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Systematics, biogeography and diversification of the Indo-Australian genus *Delias* Hübner (Lepidoptera: Pieridae): phylogenetic evidence supports an 'out-of-Australia' origin

MICHAEL F. BRABY[1,2] and NAOMI E. PIERCE[1]
[1]Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, U.S.A. and
[2]School of Botany and Zoology, The Australian National University, Canberra, Australia

Abstract. Two alternative hypotheses for the origin of butterflies in the Australian Region, that elements dispersed relatively recently from the Oriental Region into Australia (northern dispersal hypothesis) or descended from ancient stocks in Gondwana (southern vicariance hypothesis), were tested using methods of cladistic vicariance biogeography for the *Delias* group, a diverse and widespread clade in the Indo-Australian Region. A phylogenetic hypothesis of the twenty-four species-groups recognized currently in *Delias* and its sister genus *Leuciacria* is inferred from molecular characters generated from the nuclear gene elongation factor-1 alpha (*EF-1α*) and the mitochondrial genes cytochrome oxidase subunits I and II (*COI*/*COII*) and NADH dehydrogenase 5 (*ND5*). Phylogenetic analyses based on maximum parsimony, maximum likelihood and Bayesian inference of the combined dataset (3888 bp, 1014 parsimony informative characters) confirmed the monophyly of *Delias* and recovered eight major lineages within the genus, informally designated the *singhapura*, *belladonna*, *hyparete*, *chrysomelaena*, *eichhorni*, *cuningputi*, *belisama* and *nigrina* clades. Species-group relationships within these clades are, in general, concordant with current systematic arrangements based on morphology. The major discrepancies concern the placement of the *aganippe*, *belisama* and *chrysomelaena* groups, as well as several species-groups endemic to mainland New Guinea. Two species (*D. harpalyce* (Donovan), *D. messalina* Arora) of uncertain group status are currently misplaced based on strong evidence of paraphyly, and are accordingly transferred to the *nigrina* and *kummeri* groups, respectively. Based on this phylogeny, a revised systematic classification is presented at the species-group level. An historical biogeographical analysis of the *Delias* group revealed that the most parsimonious reconstruction is an origin in the Australian Region, with at least seven dispersal events across Wallacea to the Oriental Region. The eight major clades of *Delias* appear to have diverged rapidly following complete separation of the Australian plate from Gondwana and its collision with the Asian plate in the late Oligocene. Further diversification and dispersal of *Delias* in the Miocene–Pliocene are associated with major geological and climatic changes that occurred in Australia–New Guinea during the late Tertiary. The 'out-of-Australia' hypothesis for the *Delias* group supports an origin of the Aporiina in southern Gondwana (southern vicariance hypothesis), which proposes that the ancestor of *Delias* + *Leuciacria* differentiated vicariantly on the Australian plate.

Correspondence: Michael F. Braby, Biodiversity Conservation Division, Department of Natural Resources, Environment and the Arts, PO Box 496, Palmerston, NT 0831, Australia. E-mail: michael.braby@nt.gov.au

Introduction

Two main alternative hypotheses have been advanced to explain the origin, age and evolutionary history of Australian butterflies. First, all the butterflies in the Australian Region are the progeny of ancestors that dispersed relatively recently from Asia or Eurasia (northern dispersal hypothesis) (de Jong, 2003). Second, a component of the Australian fauna is more ancient, having descended from stocks in the southern lands of either remnant Gondwana (Madagascar–Greater India–Australia–Antarctica–South America) or southern Gondwana (Australia–Antarctica–South America) (southern vicariance hypothesis) (Braby *et al*., 2005). Evidence in support or against these hypotheses has been hampered by the lack of fossils from the Australian Region, and modern workers have had to rely on methods of cladistic vicariance biogeography, which integrates phylogenetic hypotheses with areas of endemism. One approach of this method is to search for sister-group (vicariant) relationships of Australian endemic taxa with those found in other Gondwanan fragments, such as South America. A second approach is to investigate historical patterns of more widespread taxa in both the Australian and Oriental Regions (de Jong, 1990, 2001, 2004). In this approach, optimization of the number and direction of dispersal events between the two zoogeographical regions can be used to infer whether movement is more likely to be 'in' or 'out' of one of the regions, provided that a reasonably robust phylogenetic framework is available and an estimate of age of the stem-group is known. A third, but perhaps less satisfactory, approach is to date the time of divergence of lineages endemic to the Australian mainland in order to establish whether the taxa arose *in situ*, that is, at a time when the colonization of Australia from adjacent landmasses, such as South-East Asia, was unlikely.

de Jong (2003) has suggested that most butterfly genera endemic to Australia have a close relationship with the Oriental Region. Although many higher taxa in Australia and mainland New Guinea also occur in South-East Asia (de Jong, 2001; Kitching *et al*., 2001), detailed phylogenies are lacking for much of the Indo-Australian fauna to ascertain whether it is more likely that taxa arose 'in' or 'out' of Australia prior to dispersal across Wallacea. Wallacea (Fig. 1) is a transitional zone between the Oriental and Australian Regions, and lies between the Sunda continental shelf of Asia (Wallace's Line) and the Sahul continental shelf of Australia–New Guinea (Lydekker's Line); it is a complex region of islands, many of recent geological age, surrounded

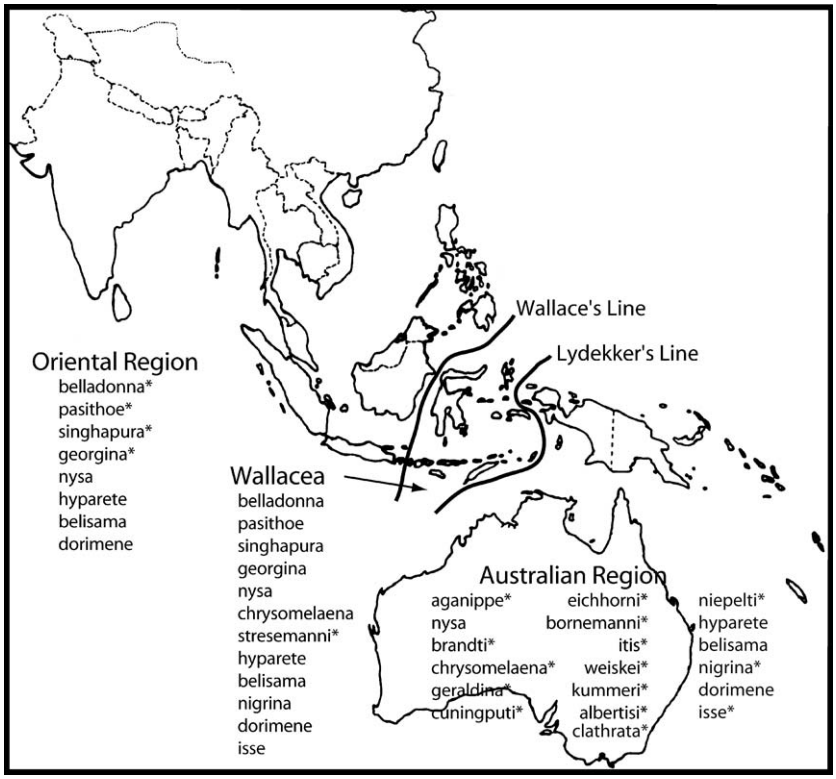

Fig. 1. Geographical distribution of *Delias*, showing the number and composition of species-groups within the Oriental and Australian zoogeographical regions. *Species-group endemic to a region. Of the twenty-four species-groups currently recognized, eight occur in the Oriental Region (four of which are endemic) and nineteen occur in the Australian Region (fifteen of which are endemic), with four shared between the two regions. Only one species-group (*stresemanni*) is restricted to Wallacea, a transitional zone between the two regions, which lies in a deep ocean trench between Wallace's Line at the eastern edge of the Sunda continental shelf and Lydekker's Line at the western edge of the Sahul continental shelf. Distributions for species-groups are based on Talbot (1928–37), Yagishita *et al*. (1993) and Müller (2001a).

by a wide deep ocean trench (Simpson, 1977; Heatwole, 1987; Cox & Moore, 2000; Hall, 2001; Kitching *et al*., 2001).

One higher butterfly taxon that has been analysed recently is the subtribe Aporiina of the Pierinae (Lepidoptera: Pieridae), which originated during or before the Palaeocene [69–54 million years ago (Mya)] and radiated in the Eocene (57–45 Mya) (Braby *et al*., 2006a, b). Several genera in this subtribe occur in the Indo-Australian Region, one of which is *Delias* Hübner, a large and widespread genus. The nearest relative of *Delias* is the Australian endemic *Leuciacria* Rothschild & Jordan (Braby *et al*., 2006b), a small genus restricted to the highlands of montane mainland New Guinea (Papua New Guinea, West Papua = Irian Jaya) and New Ireland. Within the Aporiina, these two genera comprise a monophyletic group referred to as the '*Delias* group'. However, Braby *et al*. (2006b) were unable to satisfactorily reconstruct the geographical origin of the Aporiina, and presented two equally most parsimonious hypotheses to explain the presence of the *Delias* group in the Australian Region. A southern vicariance hypothesis proposed an origin of the subtribe in southern Gondwana, with the ancestor of *Delias* + *Leuciacria* evolving vicariantly on the Australian plate; *Delias* subsequently dispersed at least once out of Australia across Wallacea to Asia. The alternative northern dispersal hypothesis postulated an origin of the subtribe in Laurasia, with *Delias* dispersing at least once out of Asia across Wallacea to Australia/New Guinea where it radiated subsequently. Each hypothesis had one serious anomaly or major weakness in which a formidable long-distance dispersal event across an ocean barrier had to be invoked. For the latter hypothesis, the anomaly concerned long-distance dispersal of the ancestor of *Leuciacria* from Asia across the Indian Ocean to Australia, followed by allopatric speciation in the late Eocene–early Oligocene. Braby *et al*. (2006b) concluded that rejecting one hypothesis over the other may be resolved only by reconstructing phylogenies of many of the larger genera/clades, and called for additional fine-scale species-level phylogenies to determine the directionality of dispersal implicit in each hypothesis. For the *Delias* group, the widely accepted view is that *Delias* originated in Asia and entered Australia only recently from northern latitudes (Dixey, 1894; Talbot, 1928–37; Klots, 1933; Roepke, 1955; Holloway, 1969, 1974, 1986; Mani, 1986). However, there is currently no adequate species-group level phylogeny available for *Delias* to test this hypothesis.

Here, we use molecular markers to derive an estimate of the evolutionary history of the *Delias* group based on phylogenetic analysis of exemplars representing all twenty-four species-groups of *Delias* and its sister genus *Leuciacria*. Our goal is to use the phylogeny as a framework to analyse patterns of historical biogeography and diversification within *Delias*, and to determine whether the genus was more likely to have evolved in the Australian Region (i.e. Australia) or Oriental Region (i.e. Asia). We also compare our phylogenetic hypothesis with that based on morphological characters, and propose a revised systematic classification. The systematic placement of four taxa of uncertain species-group status (*D. diaphana* Semper, *D. ellipsis* de Joannis, *D. harpalyce* (Donovan), *D. messalina* Arora) is also investigated.

The genus *Delias*

Delias is a distinct, large and widespread genus. Compared with other pierids, the adults are phenotypically divergent, having striking patterns and aposematic colours on the underside, especially of the hind wing (Fig. 2). It is by far the largest genus in the Pieridae, comprising about 20% of all species recognized in the family and about 10% of all species of butterflies in the Australian Region. Estimates of the number of recognized species currently vary from 165 (Parsons, 1998) to more than 250 (F. Gerrits, pers. comm., private collector, Australia; A. Yagishita, pers. comm., private collector, Japan). However, species have not been defined using consistent criteria, and some morphologically similar allopatric montane species have been delineated on the basis of extremely small genetic divergences [e.g. 0.1–0.2% for mitochondrial NADH dehydrogenase 5 (*ND5*)] (Morinaka & Nakazawa, 1999b; Morinaka *et al*., 2002). A more realistic estimate probably lies midway between these two extremes; for example, Yagishita *et al*. (1993) and Tuzov (1996) recognized 216 and 226 species, respectively. Despite variations in estimates of species richness and composition, it is agreed generally that the genus represents an example of rapid diversification of considerable proportions, particularly in New Guinea (Parsons, 1998).

