PHYLOGENY AND BIOGEOGRAPHY OF THE APORIINA

MICHAEL F. BRABY1,2*, NAOMI E. PIERCE1 and ROGER VILA1†

1Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MS 02138, USA
2School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia

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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 Pan-

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INTRODUCTION

The Australian fauna is composed of several major biogeographical elements, reflecting different evolu-

*Corresponding author. Current address: Biodiversity Conservation Division, Department of Natural Resources, Environment and the Arts, PO Box 496, Palmerston NT 0831, Australia. E-mail: michael.braby@nt.gov.au
†Current address: Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Edifici C, Campus de la UAB, 08193 Cerdanyola del Vallès, Spain.
wana. This southern element may comprise two components or subelements: (1) relicual 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 eastern 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 Afro-gondwanan 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; Vitoria, 2003); and (3) an origin in Gondwana or remnant Gondwana (Madagascar–Greater India–Australia–Antarctica–South America), but with dispersals 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 Pangaeana 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 (Palaeartic 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 Dixia 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
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 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 + 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, Malay, 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

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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).
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 Leucacia 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 sub-tribe were not well resolved. Furthermore, a phylogenetic and biogeographical analysis of the 24 species groups of Delias plus Leucacia 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 relatives 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 & Shuqin, 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 Leuciarcia 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-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 telane) 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

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<th>GenBank Accession No.</th>
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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 for 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; Rodriguez 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 confidence interval (CI) = 99.9% 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 clock-like (δ = 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
pairwise ‘p’ distance for MP trees generated for each data partition, to ascertain the extent of saturation, revealed that first positions for \( \text{wg} \) and \( \text{COI} \) showed weak saturation among the deeper level divergences, but not for \( \text{EF-1} \alpha \) (Fig. 3). Third positions, however, were strongly saturated for \( \text{COI} \) but less so for the two nuclear genes. These findings suggested that \( \text{EF-1} \alpha \) third positions were likely to contribute most of the phylogenetic signal in the combined data set. Addition of \( \text{wg} \) and \( \text{COI} \), 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 \( \text{EF-1} \alpha \), moderate signal for \( \text{wg} \) 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 \( \text{COI} \) and five nodes for \( \text{wg} \). However, the magnitude of the conflicts was not large and, within the Aporiina, only three clades of interest were in conflict (\( \text{wg} \) for node 17 (\( \text{Delias} \) group); \( \text{COI} \) for node 20 (\( \text{Catasticta} \) group); \( \text{COI} \) for node 23). Both the \( \text{EF-1} \alpha \) and \( \text{wg} \) 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 \( \text{COI} \) was in conflict. The negative interactions of \( \text{COI} \) 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 transi-

<table>
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<th>Character summary for the combined data set, with numbers of sites for each codon position for each gene partition</th>
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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 \( \text{Cepora/Prioneris} \) 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) \( \text{M. agathina + M. bernice} \); (2) \( \text{Aporia crataegi + Aporia (Metaporia) agathon} \); (3) the \( \text{Delias} \) group; and (4) the \( \text{Catasticta} \) group. The \( \text{Catasticta} \) group consisted of eight genera with the following well structured topology: \( \text{Melete + ((Pereute + Leodonta) + Neophasia + (Eucheira + (Catasticta cerberus + (Catasticta teutila + (Archonias + Charonias))))}) \). However, relationships among these four major lineages were not well resolved. The topology suggested that \( \text{Mylothris} \) was sister to the three other groups, and that the \( \text{Delias} \) and \( \text{Catasticta} \) groups were sister taxa, both of which were sister to \( \text{Aporia} \), but there was no evidence in support of these relationships. Of the five genera for which two exemplar species were included, \( \text{Mylothris, Aporia, Delias, and Leuciacia} \) were monophyletic, but \( \text{Catasticta} \) was not (Fig. 4D).

Various weighting schemes were explored, particularly for the \( \text{COI} \) 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 \( \text{COI} \) third positions (1:5) gave increased support for the monophyly of \( \text{Mylothris + Aporia + Delias} \) group + \( \text{Catasticta} \) group (bootstrap 97%), the nodes uniting these clades

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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% BI). 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

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**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*. **© 2007 The Linnean Society of London, Biological Journal of the Linnean Society, 2007, 90, 413–440**
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

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*Clades of interest within the Aporiina.
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-1\(\alpha\) 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 wug 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 + \(\Gamma\) 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 (\(\alpha\)) of Gamma distribution (\(\Gamma\) = 0.8313), with bootstrap values (100 full heuristic search replicates) shown below branches for nodes with \(\geq\)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 + \(\Gamma\) 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.
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

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**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 Muller (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. Muller (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
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, Leuciaria) 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 Leuciaria 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

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**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**

<table>
<thead>
<tr>
<th>Biogeographical hypothesis</th>
<th>Minimum number of steps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dispersals</td>
</tr>
<tr>
<td>Partial Gondwana* (Late Cretaceous): Madagascar–Greater India–Australia–Antarctica–South America</td>
<td>9</td>
</tr>
<tr>
<td>Southern Gondwana (early Tertiary): Australia–Antarctica–South America</td>
<td>12</td>
</tr>
<tr>
<td>Laurasia (early Tertiary): Eurasia–North America</td>
<td>12</td>
</tr>
</tbody>
</table>

*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).
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 Kergülen 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 Kerguelen 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 elliptis (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 Neohasia 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, Neohasia then spread into much of western North America. An additional biogeographical step is also required for the fossil taxon Oligodonta florissanensis 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 Neohasia + Eucheira + (Catasticta + (Archonias + Charonia)). 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

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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 Metoparia 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 Oli
godonta 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 Neotropical 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 extent 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 (*Prioneris* 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* + *Leucacia* 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 APORINAE

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 Aporinae, 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’Abera, 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 Palearctic (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’Abera, 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 Papuan Highlands. Species occur on New Caledonia, one of which (Delias lativitta) has a weak representation in southeastern China (A. Yagishita, pers. comm.; F. Gerrits, pers. comm.). The geographical range extends from the southern slopes of the Himalaya (Kashmir, Nepal, Sikkim, Bhutan, northern India) (Mani, 1986), southern and south-eastern China (including the eastern edge of Tibet), and Taiwan, through Central and South-east Asia, including the Malay Peninsula, the Philippines, and Indonesia, to mainland New Guinea and Australia, reaching its easternmost limits on the Solomon Islands, Vanuatu, and New Caledonia (Talbot, 1928-37; Holloway & Peters, 1976; D’Abrera, 1990; Yagishita, Nakano & Morita, 1993; Tennent, 2002, 2004). Five species groups (belladonna, postisoe, belisama, hyparete, dorimene) have a weak representation in southeastern China (Wei & Wu, 2005), with two species in the belladonna group (Delias lativitta, Delias berinda) reaching their westernmost limits at Tangmai (2200 m) near Bomi (north of Arunachal Pradesh) just east of the Plateau of Tibet in the Palaearctic Region (A. Yagishita, pers. comm.). Only two species occur on New Caledonia, one of which (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 Neotropical 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 bcyimnia (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 Texas, 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 + Leodontia (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). Leodontia 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 [Leodontia 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 terloopii 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).