We also updated the taxonomy of the *D. hyparete* group based on the molecular phylogeny.

MATERIAL AND METHODS

The butterflies of most species of the genus *Delias* (Hübner) have brightly coloured markings on the underside of the wings, and wing markings often markedly vary among *Delias* species. The bright wing markings of *Delias* are considered to be aposematic to predators; *Delias* butterflies are considered unpalatable (Morishita & Yata, 1981; Braby & Nishida, 2010). The larval host plants of *Delias* species are, to the best of our knowledge, plants of Loranthaceae, their relatives in Santalales and Euphorbiaceae (Talbot, 1928–1937; Morishita & Yata, 1981; Stirpe, 1983; Corbet & Pendlebury, 1993; Parsons, 1998; Braby, 2006, 2012; Braby & Trueman, 2006).

We sampled 18 of the 21 species in the *D. hyparete* group (Table 1, Supporting Information, Fig. S1). The three species not used were *Delias bagoe* (Boisduval) from the Bismarck Islands, *Delias schoenbergi* Rothschild from the Solomon Islands and *Delias aestiva* Butler from the Northern Territory and north-western Queensland in Australia (Braby, 2014). In order to cover a large part of the species distribution range, we included as many subspecies as possible: five out of seven subspecies for *D. periboea*, six out of seven subspecies for *Delias timorensis* (Boisduval), all five subspecies for *D. sambawana* and seven out of 20 subspecies for *D. hyparete* (Table 1). When the previous (Müller *et al.*, 2012) and present studies were combined, all known species of the *D. hyparete* group, except for *D. aestiva*, were phylogenetically analysed: *D. aestiva* was recently recognized as a distinct species by Braby (2012, 2014), inhabits the Northern Territory and Queensland, Australia, and is close to *Delias mysis* (Fabricius). The outgroup species used were as follows: *Delias eichhorni* Rothchild & Jordan, *Delias toxopei* Roepke, *Delias carstensziana* Rothchild, *Delias gabia* (Boisduval), *Cepora iudith* (Fabricius), *Aporia crataegi* (Linnaeus) and *Leuciacria acuta* Rothschild & Jordan. Sequence data for *D. eichhorni*, *D. toxopei* and *D. carstensziana* were previously published in Morinaka *et al.* (2002). Insects from countries other than Japan were obtained for years as dried specimens from Japanese amateur collectors/researchers of butterflies. Voucher specimens are kept at the laboratory of S. Morinaka (Table 1).

Based on their original descriptions, the names *Delias poecilea* (Vollenhoven) and *Delias ceneus* (Linnaeus) were used in the present study. However, these butterflies have been widely described as *D. poecila* and *D. caeneus*, respectively, in most of the literature (Talbot, 1928–1937; D'Abrera, 1990; Yagishita *et al.*, 1993).

The *Delias* species used in the present study were largely included in the previous study by Müller *et al.* (2012), but the two studies do not share sequence data.

Total DNA was obtained from butterfly bodies or legs with the standard protocol using sequential extraction with phenol, phenol/chloroform (1:1, v/v), and chloroform followed by precipitation with ethanol. The precipitants were dissolved in 200 µL TE solution (i.e. 10 mM Tris–HCl and 1 mM EDTA). Using DNA solution as the template, the 924-base pair (bp) portion of the mitochondrial NADH-dependent dehydrogenase subunit 5 (*ND5*) gene and 1087-bp portion of the nuclear elongation factor 1 alpha (EF-1α) gene were amplified by a polymerase chain reaction (PCR). The *ND5* gene was selected as a mitochondrial gene marker as in the previous phylogenetic study on *Delias* by S.M. (Morinaka *et al.*, 2002). The EF-1α gene was selected as a nuclear marker because it has frequently been used in phylogenetic analyses on various insect lineages (Reed & Sperling, 1999; Vane-Wright *et al.*, 1999; Zakharov *et al.*, 2004). The two genes show different rates of nucleotide substitution, and hence their combination is suitable to infer phylogeny with varying ages of divergence (Müller *et al.*, 2010 and references therein). To amplify *ND5*, we used primer 'a' (forward, 5′-CCTGTTTCTGCTTTAGTTCA-3′) designed by Su *et al.* (1996) and primer 'B3' (reverse, 5′-TAACCTCTATATATYTCTCTT-3′), which we designed based on the sequences of *Drosophila yakuba* Burla, *Carabus dehaanii* Chaudoir, *Apis mellifera* Linnaeus and several lepidopteran species. To amplify EF-1α, we designed the primers 'C4' (forward, 5′-CAAATGTGGTGGTATCGA-3′) and 'D5' (reverse, 5′-GTTGACAATACGAGCATC-3′) based on the sequences of several papilionid butterflies (Reed *et al.*, 1999). When the target region was not amplified as a whole, additional primers (Supporting Information, Table S1) were used to obtain the target region as a group of shorter fragments. The PCR mixture was prepared in a volume of 50 µL, using the KOD FX system (Toyobo Life Science Department, Osaka, Japan). Amplification was performed with 35 cycles of denaturation at 98 °C for 10 s, annealing at 47–51 °C for 30 s and extension at 68 °C for 1 min. The amplification product was directly sequenced by Macrogen Japan Corp., with the dye terminator cycle sequencing method using primers for PCR amplification.

Nucleotide sequences were aligned using CLUSTAL X with the default setting (Thompson *et al.*, 1994). The best model to explain nucleotide substitution for each of the ND5 and EF-1α data sets was examined using Kakusan3 (Tanabe, 2007) with the number of gamma

Table 1. Taxa used in the present study with collection localities and accession numbers

Species	Subspecies	Locality	Voucher ID	ND5*	EF1-α*
Delias eucharis	nominate	Nilgiri, India	SZM1465	AB901142	–
		Himachal, India	SZM1450	AB901143	AB899858
Delias hyparete	nominate	Nusa Penida, Indonesia	SZM176	AB901144	–
		Bali, Indonesia	SZM175	AB055837	AB899859
	luzonensis	Leyte, Philippines	SZM1456	AB901146	AB899860
	indica	Nepal	SZM1458	AB901147	AB899861
		Thailand	SZM876	AB901148	DQ082831
	metarete	Andaman, India	SZM1455	AB901149	–
	niasana	Nias, Indonesia	SZM1461	AB901150	–
	ethire	Andhra, India	SZM1453	AB901151	AB899862
	aurago	Belitung, Indonesia	SZM1464	AB901152	–
Delias rosenbergi	nominate	Central Sulawesi, Indonesia	SZM1463	AB901153	AB899863
	munaensis	Muna, Indonesia	SZM1457	AB901154	AB899864
Delias mitisi	*banggaiensis*	Peleng, Indonesia	SZM1406	AB901155	AB899865
Delias periboea	nominate	East Java, Indonesia	SZM1428	AB901156	AB899866
	atakei	Kangean, Indonesia	SZM1431	AB901157	–
	wallacei	Bali, Indonesia	SZM132	AB901158	AB899867
	livia	Lombok, Indonesia	SZM1423	AB901159	AB899868
	alorensis	Alor, Indonesia	SZM1413	AB901160	–
Delias fasciata	nominate	Sumba, Indonesia	SZM1271	AB901161	AB899869
Delias sambawana	nominate	Sumbawa, Indonesia	SZM1382	AB901162	AB899870
	minerva	Lombok, Indonesia	SZM1307	AB901163	AB899871
	everetti	Flores, Indonesia	SZM794	AB298380	AB899872
	boejanana	Bali, Indonesia	SZM15	AB901165	AB899873
	kenta	Alor, Indonesia	SZM1274	AB901166	AB899874
Delias argenthona	nominate	Queensland, Australia	SZM1273	AB901167	AB899875
			SZM1467	AB901168	AB899876
Delias eileenae	nominate	Timor, Indonesia	SZM693	AB298381	AB899877
Delias poecilea	nominate	Bacan, Indonesia	SZM1378	AB901170	–
		Halmahera, Indonesia	SZM1404	AB901171	AB899878
Delias edela	nominate	Obi, Indonesia	SZM1432	AB901172	–
Delias ceneus	nominate	Ambon, Indonesia	SZM1443	AB901173	AB899879
	philotis	Buru, Indonesia	SZM1448	AB901174	–
Delias timorensis	nominate	Timor, Indonesia	SZM1414	AB901175	AB899880
	vishnu	Wetar, Indonesia	SZM1439	AB901176	AB899881
	gardineri	Tanimbar, Indonesia	SZM1377	AB901177	AB899882
			SZM1435	AB901178	–
	moaensis	Moa, Indonesia	SZM1422	AB901179	AB899883
	andesiaca	Damar, Indonesia	SZM1425	AB901180	–
	babarica	Babar, Indonesia	SZM1437	AB901181	AB899884
Delias mysis	nominate	Queensland, Australia	SZM1471	AB901182	DQ082832
	aruensis	Aru, Indonesia	SZM1440	AB901183	AB899884
Delias lara	nominate	Fakfak, West Papua, Indonesia	SZM670	AB513912	AB899886
		Timika, West Papua, Indonesia	SZM1468	AB901184	AB899887
Delias doylei	nominate	Simbu Province, Papua New Guinea	SZM1403	AB901185	AB899888
Delias euphemia	nominate	Biak, Indonesia	SZM1436	AB901186	AB899889
Delias salvini	nominate	New Britain, Papua New Guinea	SZM1379	AB901187	AB899890
			SZM1462	AB901188	AB899891
Delias eichhorni		Western Highland Province, Papua New Guinea	SZM85	AB044618	AB069892
Delias carstensziana		Habbema, West Papua, Indonesia	SZM213	AB055826	AB069896
Delias toxopei		Ibele, West Papua, Indonesia	SZM217	AB055827	AB069897

