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Phylogeny and historical biogeography of the subtribe Aporiina (Lepidoptera: Pieridae): implications for the origin of Australian butterflies

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The Australian fauna is composed of several major biogeographical elements reflecting different spatial and temporal histories. Two groups of particular interest are the Gondwanan Element, reflecting an ancient origin in Gondwana or southern Gondwana (southern vicariance hypothesis), and the Asian Element, reflecting a more recent origin in Asia, Eurasia or Laurasia (northern dispersal hypothesis). Theories regarding the origin and evolution of butterflies (Hesperioidea, Papilionoidea) in Australia are controversial, with no clear consensus. Here, we investigate the phylogenetic and historical biogeographical relationships of the subtribe Aporiina, a widespread taxon with disjunct distributions in each of the major zoogeographical regions. Attention is paid to origins of the subtribe in the Australian Region for which several conflicting hypotheses have been proposed for the Old World genus *Delias* Hübner. Our phylogenetic reconstruction was based on analysis of fragments of two nuclear genes (*elongation factor-1α*, *wingless*) and one mitochondrial gene (*cytochrome oxidase subunit I*) for 30 taxa. Phylogenetic analyses based on maximum parsimony, maximum likelihood and Bayesian inference of the combined data set (2729 bp; 917 parsimony informative characters) recovered six major lineages within the monophyletic Aporiina, with the following topology: (*Cepora* + *Prioneris* + (*Mylothris* + (*Aporia* + *Delias* group + *Catasticta* group))). Given a probable age of origin of the stem-group near the Cretaceous/Tertiary boundary (69–54 Mya), followed by diversification of the crown-group in the early to mid Tertiary (57–45 Mya), we show that an origin of the Aporiina in either southern Gondwana or Laurasia is equally parsimonious, and that dispersal has played a major role in shaping the underlying phylogenetic pattern. We tentatively conclude that an origin in southern Gondwanan is more likely; however, neither hypothesis satisfactorily explains the present-day distribution, and additional lower-level phylogenies are needed to determine the directionality of dispersal events of several taxa and to reject one hypothesis over the other. Dispersal is inferred to have occurred primarily during cooler periods when land bridges or stepping-stones were available between many of the zoogeographical regions.

ADDITIONAL KEYWORDS: Australian origins – Cretaceous – long-distance dispersal – Gondwana – Greater India – Laurasia – Madagascar – Tertiary – vicariance.

INTRODUCTION

The Australian fauna is composed of several major biogeographical elements, reflecting different evolutionary histories in both space and time (Heatwole, 1987; Cranston & Naumann, 1991). One, the Pangaean Element, has an ancient origin dating back to the mid Jurassic before the break up of Pangaea, and comprises mostly sedentary relictual taxa. A second, the Gondwanan Element, has a southern origin in the Cretaceous when the southern landmasses of Australia, South America, Antarctica, New Zealand, New Caledonia, Greater India, Madagascar, and Africa were connected to form the supercontinent Gond-

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wana. This southern element may comprise two components or subelements: (1) relictual taxa, which are restricted to relatively cool-to-warm, humid environments (mostly closed forest), and which have not diversified much following the break up of Gondwana; and (2) a more recent Austral (autochthonous) component that has adapted and radiated in response to increasing aridity during the Miocene-Pliocene of the late Tertiary (from *c*. 23 Mya onwards) with post-Gondwanic isolation of the continent. A third, the Asian Element, has a more recent northern origin as a result of the Australian tectonic plate drifting into tropical latitudes and colliding with the Sunda Island Arc of the Asian plate. This northern element reached Australia from the Oriental Region (Central Asia, South-eastern Asia) via Wallacea and the Arafura Sea (Sahul Shelf) when the sea level was lower and emergent land (islands or stepping stones) was available during the mid- to late Miocene (*c*. 15–10 Mya) and then more recently during the Pleistocene (1 Mya) (Heatwole, 1987; de Jong, 2001; Hall, 2001). However, some components of this fauna may ultimately have a Gondwanan origin, having reached and radiated in Asia via Greater India (the Indogondwanan subelement; Barlow, 1981, 1990; see also Hall, 1998; Holloway & Hall, 1998; Holloway, 2003), or via Africa (the Afrogondwanan subelement; Eliot, 1973). Two other elements are the 'cosmopolitan element' (highly dispersive taxa) and the 'introduced element' (taxa introduced by humans), both of which are minor components in the Australian fauna and do not concern us further.

Although the evolutionary history has been particularly well documented for vertebrates such as birds (Keast, 1981; Cracraft, 2001; Barker, Barrowclough & Groth, 2002; Ericson *et al*., 2002; Barker *et al*., 2004) and some insects (Cranston & Naumann, 1991; Austin *et al*., 2004), current theories regarding the origin of butterflies (Lepidoptera: Ditrysia: Hesperioidea, Papilionoidea) in the Australia region are controversial. These theories fall into three general hypotheses: (1) an origin in Asia, Eurasia or Laurasia (northern dispersal hypothesis) (Symon, 1980; Kitching, 1981; Scott, 1985, 1986; Ackery, 1991; New, 1999; de Jong, 2003); (2) an origin in southern Gondwana (Australia–Antarctica–South America) (southern vicariance hypothesis) (DeVries, 1987; Parsons, 1998; Orr, 1999; Pierce *et al*., 2002; Viloria, 2003); and (3) an origin in Gondwana or remnant Gondwana (Madagascar–Greater India–Australia–Antarctica–South America), but with dispersal from Asia via Greater India (Indogondwanan hypothesis) (Braby, Trueman & Eastwood, 2005). Because butterflies are thought to be no older than approximately the mid Cretaceous (Braby *et al*., 2005), a Pangaean origin can be ruled out.

Although the higher taxa of Australian butterflies are likely to have independent origins and therefore different evolutionary histories, testable hypotheses have been proposed for remarkably few candidates. As pointed out by Edwards, Newland & Regan (2001) and Austin *et al*. (2004), sound phylogenies are lacking for the majority of Australian endemic butterflies, as well as widespread taxa, thereby precluding determination of their relationships and hence geographical origins. Thus, without a robust phylogenetic framework, disentangling each of the three general hypotheses outlined above is not possible for most taxa. We therefore analysed phylogenetic relationships within a higher butterfly taxon, the subtribe Aporiina, mainly because a set of clearly testable (conflicting) hypotheses have been proposed for the evolutionary relatedness and biogeography of one of its members in the Australian Region, the genus *Delias*. The Aporiina, as circumscribed by Braby, Vila & Pierce (2006), comprise a well-supported monophyletic clade of 16 lower taxa (14 genera, two subgenera) within the tribe Pierini. Although *Delias* occurs widely in both the Australian and Oriental Zoogeographical Regions of the Old World, it shows high levels of endemism within the Australian Region and its putative nearest relatives have disjunct distributions in areas of endemism.

In terms of the phylogenetic relationships of *Delias*, Parsons (1998: 297) stated succinctly, 'The origin of *Delias* and its exact relationships to other pierid groups remains open to debate'. The conventional view has been that *Delias*, together with *Prioneris* Wallace and *Cepora* Billberg, evolved in the Northern Hemisphere in the mountains of eastern India (northern Oriental Region) from an *Aporia*/*Metaporia*-like ancestor in the Himalaya (Palaearctic Region) (Dixey, 1894; Talbot, 1928–37; Yata, 1985). *Delias* and *Cepora* then dispersed and differentiated southwards through Central Asia and South-eastern Asia (including Indonesia), eventually crossing Wallacea to reach mainland New Guinea and finally Australia (Fig. 1A). Klots (1933) reached the same general conclusion as Dixey (1894) and Talbot (1928–37), and also proposed a northern dispersal hypothesis, although with slight modification (Fig. 1B). He treated *Metaporia* Butler as a subgenus of *Aporia* Hübner, and considered *Prioneris* to be more closely related to *Belenois* Hübner and *Dixeia* Talbot than to *Delias* and *Cepora*. However, Klots (1933) noted that *Delias* also showed a close relationship with *Pereute* Herrich-Schäffer and *Leodonta* Butler from Central and South America, based on similarities in genitalia and wing venation. He went on to state 'It is only reasonable to suppose that *Pereute* and *Leodonta* represent New World offshoots from the same *Aporiine* stock, which have become isolated in the tropics' (Klots, 1933: 203). Furthermore, 'It is possible that the resemblance of *Pereute* and *Leodonta* to

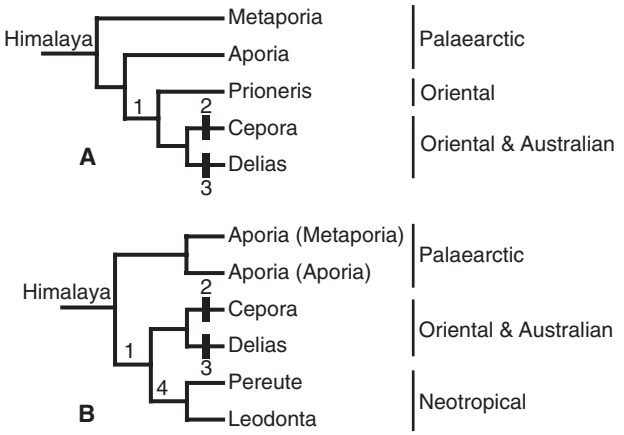

Figure 1. Northern dispersal hypothesis for origin of *Delias* in Australia, according to the early phylogenetic hypotheses proposed by: A, Dixey (1894) and Talbot (1928–37); B, Klots (1933). *Delias* is envisaged to have reached Australia by dispersal via Asia from an *Aporia*-like ancestor in the Himalaya. The minimum biogeographical steps are: 1 = dispersal from Himalaya (Palaearctic Region) to Asia (Oriental Region) resulting in allopatric speciation; 2, 3 = dispersal (range expansion) from Oriental to Australian region; 4 = long-distance dispersal from Old World (Oriental region) to New World (Neotropical Region) resulting in allopatric speciation.

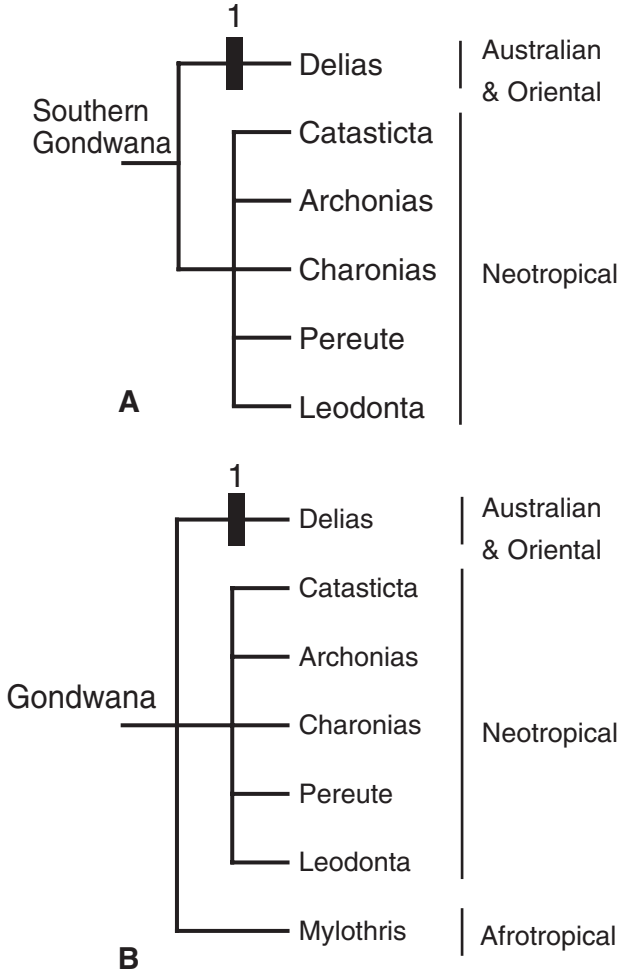

Figure 2. Southern vicariance hypothesis for origin of *Delias* in Australia, according to the recent views expressed by: A, DeVries (1987); B, J. N. Eliot (see Corbet & Pendlebury, 1978, 1992). *Delias* is envisaged to have reached Australia as a result of the break up of southern Gondwana (Australia–Antarctica–South America) or Gondwana. Minimum biogeographical step 1 represents dispersal (range expansion) from Australian region across Wallacea to Oriental region (for an alternative dispersal route for Fig. 2B, see text).

Delias is merely accidental. The fact of their isolation in the New World tropics, with no geographical connecting links to *Delias* or *Aporia* is an argument in favour of a theory of their independent origin. The author considers, however, that their [sic] similarity to *Delias* too great, and in too many structures, to be purely fortuitous' (Klots, 1933: 229). Dixey (1894), Grote (1900) and Talbot (1928–37) also drew attention to a possible connection between *Delias* and several Neotropical taxa, including *Pereute* and also *Catasticta* Butler, but they proposed an independent line of differentiation in the Northern Hemisphere. For example, Dixey (1894) assumed that an *Eucheira*-like ancestor in montane Mexico (Nearctic) gave rise to one lineage comprising *Neophasia* Behr and *Catasticta* + *Archonias* + *Charonias* + *Pereute* + *Leodonta* in the New World, and to another lineage comprising *Aporia* and *Prioneris* + (*Delias* + *Cepora*) in the Old World.