Delias occurs widely in the Australian and Oriental Regions, with a weak representation in the Palaearctic, where a few species in the *belladonna* group extend as far north as the Sichuan Province of southern China (A. Yagishita, pers. comm.; F. Gerrits, pers. comm.). The geographical range extends from the southern slopes of the Himalaya (Kashmir, Nepal, Sikkim, Bhutan, northern India) (Mani, 1986), southern and south-eastern China (including the eastern edge of Tibet) and Taiwan, through Central and South-East Asia, including the Malay Peninsula, the Philippines and Indonesia, to mainland New Guinea and Australia, reaching its easternmost limits on the Solomon Islands, Vanuatu and New Caledonia (Talbot, 1928–37; Holloway & Peters, 1976; D'Abrera, 1990; Yagishita *et al*., 1993; Tennent, 2002, 2004). Five species-groups (*belladonna*, *pasithoe*, *belisama*, *hyparete*, *dorimene*) have a weak representation in south-eastern China (Wei & Wu, 2005), with two species in the *belladonna* group (*D. lativitta* Leech and *D. berinda* Moore) reaching their westernmost limits at Tangmai (2200 m) near Bomi (north of Arunachal Pradesh), just east of the Plateau of Tibet in the Palaearctic Region (A. Yagishita, pers. comm.). Only two species occur on New Caledonia, one of which (*D. ellipsis*) is endemic to the island; the other (*D. nysa* (Fabricius)) also occurs on Vanuatu and mainland Australia. The genus is absent from New Zealand and most of the smaller oceanic islands of the south-west Pacific, as well as Tasmania, the island state of Australia. Most species occur in the mid- to upper montane cool temperate forests in

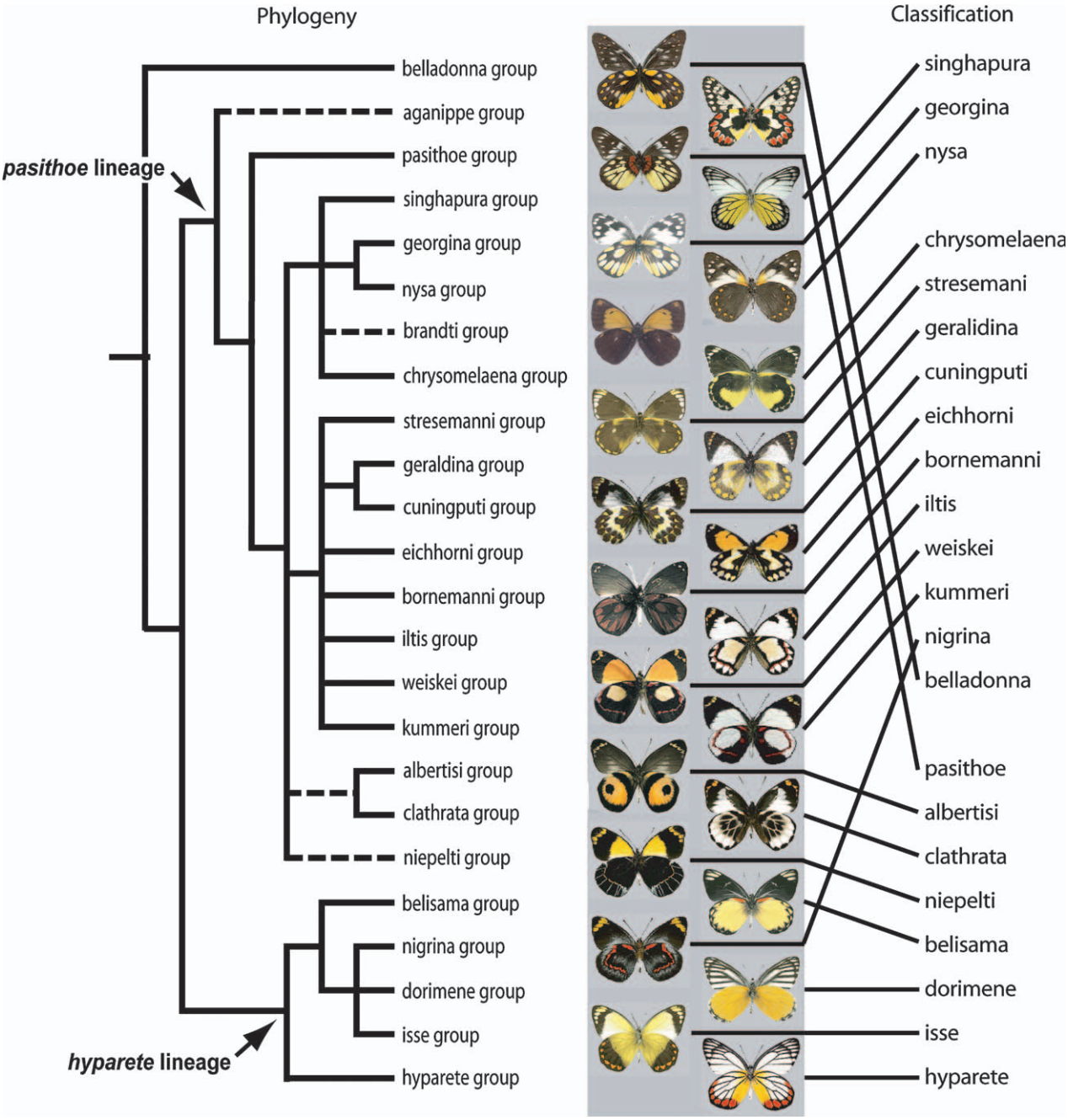

Fig. 2. Intuitive phylogeny of *Delias*, showing the relationships of the twenty-four species-groups according to the evolutionary hypothesis of Talbot (1928–37), subsequently amended by Ford (1942), Yagishita *et al*. (1993) and Müller (2001a). Broken lines indicate uncertainty in the phylogenetic position of five of the species-groups. The phylogeny is compared with the systematic classification of Talbot (1928–37) and Yagishita *et al*. (1993), shown to the right of the tree. All photographs, except *brandti*, © L. Day (2000) *Delias of the World*: http://www.delias-butterflies.co.uk/index.html

tropical latitudes, with greatest species richness in mainland New Guinea (Talbot, 1928–37; Yagishita *et al*., 1993; Parsons, 1998) where they are found predominantly at elevations above 1200 m (Jordan, 1912; Roepke, 1955; Corbet & Pendlebury, 1992; Parsons, 1998; van Mastrigt, 2001). In mainland New Guinea, the vast majority of species occur at elevations between 1600 and 2000 m, and many occur at elevations above 2400 m; some species exist as high as 3600 m or more (Parsons, 1998). Although many species-groups are represented at altitudes below 1200 m, few species are limited to the hot lowland areas (< 300 m) between the Tropics of Cancer and Capricorn. Despite

having a preference for cool temperate climates, only a single species (*D. harpalyce*) is restricted to the temperate latitudes of south-eastern Australia (Braby, 2000). The majority of species have rather limited ranges, often restricted to particular mountains and peaks on islands, and few have wide ranges. An exception is the Australian endemic, *D. aganippe* (Donovan), which occurs widely in the temperate, semiarid and arid areas of inland southern Australia (Braby, 2000).

In his extensive monograph of the genus, Talbot (1928–37), building on the earlier work of Wallace (1867), Dixey (1894) and others, originally divided *Delias* into twenty species-groups according to differences in form of the androconia, male genitalia and, to a lesser extent, wing pattern. Talbot (1928–37: 375, 423) noted, however, that the Australian endemic *D. aganippe*, provisionally placed in the *belisama* group, 'seems somewhat isolated' on structural grounds and is 'placed doubtfully in this group'. Wallace (1867: 349) similarly remarked that, 'It is difficult to locate this common Australian species', and placed *D. aganippe* in the *belladonna* group. However, Ford (1942), in a paper that has hitherto been overlooked for more than 60 years, proposed that *D. aganippe* be placed in its own species-group (*aganippe*) based on the possession of an unusual (but not unique) combination of red pigments, together with its distinct morphology, including fore wing shape. Holloway & Peters (1976) suggested that *D. ellipsis* from New Caledonia also be placed into its own species-group, but so far this proposal has not been adopted. Yagishita *et al.* (1993) subdivided a further two of Talbot's species-groups, the *nysa* and *geraldina* groups. The *nysa* group was split into two species-groups (*nysa* with thirteen species, and *georgina* with ten species), and the *geraldina* group was divided into two species-groups (*geraldina* with fifteen species, and *cuningputi* with twenty species). More recently, Müller (2001a) has described a new taxon from New Ireland, *D. brandti* Müller, which he accorded separate species-group status (i.e. *brandti* group) based on its distinctive genitalia, thus bringing the total number of species-groups to twenty-four.

Talbot (1928–37: iv) assumed that his species-groups were monophyletic, although cautioned that, 'the author is still not satisfied as to the affinities and composition of the subgroups...'. Although most species-groups were considered to be relatively 'homogeneous', at least one species-group (*belisama*) was not. The *belisama* group contained four distinct taxa, namely *D. diaphana* from the Philippines, *D. ellipsis* from New Caledonia, and *D. aganippe* and *D. harpalyce* from Australia. Talbot (1928–37: 375) stated that these taxa were 'included more for convenience ... they represent isolated forms which do not fit well into any part of our system, but seem to approximate most to the present group'. The placement of several other taxa has been revised. For example, Morishita (1981) transferred *D. benasu* Martin (from Sulawesi) from the *belladonna* group to the *pasithoe* group, and Yata (1985) transferred *D. blanca* C. & R. Felder (from the Philippines and northern Borneo) from the *nysa* group to the *belladonna* group. Yagishita *et al.* (1993) and Tuzov (1996) adopted both of these proposals in their checklists of the genus. Arora (1983) described and placed *D. messalina*, known from the Solomon Islands, Bougainville, New Britain (Arora, 1983) and New Ireland (Müller, 1999a), tentatively in the *nigrina* group. However, Parsons (1998) suggested that *D. messalina* was related more closely to members of the *weiskei* group.

Dixey (1894: 301) remarked more than 100 years ago that, 'It would be most interesting to attempt to trace in detail the phylogenetic history of the whole of this extensive genus'. Talbot (1928–37) subsequently proposed an intuitive phylogeny of *Delias* based on his species-groups (Fig. 2), but the general situation has not been revised substantially since that time.

Talbot (1928–37) considered the *belladonna* group to be the 'ancestral' group of the genus, which was envisaged to have given rise to two lineages (*pasithoe*, *hyparete*). The *pasithoe* lineage subsequently gave rise to two daughter lineages: *singhapura* + *nysa s.l.* + *chrysomelaena* and *stresemanni* + *geraldina s.l.* + *eichhorni* + *bornemanni* + *iltis* + *weiskei* + *kummeri*. The *hyparete* lineage was also envisaged to have given rise to two daughter lineages: *belisama* and *nigrina* + *dorimene* + *isse*. Talbot expressed considerable doubt about the position of the three remaining species-groups from mainland New Guinea (*albertisi*, *clathrata*, *niepelti*), which he placed between the *pasithoe* and *hyparete* lineages in his systematic classification. Talbot (1928–37: 79) also questioned the phylogenetic positions of the other montane endemic New Guinea species-groups (*stresemanni*, *geraldina s.l.*, *eichhorni*, *bornemanni*, *iltis*, *weiskei*, *kummeri*), noting that these 'were derived from mountain forms in the Papuan region, and have become so divergent that little can be suggested as to their progenitors'. Perhaps more seriously though was the fact that Talbot's order of systematic classification and ideas of evolution of the genus were incongruent with one another (see Fig. 2). For example, the *nigrina* group, which he thought was related to the *dorimene* and *isse* groups of the *hyparete* lineage in an evolutionary sense, nonetheless was placed close to *kummeri* and *weiskei* in the *pasithoe* lineage in his checklist of the species-groups. The *belladonna* group was also placed in the *pasithoe* lineage (close to *pasithoe*), rather than as a separate 'basal' lineage as envisaged in his phylogeny. Ford (1942) noted that *aganippe* was closely related to the *belladonna* and *pasithoe* groups, thereby partly supporting Wallace's (1867) earlier suggestion that *D. aganippe* was related to the *belladonna* group. Müller (2001a) suggested that *brandti* was related to either the *nysa* or *chrysomelaena* groups. These ideas regarding the phylogenetic relationships of the twenty-four species-groups are summarized in the form of a cladogram in Fig. 2.

Although *Delias* occurs widely in the Oriental and Australian Regions, the twenty-four species-groups are not evenly distributed between the two geographical areas (Fig. 1). Eight species-groups occur in the Oriental Region, four (50%) of which are endemic to the region, and all of these extend into Wallacea. Nineteen species-groups occur in the Australian Region, 15 (79%) of which are endemic to the region, with four of these extending into Wallacea. Four species-groups (*nysa*, *belisama*, *dorimene* and *hyparete*) are

more widely distributed, being common to both regions, although the *nysa* group is much less widespread, especially in the Oriental Region, where it is restricted to western Indonesia (Sumatra, Java). Twelve species-groups thus occur in Wallacea, but only one (*stresemanni*) is endemic to that area. As noted above, several species in the *belladonna* group extend into south-east China in the Palaearctic Region, treated as part of the Oriental fauna for the purposes of this study. Of the Australian endemics, twelve (80%) species-groups are restricted to montane areas of mainland New Guinea and/or its adjacent islands, where many are sympatric. Not only is the Australian Region systematically more diverse, but the high species richness and high level of endemism in New Guinea represent more than half (54%) of the higher level diversity recognized within the genus.