Table 1. *Continued*

Species	Subspecies	Locality	Voucher ID	ND5*	EF1-α*
Delias gabia	*aurantimaclua*	Timika, West Papua, Indonesia	SZM958	AB513910	AB899893
Leuciacria acuta	nominate	Pass Valley, West Papua, Indonesia	SZM34.2	AB901192	AB899894
Aporia crataegi	*adherbal*	Japan	SZM346	AB044622	AB069900
Cepora iudith	nominate	Bali, Indonesia	SZM206	AB044623	AB069901

*Accession code in the DDBJ/GenBank/EMBL database.

category set to 4 and default settings for the other parameters. Based on the BIC4 criterion, HKY+G and SYM+G were used as the best models among those available in BEAST v1.8.4 (Drummond & Rambaut, 2007) for ND5 and EF-1α data, respectively. The sequences of the two genes were concatenated using SequenceMatrix (Vaidya *et al.*, 2011).

Bayesian molecular phylogenetic and dating analyses were conducted in BEAST v1.8.4. We unlinked the substitution and clock models between the two genes, but linked the tree topology model. We used an uncorrelated lognormal relaxed clock model and a birth-death process of speciation for each gene. A UPGMA tree was used as the starting tree. Fifty million generations of the Monte Carlo Markov chains (MCMC) were run, sampling every 1000 generations. Default settings was used for the other parameters. Convergence of the stationary distribution was checked by visually inspecting plots of the posterior estimates using Tracer v1.6.0 (Rambaut *et al.*, 2014). After discarding the initial 5 million generations as burn-in, samples were summarized in the maximum clade credibility tree using TreeAnnotator v1.8.4 (Drummond *et al.*, 2016) with the "common ancestor" node height option (Heled & Bouckaert, 2013) in effect. The maximum clade credibility tree was visualized using FigTree v1.4.0 (Rambaut, 2012). Analyses were run twice to check for convergence in the topology.

After scaling up the branch lengths using Mesquite 3.03 (Maddison & Maddison, 2015), the maximum credibility tree was used as the starting tree in the second round BEAST analysis with the estimation of divergence times. Priors were generally set as in the first round. No reliable internal fossil calibration points were available for the *D. hyparete* group; therefore, we used a secondary calibration point. Based on previous molecular phylogenetic studies on the family Pieridae (Braby *et al.* 2006; Braby & Pierce, 2007), Müller *et al.* (2012) calibrated the molecular phylogeny of *Delias* using the node between *Leuciacria* and *Delias*, which are sister genera (Braby *et al.*, 2006). Following Müller *et al.* (2012), the present study used the node between *L. acuta* and *Delias* species as an external calibration point for the *D. hyparete* group. It consisted of a uniformly distributed prior between 23 and 39 Mya, which corresponds to the range of the mean ± 2SE in the prior distribution used in Müller *et al.* (2012). After 50 million generations of the MCMC chains with samples of every 1000 generations run in BEAST, the initial 5 million generations were discarded as burn-in, and samples were summarized in the maximum clade credibility tree using TreeAnnotator v1.8.4 (Drummond *et al.*, 2016) with the 'common ancestor' node height option (Heled & Bouckaert, 2013) in effect. The maximum clade credibility tree was visualized using FigTree v1.4.0 (Rambaut, 2012). Analyses were run twice to check for convergence in the topology.

We used R v3.3.2 with the R package BioGeoBEARS (Matzke, 2013a, b, 2014) to infer the possible ancestral ranges of the *D. hyparete* group on the phylogenetic tree. The biogeographical model of DEC+J was used. BioGeoBEARS was implemented with the BEAST maximum credibility tree from which outgroups were pruned. The geographical range of the *D. hyparete* group was divided into six areas (Fig. 1; also refer to the present results). These areas are Asia (the Oriental region), the Australian region including Australia, New Guinea and neighbouring islands, Sulawesi (Wallacea), North Maluku (Wallacea), South Maluku (Wallacea) and Lesser Sunda (Wallacea). The maximum number of ancestral areas was set to six. Default settings on dispersal rates between the different areas were used.

RESULTS

No deletions or insertions were detected in any of the 877 or 918 bp of the ND5 or EF-1α gene, respectively. An outline and details of the Bayesian phylogram for the *D. hyparete* group are shown in Figures 2 and 3, respectively. The *D. hyparete* group comprised four major clades, as follows. *D. sambawana* and *Delias eileenae* Joicey & Talbot formed clade A with a posterior probability of 1.0. *Delias salvini* Butler was then recognized as a singleton clade with a posterior probability of 1.0 (clade B). Clade C was composed of *D. hyparete*, *D. eucharis*, *Delias rosenbergi* (Snellen van Vollenhoven) and *Delias mitisi* Staudinger with a posterior probability of 1.0. Clade D included the other

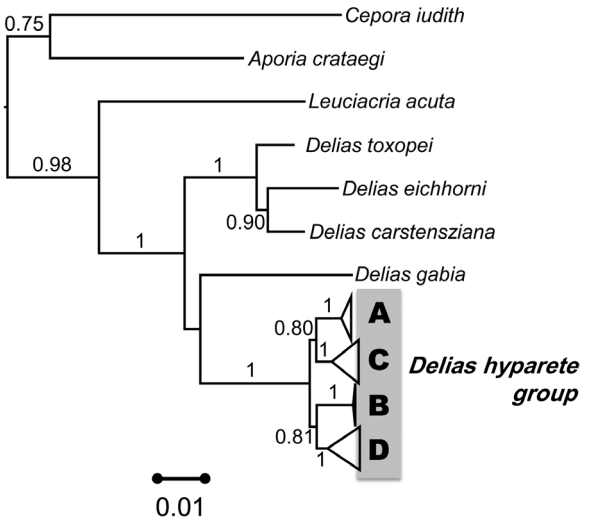


Figure 2. Bayesian phylogram of the *D. hyparete* group and other pierid butterflies, recovered in the BEAST analysis using 56 terminals with the concatenated mitochondrial ND5 and the nuclear EF-1α markers. Four major clades (A–D) in the *D. hyparete* group were reduced to the triangles. See Figure 3 for phylogenetic relationships in the *D. hyparete* group. Values on branches are posterior probabilities.

11 species (*Delias edela* Fruhstorfer was not examined in the EF-1α data) with a posterior probability of 1.0: *D. poecilea*, *D. edela*, *D. ceneus*, *D. mysis*, *Delias lara* (Boisduval), *Delias doylei* Sanford & Bonnett, *Delias euphemia* Grose-Smith, *Delias argenthona* (Fabricius), *D. periboea*, *D. timorensis* and *Delias fasciata* Rothschild. Clades A and C formed a sister group, as did clades B and D, with posterior probability supports of 0.80 and 0.81, respectively.

Clade A represented the western part of the Lesser Sunda Islands in Wallacea: *D. eileenae* is endemic to Timor, while *D. sambawana* is distributed over western Lesser Sunda and Bali. The two species are allopatric. *D. sambawana* was paraphyletic: *D. eileenae* was more closely related to the Flores sample of *D. sambawana*.

Clade C was dominated by Oriental region samples and also included Sulawesi samples. *Delias hyparete* is widely distributed over continental Asia, but not in Wallacea (except for Lombok). *Delias eucharis* is distributed in India and Sri Lanka. *Delias rosenbergi* and *D. mitisi* are endemic to Sulawesi and its neighbouring islands, and are allopatric to each other. *Delias hyparete* was paraphyletic because it included both *D. rosenbergi* and *D. mitisi*.

Clade D represented the Australian region and Wallacea other than Sulawesi. The oldest split in clade D separated the Northern Maluku taxa, i.e. *D. poecilea* + *D. edela* (subclade D-1). The others consisted of three subclades. One subclade included *D. lara*, *D. mysis*, *D. doylei* and *D. euphemia*, representing New Guinea Island, its neighbouring islands and Australia (subclade D-2). Another subclade was *D. ceneus* representing Southern Maluku (subclade D-3), which was sister to subclade D-2. The third subclade included *D. argenthona*, *D. periboea*, *D. timorensis* and *D. fasciata* (subclade D-4), including species from the entirety of Lesser Sunda, Australia, New Guinea and the eastern end of the Oriental region. *Delias timorensis* was polyphyletic, with the Tanimbar samples basal to the monophyletic group of *D. periboea*, *D. fasciata* and *D. timorensis* from elsewhere (Demar, Babar, Wetar, Sumba, Timor and Moa). Species in clade D, except for *D. argenthona*, are allopatric to each other.