Roepke (1955) and Holloway (1969, 1974a, 1986) (see also Holloway & Jardine, 1968) also concluded that *Delias* originated in Asia. However, in contrast to the hypotheses of Dixey (1894) and Talbot (1928–37), Holloway postulated an origin in mainland Asia, from whence the genus dispersed west to reach India (during the Pliocene) and east to reach New Guinea (during the Miocene), where it subsequently radiated in the Pleistocene. Similarly, Mani (1986) advocated that *Delias* reached the Himalaya from an 'eastern element' in the Oriental Region, where it differentiated in, and spread from, the mountains of mainland Asia (Burma, Yunnan, Indo-China, Thailand, Malaya, Sundaland). However, phylogenetic relationships of the genus were not considered.

Alternative hypotheses expressed by several authors suggest a southern vicariance hypothesis for origin of *Delias* in Australia (Fig. 2). For example, DeVries (1987) suggested a close relationship between *Delias* and *Catasticta*, *Archonias* Hübner, *Charonias* Röber, *Pereute*, and *Leodonta* from Central and South America based on similarities in biology, including

larval food plant specialization and behaviour of the immature stages, and habitat distribution (Fig. 2A). He suggested 'these genera form the Neotropical counterpart of the diverse Old World genus *Delias* . . . It is surprising that there has not been a biogeographical study examining these genera in the context of continental drift and speciation events' (DeVries, 1987: 90–91). Wallace (1867) independently reached the same conclusion regarding the systematic relationship between these two groups more than a century earlier. He stated '*Thyca* (an invalid name of *Delias*) appears to be closely related to the American genus *Euterpe* (a synonym of *Archonias*, but used in the broad sense to include *Catasticta*, *Archonias*, *Charonias*, *Pereute*, *Leodonta*), since it . . . hardly offers any constant structural differences' (Wallace, 1867: 344). By contrast, Trimen (1889), Talbot (1944), Holloway (1969), D'Abrera (1980), and Larsen (1991) suggested that *Delias* is most closely related to *Mylothris* Hübner from Madagascar and Africa, drawing attention to a number of similarities, including larval food plant specialization, habitat distribution, and flight behaviour. However, J. N. Eliot went further and postulated that 'The genus [*Delias*] . . . with the African genus *Mylothris* and the South American *Pereute*, *Archonias*, *Leodonta* and *Catasticta*, probably forms a good tribe' (Corbet & Pendlebury, 1978, 1992: 82), implying that the taxa from each of the three broad geographical areas comprise a monophyletic group (Fig. 2B). Eliot (1973) believed that the true butterflies originated in western Gondwana (Africa–South America), with subsequent dispersal and differentiation from Africa via Eurasia and south-eastern Asia to Australia, and this view may well have applied to *Delias* rather than a strictly southern origin in Gondwana with taxa originating in, and then dispersing out of, Australia (one step), as shown in Figure 2B. However, such a scenario is less parsimonious because it requires two biogeographical steps: (1) dispersal of the ancestor of *Delias* from Africa across the Mediterranean Sea to Laurasia resulting in allopatric speciation; and (2) dispersal (range expansion) of *Delias* from south-eastern Asia across Wallacea to Australia.

Orr (1999) and Braby & Lyonns (2003) suggested that *Delias* may have a Gondwanan origin, the latter authors basing their conclusions on larval food plant associations, physiological adaptation to cool climate, and the general restricted occurrence of the genus to moist cool temperate or montane habitats, particularly in mainland New Guinea (the Tumbunan Element, a relictual element of the Eocene rainforests of Gondwana) (Schodde, 1989; Crisp, West & Linder, 1999). The major larval food plants of *Delias*, the Loranthaceae and Santalaceae + Viscaceae, almost certainly evolved under warm, moist conditions in closed forests of the southern temperate latitudes of Gondwana, possibly during the mid- to Late Cretaceous (Barlow, 1981, 1983, 1990; Walsh & Jeanes, 1997; Macklin & Parnell, 2000; Macklin, 2000). Wikström, Savolainen & Chase (2001) estimated the age of the order Santalales, to which these three families belong, to have evolved in the early Cretaceous (118–113 Mya for stem-group), with the crown-group diverging in the Late Cretaceous (97–85 Mya). The Santalaceae + Viscaceae was estimated to have evolved in the Late Cretaceous (80–69 Mya for stem-group) and diversified in the early Tertiary (67–53 Mya for crown-group). Although the Loranthaceae were not included in the analyses of Soltis, Soltis & Chase (1999) and Wikström *et al*. (2001), a phylogenetic analysis of the Santalales by Nickrent *et al*. (1998) shows that the Loranthaceae originated at approximately the same time as the Santalaceae. The Loranthaceae have poor dispersal ability (Barlow, 1983) and, in Australia, fossil records of the family extend as far back as the Eocene (Macphail & Hill, 1994; Martin, 1994; White, 1998). The larval food plants of *Delias* are thus old enough to be of Gondwanan age, and were present in southern Gondwana during the mid Tertiary.

More recently, Braby *et al*. (2006) found that molecular evidence supported a close relationship between *Delias* and *Leuciacria* Rothschild & Jordan, a small genus endemic to the Australian Region, a finding that differs from all previous phylogenetic hypotheses. These two taxa were placed in the *Delias* group of the Aporiina, but generic relationships within the subtribe were not well resolved. Furthermore, a phylogenetic and biogeographical analysis of the 24 species groups of *Delias* plus *Leuciacria* revealed that the most parsimonious reconstruction showed an origin of the *Delias* group in the Australian Region, with multiple dispersal events across Wallacea to the Oriental Region (Braby & Pierce, 2007). However, because systematic and biogeographical relationships of the *Delias* group and its putative relataives are uncertain, higher-level phylogenies of the Aporiina are needed to determine which of the two general hypotheses outlined above regarding the origin of *Delias* is more likely (Figs 1, 2).

In the present study, we infer how *Delias* reached the Australian Region by consideration of key events such as vicariance, dispersal, and extinction using cladistic methods of historical biogeography. More specifically, we ask two questions: (1) which taxon is *Delias* most closely related to? and (2) where did *Delias* originate? The answers to these questions may help elucidate the possible role of Gondwanan or Asian origins in Australian butterflies, and whether certain components of the butterfly fauna entered the continent from the south or north.

MATERIAL AND METHODS

MOLECULAR MARKERS

Characters from fragments of two nuclear protein-encoding genes, *elongation factor-1α* (*EF-1α*) and *wingless* (*wg*), and the mitochondrial gene *cytochrome oxidase subunit I* (*COI*), were used to infer phylogenetic relationships among the Aporiina. *EF-1α* is a useful marker for resolving deeper-level divergence events of insects, especially after the mid Tertiary (Cho *et al*., 1995; Mitchell *et al*., 1997; Danforth & Shuqing, 1998). *wg* has been used successfully in several phylogenetic studies of Lepidoptera at both higher and lower taxonomic levels (Brower & Egan, 1997; Brower & DeSalle, 1998; Brower, 2000; Campbell, Brower & Pierce, 2000; Wahlberg, Weingartner & Nylin, 2003). *COI* is a widely used mitochondrial gene and has great utility for resolving more recent divergence events (Simon *et al*., 1994; Hillis *et al*., 1996; Palumbi, 1996). Several recent studies of Lepidoptera have demonstrated improved resolution and increased nodal support at most levels in combined analysis of these three genes (Caterino *et al*., 2001; Monteiro & Pierce, 2001; Wahlberg *et al*., 2003, 2005; Zakharov, Caterino & Sperling, 2004).

TAXON SAMPLING

Nineteen species representing 15 of the 16 higher taxa (genera, subgenera) recognized in the Aporiina (Braby, 2005b; Braby *et al*., 2006) were included in the study (Table 1). The only higher taxon not included was the subgenus *Aporia* (*Mesapia*) from the Himalaya. Most of the higher taxa occur in areas of endemism, with *Delias* and *Cepora* being the only genera that cross zoogeographical boundaries to any great extent (both are widespread in the Australian and Oriental Regions) (see Appendix). The Aporiina includes around 440–480 species or approximately 40% of all known species in the family Pieridae. *Delias* and *Catasticta* are relatively large genera (containing ≥100 species), whereas *Mylothris*, *Aporia*, and *Cepora* also contain many species (*c*. 20–60) (see Appendix). With the exception of *Delias* (Morinaka, Miyata & Tanaka, 2002; Braby & Pierce, 2007), the monophyly of most genera has not been established rigorously, although they have been well studied taxonomically and, in some cases, systematically monographed (Klots, 1933; Talbot, 1944; van Son, 1949; Reissinger, 1972; Yata, 1985; Eitschberger & Racheli, 1998), rendering it unlikely that they comprise paraphyletic assemblages. Nevertheless, two exemplar species were included for each of *Catasticta*, *Mylothris*, and *Aporia* to test for potential nonmonophyly. Two species of the monophyletic genus *Delias* and both species of *Leuciacria* were also included. For the first four genera, and as far as possible, the exemplars were chosen to represent a wide snapshot of the morphological diversity (i.e. subgenera, species groups, etc.).

A further nine pierid taxa, representing all the major taxonomic groups within the family (i.e. Pseudopontiinae, Dismorphiinae, Coliadinae, Pierinae) were included as distant ingroup taxa. Two species from the family Papilionidae (*Papilio rutulus*, *Troides helena*) were chosen as outgroup taxa. The final data set thus comprised 30 taxa (28 Pieridae, two Papilionidae) (Table 1). For the genus *Colias*, sequences representing different gene partitions from two closely-related species, *Colias eurytheme* (*EF-1α*, *wg*) and *Colias philodice* (*COI*), were combined into a single terminal unit.

MOLECULAR TECHNIQUES

Of the 90 sequences assembled for our 30 taxon data set, 32 (one *EF-1α*, 15 *wg*, 16 *COI*) comprised new sequences, 51 (28 *EF-1α*, 12 *wg*, 11 *COI*) were derived from our recent study of the Pieridae (Braby *et al*., 2006), whereas a further seven (one *EF-1α*, three *wg*, three *COI*) were obtained from those registered on GenBank based on previously published work (Caterino & Sperling, 1999; Brower, 2000; Campbell *et al*., 2000; Caterino *et al*., 2001; Wahlberg *et al*., 2005) (Table 1). Methods for the collection, preservation, extraction, purification, amplification, sequencing, and alignment of the 32 new sequences were similar to those described previously (Braby *et al*., 2005, 2006). The only difference was that a different and slightly longer fragment (1283 bp) of *COI* was amplified, corresponding to positions 1729–3011 relative to *Drosophila yakuba* (Clary & Wolstenholme, 1985), employing the primers Ron–Eva (Ron: 5′-GGATCACCTGATATAGCATTCCC-3′, 1729–1751; Eva: 5′-GAGACCATTACTTGCTTTCAG TCATCT-3′, 3798–3772). However, for 13 taxa, a small portion (231–320 bp) at the end of the *COI* gene was not sequenced and this region was coded as missing data for these samples.

In *wg*, five samples (*Delias belladonna*, *Delias nigrina*, *Charonias eurytele*, *Mylothris agathina*, *Mylothris bernice*) had a homologous one-codon deletion; a further two samples (*Pereute charops*, *Leodonta tellane*) had a homologous one-codon deletion but in a different position to that of the other samples. In *COI*, one sample (*Delias nigrina*) had a 1-bp insertion ('T'), 17 bp from the end of the gene (i.e. position 2992 near the start of tRNA-leucine). The additional base created a frame-shift such that the termination codon started at position 2997, instead of 3007, and the last three amino acids of *COI* were not translated. Indels in both genes were treated as characters and not as missing data.