The occurrence of widespread and narrowly restricted (endemic) species-groups in both zoogeographical regions thus makes the genus particularly attractive to investigate from an historical biogeography perspective, especially the number and direction of dispersal events across Wallacea. The presence of four widespread taxa, for instance, implies at least four independent dispersals across Wallacea. However, without a detailed estimate of the phylogeny, biogeographical hypotheses cannot be tested. Although there have been several detailed taxonomic studies and revisions of various species-groups and subgroups (Jordan, 1925; Roepke, 1955; Morishita, 1979, 1981; Yata, 1985; van Mastrigt, 1989, 2000; Yagishita, 1997b), most work over the past two decades has focused at the level of species (e.g. Orr & Sibatani, 1985, 1986; Kitahara, 1986; Nakano, 1986, 1991; Morita, 1989, 1996a, b, 2001, 2003; Yagishita, 1989, 1990; Gerrits & van Mastright, 1992; Yamamoto & Takei, 1995; van Mastrigt, 1996; Lachlan, 1999, 2000; Sakuma, 1999a; Monastyrskii & Devyatkin, 2000) or subspecies (e.g. Inomata & Nakano, 1987; Nakano, 1987, 1988, 1995a, b, 1998; Nihira & Kawamura, 1987; Samusawa & Kawamura, 1988; Parsons, 1989; van Mastrigt, 1990; Morita, 1995, 1996c, 1998; Arima, 1996; Sakuma & Morita, 1996; Sakuma, 1996, 1999b; Inayoshi & Nishimura, 1997; Nakano & Kawai, 1997; Yagishita, 1997a, c, d, 1998; Tuzov & Churkin, 1998; Gotts & Ginn, 2004, 2005), with little attempt to establish monophyly of the proposed species-groups, let alone their evolutionary relationships. An exception is the systematic study of the *eichhorni* group by S. Morinaka and coworkers (Morinaka *et al*., 1991, 1993, 2002; Morinaka & Nakazawa, 1997, 1999a, b), who confirmed its monophyly based on sequences of the mitochondrial gene *ND5* for the putative species. The gene, however, proved to be inadequate for resolving higher level relationships between the species-groups.

Materials and methods

Molecular markers

The nuclear gene, elongation factor-1 alpha (*EF-1α*), and three mitochondrial genes, cytochrome oxidase subunits I and II (*COI*/*COII*) and *ND5*, were used to infer phylogenetic relationships between the species and species-groups of *Delias*. *EF-1α* is a protein-encoding gene that has proved to be a useful marker for resolving deeper level divergence events of insects of at least mid-Tertiary age (Cho *et al*., 1995; Mitchell *et al*., 1997; Danforth & Shuqing, 1998). *COI* and *COII* are protein-encoding genes that have been widely used to resolve more recent divergence events (e.g. Simon *et al*., 1994; Hillis *et al*., 1996; Palumbi, 1996). *ND5*, another rapidly evolving protein-encoding gene, has the potential to resolve relatively recent, lower level relationships, such as those between populations within a species or between closely related species within a genus (e.g. Clary & Wolstenholme, 1985; Simon *et al*., 1994; Su *et al*., 1998; Yagi *et al*., 1999). Because of the different rates of evolution of the three genes, their combination can help to increase phylogenetic estimation and resolution of both deep and shallow nodes, provided that the data partitions are congruent (Caterino *et al*., 2000). Several recent studies of Lepidoptera have demonstrated improved resolution and increased nodal support at most levels in combined analysis of nuclear and mitochondrial genes (e.g. Caterino *et al*., 2001; Monteiro & Pierce, 2001; Wahlberg *et al*., 2003, 2005; Megens *et al*., 2004; Zakharov *et al*., 2004; Braby *et al*., 2005).

Taxon sampling

Forty-six species representing all twenty-four species-groups of the genus *Delias* were included in this study (Appendix, see 'Supplementary material'). Another fifteen species, representing thirteen genera spread across the major taxonomic groups within the family Pieridae, were included as distant ingroup taxa. An additional two genera (*Colias*, *Eurema*) from the subfamily Coliadinae were chosen as outgroup taxa in all datasets.

Different combinations of taxa were analysed separately for each data partition (Appendix, see 'Supplementary material'). For *EF-1α*, twenty-seven taxa representing twenty-one species-groups of *Delias*, plus a further twelve pierid taxa, were analysed. Two exemplar species were included for each of the *hyparete*, *cuningputi* and *geraldina* groups to test for potential non-monophyly, plus the two uncertain taxa *D. harpalyce* and *D. messalina*. Three species-groups (*albertisi*, *georgina* and *stresemanni*) were not analysed for this gene because of the difficulty of obtaining sequences from dried specimens. For *COI*/*COII*, thirty-one taxa representing twenty-two species-groups of *Delias*, plus a further nine pierid taxa, were analysed. The dataset included two exemplar species for each of the *hyparete*, *cuningputi*, *geraldina* and *dorimene* groups to test for potential non-monophyly, plus the four uncertain taxa, *D. diaphana*, *D. ellipsis*, *D. harpalyce* and *D. messalina*. Two species-groups (*brandti* and *georgina*) were not analysed for this gene because of inadequate dry material; for *D. ellipsis*, only the *COI* portion was successfully amplified. Two individuals of *D. aganippe*, sampled from populations more than 2500 km apart, were also included for both *EF-1α* and *COI*/*COII*. In seven pierid taxa (*Eurema mexicana*, *Hebomoia glaucippe*, *Appias paulina*, *Melete isandra*, *Charonias*

eurytele, *Aporia crataegi* and *Leuciacria acuta*), only the *COI* block was amplified and sequenced; the *COII* portion was coded as 'missing' data in both the separate and combined analyses. For *ND5*, twenty-one taxa, each representing different species-groups of *Delias*, plus a further eight pierid taxa, were analysed from sequences obtained from GenBank based on a published study (Morinaka *et al*., 2002). Three species-groups (*aganippe*, *brandti* and *bornemanni*) were not analysed for this gene by Morinaka *et al*. (2002).

The total dataset for the three genes combined comprised thirty-one taxa (twenty-four *Delias* species-groups; seven other Pieridae genera). With the exception of seven taxa (*D. belladonna*, *D. ladas*, *D. eichhorni*, *D. hyparete*, *D. iltis*, *Hebomoia glaucippe*, *Aporia crataegi*), for which the same species was used for each gene partition, the terminal units of the remaining twenty-four taxa (nineteen *Delias* species-groups; five other pierid genera) comprised sequences representing different exemplar species. For example, the *isse* group comprised sequences combined from two exemplar species, *D. ennia* (*EF-1α*, *COI*/*COII*) and *D. candida* (*ND5*). The taxa used to make up the other terminal units for each species-group are indicated in the Appendix (see 'Supplementary material'). Only two gene partitions were available for the *aganippe* (*EF-1α*, *COI*/*COII*), *bornemanni* (*EF-1α*, *COI*/*COII*), *albertisi* (*COI*/*COII*, *ND5*) and *stresemanni* (*COI*/*COII*, *ND5*) groups, and only single gene partitions were available for the *brandti* (*EF-1α*) and *georgina* (*ND5*) groups. The absence of gene partitions in these six taxa was coded as 'missing' in the combined dataset. Tree topologies were unaffected by the inclusion or omission of these six taxa (not shown).

Molecular techniques

One hundred and eight DNA sequences were included in this study. Of these, twenty-two (fourteen *EF-1α* and eight *COI*/*COII*) were derived from our previous studies of the Pieridae (Braby *et al*., 2006a). Another thirty-one sequences (two *COI*/*COII* and twenty-nine *ND5*) were obtained from those registered on GenBank based on previously published work (Yagi *et al*., 1999; Morinaka *et al*., 2000, 2002; Caterino *et al*., 2001) (Appendix, see 'Supplementary material'). A further fifty-five sequences (twenty-five *EF-1α* and thirty *COI*/*COII*) were added to the dataset. Protocols for the collection, preservation, extraction, purification, amplification, sequencing and alignment of DNA fragments were similar to those reported in Braby *et al*. (2005) and Braby *et al*. (2006a). For *COI*/*COII*, several additional primers were employed, including: George I (fwd) 5′-ATACCTCGACGTTATTCAGA-3′ (2773–2792), George II (fwd) 5′-ATACCTCGTCGTTAYTCTGA-3′ (2773–2792), George III (fwd) 5′-ATRCCTCGTCGTTACTCTGA-3′ (2773–2792), Phyllis (rev) 5′-GTAATAGCIGGTAARATAGTTCA-3′ (3297–3275), Strom I (fwd) 5′-TAATTTGAACTATYTTACCIGC-3′ (3270–3291), Strom II (fwd) 5′-ACTTATTTGAACTATCTTACC-3′ (3268–3288), COII19.42 (rev) 5′-AATACTTTCAATAACAATAGG-3′ (3724–3704) and Eva (rev) 5′-GAGACCATTACTTGCTTTCAGTCATCT-3′ (3798–3772). In *COI*/*COII*, the tRNA-leucine gene (approximately 64 bp) between the *COI* (1262 bp sequenced) and *COII* (683 bp sequenced) partitions was excluded in all samples. The *COI* and *COII* partitions were then combined into a single block of mitochondrial DNA (mtDNA). In four samples (*D. nigrina*, *D. harpalyce*, *D. kummeri* and *D. messalina*), there was a 1-bp insertion ('T') near the end of the *COI* block, 17 bp from the start of the tRNA-leucine gene (i.e. position 2992). The additional base created a frame-shift such that the termination codon started at position 2997, instead of 3007, so that the last three amino acids of *COI* were not translated.

Phylogenetic analysis

Maximum parsimony. Phylogenetic trees were reconstructed from the separate gene partitions and from the combined dataset using unweighted and weighted maximum parsimony (MP) as the optimality criterion, as implemented in PAUP* version 4.0b10 (Swofford, 2002). Tree estimation involved heuristic searches with the tree-bisection–reconnection (TBR) branch-swapping algorithm, stepwise addition with up to 100 random starts to check for islands of trees, and 'MulTrees' option in effect. Strict consensus trees were computed where there was more than one equally parsimonious tree. We also compared tree topology with distance methods (neighbour joining) as implemented in PAUP. In order to ascertain the extent of saturation, the transition to transversion ratio was plotted against the observed or uncorrected pairwise distance for first and second codon positions combined and for the third codon positions separately. Various weighting schemes were explored, including removing or down-weighting third codon positions over first and second positions (1 : 2, 1 : 3, 1 : 5), particularly for the mitochondrial genes, and/or weighting transversions over transitions (2 : 1, 3 : 1). Bootstrap analyses (Felsenstein, 1985, 1988), based on a full heuristic search of 1000–10 000 pseudoreplicates using TBR branch swapping and simple stepwise addition or up to fifty random additions, were carried out for each analysis to determine the level of support of each node. Only clades with bootstrap values of 50% or more were retained in analyses of the separate gene partitions. The total Bremer support (decay index) (Bremer, 1988, 1994) was also calculated to evaluate the nodal support in the combined analysis using the program TREEROT version 2. (Sorenson, 1999). To establish whether the data partitions carried substantially different phylogenetic signals, congruence was evaluated by comparing the tree topologies generated by each gene and by calculating partitioned Bremer support in the combined analysis.

Maximum likelihood. Phylogenetic reconstruction was estimated using maximum likelihood (ML) tree building methods for the combined dataset, as implemented in PAUP* version 4.0b10 (Swofford, 2002). Model selection was determined according to the hierarchical likelihood ratio test (hLRT) (Huelsenbeck & Rannala, 1997), as implemented in

MODELTEST 3.06 (Posada & Crandall, 1998), with the starting tree obtained by MP to estimate model parameters. hLRT performs a likelihood ratio test between different DNA substitution models and determines whether or not increasing the number of parameters significantly increases the fit of the model to the data. The substitution model that best fitted the observed data was the parameter-rich general time reversible substitution model (Lanave *et al*., 1984; Rodríguez *et al*., 1990) with among-site rate variation (invariable sites and Gamma distribution) (i.e. GTR + I + Γ). Analysis based on the ML optimality criterion was then performed to generate an ML tree under a heuristic search using the TBR branch-swapping algorithm with as-is stepwise addition. To determine the approximate level of support for all branching events, bootstrap analysis was performed with 100 pseudoreplicates, using a full heuristic search with TBR branch swapping and simple stepwise addition. ML tree reconstruction was also performed using PHYML version 2.4.3 (Guindon & Gascuel, 2003). PHYML builds an initial tree, using a distance-based method, which is then modified to improve its likelihood by simultaneous adjustments of topology and branch lengths. The model selected was GTR + I + Γ, according to hLRT, with model parameters optimized during tree inference. To estimate the nodal support for all branching events, 500 bootstrap pseudoreplicates were generated for each character partition, with support percentages computed by majority rule consensus.

Bayesian inference. Bayesian inference (BI) was performed on the combined dataset, partitioned according to codon position and gene partition, as implemented in the program MRBAYES 3.1 (Huelsenbeck, 2000; Huelsenbeck & Ronquist, 2001). Multiple Bayesian searches were conducted using metropolis-coupled Markov chain Monte Carlo samplings. One cold and three heated Markov chains, applying MRBAYES default heating values ($t = 0.2$), were used in the analysis, with model parameters estimated during each analysis. Each analysis was run for 1 000 000 generations with trees sampled every 100 generations. To ensure that the analyses were not trapped in local optima, we ran BI analyses for three independent runs, each starting from a random independent tree. We used the average standard deviation of split frequencies (ASD-SF) between individual runs that sampled every 2500 generations to estimate convergence between individual runs. We considered runs to converge on a single optimum solution when ASD-SF was less than 1%. The 'burn-in' value (number of sampled trees discarded) was set when ASD-SF was lower than 1%. In addition, average log-likelihood values with standard deviation at stationarity were calculated using Microsoft Excel and compared for convergence. Phylograms were created as average branch-length consensus trees, and posterior probabilities of recovered nodes were estimated based on the majority rule consensus of trees found at stationarity in MRBAYES (Larget & Simon, 1999; Huelsenbeck & Ronquist, 2001). Trees recovered in MRBAYES were viewed and rooted in TREEVIEW 1.6.6 (Page, 1996).