The chronogram obtained from the BEAST analysis is shown in Figure 4. Clades A–D emerged during a short period: the split between A/C and B/D 8.2 Mya [95% highest posterior density (HPD): 4.5–12.5 Mya], between A and C 7.2 Mya (95% HPD: 3.5–10.7 Mya), and between B and D 7.1 Mya (95% HPD: 3.5–10.7, Mya). In clade A, radiation in Lesser Sunda started 1.5 Mya (95% HPD: 0.60–2.65 Mya). In *D. sambawana*, the Bali sample split from Lesser Sunda samples 0.19 Mya (95% HPD: 0.01–0.44 Mya). In clade C, after the separation of *D. eucharis* 4.3 Mya (95% HPD: 2.0–6.8 Mya), *D. hyparete* spread over Asia from 1.9 Mya (95% HPD: 0.87–3.1 Mya), and then the Sulawesi taxa split 0.90 Mya (95% HPD: 0.31–1.34 Mya). In clade D, the clades for North Maluku, South Maluku and Lesser Sunda split 5.0 Mya (95% HPD: 2.4–8.0 Mya), 3.0 Mya (95% HPD: 1.3–4.4 Mya) and 1.7 Mya (95% HPD: 0.80–2.8 Mya), respectively. In the *D. periboea* clade, neither Asian (Bali, Jawa and Kangean) nor Lesser Sunda (Lombok and Alor) samples formed a clade: the Bali/Jawa samples split from the Alor sample 0.36 Mya (95% HPD: 0.02–0.45 Mya), and the Kangean sample split from the Lombok sample 0.08 Mya (95% HPD: 0–0.22 Mya).

The results of ancestral area analyses are shown in Figure 5. Clade A including Asian and Lesser Sunda samples was estimated to have its most recent common ancestor in Lesser Sunda. The range of the most recent common ancestor of clade C including the Oriental region and Sulawesi samples was estimated to be the Oriental region. Regarding clade D, the most recent common ancestor was most likely to have occurred in the Australian region. Subsequently, three dispersal events from the Australian region were inferred: clade D-1 to North Maluku; clade D-3 to South Maluku; and then clade D-4 to Lesser Sunda. Part of clade D-4 reached the Oriental region.

The range of the most recent common ancestor of clades B and D was estimated to be the Australian region. However, the ancestral area of clades A and C was estimated to be Asia or Lesser Sunda. The

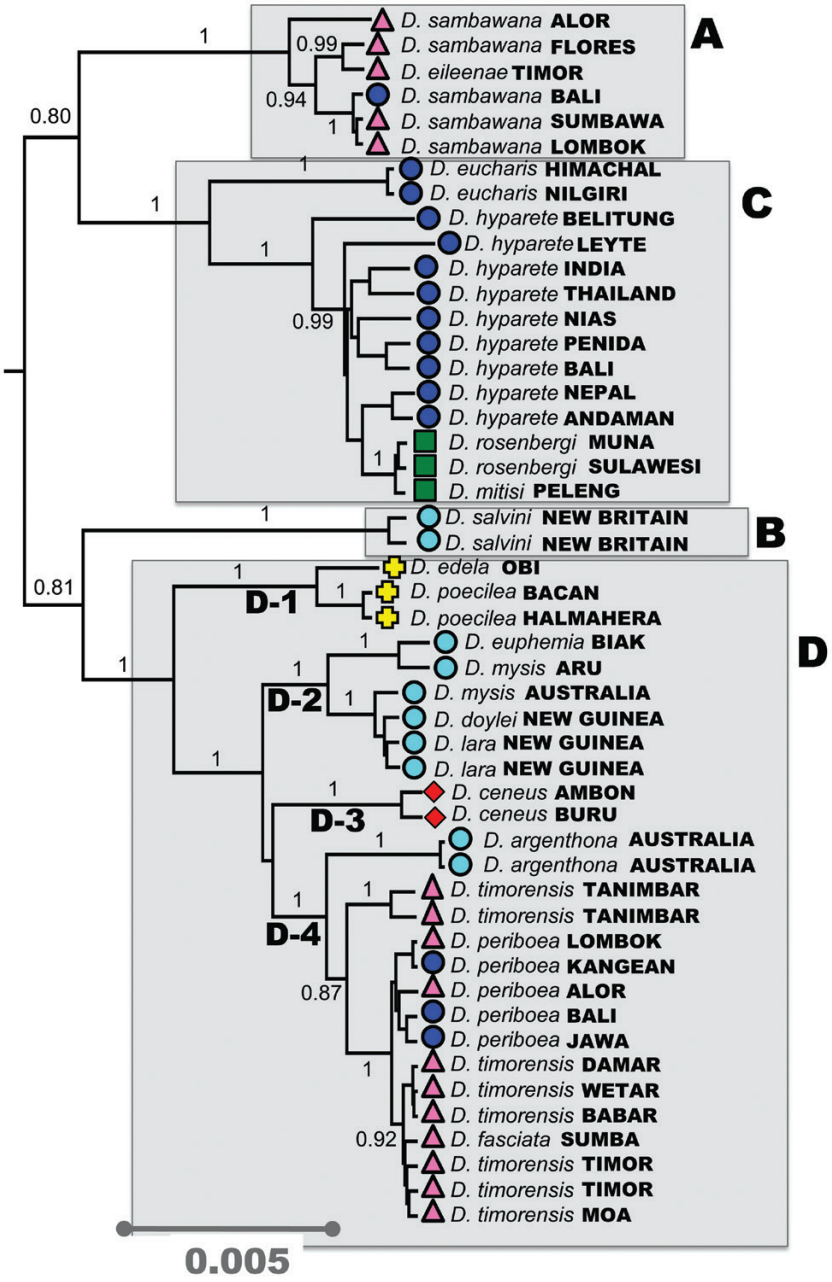


Figure 3. Phylogenetic relationships in the *D. hyparete* group. Bayesian posterior probabilities are shown under/over major branches. The collection locality is shown in bold. The symbols for the collection locality follow Figure 1. The major clades A–D are indicated.

ancestral area of the entire *D. hyparete* group was estimated to be Asia, Australian region or Lesser Sunda.

DISCUSSION

A molecular phylogenetic analysis of 138 *Delias* species by Müller *et al.* (2012) included 14 species of the *D. hyparete* group. The present study used 18 out of 21 species in the *D. hyparete* group and many subspecies, including six species that have not been used in previous phylogenetic studies of *Delias*. Therefore, the present results markedly increase the taxon sampling of phylogenetic relationships in the *D. hyparete* group, enabling inferences to be made on the diversification process in this group.