Table 1. Exemplar taxa used in this study, with collection localities and GenBank accession numbers

Taxon	MCZ voucher no.	Locality	GenBank Accession No.		
			EF-1α	*wg*	*COI*
Pseudopontiinae					
Pseudopontia paradoxa	SC-01-T380	Zambia: Zambesi Bridge, Ikelenge	AY870580	AY954549	AY954564
Dismorphiinae					
Dismorphia zathoe	MFB-00-P231	Costa Rica: Monteverde	AY870578	AY954596	AY954566
Leptidea sinapis	RV-00-T760	Spain: Vallgrassa, Parc del Garraf, Barcelona	AY870573	AY954595	AY954565
Coliadinae					
Colias philodice	NP-98-U268	USA: Harvard Forest, MA	AY870565	AY954600	
Colias eurytheme		Canada: Ontario			AF044024*
Eurema mexicana	DW-92-Z084	USA: South fork of Cave Creek, Chiricahua Mtns., Cochise Co. AZ	AY870563	AY954598	AY954568
Pierinae: Anthocharidini					
Hesperocharis crocea	MFB-00-P268	Costa Rica: Alajuela	AY870555	AY954606	AY954576
Pierinae: *Leptosia* group					
Leptosia nina	NP-95-Y248	Malaysia: Fraser's Hill, Pahang	AY870519	AY954616	AY954586
Pierinae: Pierini: Pierina					
Pieriballia viardi	MFB-00-P221	Costa Rica: Río Alondra, San Luís	AY870547	AY954612	AY954582
Pieris rapae	NM-95-Y381	USA: Nora Murphy culture	AY870550	AY954611	AY954581
Pierinae: Pierini: Aporiina					
Cepora perimale	MFB-00-P131	Australia: Brisbane Forest Park, Qld	AY870524	DQ082804	DQ082757
Prioneris philonome	DL-00-Q610	Thailand: Gaw Chan Waterfall, Ratchaburi	AY870527	AY954613	AY954583
Mylothris agathina	NP-99-T486	South Africa: Muizenberg, Western Cape	AY870528	AY954615	AY954585
Mylothris bernice	SC-01-M002	Tanzania: Mufindi, Kihansi	AY870513	DQ082805	DQ082758
Aporia crataegi	RV-00-T758	Spain: Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona	AY870529	DQ082806	DQ082759
Aporia (*Metaporia*) *agathon*	MFB-00-P481	China: Near Lijang, Yunnan Province	AY870590	DQ082807	DQ082760
Delias belladonna	DL-00-N104	Thailand: Doi Inthanon NP, Chiang Mai	AY870510	DQ082808	DQ082773
Delias nigrina	MFB-97-U341	Australia: Mogo, NSW	DQ082837		DQ082790
Delias nigrina		Australia: Lismore, NSW		AF246577‡	
Leuciacria acuta	MFB-00-P468	Indonesia: Pass Valley Wamena, Papua.	AY870591	DQ082809	DQ082761
Leuciacria olivei	MFB-00-S095	Papua New Guinea: Schleinitz Mts, south-central New Ireland	AY870592	DQ082810	DQ082762
Melete lycimnia	MFB-00-P316	Peru: 10 km SW of San Ramón, Chanchamayo district	AY870530	DQ082811	DQ082800
Pereute charops	MFB-00-P283	Costa Rica: Río Macho, Cartago Province	AY870538	DQ082812	DQ082763
Leodonta tellane	MFB-00-P265	Costa Rica: Río Orosi	AY870537	DQ082813	DQ082764
Neophasia menapia	AMS-00-R052	USA: Ice Spring Road, Glenn Co, CA	AY870536	DQ082814	DQ082765

Table 1. *Continued*

Taxon	MCZ voucher no.	Locality	GenBank Accession No. *EF-1α*	*wg*	*COI*
Eucheira socialis	MFB-00-P284	Mexico: Kilometer 64 on Highway 40, town of El Madrono, Durango	AY870535	DQ082815	DQ082766
Catasticta teutila	MFB-00-P247	Costa Rica: Copey	AY870532	*AY954614*	*AY954584*
Catasticta cerberus	MFB-00-P266	Costa Rica: Cerro de la Muerte, Villa Mills	AY870533	DQ082816	DQ082767
Archonias brassolis	MFB-00-P263	Costa Rica: Río Orosi, Cartago Province	AY870534	DQ082817	DQ082768
Charonias eurytele	MFB-00-P395	Peru: 9 km S of Tingo Mar'a	AY870531	DQ082818	DQ082801
Papilionidae					
Papilio rutulus	AF-94-A002	USA: Gold Basin, Gunnison, CO	AY954620	AF233563†	
Papilio rutulus		USA: Maryland			AF044013*
Troides helena		Malaysia	AF173418§	AY569047¶	AF170878§

*Sequences for these taxa are those published by Caterino & Sperling (1999).
†Taxon sequence published by Campbell *et al*. (2000).
‡Taxon sequence published by Brower (2000).
§Taxon sequences published by Caterino *et al*. (2001).
¶Taxon sequence published by Wahlberg *et al*. (2005).
Voucher numbers refer to those specimens deposited in the Museum of Comparative Zoology (MCZ), Harvard University, USA.

Phylogenetic analysis

Maximum parsimony

Phylogenetic trees were reconstructed from the individual data partitions (genes) and from the combined data set 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 500 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 compared results based on MP analyses of each data partition and of each codon position (first and second vs. third) to investigate whether there were conflicting signals within each data set. Various weighting schemes were then explored, including removing or down weighting third codon positions over first and second positions (1 : 2, 1 : 5), particularly for the mitochondrial gene, and/or weighting transversions over transitions (2 : 1). Bootstrap analyses (Felsenstein, 1985, 1988), based on full heuristic search of 1000 pseudoreplicates using TBR branch-swapping and simple stepwise addition, 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. Total Bremer Support (decay index) (Bremer, 1988, 1994) was also calculated to evaluate nodal support using the program TreeRot, version 2 (Sorenson, 1999). Topologies generated by both separate and combined analyses were compared to establish whether the gene partitions carried substantially different phylogenetic signals. We also compared Partitioned Bremer Support to ascertain which, if any, nodes among the gene partitions were in conflict in the cladogram. This index measures the support from each data partition in the combined data set (positive values indicate increased character support whereas negative values indicate increased character conflict in the combined analysis).

Maximum likelihood

Phylogenetic reconstruction was estimated using maximum likelihood (ML) tree building methods for the combined data set. Model selection was determined according to the hierarchical likelihood ratio test as implemented in ModelTest 3.06 (Posada & Crandall, 1998), with the starting tree obtained by MP to estimate model parameters. The 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.

Bayesian inference

Finally, we ran Bayesian inference (BI), partitioned by codon position (first and second; third) for each gene (i.e. total of six partitions), using the program MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003). The GTR + I + Γ model of sequence evolution was used for each independent partition, with unlinked model parameters preset as starting values for all partitioned analyses. Four independent Bayesian runs each with four chains (one cold and three heated) at temperature settings of 0.4–1.0 were performed on the data using Metropolis-coupled Markov chain Monte Carlo simulations, each with one million generations, and tree sampling every 100 generations. Variations in heating chain parameters did not affect final tree topology, with the replicates converging after reaching a steady plateau. Likelihood values were graphically inspected and the sampled trees with preasymptotic scores (first 1000–3000) were discarded as 'burn-in'. Bayesian topology and branch posterior probabilities were computed by majority rule consensus.

BIOGEOGRAPHY

Geographical distribution was examined as a character trait and coded at the level of zoogeographical region for each taxon to infer ancestral states and patterns of historical biogeography within the Aporiina. Character states were optimized on our best estimate of the phylogeny of the subtribe using dispersal-vicariance analysis (DIVA) (Ronquist, 1997) to establish the most parsimonious ancestral reconstruction, assuming that there was a single broad ancestral area. DIVA optimizes the ancestral distribution by minimizing the total cost at each node in the area cladogram, expressed in terms of the minimum number of dispersal and extinction events. Dispersal and extinction were assigned equal cost, as one unit per area added/deleted in the analysis, whereas speciation events caused by vicariance (allopatric speciation) or duplication (sympatric speciation) were given zero cost. DIVA tends to favour vicariance reconstructions over dispersal as the underlying mode of speciation. Cook & Crisp (2005) have recently noted that the assumption of assigning equal costs to these different evolutionary events may not be valid, particularly where there is directionality to long-distance dispersal caused by processes such as prevailing winds and ocean currents. That is, the cost should be inversely related to its likelihood: the less likely an event, the more costly it should be. However, in the absence of additional information on the extent to which directional processes affect dispersal in the Aporiina, directionality was not included in the cost matrix. The Aporiina are medium-sized butterflies (wingspans in the range 40–80 mm) and are relatively specialized ecologically; they do not appear to disperse long distances by wind currents, although several lowland species migrate irregularly within their broad areas of distribution.

AGE OF DIVERGENCE ESTIMATIONS

In our previous study of the Pieridae, we estimated the minimum age of the Aporiina to be 61 Mya [99.9% confidence interval (CI) = 69–54 Mya] for the stem-group and 50 Mya (99.9% CI = 57–45 Mya) for the crown-group based on extrapolation of fossil evidence in the Aporiina and its putative sister taxon the Pierina (Braby *et al*., 2006). Given these two mean minimum ages as calibration points, we estimated the ages of various nodes in our phylogeny of the Aporiina. To calibrate the evolutionary rate of substitution, we first assessed whether the rate was constant (i.e. clocklike) by comparing the likelihood scores of our best ML model with and without enforcing a molecular clock, using the likelihood ratio test in PAUP. The LRT test rejected the null hypothesis that the data were clocklike ($\delta = 228$, d.f. = 28, $P < 0.0001$). We therefore applied Sanderson's semiparametric rate smoothing according to the penalized likelihood method, as implemented in the r8s program (Sanderson, 2002), to correct for rate heterogeneity across the ML tree. Age estimations were calculated for each node, with the smoothing parameter λ optimized using the cross-validation method based on minimizing the chi-square error values. Error terms for each node were estimated based on the 99.9% CI 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, concatenated sequences included 2729 bp for the combined 30 taxon data set (*EF-1α*: 1066 bp, *wg*: 401 bp, *COI*: 1262 bp), of which 917 sites (33%) were parsimony informative (Table 2). Most of the informative sites for nuclear *EF-1α* were in the third codon position [282 sites (90%) parsimony informative] whereas, in the two other genes, first and second positions were far less conserved [47 sites (28%) parsimony informative for *wg*, 107 sites (25%) parsimony informative for *COI*]. A plot of the transition/transversion ratio against the observed or uncorrected

Table 2. Character summary for the combined data set, with numbers of sites for each codon position for each gene partition

	EF-1α				*wg*				*COI*				
	Codon position												
	First	Second	Third	All	First	Second	Third	All	First	Second	Third	All	Total
Number parsimony-informative	21	9	282	312	35	12	122	169	84	23	329	436	917
Number variable but parsimony-uninformative	14	9	38	61	14	15	7	36	40	38	50	128	225
Number constant	320	337	36	693	84	107	5	196	297	359	42	698	1587
Total characters	355	355	356	1066	133	134	134	401	421	420	421	1262	2729

pairwise 'p' distance for MP trees generated for each data partition, to ascertain the extent of saturation, revealed that first positions for *wg* and *COI* showed weak saturation among the deeper level divergences, but not for *EF-1α* (Fig. 3). Third positions, however, were strongly saturated for *COI* but less so for the two nuclear genes. These findings suggested that *EF-1α* third positions were likely to contribute most of the phylogenetic signal in the combined data set. Addition of *wg* and *COI*, particularly first and second positions, was likely only to improve level of support of nodes, especially those towards the tips. Indeed, bootstrapped consensus cladograms generated for each data partition indicated substantial phylogenetic signal for *EF-1α*, moderate signal for *wg* but negligible hierarchic signal for the mitochondrial gene (Fig. 4A, B, C).

The single-gene analyses thus showed that the topologies generated by each data partition (Fig. 4A, B, C) were not in conflict with one another. Moreover, partitioned Bremer support of the combined data set under MP (Fig. 4D) revealed substantial congruence between the three genes for most nodes (Table 3). Negative interactions among the data partitions were evident in only seven nodes for *COI* and five nodes for *wg*. However, the magnitude of the conflicts was not large and, within the Aporiina, only three clades of interest were in conflict [*wg* for node 17 (*Delias* group); *COI* for node 20 (*Catasticta* group); *COI* for node 23]. Both the *EF-1α* and *wg* data partitions had a strong positive effect on tree structure, often with high partitioned Bremer support. Indeed, the positive interaction of these two genes compensated at all nodes for which *COI* was in conflict. The negative interactions of *COI* were probably due to higher levels of homoplasy as a result of mtDNA being saturated at third positions because of a high A-T bias (70% in our data set), with transversions becoming equally as likely as transitions (Fig. 3) resulting in loss of deep hierarchical signal (Fig. 4C).

Results for the combined analysis of the three genes are shown in Figure 4D and Table 3. Unweighted parsimony resulted in two equally most parsimonious trees. There was considerable phylogenetic structure, with most nodes being well resolved. The Aporiina were recovered as a well-supported monophyletic group (bootstrap 91%), with *Cepora*/*Prioneris* sister to the remaining taxa, which also formed a well-supported monophyletic group. Within the latter group, four major clades were evident, each with strong support (bootstrap 82–100%): (1) *M. agathina* + *M. bernice*; (2) *Aporia crataegi* + *Aporia* (*Metaporia*) *agathon*; (3) the *Delias* group; and (4) the *Catasticta* group. The *Catasticta* group consisted of eight genera with the following well structured topology: *Melete* + ((*Pereute* + *Leodonta*) + *Neophasia* + (*Eucheira* + (*Catasticta cerberus* + (*Catasticta teutila* + (*Archonias* + *Charonias*))))). However, relationships among these four major lineages were not well resolved. The topology suggested that *Mylothris* was sister to the three other groups, and that the *Delias* and *Catasticta* groups were sister taxa, both of which were sister to *Aporia*, but there was no evidence in support of these relationships. Of the five genera for which two exemplar species were included, *Mylothris*, *Aporia*, *Delias*, and *Leuciacria* were monophyletic, but *Catasticta* was not (Fig. 4D).