Biogeographical analysis

Geographical distribution was examined as a character trait to infer ancestral states and patterns of historical biogeography within *Delias* and its sister genus *Leuciacria* in our best estimate of the tree. Geographical distribution was coded as two states at the level of the zoogeographical region for each species-group: Australian (A) or Oriental (O). Taxa that occurred predominantly in one zoogeographical region, but extended also into Wallacea, were coded as belonging only to that region. For example, the *nigrina* group occurs predominantly in the Australian Region (mainland Australia, New Guinea, New Ireland and some of the smaller nearby islands such as Biak), but five species occur west of Lydekker's Line in Halmahera (*D. funereal* Rothschild), Seram (*D. duris* Hewitson, *D. joiceyi* Talbot) and Buru (*D. buruana* Rothschild, *D. prouti* Joicey & Talbot). However, as the *nigrina* group does not cross Wallace's Line into the Oriental Region, the species-group was coded as 'A'. Similarly, the *belladonna* group occurs mainly on the Asian mainland, as well as in the Malay Peninsula and Sumatra, but one disjunct species (*D. surprisa* Martin) occurs east of Wallace's Line in Sulawesi. However, as the *belladonna* group does not cross Lydekker's Line into the Australian Region, the species-group was coded as 'O'. The *belladonna* group extends weakly into the Palaearctic Region (south-east China), treated as part of the Oriental Region for the purposes of this study. Widespread taxa, such as the *belisama* and *hyparete* groups, which occur in both regions as well as Wallacea, were coded as multistate (A, O). The *stresemanni* group comprises three species (*D. stresemanni* Rothschild, *D. schassmanni* Joicey & Talbot and *D. waterstradti* Rothschild) restricted to the eastern islands of Wallacea (Morotai, Halmahera, Bacan, Buru, Seram). Because it is the only species-group endemic to Wallacea, it was coded as missing data or equivocal rather than as a separate character state. Character states were optimized on the tree using dispersal-vicariance analysis (DIVA) (Ronquist, 1997) to establish the most parsimonious ancestral reconstruction. DIVA optimizes the ancestral distribution by minimizing the total cost at each node in the area cladogram, expressed in terms of the number of dispersal and extinction events. Dispersal is the addition of one unit area to a distribution (range expansion) or the founding of a new unit area by a unique taxon (long-distance dispersal followed by allopatric speciation), whereas extinction is the deletion of one unit area from a distribution – both events were assigned equal costs as one per area added/deleted in the analysis (but see Cook & Crisp, 2005 for methods of assigning differential costs when evolutionary events have unequal probabilities).

Age of divergence estimations

In our previous studies of the Pieridae (Braby *et al*., 2006a), we estimated the minimum age of the Aporiina to be

61 Mya for the stem-group (99.9% confidence interval, 69–54 Mya) and 50 Mya for the crown-group (99.9% confidence interval, 57–45 Mya) based on extrapolation from fossils. Given these two minimum ages as calibration points, we estimated the ages of various nodes in the crown-group of *Delias* in our best ML model using Sanderson's semi-parametric rate smoothing according to the penalized likelihood method to correct rate heterogeneity across the tree, as implemented in the r8s program (Sanderson, 2002). Age estimations were estimated with the smoothing parameter λ optimized using the cross-validation method based on the minimization of the chi-squared error values. Error terms for each node were estimated according to the 99.9% confidence interval (4 SD) calculated for each calibration point (i.e. 69–54 Mya for the stem-group, 57–45 Mya for the crown-group).

Results

The final aligned sequences for the combined dataset of thirty-one taxa included 3888 bp (1066 *EF-1α*, 1945 *COI/COII*, 877 *ND5*), of which 1014 sites (26%) were parsimony informative (Table 1). For nuclear *EF-1α*, almost all informative variable sites were in third codon positions [220 bp (93%) parsimony informative] compared with only 17 bp (7%) for first and second positions combined. In contrast, first and second positions in the two mitochondrial genes were far more variable [113 bp (22%) parsimony informative for *COI/COII*; 91 bp (36%) parsimony informative for *ND5*] than the third positions [409 bp (78%) for *COI/COII*; 164 bp (64%) for *ND5*]. A plot of the transition to transversion ratio against the uncorrected pairwise distance for MP trees generated for each data partition revealed significant saturation in all three genes, especially third positions (see Fig. 7 in 'Supplementary material'). First and second positions were also saturated in *ND5*, less so in *COI/COII*, and were not saturated in *EF-1α*. These differences in site variation and saturation level reflect differential rates of substitution between the three genes, with the nuclear gene evolving substantially slower than *COI/COII*, which in turn is slower evolving than *ND5*. We first examine results for the individual data partitions, and then consider the combined data.

Separate analyses

Cladograms (strict consensus) for each data partition inferred under MP are shown in Fig. 3(A)–(C). Nuclear *EF-1α* (Fig. 3A) recovered *Delias* as a well-supported monophyletic group (bootstrap 93%) and provided substantial resolution between the distant ingroup taxa (bootstrap > 80% for most of the basal nodes), as well as many of the shallow nodes within the genus. Mitochondrial *COI/COII* (Fig. 3B) and *ND5* (Fig. 3C) also resolved many shallow nodes, particularly the tips in the former gene (bootstrap typically > 80%). However, both *COI/COII* and *ND5* failed to recover monophyly and any deep structure within *Delias*, despite attempts to minimize the effects of saturation (homoplasy) by down-weighting third positions and/or transitions. Although useful for resolving relationships within and between closely related species, the mitochondrial markers were characterized by high levels of noise. Clearly, these genes on their own were limited in their phylogenetic utility for resolving nodes beyond genus level.

The four species-groups (*hyparete*, *cuningputi*, *geraldina* and *dorimene*), for which two exemplar taxa were included, were each recovered as well-supported monophyletic groups (bootstrap 99–100% *EF-1α*, 82–100% *COI/COII*). In addition, the two individuals of *D. aganippe* sampled more than 2500 km apart, in western and eastern Australia, showed negligible uncorrected pairwise divergence (0.09% *EF-1α*, 0% *COI/COII*).

Among the species-groups, both *EF-1α* and *COI/COII* recovered several well-supported deep clades, but this was less evident in *ND5* for which only six small clades were recovered (bootstrap > 70%) (Fig. 3A–C). Moreover, clades recovered in *EF-1α* and *COI/COII* were identical in topology, indicating concordance between these alternative markers. Similarly, most of the smaller clades recovered in *ND5* comprised exemplars from the same species-groups as those found in the two other genes. The only major difference in topology in *ND5* was the sister relationship between *D. ladas* (*chrysomelaena* group) and *D. belisama* (*belisama* group), a pairing not at all evident in the two other genes.

Among the species-groups for which only two sequences were available, *D. nais* (*bornemanni* group) showed a close relationship with *D. clathrata* (*clathrata* group) (bootstrap 94% *EF-1α*, 75% *COI/COII*), *D. discus* (*albertisi* group) was sister to the *D. nais* + *D. clathrata* clade (bootstrap 98% *COI/COII*), and *D. stresemanni* (*stresemanni* group) was

Fig. 3. Phylogenetic trees for *Delias* (taxa indicated by thick lines) inferred from unweighted or weighted maximum parsimony (MP) analyses for the three genes separately and in combination. (A) Strict consensus of four equally MP trees based on unweighted analysis of 1066 bp of elongation factor-1 alpha (*EF-1α*) [291 informative characters; length = 1260, consistency index (CI) = 0.442, retention index (RI) = 0.528]. (B) Strict consensus of four equally MP trees based on weighted analysis of 1945 bp of cytochrome oxidase subunits I and II (*COI/COII*), with transitions down-weighted 1 : 2 against transversions (604 informative characters; length = 4456; CI = 0.347, RI = 0.421). (C) Strict consensus of five equally MP trees based on weighted analysis of 877 bp of NADH dehydrogenase 5 (*ND5*), with third positions down-weighted 1 : 2 against first and second positions and transitions down-weighted 1 : 2 against transversions (288 informative characters; length = 2762, CI = 0.461, RI = 0.373). (D) Strict consensus of nine equally MP trees based on unweighted analysis of the combined dataset (3888 bp, 1014 informative characters; length = 4500, CI = 0.448, RI = 0.359). Bootstrap values (1000 full heuristic search replicates, with up to fifty random additions) are shown below branches for nodes with more than 50% support; nodes with less than 50% support are collapsed in Fig. 4(A)–(C). Total Bremer support values are shown above branches in Fig. 4(D). *Taxa of uncertain species-group status. Circled letters A–E designate major clades evident in the combined analysis. *Colias* and/or *Eurema* are outgroup taxa.

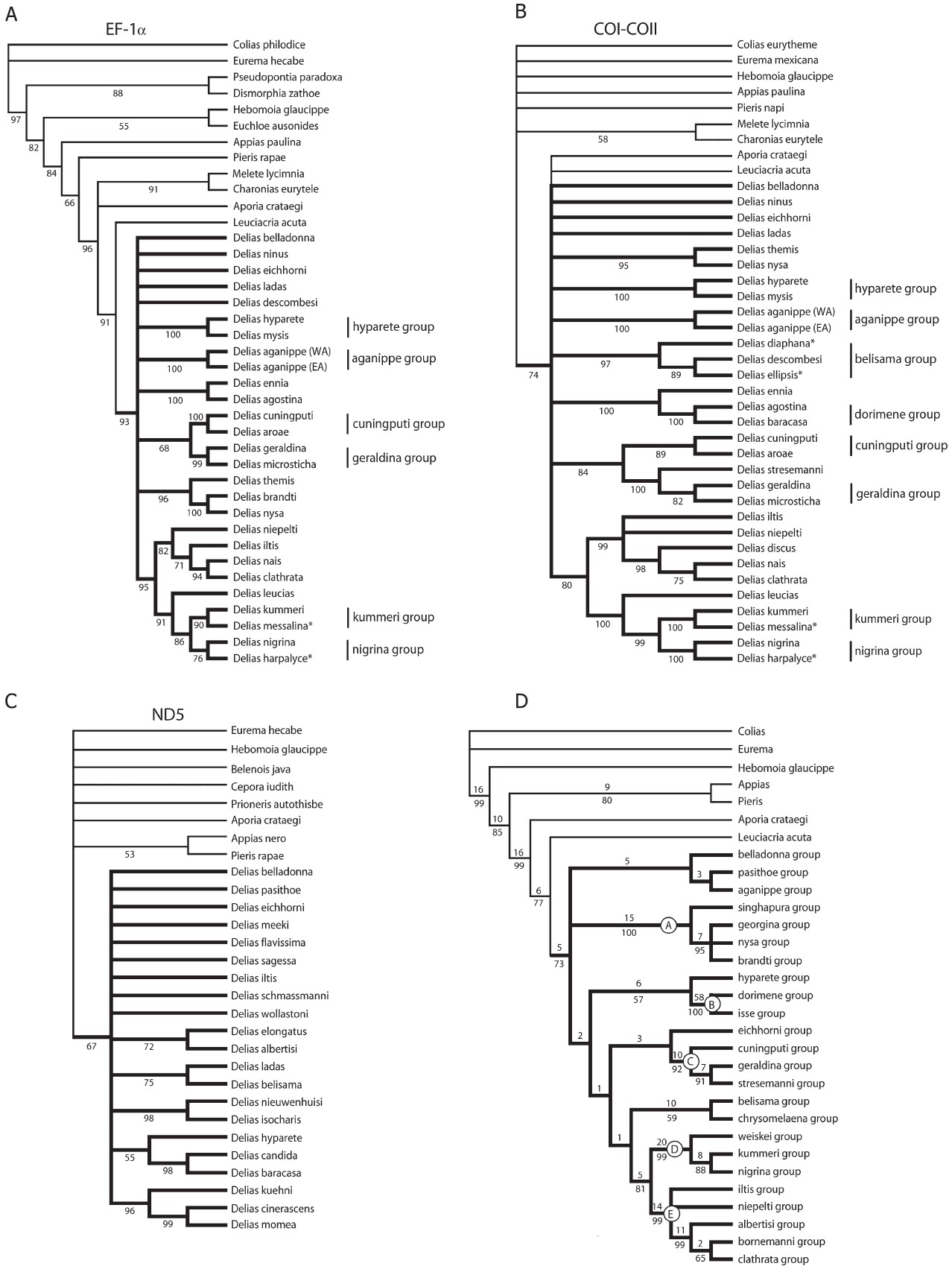

Table 1. Character summary for the combined dataset, with numbers of sites for each codon position for each gene partition.