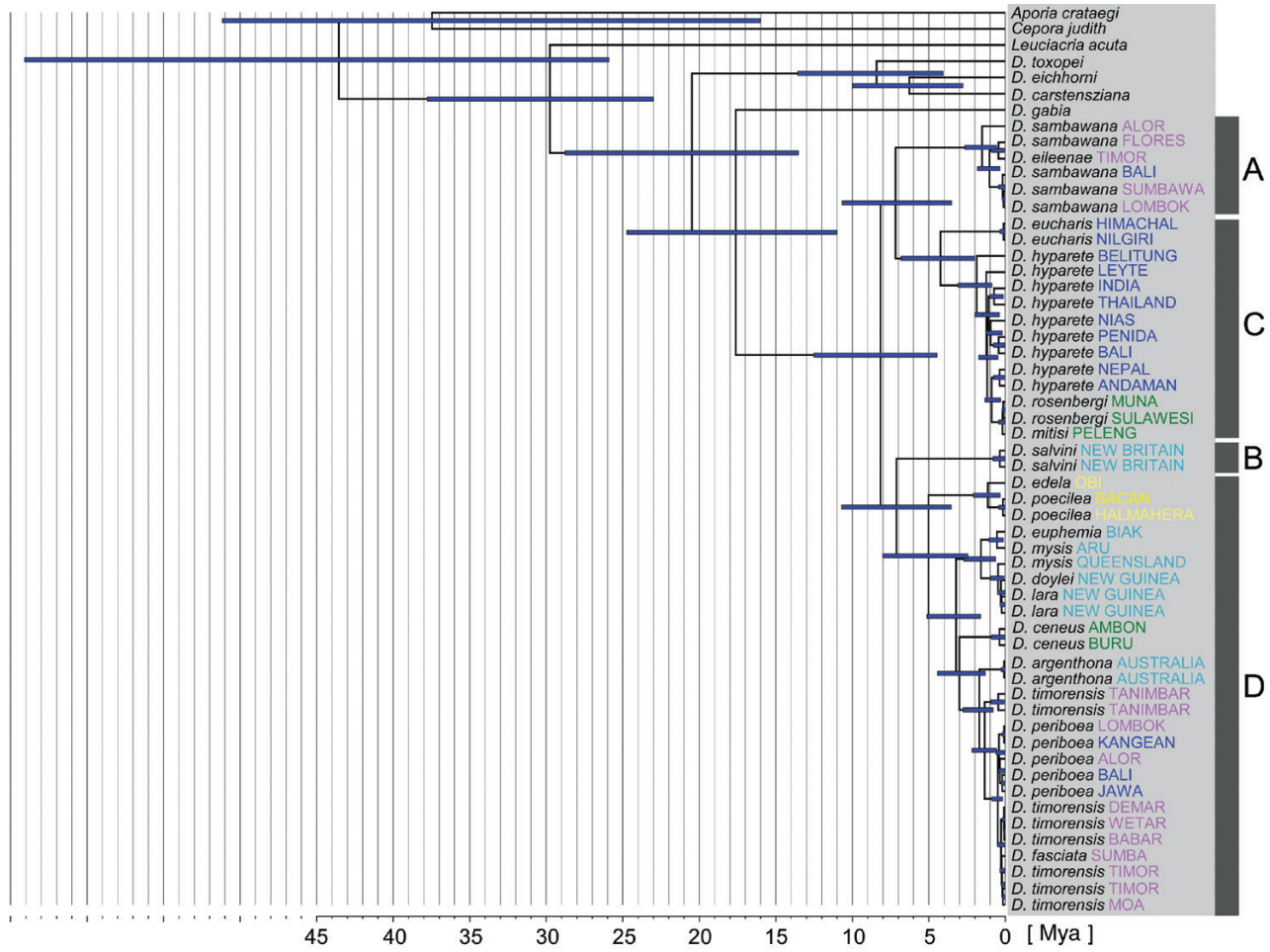


Figure 4. Chronogram inferred by a Bayesian dating analysis of the *D. hyparete* group. Mya: million years ago. Grey bars represent the 95% highest posterior density intervals around the mean age. Regarding operational taxonomic units, the collection locality and species are indicated.

Areas of endemism in Wallacea

Four major clades were identified in the *D. hyparete* group. They were composed of exclusively different groups of species and closely associated with particular geographical areas. Specifically, clade A represented the Lesser Sunda Islands. Clade B included only *D. salvini*. *D. salvini* and *D. bagoe*, which was not used in the present study, are allopatric sister species in the Bismarck Islands (Yagishita *et al.*, 1993; Müller *et al.*, 2012). Therefore, clade B represents the Bismarck Islands. Clade C included four species from the Oriental region and Sulawesi: *D. hyparete* and *D. eucharis* are principally Oriental species, and *D. rosenbergi* and *D. mitisi* are distributed in Sulawesi and the nearby islands (Yagishita *et al.*, 1993). Four subclades in clade D corresponded to particular geographical regions: clade D-1 represents northern Maluku; clade D-2 the Australian region; clade D-3 southern Maluku; and clade D-4 Lesser Sundas, the eastern end of the Greater Sundas and Australia. Thus, Wallacea was separated into four geographical areas in the phylogeny of the *D. hyparete* group: Sulawesi, Lesser Sundas, northern Maluku and southern Maluku.

A similar separation of Wallacea was previously reported for faunistic diversity. Michaux (2010) reviewed the biogeographical patterns of Wallacean fauna and proposed nine areas of endemism in Wallacea and the Philippines. Wallacea was separated into seven areas: three regions of Sulawesi, northern Maluku, southern Maluku, western Lesser Sundas and Timor. The faunistic study of various animal groups and the phylogenetic study of a particular animal lineage led to the recognition of similar areas of endemism, suggesting that a common geological event(s) is involved (cf. Ung *et al.*, 2016; Escalante, 2016).

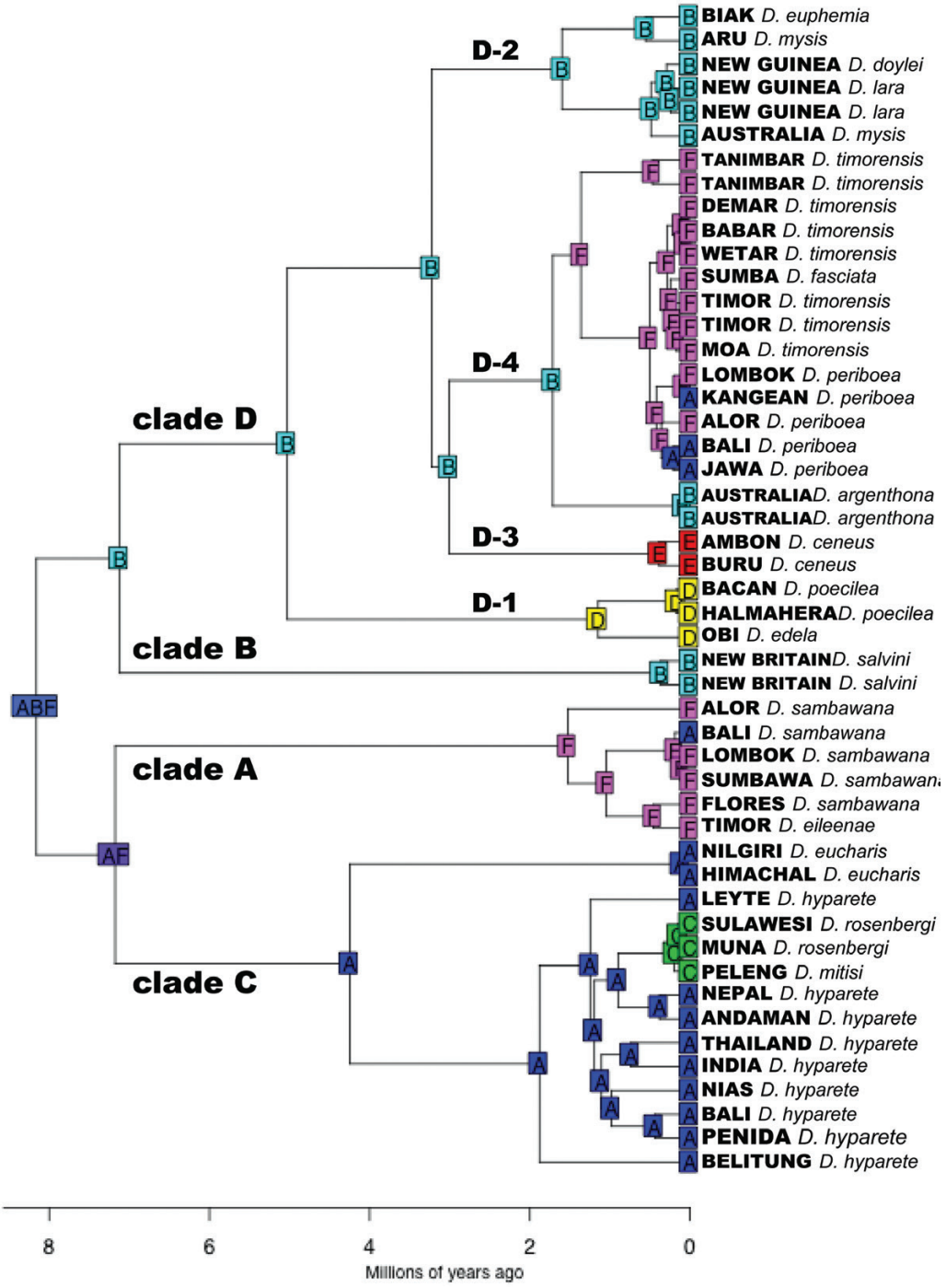


Figure 5. Reconstructed ancestral areas of the *D. hyparete* group. Characters in and colours of squares at nodes show the most likely ancestral states inferred by BioGeoBEARS. Regarding operational taxonomic units, the collection locality, corresponding zoogeographical area and the species are indicated. A, the Oriental region; B, the Australian region; C, Sulawesi region; D, North Maluku; E, South Maluku; F, Lesser Sunda Islands.

In the *D. hyparete* group, the divergence age within single areas of endemism in Wallacea was shallow, i.e. <1.2 Mya, the late Pleistocene. During the late Pleistocene, sea level was estimated to be >100 m below the present level for 30% of this epoch (Voris, 2000). According to figures 1 and 2 in the study by Voris (2000),

when the sea level was >100 m below the present level, the islands of North Maluku were connected to each other, and some of the Lesser Sunda islands were connected to each other. This may have contributed to the dispersal of *Delias* butterflies among the present islands within at least North Maluku and Lesser Sunda, which may have hampered geographical differentiation within areas of endemism. Therefore, the areas of endemism for the *D. hyparete* group in Wallacea may have finally formed during the late Pleistocene.

Historical dispersal into and out of Wallacea and diversification

The results of the ancestral area analyses conducted in the present study suggest that five events of colonization into Wallacea occurred in the *D. hyparete* group: colonization into Lesser Sunda in clade A, that into Sulawesi in clade C, that into North Maluku in clade D-1, that into South Maluku in clade D-3 and that into Lesser Sunda in clade D-4. Moreover, evidence for the historical dispersal out of Wallacea was observed, as discussed below.