Various weighting schemes were explored, particularly for the *COI* partition, in an attempt to resolve the polytomy between the four clades noted above, but these analyses produced trees identical in topology to that of the unweighted analysis shown in Figure 4D. Although down weighting *COI* third positions (1 : 5) gave increased support for the monophyly of *Mylothris* + *Aporia* + *Delias* group + *Catasticta* group (bootstrap 97%), the nodes uniting these clades

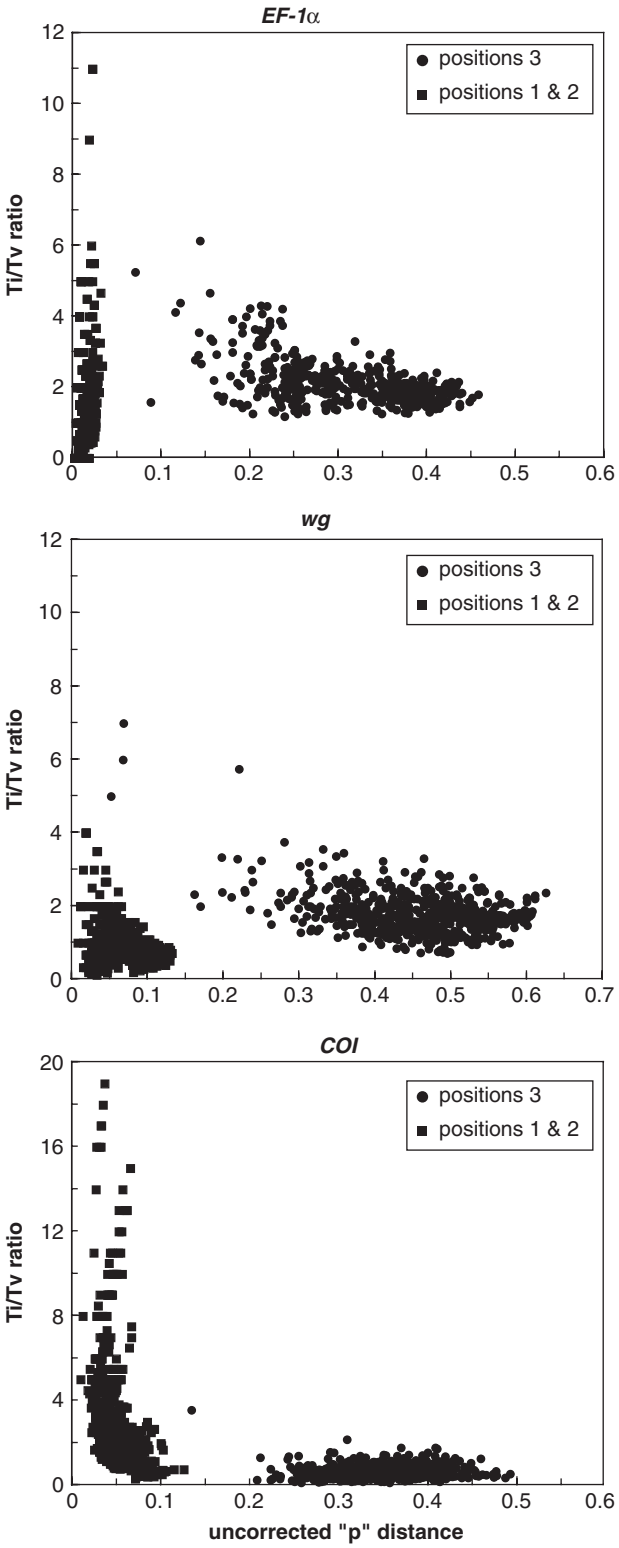

Figure 3. Saturation plots showing the relationship between transition (Ti)/transversion ratio (Tv) and uncorrected pairwise distance for third codon positions, and first and second positions, inferred under maximum parsimony for the three genes (*EF-1α*, *wg*, *COI*) for the Aporiina.

showed no greater support (Fig. 4D). Down weighting or removing all positions or third positions of *COI* consistently placed *Mylothris* sister to *Aporia* + *Delias* group + *Catasticta* group, but support for the arrangement was not strong (bootstrap 56–58%). Down weighting *COI* third positions (1 : 5) gave increased support for the monophyly of (and the basal nodes within) the *Catasticta* group.

Our ML and Bayesian (BI) trees (Fig. 5) of the combined data set essentially gave the same topology as that inferred under MP. Monophyly of the subtribe was well supported (bootstrap 97% ML, 100% BI), as was monophyly of the clade containing four major lineages (i.e. *Mylothris*, *Aporia*, *Delias* group, *Catasticta* group) (bootstrap 94% ML, 100% BI). However, although there was strong support for monophyly of each lineage (bootstrap 95–100% ML, 100% BI), relationships among these were not well resolved. Again, the topology suggested that *Mylothris* was the sister taxon to the three other groups, but evidence in support of monophyly of *Aporia* + *Delias* group + *Catasticta* group was weak (bootstrap < 50% ML, 72% BL). Within the *Catasticta* group, the topology was similar to that recovered under MP, except *Neophasia* and *Eucheira* were recovered as sister taxa (bootstrap 66% ML, 79% BI).

To understand why relationships between *Aporia*, the *Delias* group, and the *Catasticta* group were so poorly resolved, we examined the characters and branch lengths in our ML tree (Fig. 5A). The branch lengths subtending the basal nodes of the clade *Catasticta* group + (*Delias* group + *Aporia*) were extremely short, comprising 13 and ten synapomorphies, respectively. Moreover, none of these synapomorphies were uniquely derived (i.e. consistency index = 1). We also compared bootstrap support among the basal and terminal nodes for third positions, and for first and second positions combined, under MP. Bootstrap analyses of third positions for the combined data set under MP (tree not shown) revealed strong support for most nodes at the tips (bootstrap 80–100%), as well as for some of the basal nodes, particularly monophyly of the Aporiina and the sister relationship between the Aporiina and Pierina (i.e. *Pieris* + *Pieriballia*) (bootstrap 70–79%), despite saturation among the deeper divergences. However, *Aporia*, the *Delias* group and the *Catasticta* group comprised an unresolved polytomy. A similar pattern was found when first and second positions for the combined data set were analysed (tree not shown). Support at the tips was high (bootstrap 77–100%), as was support at the base for monophyly of Aporiina + Pierini (bootstrap 83%). Monophyly of *Aporia* + *Delias* group + *Catasticta* group was also well supported (bootstrap 80%), but again relationships among these three taxa were not resolved. Taken together, these findings suggest that

Table 3. Total Bremer support and partitioned Bremer support for each gene for nodes in the single MP tree of the combined data set (Fig. 4D)

Node	Clade	Total	*EF-1α*	*wg*	*COI*
1	Pieridae	33	7.7	13	12.3
2	Coliadinae	15	12.5	0	2.5
3		6	3	–3	6
4		6	3	–3	6
5		3	0	0	3
6	Pseudopontiinae + Dismorphiinae	13	4	8	1
7	Dismorphiinae	25	7.2	14.2	3.5
8	Pierina + Aporiina	12	6	5	1
9	Pierina	23	10.8	7.5	4.7
10	Aporiina	8	3	2	3
11		1	0	–1	2
12		8	5	7	–4
13	*Mylothris*	92	33	35	24
14		2	0	6	–4
15	*Aporia**	16	7	3.5	5.5
16		2	0	6	–4
17	*Delias* group*	7	11	–4	0
18	*Delias*	20	16	2	2
19	*Leuciacria*	51	23	27	1
20	*Catasticta* group*	9	1	9.5	–1.5
21		4	5	3.5	–4.5
22	*Pereute* + *Leodonta* *	48	19	16	13
23	*Neophasia* + *Eucheira* + *Catasticta* + *Archonias* + *Charonias* *	19	12.5	8.5	–2
24		4	3	0	1
25		5	10	–1.5	–3.5
26		6	0	4	2
27		11	1	2	8

*Clades of interest within the Aporiina.

Aporia, the *Delias* group and the *Catasticta* group may comprise a hard polytomy in the sense that they most likely represent rapid radiation, rather than a soft polytomy in which lack of data or multiple substitutions (homoplasy) are obscuring phylogenetic signal.

DISCUSSION

PHYLOGENY

The three methods of analysis (MP, ML, BI) of the combined data set recovered the subtribe Aporiina as a well supported monophyletic clade, with the lower taxa comprising six major subclades or lineages: *Cepora*, *Prioneris*, *Mylothris*, *Aporia*, *Delias* group, and *Catasticta* group. Our best estimate of the phylogenetic relationships of these lineages is summarized in Figure 6. We assume that *Aporia* (*Mesapia*), not included in this study, is closely related to *Aporia* (*Aporia*) or *Aporia* (*Metaporia*), and the three taxa comprise a monophyletic group. Monophyly of *Mylothris* + *Aporia* + *Delias* group + *Catasticta* group is well supported; these taxa also share a number of larval and adult morphological features, and the majority of species for which life histories are known feed as larvae on 'mistletoes' in the order Santalales. However, it is not certain whether *Cepora* and *Prioneris* form a monophyletic group sister to this clade, or represent two independent lineages that diverged early (and simultaneously) in the evolution of the Aporiina. *Cepora* and *Prioneris* both feed primarily on Brassicaceae (Brassicales) as larvae, and the pupae resemble each other morphologically more closely than they do the pupae of the four other subclades.

Klots (1933) was uncertain about the position of *Mylothris*, placing it at the end of his classification of the Pieridae in the belief that it represented an independent lineage isolated from (or at least sister to) the rest of the Pierini *s.l.* Our combined analyses, together with morphological and biological evidence of the early stages (Braby, 2005a), clearly show that *Mylothris* is closely related to *Aporia*, the *Delias* group and

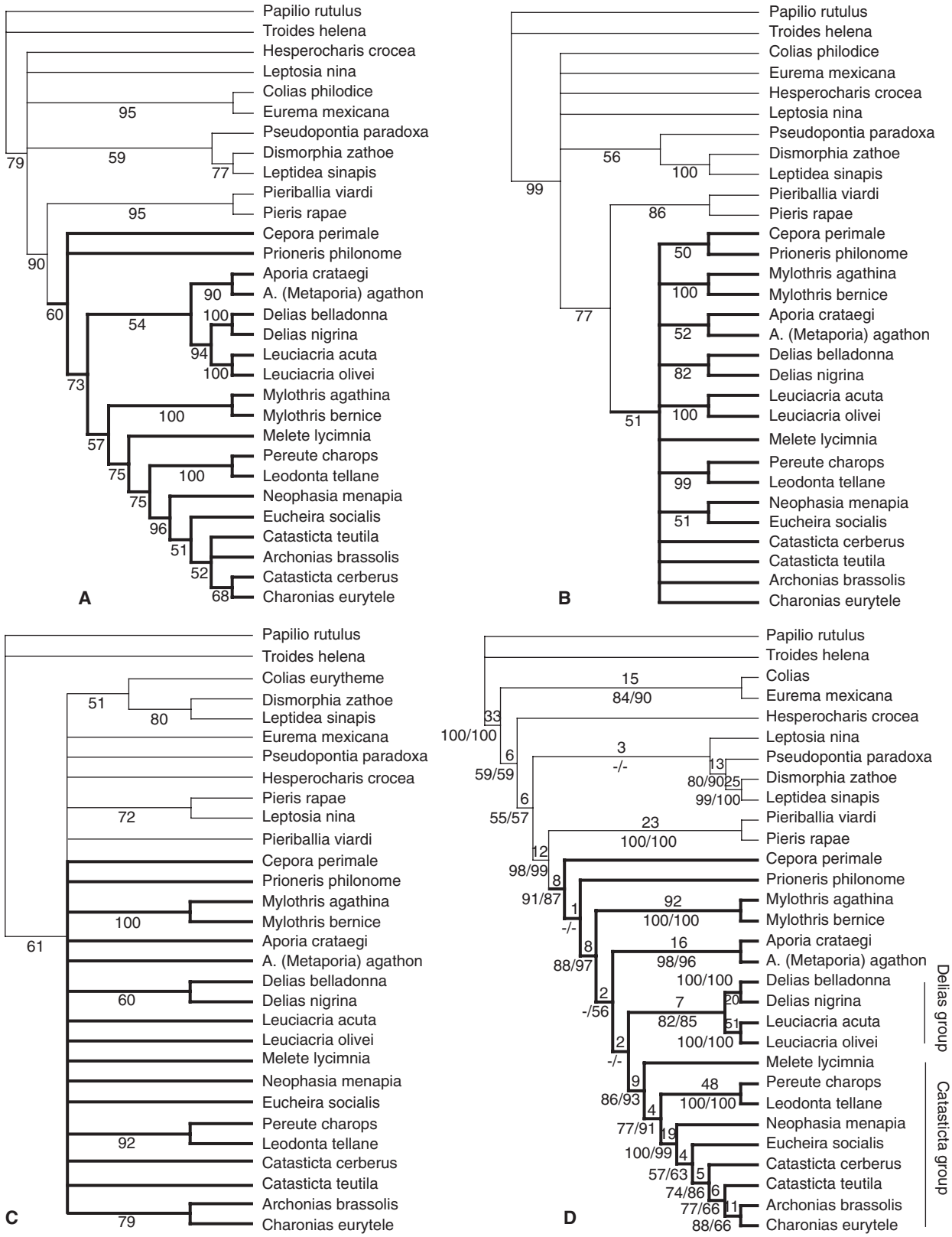

Figure 4. Phylogenetic trees for the Aporiina (taxa indicated by thick lines) inferred from equally weighted parsimony analysis for the three genes separately and in combination: A, strict consensus of three equally MP trees for *EF-1α* based on 1066 bp [312 informative characters; length 1468, consistency index (CI) = 0.404, retention index (RI) = 0.489]; B, strict consensus of three equally MP trees for *wg* based on 401 bp (169 informative characters; length 957, CI = 0.383, RI = 0.517); C, consensus of two equally MP trees for *COI* based on 1262 bp (436 informative characters; length 2584; CI = 0.341, RI = 0.308); D, one of two equally MP trees for the three genes combined based on 2729 bp (917 informative characters: length = 5093, CI = 0.358, RI = 0.399) (A, *Aporia*). Values below branches are bootstraps (1000 full heuristic search replicates, with up to 500 random additions); nodes with < 50% support are collapsed in the separate analyses. D, for the combined analysis, two bootstrap values are given: unweighted analysis and weighted analysis with *COI* third positions down weighted over first and second positions (1 : 5) (topology identical to unweighted analysis); values above branches are Total Bremer Support indices. *Papilio rutulus* and *Troides helena* (Papilionidae) are outgroup taxa.