Gene partition	*EF-1α*				*COI/COII*				*ND5*				
Codon partition	1st	2nd	3rd	All	1st	2nd	3rd	All	1st	2nd	3rd	All	Total
Number parsimony informative	13	4	220	237	95	18	409	522	62	29	164	255	1014
Number variable but parsimony uninformative	12	9	60	81	46	31	111	188	55	34	77	166	435
Number constant	330	342	76	748	508	599	128	1235	175	230	51	456	2439
Total	355	355	356	1066	649	648	648	1945	292	293	292	877	3888

COI/COII, cytochrome oxidase subunits I and II; *EF-1α*, elongation factor-1 alpha; *ND5*, NADH dehydrogenase 5.

sister to the *D. geraldina* + *D. microsticha* clade (both *geraldina* group) (bootstrap 100% *COI/COII*). *D. aganippe* (*aganippe* group) was unplaced in both *EF-1α* and *COI/COII*, supporting Ford's (1942) proposal of separate species-group status for this species rather than affiliation with the *belisama* group (Talbot, 1928–37; Yagishita *et al*., 1993). Of the taxa for which only one sequence was available, *D. brandti* (*brandti* group) showed a close relationship with *D. nysa* (*nysa* group) (bootstrap 100% *EF-1α*), and *D. cinerascens* (*georgina* group) showed a close relationship with *D. momea* (*nysa* group) (bootstrap 99% *ND5*).

Both *EF-1α* and *COI/COII* resolved satisfactorily the systematic position of the four species of uncertain species-group status (Fig. 3A, B). *D. harpalyce* and *D. messalina* were consistently recovered in the *nigrina* and *kummeri* groups, respectively, rather than with the groups in which they had been placed previously (i.e. *belisama* group for *D. harpalyce*, *nigrina* group for *D. messalina*) (Talbot, 1928–37; Yagishita *et al*., 1993). On the other hand, *D. diaphana* and *D. ellipsis* were both placed in a well-supported clade that included *D. descombesi* from the *belisama* group (bootstrap 97% *COI/COII*), corroborating their previous (tentative) placement in this species-group (Talbot, 1928–37). Thus, *D. ellipsis* was not supported as a separate species-group, as suggested by some authors (Holloway & Peters, 1976).

Combined analysis

Bootstrap analyses revealed little conflict in tree topology between the individual data partitions. In all partitions, the basal nodes of *Delias* were unresolved, and clades among the shallow nodes generally comprised the same species-groups, particularly for *EF-1α* and *COI/COII*, indicating congruence between the two genes. Partitioned Bremer support of the combined dataset under MP (Fig. 3D) also revealed general congruence between the partitions for the twenty-five nodes in the strict consensus tree (Table 2). In six nodes (1, 9, 13, 16, 23 and 24), all partitions contributed positively. The major source of conflict was with nuclear *EF-1α*, in which the support values interacted negatively in eleven nodes. Negative interactions were also recorded in the two mitochondrial partitions (six nodes for *COI/COII*, five nodes for *ND5*) and, in two nodes (11 and 17), both nuclear and mitochondrial genes were in conflict. However, in all cases, the magnitude of conflict was relatively small (no greater than – 2.7) and, apart from six nodes (8, 11, 14, 15, 18 and 25) that were poorly supported (total Bremer support < 5), most negative interactions were compensated by strong positive support by one of the other or both partitions, which contributed to an overall high total Bremer support (≥ 5) in the remaining thirteen nodes.

Unweighted MP of the combined dataset resulted in nine equally most parsimonious trees, the strict consensus of which is shown in Fig. 3(D). In general, the combined analysis improved the resolution and nodal support for many of the clades identified in the separate analyses. The genus *Delias* was recovered as a reasonably well-supported monophyletic group (bootstrap 73%), with *Leuciacria* as its sister genus. Within *Delias*, five major clades (A–E) with high support (bootstrap 92–100%, total Bremer support 10–58) were evident among the twenty-four species-groups (Fig. 3 D; Table 2). One clade (A) comprised four species-groups (*singhapura*, *georgina*, *nysa* and *brandti*). A second clade (B) comprised the *dorimene* and *isse* groups. A third clade (C) comprised the *cuningputi*, *geraldina* and *stresemanni* species-groups. The fourth clade (D) comprised the *weiskei*, *kummeri* and *nigrina* species-groups, and the fifth clade (E) comprised five species-groups (*niepelti*, *iltis*, *albertisi*, *bornemanni* and *clathrata*). A sister relationship between clades D and E was also evident (bootstrap 81%, $P = 0.0058$).

Relationships between the remaining seven species-groups were not well resolved and consequently uncertain. The *belisama* and *chrysomelaena* groups formed a weakly supported clade. Similarly, three species-groups (*belladonna*, *pasithoe* and *aganippe*) formed a cluster, but without support. The *hyparete* and *eichhorni* groups appeared to be sister to clades B and C, respectively, but again supporting evidence was weak. A test for monophyly, employing a topology-dependent permutation tail probability test (T-PTP) with 10 000 randomizations as implemented in PAUP (Faith, 1991; Trueman, 1996), was therefore undertaken to evaluate whether clades with poor nodal support were a result of random error or a hierarchical signal in the data. These tests revealed significantly more evidence in support of monophyly than would be expected by chance alone for the following three clades: (1) *belladonna* +

Table 2. Total Bremer support and partitioned Bremer support for each gene for nodes in the strict consensus maximum parsimony cladogram of the combined dataset (Fig. 4D). Rows in bold refer to major clades (A–E) with high Bremer/bootstrap (>90%) support.

Node	Clade	Species-group	Total	*EF-1α*	*COI/COII*	*ND5*
1			16	16	0	0
2			10	9	−5	6
3			9	−1.2	0.2	10
4			16	8.3	8.7	−1
5		*Delias* + *Leuciacria*	6	6.3	−1.3	1
6		*Delias*	5	6.3	−2.3	1
7			5	−1.7	3.7	3
8			3	−2.7	1.7	4
9	**A**	***singhapura* + *nysa* + *brandti* + *georgina***	**15**	**13.3**	**0.7**	**1**
10			7	−1.7	0.7	8
11			2	−0.7	−0.3	3
12			6	−0.7	5.7	1
13	**B**	***dorimene* + *isse***	**58**	**43**	**10**	**5**
14			1	−2.2	0.2	3
15			3	−0.7	1.7	2
16	**C**	***cuningputi* + *geraldina* + *stresemanni***	**10**	**4**	**4**	**2**
17			7	−1.7	11.7	−3
18			1	−2.2	0.2	3
19			10	3.3	−1.3	8
20			5	7.3	−1.3	−1
21	**D**	***weiskei* + *kummeri* + *nigrina***	**20**	**1.6**	**19.1**	**−0.7**
22			8	2.3	13.7	−8
23	**E**	***iltis* + *niepelti* + *albertisi* + *bornemanni* + *clathrata***	**14**	**3.5**	**9.3**	**1.2**
24			11	1.3	7.7	2
25			2	−1.3	2.3	1

COI/COII, cytochrome oxidase subunits I and II; *EF-1α*, elongation factor-1 alpha; *ND5*, NADH dehydrogenase 5.

aganippe + *pasithoe* ($P = 0.0394$); (2) *hyparete* + (*dorimene* + *isse*) ($P = 0.0338$); and (3) *eichhorni* + (*cuningputi* + (*geraldina* + *stresemanni*)) ($P = 0.0256$). Evidence for the monophyly of *belisama* + *chrysomelaena*, however, was rejected ($P > 0.05$). Although there was strong support for clades A–E and many of the smaller subclades within each, there was no resolution between the deeper divergences in *Delias*, with the major lineages forming a basal polytomy.

ML analysis of the combined dataset under PAUP yielded a single tree (Fig. 4A), the topology of which was broadly similar to that obtained with MP (Fig. 3D). The five major clades (A–E) identified under MP were recovered with high support under ML (bootstrap 97–100%). Moreover, as observed under MP, the *hyparete* group clustered with clade C, and clades D and E were reciprocally monophyletic. The *eichhorni* group united with clade B, but there was no support for this arrangement. The *belisama* and *chrysomelaena* groups appeared to be quite distantly related and comprised unresolved lineages. The only major inconsistency in the ML tree was the position of the *aganippe* group, a relatively long-branched taxon that did not cluster with *belladonna* + *pasithoe* as observed under MP. ML analysis of the combined dataset implemented under PHYML yielded a tree similar to that generated under PAUP (tree not shown), but with two minor differences. First, the *eichhorni* group did not cluster with clade B, but was unplaced. Second, the *belladonna*, *pasithoe* and *aganippe* groups clustered together but without support. There was, however, increased support for the *hyparete* group uniting with clade C (bootstrap 79%), as well as for the monophyly of *Delias* (bootstrap 95%).

Results for the Bayesian analyses partitioned by codon position are shown in Fig. 4(B). In general, arrangements between the tips were similar to those estimated under MP and ML. *Delias* was recovered as a well-supported monophyletic group and sister to *Leuciacria* (posterior probability 1.00 for both nodes). The monophyly of clades A–E was also strongly supported (posterior probability 1.00 for each node), with clades D and E reciprocally monophyletic (posterior probability 0.99). As observed under MP and ML, the *chrysomelaena* group was placed as an unresolved lineage, and the *belladonna*, *pasithoe* and *aganippe* groups were recovered as a monophyletic group but with low support. In contrast with MP, there was no evidence in support of the *eichhorni* group being united with clade B; rather, the lineage showed no close relationship with any of the other species-groups, similar to that observed under ML using PHYML. The most striking features of the Bayesian tree, however, were the increased levels of support for the monophyly of *hyparete* group + clade B and *belisama* group + (clade D + clade E) (posterior probability 1.00 for both nodes).

In both ML trees (PAUP, PHYML), and in the Bayesian tree, there was no support for the backbone of *Delias*, with very short branch lengths and no unique synapomorphies [i.e.

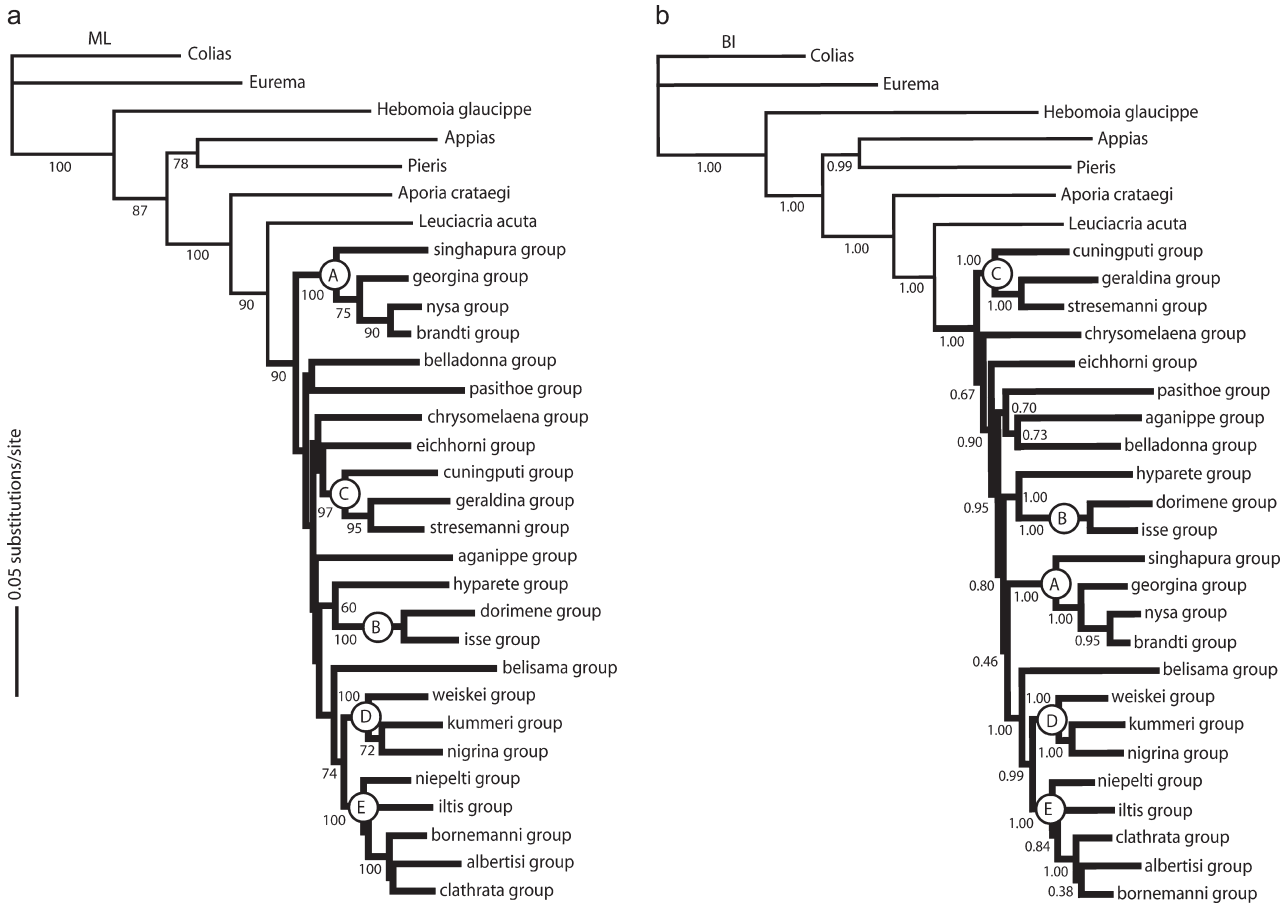

Fig. 4. Phylogenetic trees for *Delias* (taxa indicated by thick lines) for the three genes combined. (A) Maximum likelihood (ML) tree according to GTR + I + Γ substitution model [log likelihood score = –26023.256; relative rate matrix A↔C 1.7014, A↔G 8.9851, A↔T 2.8110, C↔G 2.4304, C↔T 14.8314, G↔T 1.0; base frequencies A = 0.3207, C = 0.1387, G = 0.1352, T = 0.4054; proportion of invariable sites (I) = 0.5074; shape parameter (α) of Gamma distribution (Γ) = 0.7723], with bootstrap values (100 full heuristic search replicates) shown below or next to branches for nodes with more than 50% support. (B) Bayesian inference (BI) tree (likelihood score –24869.80), partitioned by codon position; unlinked substitution model is GTR + I + Γ for each partition at a sampling temperature of 0.2; values below or adjacent to nodes are posterior probabilities estimated from majority rule consensus of 5600 trees. Circled letters A–E designate the major clades in common for both analyses of the combined dataset. *Colias* and *Eurema* are outgroup taxa.

consistency index (CI) = 1] subtending the basal nodes of the major clades (Fig. 4). Moreover, there was little consistency in the deep branching order of the ML and Bayesian trees. However, there was strong support (bootstrap frequently ≥ 90%) for all nodes below, and most nodes above, the basal polytomy, despite saturation of third positions in all genes. This suggests that lack of resolution among the basal lineages of *Delias* may comprise a hard polytomy (rapid radiation) rather than a soft polytomy in which a lack of data or multiple substitutions (homoplasy) is obscuring the phylogenetic signal.