Clade C in Sulawesi

Delias rosenbergi and *D. mitisi* are endemic to Sulawesi and the nearby islands. The two species were nested in Asia-dominant clade C and are more closely related to *D. hyparete* than to *D. eucharis*. The results of ancestral area and molecular dating analyses suggest that the ancestor of *D. mitisi and D. rosenbergi* colonized the Sulawesi area from the Oriental region 0.90 Mya. Considering that *D. hyparete* is paraphyletic to *D. mitisi/rosenbergi*, the colonizer to the Sulawesi area was the ancestor of *D. hyparete* or a population of extant *D. hyparete*. Sulawesi is considered to have taken its present form due to the collision of different islands *c*. 15–5 Mya (Lohman *et al.*, 2011). Therefore, the colonization of Sulawesi by the ancestor of *D. mitisi* and *D. rosenbergi* may have occurred much later after the island collision event. These findings are concordant with the view that Sulawesi's fauna is mostly of an Oriental origin (Lohman *et al.*, 2011). On the other hand, two other butterfly genera distributed in Sulawesi showed different geographical origins: sister relationships between Sulawesi and Timor were found in the phylogeny of *Charaxes* and *Cethosia* (Müller *et al.*, 2010). Müller *et al.* (2010) concluded that the relationship between Sulawesi and Timor was attributable to vicariance rather than historical dispersal.

Clades D-1, D-3 and D-4 in North Maluku, South Maluku and Lesser Sunda

Clade D included six Wallacean species. The results of the ancestral area analyses suggest repeated colonization of Wallacea from the Australian region. Clade D-1 was suggested to have colonized North Maluku 5.0 Mya. Clade D-3 may have colonized South Maluku 3.0 Mya. This is congruent with the geological suggestion that South Maluku emerged above the sea in the past 2 Myr (Lohman *et al.*, 2011). Based on their geological proximity, North Maluku and South Maluku may have been colonized from New Guinea. Clade D-4 was suggested to have colonized Lesser Sunda 1.7 Mya. This is congruent with geological evidence that Timor and other islands in the eastern part of Lesser Sunda are geologically young islands that are *c*. 2 Myr old (Hall, 2002; Lohman *et al.*, 2011). In clade D-4, *D. argenthona* was a sister to the Lesser Sunda taxa. This suggests that the ancestor of *D. argenthona* colonized Lesser Sunda and then subsequently speciated.

Clade A in Lesser Sunda

D. eileenae is endemic to Timor, and *D. sambawana* is mainly distributed in the western part of Lesser Sunda (Yagishita *et al.*, 1993). The results of the ancestral area analysis suggest that the ancestor of clade A colonized Lesser Sunda independently from clade D; however, these results do not allow us to clarify the geographical origin of clade A because Asia and Lesser Sunda were suggested with similar probabilities. Clades A and C were estimated to have split 7.2 Mya, suggesting that clade A colonized Lesser Sunda before clade D.

Dispersal at the early divergence of the D. hyparete *group*

The present study found that Asia-, Wallacea- and Australasia-dominant clades (C, A and D, respectively) in the *D. hyparete* group diverged 7.1–7.2 Mya: this represents early timing in the group's diversification history. This result suggests that the Oriental region, Wallacea and the Australian region were occupied by the *D. hyparete* group during the late Pleistocene, implying that the ancestors of the *D. hyparete* group had passed through both Wallace's and Lydekker's Lines before divergence to clades A–D. This implication is congruent with a suggestion by Müller *et al.* (2012) that Wallacea was colonized during early stages of the evolutionary history of the *D. hyparete* group. Therefore, was the Oriental or Australian region the geographical origin of this species group? The results of ancestral area analyses suggest the Australian region with the highest probability. Müller *et al.* (2012) previously reconstructed evolutionary changes in the distributional range of the genus *Delias*: the common ancestor of the *D. hyparete* group was inferred to have occurred in Australasia or Australasia+Wallacea. Collectively, these findings suggest that the common ancestor of the

D. hyparete group dispersed from the Australian region toward the Oriental region by way of western Lesser Sunda during the late Pleistocene. This speculation is relevant to two suggestions in geology. Western Lesser Sunda, which was formed by volcanic activity since the Miocene, is considered to be older than eastern Lesser Sunda, which emerged during the Quaternary (Hall, 2002): this may explain clade A butterflies not being distributed in eastern Lesser Sunda. Furthermore, according to figure 2 in Lohman *et al.* (2011), northern New Guinea (specifically Bird Head) was closer to western Lesser Sunda 5 Mya than it is today: this implies that the geographical origin of the supposed ancestral dispersal toward the Oriental Region in the *D. hyparete* group may be northern New Guinea.

Dispersal out of Wallacea

When a species is distributed on both sides of Wallace's or Lydekker's Line, the direction and timing of transborder dispersal are of interest. In the *D. hyparete* group, *D. sambawana* and *D. periboea* are distributed in Lesser Sunda and in the eastern part of Greater Sunda. Our results suggest that the ranges of *D. sambawana* and *D. periboea* expanded from Lesser Sunda to Greater Sunda. On the chronogram, the Bali sample of *D. sambawana* split from Lesser Sunda samples 0.19 Mya, and Greater Sunda samples of *D. periboea* split from Lesser Sunda samples 0.08–0.36 Mya. These results suggest that the two species expanded the range across Wallace's Line at the end of the middle Pleistocene or the beginning of the late Pleistocene.

Historical dispersal summary

The inferences obtained on the historical dispersal of the *D. hyparete* group into and out of Wallacea may be summarized as follows (Fig. 6). The most recent common ancestor of the *D. hyparete* group was presumably distributed in the Australian region. The ancestral species may have dispersed across Lydekker's and Wallace's Lines toward the Oriental region by way of the western Lesser Sunda Islands around 7–8 Mya. The colonizers in Lesser Sunda, now recognized as clade A,

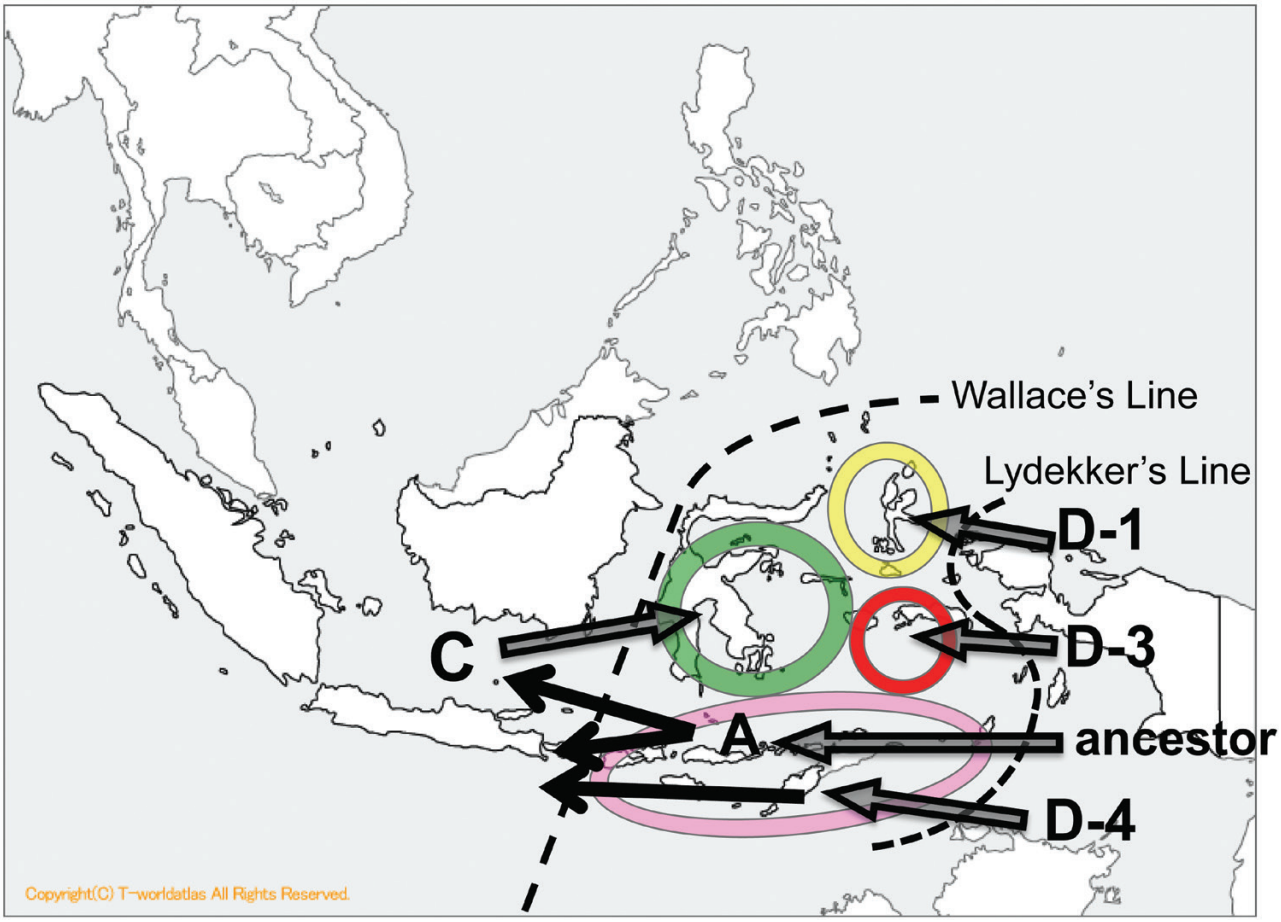


Figure 6. Inferred historical dispersal of the *D. hyparete* group around Wallacea.

have remained there to the present day and have differentiated into two species. The founder population in continental Asia (i.e. clade C) then diverged into *D. hyparete* and *D. eucharis*. The ancestor of *D. hyparete* may have then dispersed back across Wallace's Line much later and colonized Sulawesi, evolving into present-day *D. rosenbergi* and *D. mitisi*. The ancestral species of the *D. hyparete* group in the Australian region may have dispersed across Lydekker's Line to colonize North Maluku and South Maluku separately: clades D-1 and D-3 colonized 5.0 and 3.0 Mya, respectively. The former colonizers evolved into *D. edela* and *D. poecilea* in North Maluku, and the latter into *D. ceneus* in South Maluku. Lesser Sunda was subsequently colonized (1.7 Mya) from the Australian region, possibly by the ancestor of *D. argenthona*, from which *D. timorensis*, *D. periboea* and *D. fasciata* evolved. *D. periboea* and *D. sambawana* subsequently (*c*. 0.3 Mya) expanded the range across Wallace's Line to Greater Sunda.