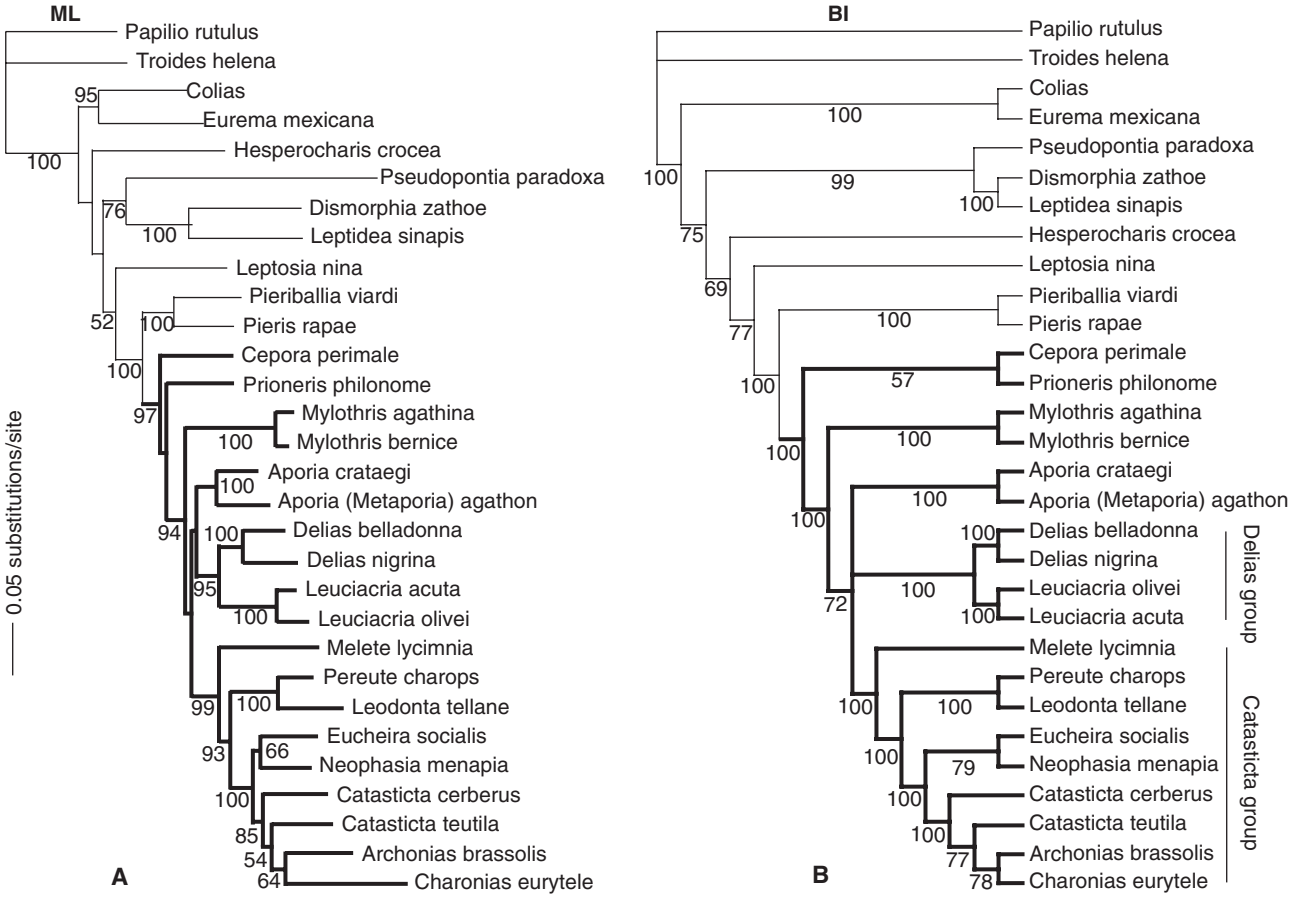

Figure 5. Phylogenetic trees for the Aporiina (taxa indicated by thick lines) based on the combined analysis of the three genes: A, maximum-likelihood (ML) tree according to GTR + I + Γ substitution model [log likelihood score = –25169.64; relative rate matrix A–C 2.4919, A–G 8.4701, A–T 5.9514, C–G 2.6362, C–T 16.1950, G–T 1.0; base frequencies: A = 0.2763, C = 0.2115, G = 0.1809, T = 0.3313; proportion of invariable sites (I) = 0.4772; shape parameter (α) of Gamma distribution (Γ) = 0.8313], with bootstrap values (100 full heuristic search replicates) shown below branches for nodes with ≥50% support; B, Bayesian inference (BI) tree (likelihood score: –23341.95), partitioned by gene and codon position (first and second; third); unlinked model is GTR + I + Γ for each partition at sampling temperature of 0.4; values below nodes are posterior branch supports estimated from majority rule consensus of 9000^2 trees (10^6 generations, 10^5 burned). *Papilio rutulus* and *Troides helena* (Papilionidae) are outgroup taxa.

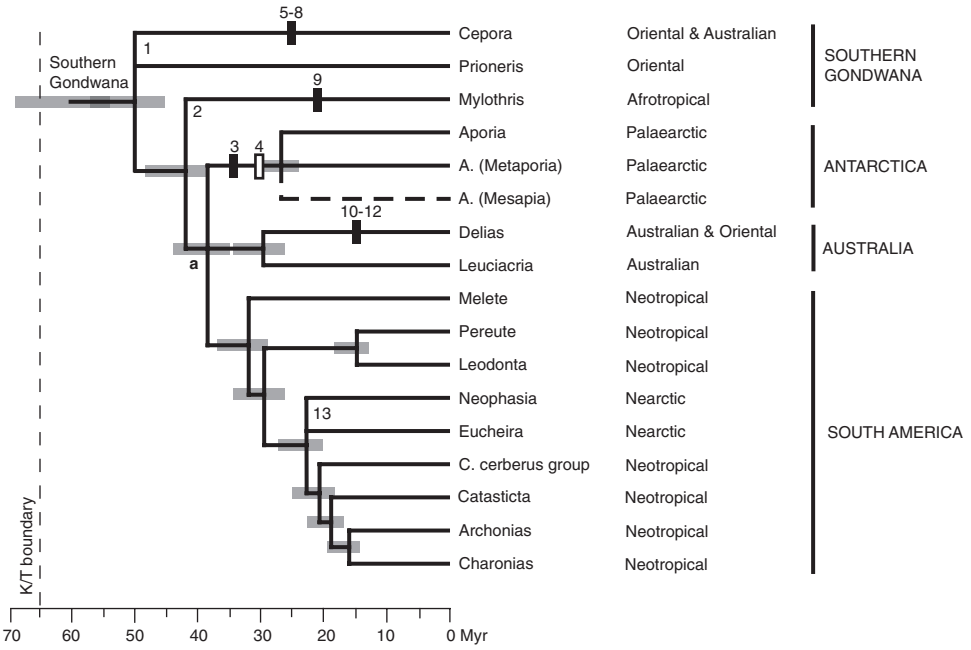

Figure 6. Phylogenetic hypothesis of the Aporiina according to the analysis of the combined data set in this study. Nodes which are well supported or consistently recovered under different methods of analysis (maximum parsimony, maximum likelihood, Bayesian inference) are shown (A., *Aporia*; C., *Catasticta*). The dashed line indicates putative relationship of *Aporia* (*Mesapia*). Divergence times and branch lengths have been corrected in the chronogram using semiparametric rate smoothing according to penalized likelihood, with age of the stem-group (61 Mya) and crown-group (50 Mya) of the Aporiina used as calibration points based on estimates extrapolated for the Pieridae (Braby *et al*., 2006). Error bars for each node (shaded rectangles) give 99.9% confidence interval based on four standard deviations of mean estimate for calibration points (69–54 Mya for stem-group, 57–45 Mya for crown-group). To the right of the chronogram are the broad zoogeographical regions of each taxon (for further details on geographical distribution, see Appendix). Also shown is historical biogeographical hypothesis according to an origin in southern Gondwana in the early Tertiary, with biogeographical steps optimized to reconcile area cladogram. Letter a designates vicariance event between *Delias* group (Australia), *Catasticta* group (South America), and *Aporia* (Antarctica). Numbers designate major biogeographical events: 1 = long-distance dispersal of the common ancestor of *Cepora* and *Prioneris* from southern Gondwana across the Indian Ocean to Greater India resulting in allopatric speciation; 2 = long-distance dispersal of the ancestor of *Mylothris* from southern Gondwana across the Indian Ocean to Madagascar resulting in allopatric speciation; 3 = long-distance dispersal of the ancestor of *Aporia* from Antarctica across the Indian Ocean to Eurasia (Himalaya); 4 = extinction of *Aporia* in Antarctica; 5 = dispersal (range expansion) of *Cepora* from South-eastern Asia across Wallacea to mainland New Guinea/Australia; 6–8 = dispersal (range expansion) of *Cepora perimale* from Australia/mainland New Guinea to New Caledonia and Vanuatu, from New Caledonia/Vanuatu to Fiji, and from Australia/New Caledonia to Norfolk Island; 9 = dispersal (range expansion) of *Mylothris* from Madagascar across Mozambique Channel to Africa; 10 = dispersal (range expansion) of *Delias* from Australia across Wallacea to Asia; 11 = dispersal (range expansion) of *Delias nysa* from Australia across the Coral Sea to New Caledonia and Vanuatu; 12 = dispersal of the ancestor of *Delias ellipsis* from Australia/mainland New Guinea across the Coral Sea to New Caledonia resulting in allopatric speciation; 13 = dispersal of the common ancestor of *Neophasia* and *Eucheira* from South to North America (montane central Mexico) resulting in allopatric speciation.

the *Catasticta* group. The three methods of analysis consistently placed *Mylothris* sister to these three taxa, although evidence for the monophyly of *Aporia* + *Delias* group + *Catasticta* group is weak. However, a test for monophyly of the latter clade, employing a topology dependent permutation tail probability test (T-PTP) with 1000 randomizations under MP in PAUP (Faith, 1991; Trueman, 1996), revealed significantly more evidence for monophyly of these three taxa than would be expected by chance alone ($P = 0.0048$). In other words, this test supports the topology of Figure 4D; hence, we conclude that *Mylothris* and (*Aporia* + *Delias* group + *Catasticta* group) are reciprocally monophyletic.

All three analytical methods were unable to resolve relationships among *Aporia*, the *Delias* group and the *Catasticta* group. Morinaka *et al*. (2002) concluded that *Delias* and *Aporia* were most closely related based on limited analysis of *EF-1α* sequences, but that study did not include *Leuciacria* or members of the

Catasticta group. Our MP tree of the combined data (Fig. 4D) placed the *Delias* and *Catasticta* groups as sister taxa, but without support. A T-PTP test for monophyly of *Delias* group + *Catasticta* group under MP again provided significantly more evidence in favour of this arrangement than would be expected by chance ($P = 0.008$). However, because the trees generated by the two other methods show somewhat contrasting patterns, we prefer to treat relationships between *Aporia*, the *Delias* group and the *Catasticta* group as an unresolved trichotomy, which we interpret as rapid radiation until further evidence is made available (Fig. 6).

The suspected close relationship between *Delias* and *Leuciacria* (Braby & Pierce, 2007; Braby *et al*., 2006) is confirmed in the present study, the two genera of which we have termed the *Delias* group. The phylogenetic position of *Leuciacria* has long remained uncertain. Klots (1933: 216) noted that various authors had noted resemblances of *Leuciacria* to *Elodina* and *Leptophobia*, but stated 'It is possible that there is such a relationship, but this possibility is not borne out by any characters other than superficial ones. Neither the venation nor the genitalia of *Leuciacria* point out definite relationships of any sort, and it must for the present at least be regarded as a somewhat isolated genus'. The morphological study by Müller (1999) of the two known species of *Leuciacria* showed that the male genitalia are remarkably similar to *Delias* but quite distinct from *Elodina* Felder & Felder. Furthermore, C. J. Müller (pers. comm.) has observed that *Leuciacria* is sympatric with *Delias* in montane New Guinea, and that adult behaviour of the two genera is similar.

