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A classification of *Danaus* butterflies (Lepidoptera: Nymphalidae) based upon data from morphology and DNA

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Classification of the cosmopolitan butterfly genus *Danaus* (Nymphalidae: Danainae) is revised at subgeneric, specific and subspecific levels, combining for the first time mitochondrial and nuclear DNA sequence information with morphological data. Tree topologies based on the nuclear genome (allozymes, pheromone components, the morphology of all life history stages and nuclear DNA sequences), on the one hand, and mitochondrial DNA, on the other, are incongruent and challenge the current taxonomy of the genus. Although earlier classifications, based on adult morphology alone, are, in general, well supported by an analysis of total evidence, the mitochondrial phylogeny shows that the species *D. chrysippus* and its subgenus *Anosia* are deeply paraphyletic. Subspecies *dorippus* of *D. chrysippus* is the basal clade of the genus and is reinstated as the species *D. dorippus*. The former species *D. plexaure* is demoted to a subspecies of *D. eresimus*. The specific status of *D. erippus*, as distinct from *D. plexippus*, is tentatively supported. On the strength of the new data, division of the monophyletic genus *Danaus s.l.* into three subgenera *Danaus s.s.*, *Salatura* and *Anosia* is unsustainable and is abandoned. Of the 15 terminal clades (taxa) of *Danaus s.l.* included in the study, 11 are species that broadly conform to the biological species concept. (The West Indian species *D. cleophile*, missing from our analysis, is the twelfth species). The remaining terminal clades are subspecies of *D. chrysippus* **comb. nov.** and *D. dorippus* **stat. rev.** Two sympatric Neotropical species, *D. eresimus* and *D. gilippus*, are morphologically distinct and sexually isolated but have nearly identical mitochondrial genomes. In contrast, two partially sympatric Palaeotropical species, *D. chrysippus* and *D. dorippus*, are cryptic species that share structural morphology and hybridize but have highly differentiated mitochondrial genomes. *D. dorippus* is polymorphic for two anciently diverged haplotypes and its history has possibly involved recombinational speciation and/or hybridism.

ADDITIONAL KEYWORDS: *Anosia* – cryptic species – hybridism – male killing – paraphyly – recombinational speciation – *Salatura* – *Spiroplasma* – subspecies – sympatric speciation.

INTRODUCTION

Danaus species are among the most apparent and abundant of all butterflies. A predominantly tropical group, all the species are medium to large in size and, with the exception of *ismare* (Cramer) and some subspecies of *melanippus* (Cramer) and *affinis* (Fabricius), sport gaudy colour schemes comprising an orange, yellow or brown ground colour with bold black and white markings. The genus has enjoyed icon status ever since *D. chrysippus* (L.) was depicted in a fresco on an Egyptian tomb some 3500 years ago (Larsen, 1977, 1984) and became the first recorded butterfly in history. Of 11 *Danaus* species recognized today (Ackery & Vane-Wright, 1984; hereinafter A & V-W), Linnaeus (1758) was able to name among his Danai Festivi only *plexippus* (L.) and *chrysippus* (L.) (as species of *Papilio*). These two, together with *genutia* (Cramer) (which Linnaeus confounded with *plexippus*), *eresimus* (Cramer) and *gilippus* (Cramer), frequently abound in the vicinity of human settlements.

The confident lifestyle that combines advertisement, relaxed flight and pungent odour serves to deter

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potential predators from launching damaging attacks. Defences of last resort are the exceptionally tough exoskeleton and acrid flavour that birds encounter when attempting to remove the wings as a prelude to devouring the body. The repellent and toxic qualities of *Danaus* butterflies derive in part from cardiac glycosides or cardenolides (CGs) that are sequestered by the larvae of some species from their predominantly milkweed (Asclepiadaceae) foodplants and subsequently stored in all life history stages (Brower, Brower & Corvino, 1967; Rothschild *et al*., 1975). CGs are not only enduringly distasteful, implanting an indelible memory in the predator's brain, but are also powerful emetics (Brower, 1984) and dangerous heart stimulants (Parsons, 1965).