Thus, we infer that the *D. hyparete* group colonized Wallacea repeatedly and from Australian and Oriental regions, and also that dispersals out of Wallacea also occurred repeatedly. This reinforces the recent argument that dispersal into/out of Wallacea was more frequent than classical expectations (Evans *et al.*, 1999; Jønsson *et al.*, 2008, 2010; Balke *et al.*, 2009; Lohman *et al.*, 2011; Tänzler *et al.*, 2013, 2016; Condamine *et al.*, 2015; Toussaint *et al.*, 2015; Toussaint & Balke, 2016). The present study also provides additional evidence for reverse colonization from islands to continents (Bellemain & Ricklefs, 2008; Toussaint *et al.*, 2016). Phylogenetic studies on other animal lineages that diverged in Wallacea may reveal further cases of complex dispersal histories across Wallace's and/or Lydekker's Lines.

The genus *Charaxes* is another butterfly group that is diverse over a wide geographical range of Wallacea. Müller *et al.* (2010) suggested that Sulawesi was the origin of all *Charaxes* species in the Australian region and Wallacea other than *Charaxes solon*. Namely, a single colonization event into Wallacea was suggested, which is in contrast to the *D. hyparete* group.

Systematics of the *D. hyparete* group

Yagishita *et al.* (1993) classified the *D. hyparete* group into seven subgroups (Table 2). We revised their classification based on the present results and previous findings obtained from molecular phylogenetic analyses.

The wing markings of *D. eileenae* are so unusual in the *D. hyparete* group that its systematic position has remained controversial. Yagishita *et al.* (1993) treated *D. eileenae* as an independent subgroup, and also stated 'Yasusuke Nishiyama speculates that *D. eileenae* is the sister species of *D. sambawana*' (caption to plate 196, which we translated into English). *Delias sambawana* was treated as a member of the *D. periboea* subgroup by Yagishita *et al.* (1993). Morinaka (2009) suggested a close relationship between *D. sambawana* and *D. eileenae* based on the mitochondrial ND5 sequences of a small number of samples. The present study confirmed the sister relationship between *D. sambawana* and *D. eileenae* using mitochondrial and nuclear genes of a larger sample size.

Delias timorensis was regarded as an independent subgroup by Yagishita *et al.* (1993) due to its unique wing markings in the species group. However, Morinaka (2009) suggested a close relationship between *D. periboea* and *D. timorensis* based on the mitochondrial ND5 sequences of a few samples per species. Similarly, *D. timorensis* appeared to be the closest to *D. periboea* in the phylogenetic tree of mitochondrial and nuclear sequences by Müller *et al.* (2012). The present study confirmed that *D. timorensis* is close to *D. periboea* and *D. fasciata* based on nuclear and mitochondrial sequences of larger sample sizes.

Table 2. Classification of the *Delias hyparete* group revised

Subgroup	Species	
Hyparete subgroup	*D. hyparete* (Linnaeus, 1758)	[Hy]*
	D. eucharis (Drury, 1773)	[Hy]
	D. rosenbergi (Vollenhoven, 1865)	[Hy]
	D. mitisi Staudinger, 1894	[Hy]
Periboea subgroup	*D. argenthona* (Fabricius, 1793)	[Pe]
	D. periboea (Godart, 1819)	[Pe]
	D. timorensis (Boisduval, 1836)	[Ti]
	D. fasciata Rothschild, 1894	[Pe]
Schoenbergi subgroup	*D. schoenbergi* Rothschild, 1895	[Pe]
Poecilea subgroup	*D. poecilea* (Vollenhoven, 1865)	[Po]
	D. edela Fruhstorfer, 1910	[Po]
Mysis subgroup	*D. mysis* (Fabricius, 1775)	[My]
	D. lara (Boisduval, 1836)	[My]
	D. aestiva Butler, 1897	[My]
	D. euphemia Grose-Smith, 1894	[My]
	D. doylei Sanford & Benett, 1955	[My]
Ceneus subgroup	*D. ceneus* (Linnaeus, 1758)	[Po]
Sambawana subgroup	*D. sambawana* Rothschild, 1894	[Pe]
	D. eileenae Joicey & Talbot, 1926	[Ei]
Bagoe subgroup	*D. bagoe* (Boisduval, 1832)	[Ba]
	D. salvini Butler, 1882	[Ba]

*The square brackets show the subgroup classification by Yagishita *et al.* (1993). Hy, *hyparete* subgroup; Pe, *periboea* subgroup; Ei, *eileenae* subgroup; Po, *poecilea* subgroup; Ce, *ceneus* subgroup; Ti, *timorensis* subgroup; My, *mysis* subgroup; Ba, *bagoe* subgroup. The names *D. lara* and *D. aestiva* were not used, but were included in *D. mysis* in Yagishita *et al.* (1993).

Based on phylogenetic trees in the present and previous (Müller *et al.*, 2012) studies, we propose eight subgroups of species, as shown in Table 2: *D. timorensis* is moved to the *D. periboea* subgroup; *D. schoenbergi* is raised to an independent subgroup, based on the findings of Müller *et al.* (2012); *D. ceneus* is raised to an independent subgroup, based on the results of the present study and previous findings by Müller *et al.* (2012); the *D. sambawana* subgroup is established, and *D. eileenae* is moved to this subgroup.

In addition, the present phylogenetic analysis showed non-monophyletic status for four species: *D. sambawana*, *D. hyparete*, *D. mysis* and *D. timorensis*. *Delias sambawana* was paraphyletic because *D. eileenae* was included within it in clade A. Similarly, *D. hyparete* was paraphyletic because *D. rosenbergi* and *D. mitisi* were included within it in clade C. One explanation for these cases of paraphyly is that *D. eileenae* and *D. rosenbergi/mitisi* were recenly established from *D. sambawana* and *D. hyparete*, respectively, by peripheral speciation events. Another interpretation is that *D. eileenae* and *D. rosenbergi/mitisi* are not valid species but geographical races of *D. sambawana* and *D. hyparete*, respectively. We argue that the former hypothesis is more reasonable given that the wing markings of *D. eileenae* and *D. sambawana*, and those of *D. rosenbergi/mitisi* and *D. hyparete* are so different and the species status of *D. eileenae* and *D. rosenbergi/mitisi* has not yet been challenged (Supporting Information, Fig. S1).

More interestingly, *D. mysis* and *D. timorensis* were each polyphyletic in clade D. *D. mysis* was polyphyletic because the two subspecies (*D. m. mysis* and *D. m. aruensis*) were included in different subclades, with *D. m. aruensis* sister to *D. euphemia*. *D. timorensis* was polyphyletic because the Tanimbar samples (*D. t. gardineri*) were phylogenetically separated from the others. The subspecies *D. t. gardineri* has a smaller body/wing than the other subspecies of *D. timorensis* (Yagishita *et al.*, 1993). It is possible that *D. m. aruensis* and *D. t. gardineri* represent distinct species that differentiated slightly in external morphology from the other subspecies of *D. mysis* and *D. timorensis*, respectively. This hypothesis needs to be investigated by more closely studying the molecular phylogeny of *D. mysis* and *D. timorensis* using more samples and by critically examining external morphology including the male genitalia.

ACKNOWLEDGEMENTS

We are grateful to Yukio Ishikawa, Takashi Matsuo, Takeshi Fujii, Hari Sutrisno, Harumi Shibuya and Tadao Matsumoto for their support of our work. We thank Osamu Yata, Isao Makibayashi, Yasusuke Nishiyama, Akira Yagishita, Katsuyuki Funahashi, Yasuo Shibatani, Yasushi Sorimachi, Kaoru Adachi and Niklas Wahlberg for providing information and/or materials. We thank Hirohisa Kishino for technical advice on the use of BEAST. We also thank Chris Müller and four anonymous reviewers for their helpful comments. This study was partly funded by the University of Tokyo.