Discussion

Systematic relationships

A phylogenetic hypothesis for the twenty-four species-groups of *Delias* is presented in Fig. 5, which summarizes the results of the combined analyses of the three genes. Only nodes that are well supported or recovered consistently under different methods of analysis (MP, ML-PAUP, ML-PHYML, BI), or for which there was further evidence in support of monophyly, are shown. Our analysis suggests that the species-groups fall into eight major clades or lineages, each with the following informal names and topologies: (1) *singhapura* clade = *singhapura* + (*georgina* + (*nysa* + *brandti*)); (2) *belladonna* clade = *belladonna* + *pasithoe* + *aganippe*; (3) *hyparete* clade = *hyparete* + (*dorimene* + *isse*); (4) *chrysomelaena* clade = *chrysomelaena*; (5) *eichhorni* clade = *eichhorni*; (6) *cuningputi* clade = *cuningputi* + (*geraldina* + *stresemanni*); (7) *belisama* clade = *belisama*; and (8) *nigrina* clade = (*weiskei* + (*kummeri* + *nigrina*)) + (*niepelti* + *iltis* + (*albertisi* + *bornemanni* + *clathrata*)). Clade 1 corresponds to clade A, clade 6 corresponds to clade C, and clade 8 corresponds to clade D + clade E of Table 2. Deep-level relationships between

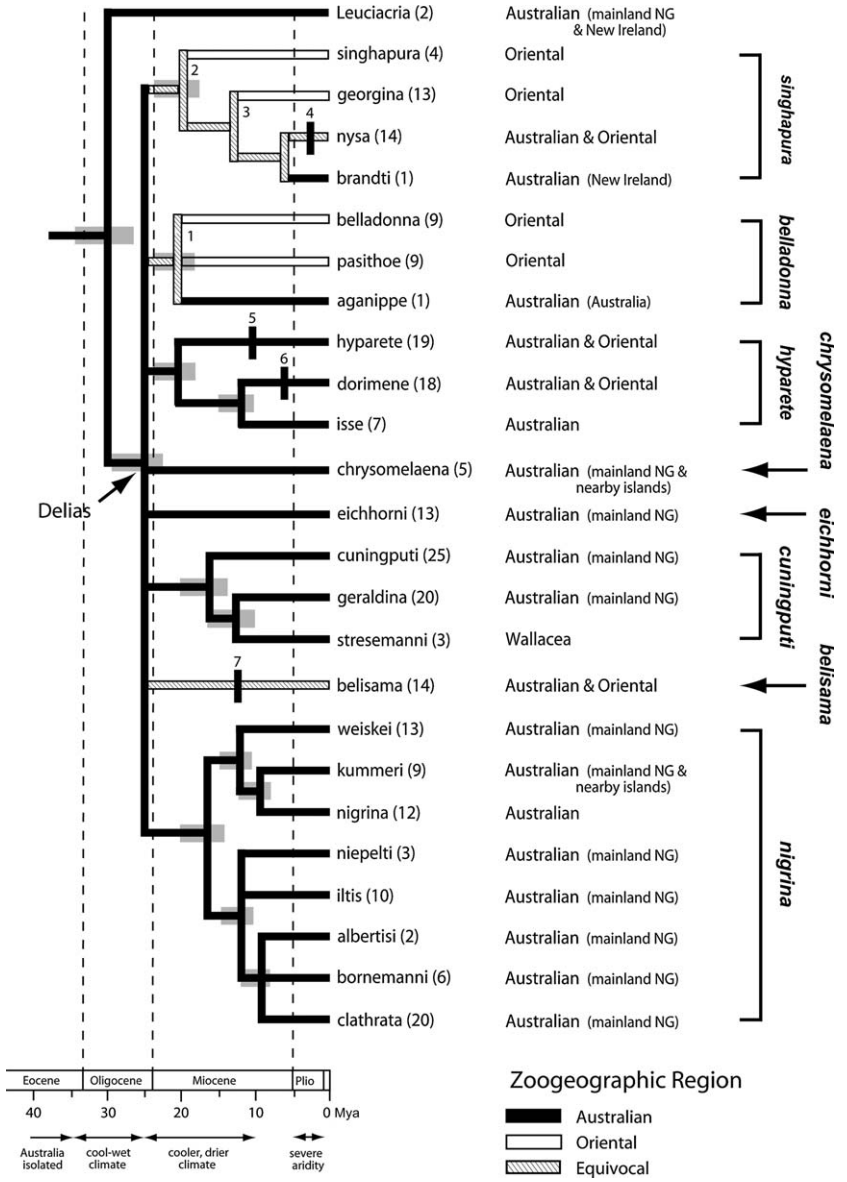

Fig. 5. Phylogenetic hypothesis of the *Delias* group (i.e. *Delias* + *Leuciacria*) according to the analysis of the combined dataset in this study. Only nodes which are well supported or consistently recovered under different methods of analysis [maximum parsimony (MP), maximum likelihood (ML)-PAUP, ML-PHYML, Bayesian inference (BI)] are shown. Numbers in parentheses after taxon names (species-groups) indicate approximate number of species (A. Yagishita, unpublished data; F. Gerrits, unpublished data). Divergence times and branch lengths have been corrected in the chronogram using semiparametric rate smoothing according to penalized likelihood, with the ages of the stem-group (61 Mya) and crown-group (50 Mya) of the Aporiina used as calibration points extrapolated from fossils in the Pieridae (see Braby *et al*., 2006a). Error bars for each node (shaded rectangles) give the 99.9% confidence interval based on 4 SD of the mean estimate for the calibration points (69–54 Mya for the stem-group, 57–45 Mya for the crown-group). Note that the branch lengths for the *georgina* + (*nysa* + *brandti*) clade have not been corrected because of insufficient data. To the right of the chronogram are the broad area relationships for each of the twenty-four species-groups, and the informal names of the eight major clades. NG, New Guinea. Climatic profile under the geological time-scale refers to mainland Australia. The most parsimonious optimization suggests an origin in the Australian Region, with at least seven biogeographical (dispersal) steps required to explain the present-day distribution (one of several optimal solutions shown for the *singhapura* clade). The steps are as follows: 1–3, long-distance dispersal from the Australian Region across Wallacea to the Oriental Region, followed by allopatric speciation of the *belladonna* + *pasithoe*, *singhapura* and *georgina* groups; 4–7, dispersal (range expansion) of the *nysa*, *hyparete*, *dorimene* and *belisama* groups from the Australian Region across Wallacea to the Oriental Region. Geographical distributions for the area cladogram are based on Talbot (1928–37), Yagishita *et al*. (1993), Müller (2001a) and Braby (2000).

these eight clades were not resolved, and we interpret this as possible evidence for an explosive radiation that occurred early in the evolution of the genus, particularly as there was little conflict between the different methods of analyses and between the data partitions. The order of clades, and order of taxa within each clade (listed from top to bottom in Fig. 5), follows the approximate branching order in our ML (PAUP) tree. Despite a lack of nodal support for the deep-level divergences, and uncertainty in the branching order of the major clades, we suggest that this ordering is followed as a tentative systematic classification of the genus until further evidence indicates a contrary pattern.

Talbot (1928–37) assumed that his proposed species-groups were monophyletic and, apart from the two Australian species (*D. aganippe* and *D. harpalyce*) that he 'dumped' in the *belisama* group, limited evidence suggests that this assumption holds true for the *eichhorni* group (Morinaka *et al.*, 2002) and the *hyparete*, *cuningputi*, *geraldina* and *dorimene* groups (Fig. 3A, B). With perhaps one exception, there is no evidence to indicate that the currently recognized species-groups should be subsumed. The level of divergence (corrected pairwise distance) between the species-groups is quite large in the combined dataset, varying from 4.2 to 15.9%, and the branch lengths in our ML phylogram are relatively long, indicating substantial differentiation between the groups. The subdivision of both the *geraldina* and *nysa* groups into two smaller groups (Yagishita *et al.*, 1993) is supported by the phylogenetic results of this study. The *geraldina* group was divided into two species-groups (*geraldina s.s.* and *cuningputi*) and, although closely related, they are not sister taxa, with the *geraldina* group *sensu stricto* more closely related to the *stresemanni* group phylogenetically (Fig. 5). The *nysa* group was divided into two species-groups (*nysa s.s.* and *georgina*). Again, although closely related, the two groups are not sister taxa, with the *nysa* group *sensu stricto* more closely related to the *brandti* group phylogenetically, supporting, in part, Müller's (2001a) supposition of a close relationship between these two species-groups. Thus, retention of the *geraldina* and *nysa* groups in their broad sense would render both species-groups paraphyletic. However, further work is needed to resolve the status of the monotypic *brandti* group for which only a single sequence (*EF-1α*) was obtained. In the separate gene analysis, support for the sister-group relationship between the *brandti* and *nysa* groups was extremely high (bootstrap 100%), and the uncorrected pairwise distance was relatively small (1.03%), suggesting that *brandti* may well belong to the *nysa* group. Müller (2001a) gave separate species-group status for *D. brandti* because of the divergent male genitalia, but he did not compare the androconia or other characters.

The species-group level cladogram of *Delias* based on molecular characters (Fig. 5) compares reasonably well with Talbot's (1928–37) classification and, to some extent, his intuitive phylogeny inferred from morphological characters (Fig. 2). For example, the close relationships between the *singhapura*, *georgina* and *nysa* groups, and the *hyparete*, *dorimene* and *isse* groups postulated by Talbot, are corroborated by this study. The eleven species-groups predominantly from mainland New Guinea (i.e. *eichhorni*, *cuningputi*, *geraldina*, *stresemanni*, *weiskei*, *kummeri*, *niepelti*, *iltis*, *albertisi*, *bornemanni* and *clathrata*), which Talbot placed together despite the uncertainty about their precise relationships (Fig. 2), fall into three major clades (*eichhorni*, *cuningputi* and *nigrina*) in our cladogram, confirming the putative close relationships of many of these taxa. The major discrepancies concern the systematic positions of the *aganippe*, *belisama* and *chrysomelaena* groups. Discrepancies also exist in the phylogenetic position of the *belladonna*, *pasithoe* and *nigrina* groups, although our results are in agreement with Talbot's classification, which partly contradicted his own evolutionary hypothesis.

The sister or 'basal' position of the *belladonna* group is rejected (Fig. 2). Indeed, our results support Ford's (1942) hypothesis that *belladonna* is closely related to the *pasithoe* group, and that these two groups, together with *D. aganippe* (treated as a separate species-group), comprise a monophyletic group (i.e. *belladonna* clade). Although Talbot (1928–37) placed the *belladonna* and *pasithoe* groups together in his classification, they were separated phylogenetically (Fig. 2). Ford (1942) demonstrated that, although most species-groups of *Delias* were characterized by a particular red pigment (pigment 'D'), the *pasithoe* group possessed a different (and unique) red pigment (pigment 'E'). Although the *belladonna* group was not assayed, Ford discovered that the *aganippe* group possessed both pigments 'D' and 'E'. The internal relationships of these three species-groups were unresolved in our analyses, although the *aganippe* and *pasithoe* groups are possibly sister taxa, in which case pigment 'E' would be a synapomorphy for their monophyly.

Talbot (1928–37) believed that the *nigrina* group was related to the *dorimene* and *isse* groups in a phylogenetic sense (Fig. 2), but nonetheless placed it close to the *kummeri* and *weiskei* groups in his classification. Our results support Talbot's systematic classification and not his phylogenetic hypothesis. Talbot (1928–37) also suggested that the *belisama* group was related to the *dorimene*, *isse* and *hyparete* groups (Fig. 2). In our analyses, however, the *belisama* group comprised a separate lineage (*belisama* clade) that was not obviously related to any of the other species-groups, other than possibly being the sister taxon to the *nigrina* clade (Fig. 4A, B). Talbot (1928–37) considered the *chrysomelaena* group to be related to the *singhapura–nysa s.l.* branch of the *pasithoe* lineage (Fig. 2). However, our results show no close relationship of this taxon to these or the other species-groups; it is therefore treated as a separate lineage (*chrysomelaena* clade) of unknown systematic affinity (Fig. 5). Talbot drew attention to the distinct and apparently uniquely derived uncus of the male genitalia in this species-group, in which the two outer lobes are large, broadly rounded and curve in towards their apices.