CG defence may be supplemented or supplanted by bitter-tasting pyrrolizidine alkaloids (PAs) that are sequestered by adults of both sexes (but especially avidly by males) from plant genera such as *Parsonsia* (Apocynaceae), *Tournefortia* and *Heliotropium* (Boraginaceae), *Crotalaria* (Fabaceae), *Erechtites*, *Gynura* and *Senecio* (Asteraceae). PAs are precursors for intrinsic components of the male pheromone or 'love-dust' that is transferred by abdominal hair-pencils to the antennae of the female during courtship (Boppré, 1984). However, nonmetabolized PAs are also stored in adult tissues, are passed to females in spermatophores and comprise up to 5% of male body mass in *D. chrysippus* (J. Edgar, pers. comm.); as potent liver toxins in vertebrates (Bull, Culvenor & Dick, 1968), PAs undoubtedly fulfil a defensive as well as a sexual role (Edgar *et al*., 1976, 1979).

When Bates (1862) in South America, followed by Wallace (1865) in the Orient, Trimen (1869) in Africa and Walsh & Riley (1869) in North America, established the notion of Batesian mimicry (as it is now known), *Danaus* butterflies were immediately recognized as models with many mimics. After Müller (1878) had extended the mimicry theme to include the convergent evolution of warning coloration among distasteful species (now known as Müllerian mimicry), Moore (1883) demonstrated in extensive comparative tables that the latter concept (to which he made no explicit reference) was highly relevant to danaine butterflies.

Various names for *Danaus* butterflies in vernacular English include monarch, queen, wanderer and tiger. The generic name *Danaus* Kluk, 1802 (family Nymphalidae, subfamily Danainae, tribe Danaini, subtribe Danaina) (Harvey, 1991; see also Wahlberg, Weingartner & Nylin, 2003b) was unstable until Hemming (1933) established it. Prior to the latest generic level revision of the milkweed butterflies (tribe Danaini) by A & V-W (as subfamily Danainae), in most previous presentations the *Danaus* umbrella had embraced the majority of *c*. 81 species now belonging to subtribes

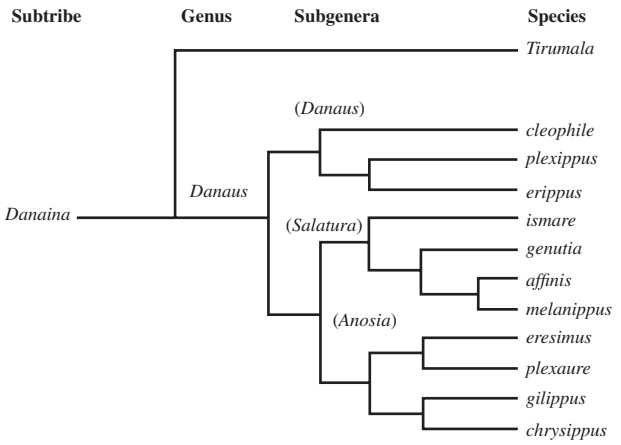

Figure 1. Cladistic reconstruction of the genus *Danaus*, after Ackery & Vane-Wright (1984).

Amaurina and Danaina. The only exceptions were some 15 species of *Amauris* Hübner and four *Ideopsis* Horsfield (Talbot, 1940a, b, 1943). However, A & V-W, in consensus with some Japanese workers, promoted the *Danaus* subgenera *Parantica* Moore and *Tirumala* Moore to generic rank and transferred to them, respectively, 38 and nine former *Danaus* species. Four *Danaus* (*Radena* Moore) were moved to *Ideopsis*; thus enlarging the latter to eight species. *Danaus s.l*. remained with just 11 species divided among three noncoordinate subgenera, *Danaus s.s*. (three species), *Salatura* Moore (four species) and *Anosia* Hübner (four species).