REFERENCES

Balke M, Ribera I, Hendrich L, Miller MA, Sagata K, Posman A, Posman A, Vogler AP, Meier R. 2009. New Guinea highland origin of a widespread arthropod supertramp. *Proceedings of the Royal Society of London, Biological Sciences* **276:** 2359–2367.

Beck J, Kitching IJ, Linsenmair KE. 2006. Wallace's line revisited: has vicariance or dispersal shaped the distribution of Malesian hawkmoths (Lepidoptera: Sphingidae)? *Biological Journal of the Linnean Society* **89:** 455–468.

Bellemain E, Ricklefs RE. 2008. Are islands the end of the colonization road? *Trends in Ecology & Evolution* **23:** 461–468.

Braby MF. 2000. *Butterflies of Australia: their identification, biology, and distribution*. Melbourne: CSIRO Publishing.

Braby MF. 2006. Evolution of larval food plant associations in *Delias* Hübner butterflies (Lepidoptera: Pieridae). *Entomological Science* **9:** 383–398.

Braby MF. 2012. The taxonomy and ecology of *Delias aestiva* Butler, 1897 stat. rev. (Lepidoptera: Pieridae), a unique mangrove specialist of Euphorbiaceae from northern Australia. *Biological Journal of the Linnean Society* **107:** 697–720.

Braby MF. 2014. Taxonomic status of *Delias aestiva smithersi* Daniels, 2012 (Lepidoptera: Pieridae) comb. nov. from the gulf country of northern Australia, with description of the female. *Records of the Australian Museum* **66:** 241–246.

Braby MF, Nishida K. 2010. The immature stages, larval food plants and biology of Neotropical mistletoe butterflies (Lepidoptera: Pieridae). II. The *Catasticta* group (Pierini: Aporiina). *Journal of Natural History* **44:** 1831–1928.

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

Braby MF, Trueman JW. 2006. Evolution of larval host plant associations and adaptive radiation in pierid butterflies. *Journal of Evolutionary Biology* **19:** 1677–1690.

Braby MF, Pierce NE, 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.

Braby MF, Vila R, Pierce NE. 2006. Molecular phylogeny and systematics of Pieridae (Lepidoptera: Papilionoidea):

higher classification and biogeography. *Zoological Journal of the Linnean Society* **147:** 239–275.

Clouse RM, Janda M, Blanchard B, Sharma P, Hoffmann BD, Andersen AN, Czekanski-Moir JE, Krushelnycky P, Rabeling P, Wilson EO, Economo EP, Sarnat EM, General DM, Alpert GD, Wheeler WC. 2015. Molecular phylogeny of Indo-Pacific carpenter ants (Hymenoptera: Formicidae, *Camponotus*) reveals waves of dispersal and colonization from diverse source areas. *Cladistics* **31:** 424–437.

Condamine FL, Toussaint EF, Clamens AL, Genson G, Sperling FA, Kergoat GJ. 2015. Deciphering the evolution of birdwing butterflies 150 years after Alfred Russel Wallace. *Scientific Reports* **5:** 11860.

Corbet AS, Pendlebury HM. 1993. *The butterflies of the Malay Peninsula*, *4th revised edn.* Kuala Lumpur: Malaysian Nature Society.

D'Abrera B. 1990. *Butterflies of the Australian region*, *3rd edn*. Melbourne: Lansdowne Press.

Davenport C, van Mastrigt HJG. 2008. Revision of *Delias mysis* (Fabricius, 1775) and closely related species (Lepidoptera: Pieridae). *Suara Serangga Papua* **3:** 15–31.

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

Escalante T. 2016. A natural regionalization of the world based on primary biogeographic homology of terrestrial mammals. *Biological Journal of the Linnean Society* (in press). DOI: 10.1111/bij.12898.

Evans BJ, Morales JC, Supriatna J, Melnick DJ. 1999. Origin of the Sulawesi macaques (Cercopithecidae: *Macaca*) as suggested by mitochondrial DNA phylogeny. *Biological Journal of the Linnean Society* **66:** 539–560.

Grismer LL, Wood Jr PL, Aowphol A, Cota M, Grismer MS, Murdoch ML, Aguilar C, Grismer JL. 2016. Out of Borneo, again and again: biogeography of the stream toad genus *Ansonia* Stoliczka (Anura: Bufonidae) and the discovery of the first limestone cave-dwelling species. *Biological Journal of the Linnean Society* (in press). DOI: 10.1111/bij.12886.

Hall R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences* **20:** 353–431.

Heled J, Bouckaert RR. 2013. Looking for trees in the forest: summary tree from posterior samples. *BMC Evolutionary Biology* **13:** 221.

Jønsson KA, Irestedt M, Fuchs J, Ericson PG, Christidis L, Bowie RC, Norman JA, Pasquet E, Fjeldså J. 2008. Explosive avian radiations and multi-directional dispersal across Wallacea: evidence from the Campephagidae and other Crown Corvida (Aves). *Molecular Phylogenetics and Evolution* **47:** 221–236.

Jønsson KA, Bowie RCK, Moyle RG, Christidis L, Norman JA, Benz BW, Fjeldså J. 2010. Historical biogeography of an Indo-Pacific passerine bird family (Pachycephalidae): different colonization patterns in the Indonesian and Melanesian archipelagos. *Journal of Biogeography* **37:** 245–257.

Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shih HT, Carvalho GR, von Rintelen T. 2011. Biogeography of the Indo-Australian Archipelago. *Annual Review of Ecology, Evolution and Systematics* **42:** 205–226.

Maddison WP, Maddison DR. 2015. *Mesquite: a modular system for evolutionary analysis, Ver. 3.03*. Available at: http://mesquiteproject.org

Matzke NJ. 2013a. *BioGeoBEARS: BioGeography with Bayesian (and Likelihood) Evolutionary Analysis in R scripts*. Berkeley, CA: University of California. Available at: http://cran.R-project.org/package=BioGeoBEARS

Matzke NJ. 2013b. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5:** 242–248.

Matzke NJ. 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in Island Clades. *Systematic Biology* **63:** 951–970.

Michaux B. 2010. Biogeology of Wallacea: geotectonic models, areas of endemism, and natural biogeographic units. *Biological Journal of the Linnean Society* **101:** 193–212.

Morinaka S. 2009. A few notes on mimicry in butterflies. In: Tera A, Matsui M, Kogure M, eds. *Collection of papers on ropalocerology of "Takao-seminar", Part 3*. Tokyo: Takao-Seminar, 173–180 (in Japanese).

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.

Morishita K, Yata O. 1981. Pieridae and Danaidae. In: E. Tsukada ed. *Butterflies of the South East Asian islands*, *Vol.* **2**. Tokyo: Plapac, 1–628.

Müller CJ, Wahlberg N, Beheregaray LB. 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.

Müller CJ, Matos-Maraví PF, Beheregaray LB. 2012. Delving into *Delias* Hübner (Lepidoptera: Pieridae): fine-scale biogeography, phylogenetics and systematics of the world's largest butterfly genus. *Journal of Biogeography* **40:** 881–893.

Ng EYX, Eaton JA, Verbelen P, Hutchinson RO, Rheindt FE. 2016. Using bioacoustic data to test species limits in an Indo-Pacific island radiation of *Macropygia* cuckoo doves. *Biological Journal of the Linnean Society* **118:** 786–812.

Parsons MJ. 1998. *The butterflies of Papua New Guinea: their systematics and biology*. London: Academic Press.

Rambaut A. 2012. *FigTree v1.4.0*. Available at http://tree.bio.ed.ac.uk/software/figtree

Rambaut A, Drummond AJ. 2016. *TreeAnnotator v1.8.4*. Available at: http://beast.bio.ed.ac.uk/downloads

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. *Tracer v1.6*. Available at: http://beast.bio.ed.ac.uk/Tracer

Reed RD, Sperling FA. 1999. Interaction of process partitions in phylogenetic analysis: an example from the swallowtail

butterfly genus Papilio. *Molecular Biology and Evolution* **16:** 286–297.

Stirpe F. 1983. Mistletoe toxicity. *Lancet (London, England)* **1:** 295.

Su ZH, Ohama T, Okada TS, Kajiwara E, Nakamura K, Ishikawa R, Osawa S. 1996. Geography-linked phylogeny of the *Damaster* ground beetles inferred from mitochondrial ND5 gene sequences. *Journal of Molecular Evolution* **42:** 130–134.

Talbot G. 1928–1937. *A monograph of the pierine genus* Delias, *Parts 1–6*. London: British Museum (Natural History).

Tanabe AS. 2007. KAKUSAN: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. *Molecular Ecology Notes* **7:** 962–964.

Tänzler R, Toussaint EFA, Suhardjono YR, Balke M, Riedel A. 2014. Multiple transgressions of Wallace's Line explain diversity of flightless *Trigonoperus* weevils on Bali. *Proceedings of the Royal Society B* **281:** 20132528.

Tänzler R, Van Dam MH, Toussaint EF, Suhardjono YR, Balke M, Riedel A. 2016. Macroevolution of hyperdiverse flightless beetles reflects the complex geological history of the Sunda Arc. *Scientific Reports* **6:** 18793.