The *Catasticta* group comprises eight genera from the New World (predominantly South America), the monophyly of which is well supported. Klots (1933: 225) previously treated these taxa in three unrelated groups in his classification: one comprised *Catasticta*, *Archonias*, *Charonias*, *Neophasia*, and *Eucheira*, a second consisted of *Pereute* and *Leodonta*, whereas a third contained *Melete* Swainson but with the comment that 'The exact relationships of *Melete* are vague. Because of the form of the male genitalia the author considers it to be descended from some stock related to *Ascia*, but the matter is open to question'. Our combined analysis not only corroborates these three groupings (Table 3), but indicates that all of these genera are closely related and have descended from a common ancestor, a fact not appreciated in previous studies (Figs 1, 2). Within this clade, there is evidence to suggest that *Catasticta* itself is paraphyletic or polyphyletic, and that *Neophasia* and *Eucheira* are sister taxa (under ML and BI). However, a T-PTP test under MP indicated no support for the monophyly of *Neophasia* and *Eucheira* ($P = 0.287$). These two genera are shown in Figure 6 as an unresolved polytomy with *Catasticta* + (*Archonias* + *Charonias*).

In terms of the hypotheses put forward regarding the systematic relationships of *Delias*, our phylogeny estimate (Fig. 6) neither supports nor refutes prior hypotheses (Figs 1, 2). Our estimate essentially integrates the two opposing views expressed by Dixey (Fig. 1A) and J. N. Eliot (Fig. 2B) (except that these earlier hypotheses did not consider *Leuciacria*). That is, our study shows that *Delias* + *Leuciacria* is closely related to both *Aporia* and the *Catasticta* group in an unresolved trichotomy, but it is also closely related to *Mylothris*, and more distantly related to *Cepora* and *Prioneris*. Interestingly, Schultze-Rhonhof (1933), who studied and compared the early stages, noted similar connections between *Delias*, some members of the *Catasticta* group (i.e. *Melete*, *Pereute*, *Leodonta*, *Catasticta*) and *Mylothris*, but suggested that similarities in morphology between the three lineages were due to convergence rather than common ancestry. Such an integration of previous ideas has considerable bearing on the biogeographical relationships of the Aporiina, *Delias* in particular. Clearly, the evolutionary history of the *Delias* group in the Old World is far more complex than a simple sister relationship between taxa in Asia or South America, and a detailed biogeographical analysis of the Aporiina is required.

Historical biogeography

The estimated divergence times for each node, and their confidence intervals are shown as a chronogram for our best estimate of the phylogeny of the Aporiina (Fig. 6). The two mean calibration points of the Aporiina (61 Mya for stem-group, 50 Mya for crown-group) suggest the subtribe evolved and diversified during the early Tertiary (Palaeocene and Eocene, respectively). However, because the calibration points are minimum estimates based on extrapolations from fossils in which the nodes were fixed and not free to vary, the Aporiina almost certainly originated and radiated before the Palaeocene-Eocene. The upper estimates of the stem- and crown-groups of the Aporiina are 69 Mya and 57 Mya, respectively (Braby *et al*., 2006), suggesting the origin was more likely towards the end of the Late Cretaceous (Maastrichtian), with diversification commencing in the early Tertiary (Palaeocene) (Fig. 6). An origin of the Aporiina in Gondwana before fragmentation can be ruled out since rifting of the southern supercontinent commenced in the Late Jurassic (*c*. 160 Mya, with Africa being the first continental landmass to become completely detached by 100–90 Mya), well before the ancestor of the subtribe had evolved. An origin in remnant Gondwana (Madagascar–Greater India–Australia–Antarctica–South America) can also probably be ruled out because

Table 4. Area cladogram of the Aporiina reconciled with minimum number of assumptions for different vicariant biogeographical hypotheses of origin in the Late Cretaceous and early Tertiary

Biogeographical hypothesis	Minimum number of steps		
	Dispersals	Extinctions	Total
Partial Gondwana* (Late Cretaceous): Madagascar–Greater India–Australia–Antarctica–South America	9	0	9
Southern Gondwana (early Tertiary): Australia–Antarctica-South America	12	1	13
Laurasia (early Tertiary): Eurasia-North America	12	1	13

*Conventional hypothesis of Gondwanan break up assumes following area cladogram: (Africa + ((Madagascar + Greater India) + ((New Zealand + New Caledonia) + (Australia + (Antarctica + South America))))) (Sanmartín & Ronquist, 2004).

Madagascar and Greater India became detached and isolated from Gondwana during the Late Cretaceous. Thus, given a period of differentiation of the Aporiina in the early Tertiary, two possible vicariant hypotheses are: (1) an origin in southern Gondwana (Australia–Antarctica–South America) or (2) an origin in Laurasia (Eurasia–North America) because both supercontinents were still partly intact at this time.

The broad zoogeographical regions of each taxon are summarized on our best estimate of the phylogeny of the Aporiina to produce a taxon-area cladogram (Fig. 6). Further detailed information on geographical distribution, habitat and phylogenetic diversity of each taxon are given in the Appendix. It is clear that the Aporiina have attained worldwide distribution, with endemic taxa occurring in all of the major zoogeographical regions. However, apart from *Cepora* and *Delias*, both of which are widely distributed in the Australian and Oriental Regions, most clades/genera are restricted to areas of endemism, and the Aporiina have a disjunct distribution. Only two taxa at the generic level (*Eucheira*, *Leuciacria*) show relictual patterns in that they have small areas of distribution and low numbers of species. *Eucheira* Westwood contains a single species restricted to montane western and northern Mexico (Northern Hemisphere), whereas *Leuciacria* contains two species restricted to montane mainland New Guinea and New Ireland (Southern Hemisphere). Apart from *Cepora*, which inhabit tropical lowlands, all taxa, as a general rule, are limited to high altitudes where they occur in montane habitats, often evergreen or cloud forest. Thus, the patterns of distribution do not provide any obvious clues regarding the subtribes' geographical origin, other than the possibility that adaptation to cool temperate climate is an ancestral trait, suggesting the Aporiina probably evolved during a period of global cooling or in high latitudes towards the end of the Late Cretaceous. The climate throughout the Late Cretaceous was warm and moist, although the Cretaceous/Tertiary boundary was characterized by a marked cooling event (White, 1994), which interestingly coincides with the estimated time of origin of the stem-group (69–61 Mya for upper limit and mean).

The two different historical vicariant biogeographical hypotheses in the Southern Hemisphere (i.e. southern Gondwana; 'southern vicariance hypothesis') and Northern Hemisphere (i.e. Laurasia; 'northern dispersal hypothesis') are compared numerically in Table 4 in terms of the minimum number of assumptions or biogeographical steps (i.e. dispersal, extinction events) required to reconcile the area cladogram under DIVA. From Table 4, it can be seen that both hypotheses are equally parsimonious with at least 13 steps. Other biogeographical hypotheses, such as an origin in Africa or Greater India in the early Tertiary, are far less parsimonious (not shown). An origin in remnant Gondwana after the rifting of Africa, New Zealand, and New Caledonia (i.e. 'Indogondwanan hypothesis') requires fewer steps (at least nine) but, because this hypothesis requires an origin/radiation in the Late Cretaceous, it is considered to be temporally incongruent, unless our age estimates for the Aporiina are severely underestimated. The two contrasting biogeographical hypotheses, southern Gondwana and Laurasia, are depicted in Figures 6, 7. We discuss the events and merits of each hypothesis in turn.

Southern Gondwana

An origin of the Aporiina in southern Gondwana (Fig. 6) would require at least one vicariance event (step a), three long-distance dispersal events (steps 1–3) frequently leading to allopatric speciation, and one extinction event (step 4). This scenario would also require nine dispersal events (steps 5–13), mostly involving range expansion.

We assume the vicariance event (step a) giving rise to the *Delias* group (Australia), the *Catasticta* group (South America), and *Aporia* (Antarctica) occurred when southern Gondwana broke up into three relatively isolated landmasses in the mid Tertiary. Our chronogram (Fig. 6) suggests this event occurred in

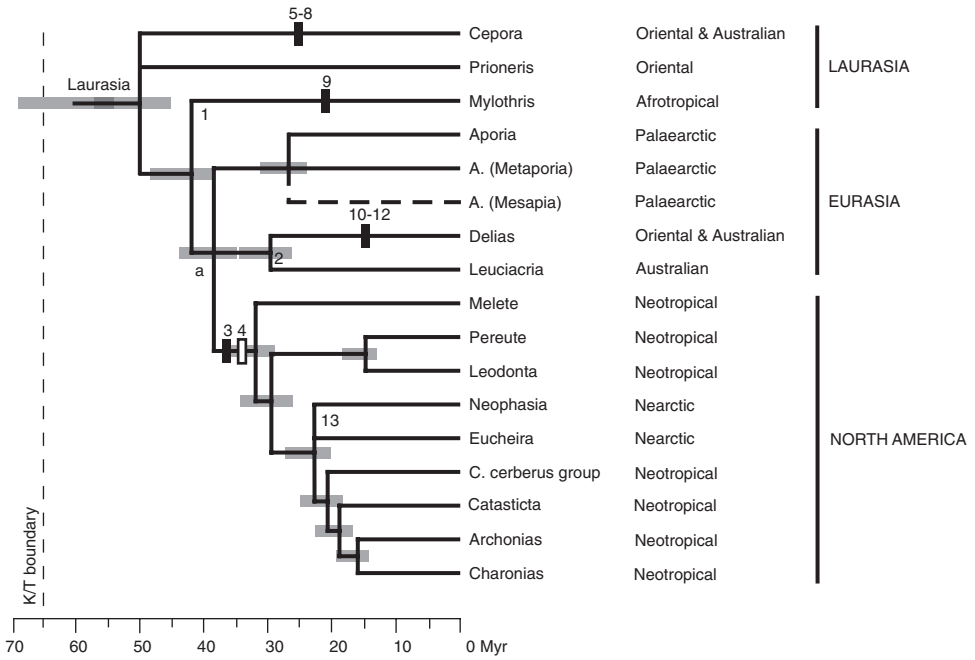

Figure 7. Chronogram of Fig. 6 showing alternative equally parsimonious historical biogeographical hypothesis according to an origin in Laurasia in the early Tertiary, with biogeographical steps optimized to reconcile area cladogram. Letter a designates vicariance event between *Aporia* + *Delias* group (Eurasia) and *Catasticta* group (North America). Numbers designate major biogeographical events: 1 = long-distance dispersal of the ancestor of *Mylothris* from Laurasia across the Mediterranean Sea to Africa resulting in allopatric speciation; 2 = long-distance dispersal of the ancestor of *Delias* group from Eurasia across the Indian Ocean to Australia resulting in allopatric speciation of *Leuciacria* in mainland New Guinea; 3 = dispersal (range expansion) of the ancestor of *Catasticta* group from North to South America; 4 = extinction of the ancestor of *Catasticta* group in North America. Steps 5–13 as per Fig. 6, except the direction of dispersal is from Africa to Madagascar for step 9 and from Asia to Australia for step 10.

the Eocene (38 Mya, 99.9% CI = 44–35 Mya), an estimate that closely agrees with the time when both Australia and South America finally broke away from Gondwana (Antarctica) (*c*. 35–32 Mya) (McLoughlin, 2001; Crisp, Cook & Steane, 2004). Such a scenario would explain the putative radiation between these three lineages.

The three long-distance dispersal events include: (1) the common ancestor of *Cepora* and *Prioneris* from southern Gondwana across the Indian Ocean to Greater India resulting in allopatric speciation; (2) the ancestor of *Mylothris* from southern Gondwana across the Indian Ocean to Madagascar leading to allopatric speciation; and (3) the ancestor of *Aporia* from southern Gondwana (Antarctica) across the Indian Ocean to Eurasia (Himalaya), followed by extinction of *Aporia* in Antarctica (step 4).