Of the four taxa of uncertain species-group status, our results show that *D. ellipsis* and *D. diaphana* belong to the *belisama* group based on strong evidence of monophyly, supporting their current (tentative) arrangement. In contrast, *D. harpalyce* clearly belongs in the *nigrina* group and not in the *belisama* group in which it has been placed.

Accordingly, we transfer *D. harpalyce* to the *nigrina* group; otherwise, the *belisama* group is polyphyletic and the *nigrina* group paraphyletic. Similarly, the placement of *D. messalina* in either the *nigrina* (Arora, 1983; Yagishita *et al*., 1993) or *weiskei* (Parsons, 1998) groups is not supported and, according to the results of this study, it should be transferred to the *kummeri* group. The *weiskei*, *nigrina* and *kummeri* groups are closely related and comprise a well-supported monophyletic group within the *nigrina* clade.

Historical biogeography

The estimated divergence times for each node, and their confidence intervals, are shown as a chronogram for our phylogenetic hypothesis of the *Delias* species-groups (Fig. 5). *Delias* is estimated to have originated in the early Oligocene (30 Mya for the stem-group; 99.9% confidence interval, 35–27 Mya), and then diversified soon afterwards in the late Oligocene (25 Mya for the crown-group; 99.9% confidence interval, 29–23 Mya). The age estimate, if accurate, implies that the genus must have evolved either in the Australian or the Oriental Region. Clearly, it cannot be both (i.e. Wallacea) because the estimated time of origin occurred well before collision of the Australian and Asian plates.

The broad zoogeographical distributions of each species-group of *Delias*, plus its sister genus *Leuciacria*, are mapped on our phylogeny to produce a taxon-area cladogram (Fig. 5). The fifteen species-groups endemic to the Australian Region are distributed across seven of the eight major clades of *Delias*, whereas the four species-groups endemic to the Oriental Region are restricted to only two clades. Moreover, *Leuciacria* and the *chrysomelaena*, *eichhorni*, *cuningputi* and *nigrina* clades are largely endemic to the Australian Region, but no major clades are endemic to the Oriental Region. Although three clades have differentiated to produce three to eight species-groups in the Australian Region, there is little evidence of cladogenesis at the species-group level in the Oriental Region. Of the nine major clades, eight (89%) are represented in mainland New Guinea, five (55%) are represented in the Australian mainland (which has only eight species), whereas four (44%) are represented in mainland Asia (which has about seventeen species). These biogeographical patterns are strongly indicative of an Australian origin. Indeed, the most parsimonious reconstruction of the ancestral area of *Delias* + *Leuciacria* under DIVA is an origin in the Australian Region (Fig. 5). This hypothesis requires a minimum of seven biogeographical (dispersal) steps to explain the present-day distribution. The alternative hypothesis, the ancestor of *Delias* + *Leuciacria* evolved in the Oriental Region, is far less parsimonious as it requires a minimum of thirteen biogeographical steps (twelve dispersal, one extinction), assuming that jump dispersal of the ancestor of the *Delias* group (or the ancestors of *Delias* and *Leuciacria*) from Asia across the Indian Ocean to Australia was not possible in the Eocene or early Oligocene.

For the 'out-of-Australia' hypothesis, three of the seven steps involve long-distance dispersal from Australia across Wallacea to Asia followed by allopatric speciation: (1) ancestor of *belladonna* clade, giving rise to the *belladonna* and *pasithoe* groups in the early Miocene (*c*. 20 Mya); (2) ancestor of *singhapura* clade, giving rise to the *singhapura* group in the early Miocene (*c*. 20 Mya); and (3) ancestor of *georgina* + (*nysa* + *brandti*) subclade, giving rise to the *georgina* group, probably in the mid-Miocene. The four remaining steps (4–7) each involve independent dispersals (range expansions) of the four widespread species-groups from Australia/New Guinea across Wallacea to Indonesia/Asia: (4) *nysa* group; (5) *hyparete* group; (6) *dorimene* group; and (7) *belisama* group. Presumably, these four dispersal steps occurred sometime during the mid-Miocene (*c*. 15–10 Mya) or even Pleistocene (1 Mya) when opportunities for range expansion were favourable. Optimization of the *stresemanni* group, the only taxon endemic to Wallacea, suggests an origin in the Australian Region of the *cuningputi* clade, followed by post-speciation dispersal of the ancestor of *geraldina* + *stresemanni* from proto- New Guinea to the nearby islands in eastern Wallacea during the mid-Miocene (Fig. 5). There are several optimal solutions for the *singhapura* clade, and an alternative equally parsimonious reconstruction is: (2) long-distance dispersal of the ancestor of the *singhapura* clade from the Australian Region across Wallacea to the Oriental Region, followed by allopatric speciation in the late Oligocene; (3) dispersal of the ancestor of the *nysa* + *brandti* subclade from the Oriental Region across Wallacea to the Australian Region, followed by allopatric speciation in the Miocene; and (4) dispersal (range expansion) of the *nysa* group from the Australian Region back across Wallacea to Indonesia (Sumatra, Java). However, if *brandti* proves to belong to the *nysa* group, only two steps are needed for the *singhapura* clade, with steps 3 and 4 being subsumed to involve only a single step: dispersal (range expansion) of the *nysa* group from Indonesia across Wallacea to the Australian Region.

We assume that the four widespread species-groups (*nysa*, *hyparete*, *dorimene* and *belisama*) dispersed only once across Wallacea, but as each of these groups contains a large number of species (fourteen to nineteen), further fine-scale species-level phylogenies are required to establish the minimum number (and timing) of dispersals. However, as these species-groups are rather poorly represented in the Oriental Region (each containing one to six species), compared with their representation in the Australian Region, they probably entered the Oriental Region fairly recently.

Collision of the northern margin of the Australian plate with the Asian plate in the late Tertiary facilitated the exchange of biota between the two zoogeographical regions, which had been isolated previously from one another. Long-distance dispersal across relatively large water gaps may have been possible as early as the late Oligocene (*c*. 25 Mya) because of the proximity of continental Australia and Asia and emergent land in Wallacea (East Sulawesi, Vogelkop) (Hall, 1998; de Jong, 2001), although generally it is believed that opportunities for dispersal were not favourable until

the mid-Miocene (*c*. 15–10 Mya) when continental shelves became exposed as a result of falling sea-level (by as much as 120 m) caused by expansion of the Antarctic icecap (White, 1994; Hall, 1998, 2001). At this time, island hopping was possible across Wallacea as a result of the increasing availability of land in the area (Sulawesi, West Maluku, Vogelkop), increasing vulcanism in East Indonesia (Lesser Sunda Island arc), accretion of microcontinental island arc fragments at the northern edge of the Australian craton in New Guinea, and exposure of the Arafura and Sunda Shelves with decreasing sea level (de Jong, 2001; Hall, 2001). The best opportunity for faunal exchange between the two zoogeographical regions, however, was during the Ice Ages of the Pleistocene (*c*. 1 Mya) (de Jong, 2001). Several studies of Lepidoptera (de Jong, 1990, 2001, 2004; Holloway, 1998; Kitching *et al*., 2001; Braby *et al*., 2005) have concluded that dispersal out of the Australia Region across Wallacea probably occurred multiple times during different epochs of the late Tertiary.

As a general rule, *Delias*, unlike many other Pieridae, do not migrate (Talbot, 1928–37). The few migratory *Delias* belong to species mainly restricted to lowland mainland Australia (Braby, 2000). However, from a consideration of the above scenarios, it is clear that there have been multiple dispersal events across Wallacea in the past. Moreover, two unrelated species (*D. nysa* and *D. ellipsis*) have colonized the remote oceanic islands of Vanuatu and/or New Caledonia in the south-west Pacific (Talbot, 1928–37; Holloway, 1979). Our chronogram suggests that these colonization events occurred sometime in the late Tertiary, and presumably involved long-distance dispersal across stepping stones from mainland Australia, or from mainland New Guinea via the Inner Melanesian Arc or, more recently, via the Solomons Archipelago, as postulated by Holloway (1979). The presence of a distinct endemic taxon (*D. ellipsis*) on New Caledonia suggests that its ancestor reached the island, where it subsequently differentiated allopatrically relatively early in the evolution of the *belisama* group. The extra dispersal steps to Vanuatu/New Caledonia do not affect the differences in the total (minimum) number of steps between an origin of *Delias* in Australia or in Asia, but it is important to consider that *Delias* is capable of long-distance dispersal when opportunities are favourable, and that this may well be a significant mode of speciation, perhaps equal to or even greater than vicariance, as suggested by some authors (e.g. de Queiroz, 2005). Indeed, Braby *et al*. (2006b) concluded that dispersal in the Aporiina has played a greater role over vicariance in shaping much of the phylogenetic pattern of the subtribe.

An origin of *Delias* + *Leuciacria* in southern Gondwana (Australia) corresponds with their specialization on 'mistletoe' larval food plants in the Gondwanan families Loranthaceae, Viscaceae (Barlow, 1983, 1990) and Santalaceae (Macklin, 2000; Macklin & Parnell, 2000), and the general restricted occurrence of the group to cool temperate, moist montane cloud forests, particularly in the highlands of New Guinea where most of the phylogenetic diversity is centred. These habitats comprise a relictual element of the Gondwanan rainforest that once covered much of Australia during the Eocene (e.g. Barlow, 1981a; Schodde, 1989; White, 1994, 1997, 1998; Crisp *et al*., 1999; Hill *et al*., 1999). During the Miocene, the Eocene Gondwanan flora contracted to small areas along the eastern coast of mainland Australia as the continent progressively dried out; in the Pliocene, the flora colonized the New Guinea highlands (1200–2100 m) when suitable climatic conditions were created with extensive mountain uplifting.

The absence of *Delias* in Tasmania is a result, in a proximal sense, of the absence of both the mistletoe larval food plants and their vectors (Mistletoebird, *Dicaeum hirundinaceum*, and Painted Honeyeater, *Grantiella picta*) on the island. However, western Tasmania is considered to be a living piece of Gondwana, and fossil evidence from pollen records shows that the Loranthaceae, the primary larval food plant of *Delias*, was part of the regional flora from late in the early Eocene to the mid-Pleistocene (Macphail *et al*., 1993; Macphail & Hill, 1994; Martin, 1994). Barlow (1981a, 1981b) suggested that the Mistletoebird, in particular, became locally extinct during the glacial episodes of the Pleistocene and, after the formation of the Bass Strait, has been unable to successfully recolonize Tasmania from mainland Australia. As a consequence, local extinction of the loranthaceous mistletoes (and presumably *Delias*) in Tasmania is likely to have been recent.

Diversification

Our phylogenetic hypothesis of *Delias* indicates that there have been at least four major periods of diversification within the genus (Fig. 5). The first was an initial rapid radiation, resulting in eight major clades. The second was early differentiation of three of these clades (*singhapura*, *belladonna* and *hyparete*). The third was differentiation and radiation of two clades in mainland New Guinea (*cuningputi* and *nigrina*), resulting in eleven species-groups, as well as further differentiation of the *hyparete* clade. The fourth was an explosive radiation of twelve species-groups (*cuningputi*, *geraldina*, *clathrata*, *hyparete*, *dorimene*, *nysa*, *belisama*, *weiskei*, *eichhorni*, *georgina*, *nigrina* and *iltis*), leading to exceptionally high species richness ($\geq$ 10 species) within each group.

Although our age estimations are approximate, and should be viewed with caution, these four diversification events correspond with the following geological time periods: (1) late Oligocene; (2) early Miocene; (3) mid-Miocene; and (4) Pliocene and/or Pleistocene. Diversification of the *Delias* group over this period shows a steady increase in the cumulative total number of species during the Oligocene and Miocene, followed by an exponential increase during the Pliocene–Pleistocene (Fig. 6). The fourth putative explosive radiation of the twelve species-groups appears to have been centred in mainland New Guinea of the Australian Region, where the rate of increase was far greater than in the Oriental Region. Of these twelve species-groups, seven (58%) have their main area of distribution in New Guinea, whereas four groups occur more widely in both regions; only one of these species-groups (*georgina*) is restricted to the

Oriental Region. In the Oriental Region, only three species-groups (*georgina*, *belladonna* and *pasithoe*) have diversified to any extent (nine to thirteen species).