Thompson JD, Higgins DG, Gibson TJ. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4623–468.

Toussaint EFA, Balke M. 2016. Historical biogeography of *Polyura* butterflies in the oriental Palaeotropics: trans-archipelagic routes and South Pacific island hopping. *Journal of Biogeography* **43:** 1560–1572.

Toussaint EFA, Condamine FL. 2016. To what extent do new fossil dicoeries change our understanding of clade evolution? A cautionary tale from burying beetles (Coleoptera: *Nicrophorus*). *Biological Journal of the Linnean Society* **117:** 686–704.

Toussaint EFA, Hendrich L, Hájek J, Michat MC, Panjaitan R, Short AEZ, Balke M. 2016. Evolution of Pacific Rim diving beetles sheds light on Amphi-Pacific biogeography. *Ecography* **39:** 1–11.

Toussaint EFA, Tänzler R, Rahmadi C, Balke M, Riedel A. 2015. Biogeography of Australian flightless weevils (Curculionidae, Celeuthetini) suggests permeability of Lydekker's and Wallace's Lines. *Zoologica Scripta* **44:** 632–644.

Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27:** 171–180.

Ung V, Zaragueta-Bagils R, Williams DM. 2016. Comparative biogeography of Southeast Asia and the West Pacific region. *Biological Journal of the Linnean Society* **117:** 372–385.

Vane-Wright RI, Raheem DC, Cieslak A, Vogler AP. 1999. Evolution of the mimetic African swallowtail butterfly *Papilio dardanus*: molecular data confirm relationships with *P. phorcas* and *P. constantinus*. *Biological Journal of the Linnean Society* **66:** 215–229.

Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27:** 1153–1167.

Wallace AR. 1867a. *The geographical distribution of animals*. New York: Harper.

Wallace AR. 1867b. On the Pieridae of the Indian and Australian regions. *Transactions of the Entomological Society of London* **4:** 301–415.

Yagishita A, Nakano S, Morita S. 1993. *An illustrated list of the genus* Delias *Hübner of the world*. Nishiyama Y, ed. Tokyo: Khepera Publishers (in Japanese).

Zakharov EV, Caterino MS, Sperling FA. 2004. Molecular phylogeny, historical biogeography, and divergence time estimates for swallowtail butterflies of the genus *Papilio* (Lepidoptera: Papilionidae). *Systematic Biology* **53:** 193–215.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

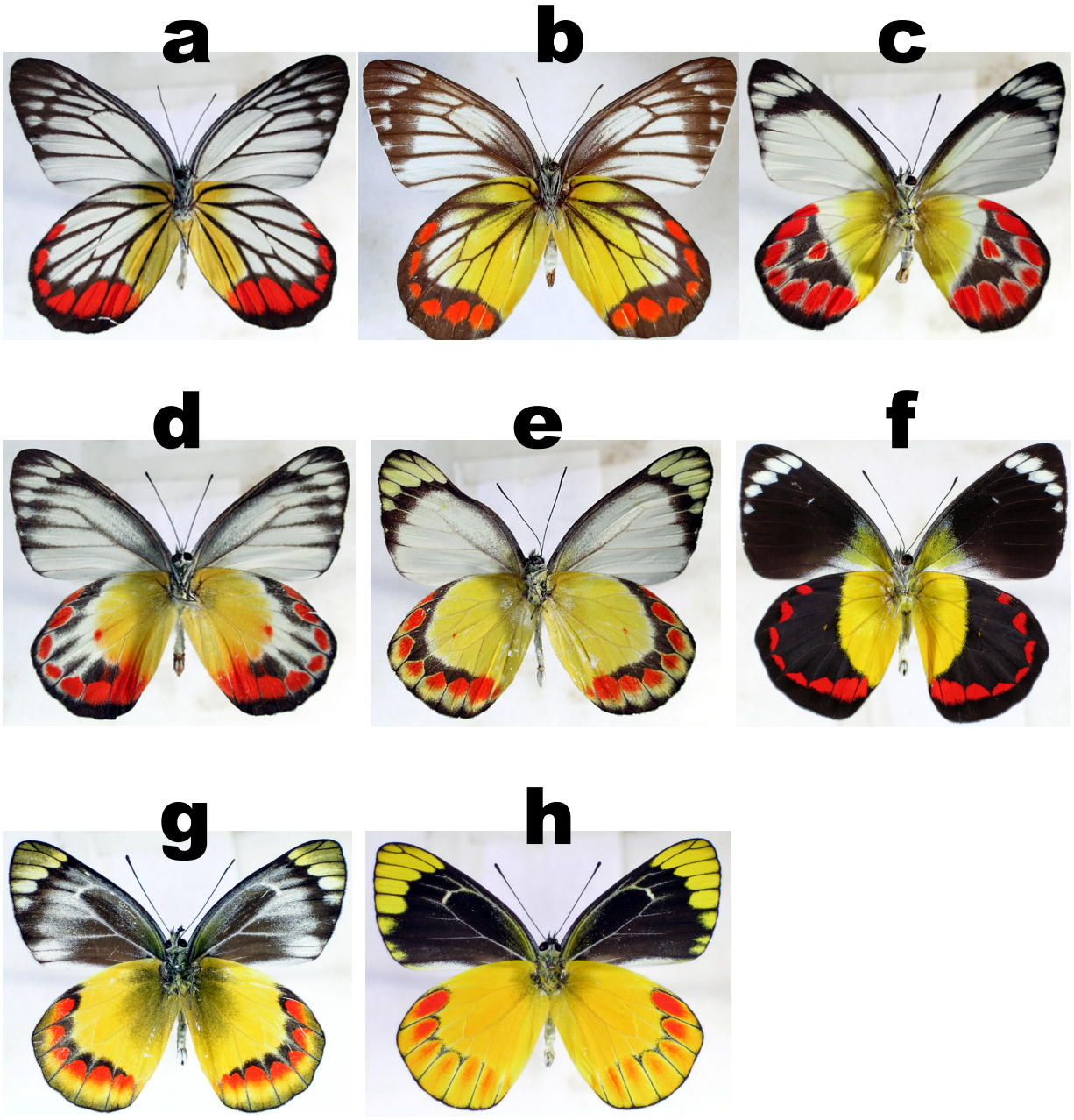
Table S1. A list of additional primers used for PCR amplification and its direct sequencing reaction.
Figure S1. The diversity of markings on the underside of wings in the *D. hyparete* group. (a) *D. hyparete*, (b) *D. rosenbergi*, (c) *D. argenthona*, (d) *D. periboea*, (e) *D. fasciata*, (f) *D. timorensis*, (g) *D. sambawana*, (h) *D. eileenae*.

Supplementary Table 1. A list of additional primers used for PCR amplification and its direct sequencing reaction.

Gene	Direction	Name	Nucleotide sequence
ND5	Forward	E2	5'-TGTGGAATTCCTTTTTTAGC-3'
		E4	5'-TTTTTTCATTTATTAACTCATGC-3'
		E6	5'-ATCCTTATATGATTTTTTTACC-3'
		E11	5'-TCGAATTTAGCTTTATGTGG-3'
	Reverse	F2	5'-TACTATTAAATATGATAATAAACG-3'
		F3	5'-AAGGTAAAAAAATCATATAAGG-3'
		F5	5'-GCTAAAAATGGYATTCCACA-3'
		B7	5'-ATTTCTCATCATCCAATATC-3'
		B9	5'-TAACCTCTATATATTTCTCTTCA-3'
		B10	5'-ACCCTCTATATATTTCTCTTC-3'
		rosF1	5'-TTGAAAAATAATATAAATAAAAAATTA-3'
EF1-a	Forward	C9	5'-GTGGTGGTATCGATAAACGTACC-3'
		C10	5'-ACCAAAATGCCTTGGTTCAAGGG-3'
		C11	5'-TGATTGTAGGAGTCAACAAAATGGA-3'
		C14	5'-ATGTTGGATTCAACGTTAAGAACGT-3'
		C15	5'-GAAGCGGGTATCTCAAAGAAYGG-3'
		C19	5'-GATGGATGGTTGAACGTAAGGAA-3'
	Reverse	D10	5'-TCTTCGGTGGATTTACCAGTACG-3'
		D11	5'-CCGCCGATTTTGTATACATCCTG-3'
		D12	5'-GGCTCAGTGGAGTCCATTTTGTT-3'
		D13	5'-TCTTAACGTTGAAACCAACATTGTC-3'
		D14	5'-AGGTAACCGTAAAGGCTTATCTGT-3'
		D16	5'-CTGTTCCAATACCRCCGATTTTGT-3'
		D19	5'-GCATCACCAGATTTAATAGATTTAG-3'

Supplementary Figure 1.

The diversity of markings on the underside of wings in the *D. hyparete* group. (a) *D. hyparete*, (b) *D. rosenbergi*, (c) *D. argenthona*, (d) *D. periboea*, (e) *D. fasciata*, (f) *D. timorensis*, (g) *D. sambawana*, (h) *D. eileenae*.



Suppl. Fig. 1