The estimated time frame for the early differentiation of *Cepora* and *Prioneris* (step 1) is during the early Eocene (50 Mya, 99.9% CI = 57–45 Mya) (Fig. 6). Although Greater India is commonly believed to have broken away from Gondwana (Antarctica) well before both Australia and South America were completely detached (Scotese, 2001; Sanmartín & Ronquist, 2004), uncertainties exist regarding the time when the terrestrial fauna of Greater India became separated from the rest of Gondwana as the landmass drifted northwards (Hay *et al*., 1999; Cracraft, 2001; Raxworthy, Forstner & Nussbaum, 2002). It is possible that the common ancestor of *Cepora* and *Prioneris* dispersed from southern Gondwana to Greater India during the northward drift of the subcontinent before the landmass collided with Eurasia in the Eocene (*c*. 50–45 Mya) (Hall, 1998; Cox & Moore, 2000; Briggs, 2003; Sanmartín & Ronquist, 2004) and then differentiated allopatrically. Such dispersal may have been facilitated by the now largely submerged Kerguélen Plateau in the Indian Ocean, which possibly served as an extensive but temporary land connection above water between Greater India and Antarctica (Sampson *et al*., 1998; Krause *et al*., 1999; Krause, 2001). Following the collision and accretion of Greater India with Eurasia, we assume *Cepora* and *Prioneris* then spread out of the Indian subcontinent into Asia. From Asia, they spread into South-eastern Asia via the Sunda Shelf when the sea level was lower and land connec-

tions existed between the Malay Peninsula and the islands of Sundaland, such as during the Pleistocene glaciations (Voris, 2000). *Cepora* then dispersed from south-eastern Asia across Wallacea at least once to reach New Guinea and Australia (step 5) and the islands of the south-west Pacific (steps 6–8), probably quite recently in the late Tertiary. However, because three of four species groups of *Cepora* (i.e. *perimale*, *nadina*, *aspasia*) occur in the Australian region (Appendix), the genus may have dispersed multiple times across Wallacea.

The estimated time of divergence of *Mylothris* (step 2) is during the late Eocene (42 Mya, 99.9% CI = 48–38 Mya) (Fig. 6), well after the fragmentation of both Africa and Madagascar from Gondwana. Isolation of these two landmasses took place in the late Cretaceous, with Madagascar separating from Greater India approximately 84 Mya with the opening of the Mascarene Basin (Cracraft, 2001; Briggs, 2003; Sanmartín & Ronquist, 2004). *Mylothris* must have therefore reached Madagascar from southern Gondwana by long-distance dispersal across the southern Indian Ocean (possibly facilitated by the Kerguélen Plateau), rather than by vicariance, and then differentiated allopatrically. We assume *Mylothris* then dispersed at least once from Madagascar across the Mozambique Channel to Africa (step 9), where it subsequently radiated in the late Tertiary. A species-level molecular phylogeny of *Mylothris* may provide additional evidence to investigate this 'out-of-Madagascar' hypothesis, and would help determine the timing of the dispersal event to Africa. *Mylothris* is currently divided into two species groups (*chloris*, *trimenia*), both of which occur in Madagascar and Africa (Appendix); hence, there may have been two dispersal events across the Mozambique Channel.

The estimated time of divergence of *Aporia* is during the Eocene (38 Mya, 99.9% CI = 44–35 Mya) (Fig. 6). Following the final separation of Australia and South America from Antarctica in the mid Tertiary, a substantial seaway developed and a deepwater circumpolar current became established around Antarctica. This circumpolar current steepened the latitudinal temperature gradient, which initiated the glaciation of Antarctica and concomitant global cooling in the late Eocene and early Oligocene (34–30 Mya) (Barlow, 1981; White, 1994; Cox & Moore, 2000). Once an ice cap developed over Antarctica, most of the terrestrial biota died out. Thus, if the ancestor of *Aporia* dispersed from Antarctica across the Indian Ocean to Eurasia, this event must have occurred sometime between 44/35 Mya and 34/30 Mya (step 3). That is, dispersal occurred after the estimated time of origin but before conditions became too severe and the taxon became extinct in Antarctica (step 4). Such long-distance dispersal may have possibly occurred when the climate was substantially cooler so that the sea level was lower, thereby facilitating island hoping via Greater India. However, the distance involved would have been formidable to say the least, and this event is a major weakness in the southern vicariance hypothesis. The alternative explanations are that our age estimations are too conservative, or that *Aporia* differentiated on another Gondwanan fragment. Whatever the true course of events of *Aporia*, its restricted occurrence in the Palaearctic does not fit well with the Southern Hemisphere model.

The four other dispersal events (steps 10–13) are more easily reconciled. *Delias* has dispersed at least once across Wallacea to reach Asia via Sundaland during or soon after the Miocene (step 10), but also to the islands of the South Pacific (steps 11, 12) (Fig. 6). A phylogenetic and historical biogeographical analysis of the 24 species groups of *Delias* plus *Leuciacria* (Braby & Pierce, 2007) suggests the most parsimonious reconstruction is an origin of the *Delias* group in the Australian Region, but with at least seven dispersal events across Wallacea to the Oriental Region. The presence of two species of *Delias* on New Caledonia, each representing different species-groups, is interpreted to represent recent independent dispersal events from Australia/New Guinea (steps 11, 12). Holloway (1974b, 1979) and Holloway & Peters (1976) reached the same conclusion and suggested that the ancestor of *Delias ellipsis* (which is endemic to the island) dispersed in the late Miocene from New Guinea to New Caledonia where it subsequently differentiated.

The common ancestor of *Neophasia* and *Eucheira* dispersed from South to North America (montane central Mexico) where it subsequently differentiated allopatrically in the Oligocene (23 Mya, 99.9% CI = 27–20 Mya) (step 13) (Fig. 6). From Central America, *Neophasia* then spread into much of western North America. An additional biogeographical step is also required for the fossil taxon *Oligodonta florissantensis* Brown from the Florissant Shale deposits (late Eocene, 34 Mya) of North America (Colorado, USA). This species belongs to the *Catasticta* group, and closely resembles *Leodonta* and *Catasticta* (Brown, 1976). According to our phylogeny (Fig. 6), *Oligodonta* is either the immediate common ancestor (or sister taxon) of *Pereute* + *Leodonta* or *Neophasia* + *Eucheira* + (*Catasticta* + (*Archonias* + *Charonias*)). Therefore, an origin of the *Catasticta* group in southern Gondwana (South America) requires at least one step to account for the presence of *Oligodonta* in North America: dispersal of the ancestor of *Oligodonta* from North to South America resulting in allopatric speciation. This event may have occurred during the mid Tertiary (> 34 Mya) when there was a temporary land bridge between North and South America that is believed to

have facilitated the general movement of butterflies between the two continents (Shields & Dvorak, 1979; Miller & Brown, 1989; Viloria, 2003; Hall, Robbins & Harvey, 2004).

Further differentiation of *Aporia* (into *Metaporia* and *Mesapia*), the *Delias* group (into *Leuciacria* and *Delias*), and the *Catasticta* group (into eight genera) in the Himalaya, Australia, and South America, respectively, presumably represent duplication events that occurred over a relatively long period during the mid Tertiary within each area of endemism as a consequence of sympatric speciation or local spatial heterogeneity leading to allopatry.

Laurasia

An origin of the Aporiina in Laurasia (Fig. 7) would require at least one vicariance event (step a), two long-distance dispersal events leading to allopatric speciation (steps 1, 2), and one extinction event (step 4). This scenario would also require nine dispersal events (steps 5–13), all of which are similar to those in the Southern Hemisphere model (Fig. 6).

Vicariance between *Aporia* + *Delias* group (Eurasia) and the *Catasticta* group (North America) (step a), estimated to have occurred in the Eocene (38 Mya, 99.9% CI = 44–35 Mya), partly explains the presence of these lineages in the Northern Hemisphere (Fig. 7). The estimated time of speciation coincides well with the time when the connection between Europe and North America was severed following the final separation of Greenland at the end of the Eocene (Cox & Moore, 2000). However, vicariance does not satisfactorily explain the origin of the *Delias* group in Asia, which differentiated simultaneously with *Aporia* and the *Catasticta* group. The only possible explanation for such a pattern under vicariance is that there was spatial heterogeneity between the European and Asian blocks of Eurasia such that differentiation of the *Delias* group (Asia) occurred around the same time that the ancestors of *Aporia* (Europe) and the *Catasticta* group (North America) separated.

Three dispersal events that occurred relatively early in the differentiation of the Aporiina include: (1) long-distance dispersal of the ancestor of *Mylothris* from Laurasia across the Mediterranean Sea to Africa resulting in allopatric speciation; (2) long-distance dispersal of the ancestor of the *Delias* group from Eurasia across the Indian Ocean to Australia resulting in allopatric speciation of *Leuciacria* in mainland New Guinea; and (3) dispersal (range expansion) of the ancestor of the *Catasticta* group from North to South America, followed by extinction of the *Catasticta* group in North America (step 4).

Long-distance dispersal of the ancestor of *Mylothris* from Laurasia across the Mediterranean Sea to northern Africa (step 1) in the mid Tertiary (42 Mya, 99.9% CI = 48–38 Mya) (Fig. 7) would have been possible because of the proximity of the African continent to southern Eurasia at that time. Although land connections between Africa and Eurasia did not arise until the Miocene, Africa probably acted as a giant stepping-stone for the dispersal of elements from western Gondwana to tropical Asia, and as a sink for the dispersal of biota from Asia/North America throughout much of the Tertiary (Cox & Moore, 2000). The other dispersal event in *Mylothris* (step 9) is similar to that in the Southern Hemisphere model, except it is in the opposite direction.

The estimated time of divergence of *Leuciacria* (step 2), the only taxon endemic to the Australian Region, is during the Oligocene (30 Mya, 99.9% CI = 35–27 Mya) (Fig. 7). Compared with the species rich sister genus *Delias*, *Leuciacria* is a relictual taxon confined to the highlands of montane mainland New Guinea (Papua, Papua New Guinea) and New Ireland. The restricted presence of *Leuciacria* in these montane areas is difficult to explain under a scenario of long-distance dispersal from Eurasia across the Indian Ocean given a probable age of differentiation in the early Oligocene (with an upper estimate in the late Eocene) because opportunities for dispersal (i.e. emergent land in Wallacea) were not available between the Oriental and Australian Zoogeographical Regions at that time (Hall, 1998; de Jong, 2001). Moreover, in the Oligocene, most if not all of New Guinea was still submerged. Southern New Guinea forms part of the northern margin of the Australian plate, but emergent land was not available for colonization until about the mid Miocene (*c.* 15 Mya), and major uplifting giving rise to New Guinea's central mountain ranges did not begin until the Pliocene (5–2 Mya) (de Boer, 1995; Hall, 1998; Parsons, 1998; Cox & Moore, 2000). The alternative explanation, that the ancestor of *Leuciacria* originated in Eurasia, dispersed from southeastern Asia across Wallacea to New Guinea during the mid- to late Miocene, and then became extinct in Asia, is possible but less parsimonious as it requires an extra step. The three other dispersal events in the *Delias* group (steps 10–12) are similar to those in the Southern Hemisphere model, except dispersal is in the opposite direction for step 10.

The most parsimonious explanation to account for the *Catasticta* group in South America is dispersal (range expansion) of its ancestor from North to South America (step 3), estimated to have occurred during the late Eocene–early Oligocene, followed by extinction of the ancestral population in North America (step 4) (Fig. 7). The common ancestor of *Neophasia* and *Eucheira* then dispersed back to North America and differentiated allopatrically in montane central Mexico sometime later in the late Oligocene–early Miocene (step 13). The ancestor of the fossil taxon *Oli-*

godonta either dispersed from South to North America and speciated allopatrically (one step required) or originated in North America (no step required) depending upon its phylogenetic position. If *Oligodonta* proves to be the sister taxon of the *Catasticta* group, rather than the ancestor of *Pereute* + *Leodonta* or *Neophasia* + *Eucheira* + *Catasticta* + *Archonias* + *Charonias*, then an origin in North America is the most parsimonious explanation.

CONCLUSIONS

The Aporiina are worldwide in distribution with endemic taxa (genera) occurring in all the major zoogeographical regions. The subtribe consists of six major lineages, with the *Delias* group (two genera, predominantly from the Australian Region) closely related to both *Aporia* (three subgenera, predominantly from the Himalaya of the Palaearctic Region) and the *Catasticta* group (eight genera from the New World, but predominantly from the Neoropical Region) in an unresolved trichotomy. The subclade *Aporia* + *Delias* group + *Catasticta* group is sister to *Mylothris* (from Madagascar and Africa of the Afrotropical Region), and this clade is closely related to *Cepora* and *Prioneris* (mainly from Asia of the Oriental Region). In this sense, our phylogeny estimate neither supports nor refutes prior hypotheses regarding the systematic and biogeographical relationships of *Delias*, but essentially integrates two major opposing views (Figs 1, 2) into a new combined hypothesis (Fig. 6). However, in this new phylogenetic hypothesis, we show that the closest relative of *Delias* is *Leuciacria*.

Given this phylogenetic estimate of the Aporiina, their diversification in the early Tertiary, and present-day spatial distribution, we demonstrate that two equally most parsimonious biogeographical reconstructions (southern Gondwana, Laurasia) can account for their distributions, with dispersal playing a significant role in shaping the underlying phylogenetic pattern regardless of the ancestral area of origin (Figs 6, 7). Indeed, speciation of several of the basal nodes appears to have been driven more by dispersal than by vicariance. If the currently recognized species groups of several genera (*Cepora*, *Mylothris*, *Delias*) are added to the cost matrix, a total minimum of 22 dispersal events is required to explain the extant distribution of the Aporiina in either biogeographical hypothesis. de Queiroz (2005) has argued that long-distance dispersal is probably more frequent in a wide variety of taxa than has previously been realized, particularly among plants (Sanmartín & Ronquist, 2004; Cook & Crisp, 2005; but see also Heads, 2005). Although dispersal almost certainly occurs to some extent in the Pieridae (many species are migratory), long-distance (jump) dispersal across wide ocean barriers probably does not occur in the Aporiina given their ecological specialization and general restricted occurrence to cool temperate montane environments. Nevertheless, dispersal between zoogeographical regions has occurred frequently in the past in this group of butterflies. Such dispersal events probably occurred during periods of global cooling when sea levels were lower and temporary land bridges or stepping-stones were available between the major landmasses.