The four major diversification events are associated with major geological, climatic and floristic changes that occurred in Australia–New Guinea during the late Tertiary, which may have provided ecological opportunity for rapid speciation. After a long period of sea floor spreading and rifting, the Australian tectonic plate finally severed its links with Gondwana towards the end of the Eocene (*c.* 35 Mya). Profound climatic changes then took place. Following the separation of Australia from Antarctica, a substantial seaway (Southern Ocean) developed between the two continents, and a deep-water circumpolar current became established, steepening the latitudinal temperature gradient, which initiated the glaciation of Antarctica and concomitant global cooling in the late Eocene and early Oligocene (34–30 Mya) (e.g. Barlow, 1981a; White, 1994; Cox & Moore, 2000). At this time, Australia, which was still at high latitudes, also experienced a dramatic decline in temperature (by as much as 7 °C), although rainfall remained high (Hill *et al.*, 1999; Crisp *et al.*, 2004; Hill, 2004). By the late Oligocene (25 Mya), however, the climate had become progressively drier, and the northern margin of the Australian plate came into contact with the island arcs of Melanesia and eastern Indonesia as it collided with the Pacific and Asian plates (Hall, 1998, 2001). The early to mid-Miocene (23–10 Mya) in Australia was characterized by cooler, drier, more seasonal climates, leading to the progressive contraction of the non-seasonal, wet Eocene Gondwanan rainforest and expansion of the unique Australian sclerophyll flora, as the continent moved north into the subtropical high-pressure ridge (Barlow, 1981a; White, 1994; Crisp *et al.*, 2004). More significantly, though, was the formation of mainland New Guinea during the mid- to late Miocene. At the northern margin of the Australian continental plate, a complex series of fragments of continental crust and emergent land developed, including the Sepik Arc Terrane, followed by the East Papua, Irian Jaya and the central Island Arc Terranes (de Boer, 1995; Hall, 1998; de Jong, 2001). Fusion of these terranes and mountain building through subduction of the northern margin during the mid- to late Miocene created a single landmass. The major uplifting of mainland New Guinea occurred during the Pliocene (5–2 Mya), creating a central range that today is the largest montane area in the Indo-Australian tropics after the Himalaya (Holloway, 1986). The uplift in New Guinea in the Pliocene also coincided with a period of severe aridity in Australia, which led to the formation of the extensive central arid zone and desert-adapted flora (Crisp *et al.*, 2004).

The split between *Delias* and *Leuciacria* (early Oligocene) thus coincides with a marked worldwide decline in temperature following the severance of Australia from Antarctica. The first explosive radiation of *Delias* – eight major clades (late Oligocene) – coincides with subsequent collision of the Australian and Asian plates and the formation of island arc fragments at the northern margin, whereas the second period of differentiation of *Delias* – the *singhapura*, *bella-*

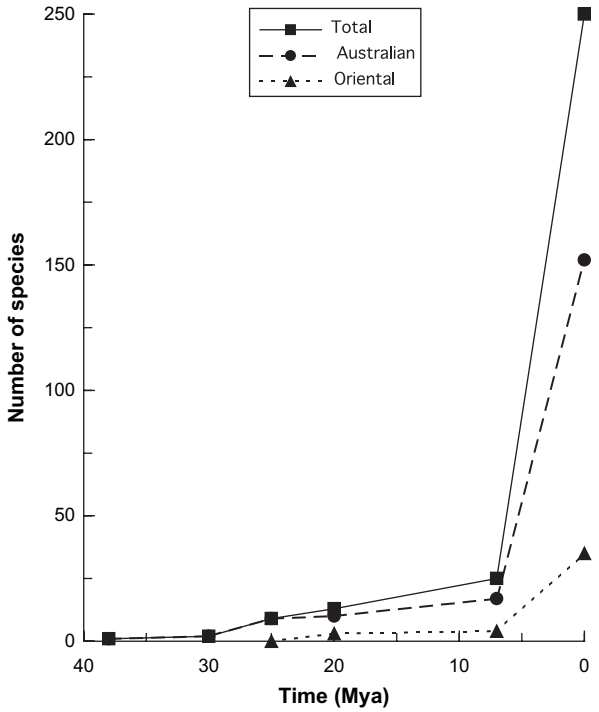

Fig. 6. Diversification of the *Delias* group, expressed as the cumulative number of species over time according to the phylogeny in Fig. 5. Radiation within most species-groups is assumed to have occurred during the Pliocene–Pleistocene. The four widespread species-groups (*nysa*, *dorimene*, *hyparete* and *belisama*) are excluded from plots for the Australian and Oriental Regions because of uncertainty in the timing of their dispersal across Wallacea.

donna and *hyparete* clades (early Miocene) – appears to be associated mainly with early long-distance dispersal across Wallacea. The third period of differentiation and radiation of *Delias* – the *cuningputi* and *nigrina* clades (mid- to late Miocene) – coincides with the widespread contraction and fragmentation of the Gondwanan forests in Australia, and the geological development of the island arcs of New Guinea. The fourth explosive radiation of *Delias* – seven species-groups (*cuningputi*, *geraldina*, *clathrata*, *weiskei*, *eichhorni*, *nigrina* and *iltis*) in New Guinea (putatively Pliocene) – coincides with the development of the New Guinea central highlands, although differentiation of these species-groups may have occurred more recently during the glacial episodes of the Pleistocene (see below).

What mechanism(s) may have facilitated the diversification of *Delias* multiple times and to such a considerable degree? Most species of *Delias* today are restricted to cool, moist, temperate, equatorial montane cloud forest habitats, typically at altitudes between 1200 and 2800 m (see fig. 19 in Parsons, 1998: 83). Much of this habitat in the Indo-Australian tropics is highly disjunct, occurring on isolated mountain ranges and peaks, and frequently on islands of varying size. These high-altitude mountains are geologically young (mid-Miocene–Pliocene) (Holloway, 1986) and, within these areas, *Delias* shows a high level of specific

and subspecific differentiation, particularly in mainland New Guinea (Roepke, 1955; Yagishita *et al*., 1993; Parsons, 1998). At least three modes of speciation could account for such a pattern of diversification. One possible mode is cospeciation with their mistletoe larval host plants, particularly the genus *Amyema* which is diverse in montane mainland New Guinea (Barlow, 1990); however, detailed phylogenies of both plants and the butterflies are currently lacking, and very little information is available on host use by *Delias* in New Guinea to test this hypothesis. The second mode involves high-altitude dispersal between adjacent mountain ranges within and between islands, followed by allopatric speciation (i.e. mountain or island hopping) (Talbot, 1928–37; Roepke, 1955; Holloway, 1986; Parsons, 1998; Morinaka *et al*., 2001). The third mode involves low-altitude movement and range expansion along intervening valleys between mountain ranges and along temporary land bridges between islands, followed by vicariance. Given the general preference and adaptation to low temperature (Braby & Lyonns, 2003), the latter mode could only have occurred when palaeotemperatures were considerably lower than at present (Holloway, 1986). At such times, the sea-level would have been lower, resulting in emergent land between many of the islands in Indonesia, the northern Australian plate and Melanesia. The altitudinal (vertical) distribution of *Delias* and its montane forest habitat could have been as much as 1000 m lower, allowing colonization of the extensive lowlands and areas of land between islands that were previously under water. When the climate warmed, the butterflies' distribution would have contracted vertically upwards, tracking the cooler temperatures and habitats at higher elevations on mountain peaks, resulting in many populations effectively being segregated by the formation of hot lowland and/or ocean barriers. If the populations remained reproductively isolated before the next episode of climate cooling, speciation might have occurred as a result of vicariance. Such a mechanism would not only lead to diversification, but could potentially result in simultaneous differentiation (radiation) of a formerly widespread taxon into many daughter species, each isolated and restricted to different mountain peaks/islands. The most dramatic climatic (temperature) fluctuations in the late Tertiary occurred during the glacial episodes of the Pleistocene. It is therefore possible that the development of a heterogeneous montane landscape, together with marked fluctuations in temperature and concomitant shifts in altitudinal range leading to vicariance in the Pleistocene, may have been a major driving force in the evolution of *Delias*. In other words, the final explosive radiation in *Delias* may have occurred relatively recently.

In contrast, *Leuciacria* has neither diversified nor dispersed to any great extent, containing two rare species restricted to the Tumbunan Element of mainland New Guinea and New Ireland (and possibly New Britain), at altitudes between 1200 and 2400 m (Parsons, 1998; Müller, 1999b, 2001b; Gotts & Pangemanan, 2001). Differences in diversification rates between the two genera are almost certainly tied to differences in ecology. *Leuciacria* probably specializes on a non-mistletoe host tree restricted to the cool temperate high-altitude forests of mainland New Guinea and nearby islands. The Tumbunan Element, which comprises cool, moist montane temperate and subtropical forests, is an *in situ* relictual element of the Gondwanan forests that once covered much of Australia during the Eocene (e.g. Schodde, 1989; Crisp *et al*., 1999, 2004; Hill *et al*., 1999). Given that *Leuciacria* is estimated to have evolved no later than the early Oligocene, the genus presumably colonized the mountains of New Guinea with the progressive drying and contraction of the moist Gondwanan forests in Australia during the Miocene. *Leuciacria* would therefore qualify as a relict taxon, its range having contracted from Australia to a small area in mainland New Guinea and New Ireland.

Conclusions

Our phylogenetic hypothesis of *Delias* has revealed that this large genus (which is currently composed of approximately 250 species arranged in twenty-four species-groups) comprises eight major clades or lineages, all of which diverged explosively. These clades contain between one and eight species-groups. In general, phylogenetic relationships between these species-groups corroborate their current systematic arrangement. The only discrepancies concern the placement of the *aganippe*, *belisama* and *chrysomelaena* groups, as well as some of the species-groups endemic to mainland New Guinea. Limited phylogenetic evidence suggests that most species-groups are discrete and monophyletic, but at least two species (*D. harpalyce* and *D. messalina*) of uncertain group status are misplaced, rendering the *belisama*, *nigrina* and *kummeri* groups paraphyletic or polyphyletic. Further evidence may show that the *brandti* group should be subsumed with the *nysa* group.

An historical biogeographical analysis of the *Delias* group (i.e. *Delias* + *Leuciacria*) revealed that the most parsimonious reconstruction supports an origin in the Australian Region, with multiple dispersal events across Wallacea to the Oriental Region, followed by limited differentiation in Asia (mainly in the *georgina*, *belladonna* and *pasithoe* groups). At least seven dispersal events are required to explain the present-day distribution, but further fine-scale phylogenetic analyses of four widespread species-groups (*nysa*, *hyparete*, *dorimene* and *belisama*) are needed to confirm the direction and to establish the minimum number (and timing) of dispersals. Our biogeographical model thus provides evidence in favour of the 'southern vicariance hypothesis' of the Aporiina, that is, the subtribe originated in southern Gondwana, with *Delias* + *Leuciacria* evolving vicariantly on the Australian plate (Braby *et al*., 2006b). We therefore reject the 'northern dispersal hypothesis' for an origin of the subtribe in Laurasia, which proposes that *Delias* originated in Asia from where it dispersed and differentiated southwards through Indonesia to reach New Guinea and finally Australia (e.g. Dixey, 1894; Talbot, 1928–37; Klots, 1933; Roepke, 1955; Holloway, 1969, 1974, 1986; Mani, 1986; Vane-Wright & de Jong, 2003). An 'out-of-Australia' hypothesis has been proposed recently

for the *Taractrocera* group of skippers (Hesperiidae) (de Jong, 2004) and troidine swallowtails (Papilionidae) (Braby *et al*., 2005) as the most likely explanation for the present-day distribution of these taxa, although the former study was severely hampered by a poorly resolved phylogeny. Nevertheless, concordance between such disparate taxonomic groups suggests that a southern origin, followed by dispersal to, and radiation in, Asia, may be more widespread than hitherto believed among butterflies in the Indo-Australian Region.

Our phylogenetic hypothesis indicates that there have been four major periods of diversification within *Delias*: late Oligocene, early Miocene, mid- to late Miocene and, putatively, Pliocene–Pleistocene. These periods of diversification are broadly associated with major geological and climatic changes that occurred in Australia–New Guinea during the late Tertiary following the separation of the Australian plate from Antarctica. The most significant changes were the development of a complex series of island arc fragments (terranes) at the northern margin of the Australian continental plate following collision with the Asian plate in the late Oligocene, and the formation of New Guinea in the Miocene and its uplifting in the Pliocene. Pronounced declines in temperature and rainfall on the Australian mainland in the Oligocene–Miocene led to the progressive contraction of the Eocene Gondwanan rainforest, and a decrease in the sea-level, providing opportunities for dispersal across Wallacea, particularly in the mid-Miocene. Unlike many insects in Australia, which radiated in response to expansion of the unique sclerophyll vegetation with decreasing moisture in the Miocene and increasing aridity in the Pliocene (Austin *et al*., 2004), *Delias* butterflies occur predominantly in cool temperate, moist, montane cloud forest habitats of the Indo-Australian tropics. Only a single species (*D. aganippe*) has adapted to the vast arid and semiarid environments of inland southern Australia. This implies that there may have been considerable movement and diversification out of Australia with contraction of the Eocene Gondwanan rainforest, and subsequent colonization of New Guinea in the Miocene–Pliocene. In more recent geological times, the development of the heterogeneous montane landscape in the Indo-Australian tropics, and the dispersal (island/mountain hopping) and vicariance caused by climatic (temperature) fluctuations during the Pleistocene leading to pronounced shifts in altitudinal range, have probably played a significant role in the evolution of *Delias*, culminating in the most diverse genus in the Pieridae.

Supplementary material

Appendix and Fig. 7 are available online at http://www.blackwell-synergy.com under DOI reference doi: 10.1111/j.1365-3113.2006.00349.x

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