Each biogeographical hypothesis of origin has one serious anomaly that does not satisfactorily explain the present-day distribution of the Aporiina, although the southern vicariance hypothesis appears more likely. A major weakness of the northern dispersal hypothesis is that it does not readily explain the presence of *Leuciacria* in the Australian Region (New Guinea) and its early differentiation from *Delias* in the late Eocene–early Oligocene. Movement from Asia to Australia at that time would have involved dispersal across a formidable water barrier (Indian Ocean), an event that we consider most unlikely. Moreover, vicariance of Laurasia does not readily explain the trichotomy between *Aporia*, *Delias* group, and *Catasticta* group. On the other hand, a major weakness of the southern vicariance hypothesis is that it does not satisfactorily explain the presence of *Aporia* in the Palaearctic, particularly the Himalaya. If *Aporia* did differentiate vicariantly on a major landmass of southern Gondwana, such as Antarctica, at the same time when the *Delias* group (Australia) and the *Catasticta* group (South America) also became isolated in the Eocene, it is by no means clear how the taxon dispersed across the Indian Ocean to reach its present-day position in the Himalaya. The most likely route was via Greater India, or across a series of stepping-stones to Greater India before it was too far out of reach and had not yet collided with Asia, but this would imply an origin of the ancestor of *Aporia* closer to the K/T boundary, at least 20 Mya before our minimum estimation of 44–35 Mya based on likelihood models of molecular substitution. An earlier origin of the Aporiina is possible, particularly because estimations based on sequence divergences frequently vary due to differences in data sets, calibration points and inference methods (Sanderson *et al*., 2004), but additional (older) fossils are needed to estimate the age of the Pieridae more accurately. As noted by Heads (2005), the break up of Gondwana was a vicariance process taking millions of years, and involved complex processes of uplift, subsidence, vulcanism, erosion, terrane accretion, etc. As the continents gradually drifted apart, connections between them probably became a filter route that was gradually more difficult to cross with time. Hence, some taxa may well have

crossed relatively narrow ocean gaps to landmasses, such as Greater India, after their severance from Gondwana.

Rejecting one hypothesis over the other may only be resolved by reconstructing species-level phylogenies of many of the larger genera/clades. Such shallow-level phylogenies may indicate the directionality of the dispersal events implicit in each hypothesis. For example, for the Southern Hemisphere model, species-level phylogenies are needed to test the 'out-of-Madagascar' hypothesis (*Mylothris* to Africa), the 'out-of-Greater India' hypothesis (*Cepora* to Asia/Australia/Pacific islands; *Prioneris* to Asia), and the 'out-of-Australia' hypothesis (*Delias* to Asia). To date, detailed species-level phylogenies are available only for the *Delias* group (Braby & Pierce, 2007). This phylogeny supports an origin of *Delias* + *Leuciacria* in the Australian Region in the early Oligocene followed by several dispersal events across Wallacea to the Oriental Region as the most parsimonious biogeographical reconstruction. We therefore tentatively conclude that the southern vicariance hypothesis is probably the more likely of the two possible biogeographical models of origin of the Aporiina.

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APPENDIX

GEOGRAPHICAL DISTRIBUTION, HABITAT AND PHYLOGENETIC DIVERSITY OF THE APORIINA

Cepora (20 species)

Cepora is widespread in the Oriental and Australian Regions and was provisionally divided by Yata (1985) into four species-groups (*nerrisa*, *perimale*, *nadina*, *aspasia*) according to differences in male and female genitalia and androconia. The genus is particularly rich in south-eastern Asia, especially Sulawesi (Yata, 1985; Vane-Wright & de Jong, 2003), but only three species (each representing different species groups) occur east of Wallacea in the Australian Region. One of these species, the highly dispersive *Cepora perimale* (Donovan), extends to Australia, New Caledonia, Vanuatu, Fiji and Norfolk Island (Parsons, 1998; Braby, 2000). Unlike other genera in the Aporiina, *Cepora* occurs predominantly in lowland areas where the adults fly close to the ground. Oceanic dispersal has clearly been an important factor in the distribution of this genus, especially the islands of the south-west Pacific. Assuming the species groups are monophyletic, the phylogenetic diversity and species richness in Asia suggests the genus originated in the Oriental Region.

Prioneris (seven species)

This small genus is endemic to the Oriental Region, occurring from northern India, Nepal, Sikkim, Burma, southern China, and Taiwan through the Malay Peninsula to Indonesia (Borneo, Java) (Yata, 1985; Corbet & Pendlebury, 1992), but is absent from the Philippines and Sulawesi. *Prioneris* is restricted to evergreen forest habitats in montane areas, generally between 500 m and 1400 m, but at lower altitudes than *Aporia* (Bell, 1912; Corbet & Pendlebury, 1992; O. Yata, pers. comm.).

Mylothris (57 species)

Mylothris is a large genus endemic to the Afrotropical Region (Ackery, Smith & Vane-Wright, 1995; Hecq, 2001; Collins, Larsen & Warren-Gash, 2003). van Son (1949) divided the genus into two species groups (*trimenia*, *chloris*) according to differences in wing colour and form of the valva of the male genitalia. The greatest concentration of species occurs in the region embraced by the Rift Valleys (i.e. eastern Congo, Uganda, western Kenya, western Tanzania), with a secondary peak in species richness in the mountains near the Gulf of Guinea (Cameroon) (Talbot, 1944). Very few species occur outside the African continent: three occur in Madagascar (Talbot, 1944; Ackery *et al*., 1995), all of which are endemic to the island, and another is limited to the south-western Arabian Peninsula (mountains of Asir and Yemen) (Larsen, 1984). The two species groups occur in both Madagascar and Africa. The vast majority of species are limited to cool-temperate montane evergreen forest, typically between 1000 m and 2000 m with at least one species occurring up to 3200 m (Talbot, 1944; Kielland, 1990; Larsen, 1991; Ackery *et al*., 1995); few species have adapted to lowland habitats (< 500 m).

Aporia (c. 25 species)

Aporia is restricted chiefly to the mountains and northern plateau of the Himalaya (Tibet, Kashmir, northern India, Nepal, Sikkim, Bhutan), at altitudes between 2000 m and 4500 m (Klots, 1933; Mani, 1986; D'Abrera, 1990; Smith, 1994). The genus is currently divided into three subgenera: *Aporia*, *Metaporia*, and *Mesapia*, all of which co-occur in the Himalaya, with *Aporia* (*Mesapia*) containing a single species (*Aporia peloria* Hewitson) restricted to the Himalaya (above 3900 m). A few species occur more widely in the Palaearctic (typically at lower elevations in the higher latitudes), and several occur in the Oriental Region adjacent to the Himalaya [mountains of north-eastern India, northern Myanmar (Burma) and southern China, but also Taiwan, above 1300 m] (D'Abrera, 1982; Igarashi & Fukuda, 1997; Robinson, Ackery, Kitching, Beccaloni & Hernández, 2001). Most species occur above the tree-line where they are cold-adapted to the high elevations (Mani, 1986).

Delias + Leuciacria (c. 250 species)

Delias is the largest genus in the Pieridae, with more than 250 recognized species (F. Gerrits & A. Yagishita, unpubl. data) divided into 24 species groups (Braby & Pierce, 2007). It occurs widely in the Oriental and Australian 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 eastern most limits on the Solomon Islands, Vanuatu, and New Caledonia (Talbot, 1928–37; Holloway & Peters, 1976; D'Abrera, 1990; Yagishita, Nakano & Morita, 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 (*Delias lativitta*, *Delias berinda*) reaching their western most 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 (*Delias ellipsis*) is endemic to the island, the other of which (*Delias nysa*) also occurs on Vanuatu and mainland Australia. The genus is absent from New Zealand and most of the smaller 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 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 even higher (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. By contrast, *Leuciacria* is endemic to the Australian region, containing two rare, poorly known and allopatric species restricted to mainland New Guinea (Papua New Guinea, Papua) and New Ireland (Müller, 1999, 2001). Similar to *Delias*, the species are limited to high altitude montane areas between 1200 and 2400 m (typically above 1800 m) (Parsons, 1998; Müller, 1999, 2001; Gotts & Pangemanan, 2001).

Melete (six species)

Melete is restricted largely to the Neotropcial region, with temporary incursions into the Nearctic. Most species occur in South America (Lamas, 2004). The breeding distribution extends from lowland southern Mexico (de la Maza, 1987; Salinas, Luis & Llorente, 2004) and the West Indies (Cuba, Haiti, Dominican Republic) (Riley, 1975; Smith, Miller & Miller, 1994) to Bolivia and Brazil (D'Abrera, 1981; Lamas, 2004). Only two species occur in Central America, one of which [*Melete salacia* (Godart)] is restricted to the West Indies. The other species [*Melete lycimnia* (Cramer)] has been regularly recorded as far north as Ciudad Victoria, Tamaulipas, in central eastern Mexico, and sporadically in the lower Rio Grande Valley of southern TX, USA. (Dauphin *et al*., 2005). Adults from the latter locality are believed to represent vagrants outside the breeding area, possibly during northern migration/dispersal from Mexico.

Pereute + Leodonta (14 species)

These two closely-related genera are restricted to the Neotropical Region (Lamas, 2004). Most species occur in cool montane cloud forests of the eastern slopes of the Andes of northern South America. *Pereute* extends from montane and lowland southern Mexico (de la Maza, 1987; Salinas *et al*., 2004) to Bolivia and Brazil (D'Abrera, 1981; Lamas, 2004). Only two species occur in Central America: one (*Pereute charops* Boisduval) is widespread in the region, as well as in northern South America, the other (*Pereute cheops* Staudinger), which is closely related to *P. charops*, is endemic to Panama and Costa Rica (DeVries, 1987). *Leodonta* is more limited in distribution, extending from Costa Rica (D'Abrera, 1981; DeVries, 1987; Robert, 1987) to Peru and Bolivia (Robert, 1987; Lamas, 2004). Only a single widely distributed species [*Leodonta tellane* (Hewitson)] reaches Central America (Costa Rica, Panama).

Neophasia + Eucheira (three species)

These two small genera are restricted to the Nearctic Region. *Neophasia* includes two allopatric species: one (*Neophasia menapia* Felder & Felder) occurs widely in montane areas (up to 2200 m) of western North America (western USA; southern British Columbia, Canada), extending to sea-level in the more northern, temperate areas of the range (Howe, 1975; Scott, 1986; D'Abrera, 1990; Layberry, Hall & Lafontaine, 1998); the other (*Neophasia terlooii* Behr) is limited to the mountains of south-western USA (south-eastern Arizona) (Scott, 1986; D'Abrera, 1990) and northern and central western Mexico (de la Maza, 1987), between 2000 m and 2300 m (Howe, 1975). *Eucheira* is

monobasic, containing a single species (*Eucheira socialis* Westwood) endemic to Mexico, where it occurs mainly in the cooler mountainous regions of central western Mexico and the Sierra Madre Occidental in the northern half of the country (between 2000 and 2700 m) (de la Maza, 1987; Kevan & Bye, 1991; Underwood, 1994; Fitzgerald & Underwood, 2000).

Catasticta + Archonias + Charonias (c. 100 species)
These three genera occur in the Neotropical Region. *Catasticta* is a very large genus, with more than 90 species currently recognized, whereas *Archonias* and *Charonias*, which comprise two small closely related genera, embrace a total of only three species (Lamas, 2004). *Catasticta* extends from the mountains of Mexico to Brazil, with highest species diversity in montane cloud forests of the eastern Andes of South America (Colombia, Ecuador, Peru, Bolivia) (D'Abrera, 1981; Lamas & Bollino, 2004); it ranges between 700 m and approximately 3900 m, but most species occur between 1200–2500 m (Röber, 1908–09; DeVries, 1987; Eitschberger & Racheli, 1998). In Central America, there are eight species (DeVries, 1987), of which three are widespread and extend to southern and/or central Mexico (de la Maza, 1987). The monobasic *Archonias*, containing the species *Archonias brassolis* (Fabricius), occurs from lowland southern Mexico (de la Maza, 1987; Salinas *et al*., 2004) to Bolivia and Brazil (Lamas, 2004). The two species of *Charonias* are allopatric: one [*Charonias eurytele* (Hewitson)] extends from lowland southern Mexico (de la Maza, 1987) to the eastern Andes of central Peru (Tingo María) (D'Abrera, 1981; G. Lamas, 2004; pers. comm.); the other [*Charonias theano* (Boisduval)] is restricted to southern Brazil (D'Abrera, 1981; Lamas, 2004). Both *Archonias* and *Charonias* generally occur at lower altitudes (low- to mid-elevation forests) than most species of *Catasticta* (DeVries, 1987).