A & V-W explicitly sought to provide a sound generic classification of the subfamily Danainae (now tribe Danaini); for the most part they accepted previous decisions by Talbot (1940a, b, 1943), Corbet (1943) and others on what, in their words, 'constitutes a danaine species'. They did, however, provide a cladistic analysis of relationships among *Danaus* species (Fig. 1) and commented on relationships that were indistinguishable in their analysis, for example between *D. affinis* (Fabricius) and *D. philene* (Stoll). Here, our principal aim is to reappraise the classification of *Danaus s.l.* at subgeneric, specific and subspecific levels, bringing together for the first time the morphological characters used by A & V-W, data on allozymes, early stages and colour genes published subsequently to A & V-W, and our own DNA sequence data from both mitochondrial and nuclear genomes.

MATERIAL AND METHODS

MORPHOLOGICAL DATABASE

The morphological data-base (Appendix 1A, B) is assembled from published sources as follows: characters from adult butterflies, including dihydropyr-

Table 1. Provenance, voucher numbers [OUMNH] of individual butterflies used for analysis of DNA sequences and sample sizes for each sequence[1] included in this paper

Species & subspecies	Provenance and voucher numbers […][1]	Sample Sizes		
		12S + COI[7]	18S	EF1-α
Amauris niavius niavius	Tanzania [640]	–	–	1
A. dannfelti dannfelti	Congo Republic [559]	–	1	–
Tirumala limniace leopardus	Maldive Is. [671]	1[4]	–	–
T. septentrionis septentrionis	Malay Peninsula [216]	8	8	–
Danaus (Danaus) plexippus	Australia [396], USA	12 (1)	12	1
D. (D) erippus	Argentina [CUIC]	1[2]	1	–
D. (Salatura) genutia genutia	Thailand [430], Malaysia	11 (1)	3	1
D. (S) melanippus hegesippus	Malaysia (Langkawi I) [495]	5	3	–
D. (S) affinis affinis	Australia [532/533]	1[3]	1	–
D. (S) ismare ismareola	Indonesia (Talaud Is.) [539]	3[5]	3	1[5]
D. (Anosia) eresimus tethys	Cayman Is. [46/48]	8	8	1
D. (A) gilippus berenice	Cayman Is. [21]	8	8	–
D. (A) petilia	Australia [373]	9	8	–
D. (A) chrysippus chrysippus	Oman [262], India, Kenya	22 (2)	27	2
D. (A) c. bataviana f. alcippoides	Malay Peninsula [106]	8	8	–
D. (A) c. orientis	Uganda, Tanzania, Zambia [257], La Réunion	25	22	2
D. (A) c. alcippus	Ghana [163], Uganda, Oman	21 (2)	16	–
D. (A) c. dorippus-1	Kenya [52]	15 (2)	6	2
D. (A) c. dorippus-2	Oman, Uganda, Kenya [55][6], Tanzania	16 (2)	9	2
Totals		174 (10)	144	13

[1]Sequence(s) used for phylogenetic analysis are represented by vouchers deposited in the OUMNH; GenBank numbers are given in Lushai *et al*. (2003a, b, c, 2005b).
[2]COI sequence courtesy of Brower & Jeansonne (2004), voucher deposited in the Cornell University Insect Collection (CUIC).
[3]Not sequenced for COI.
[4]Not sequenced for 12S.
[5]COI (GenBank AY855061) and EF1-α (GenBank AY855062) sequences provided by Niklas Wahlberg (unpublished).
[6]Samples taken from a single site in the countries specified except for *dorippus-2* from Kenya, which was collected at six locations, Athi River (near Nairobi), Masai Mara, Amboseli, Mombasa, Malindi and Galana River.
[7]Numbers in parentheses are specimens sequenced twice for COI using different primers.

rolizines (Ackery & Vane-Wright, 1984), allozymes, Kitching (1986); characters from eggs, larvae and pupae (Kitching (1985); adult colour gene characters (Smith, 1975, 1998; Smith & Owen, 1997; Smith *et al*., 2002; Lushai *et al*., 2003b). Voucher specimens of butterflies used for DNA sequence analysis ($N = 174$, Table 1) are deposited in the Oxford University Museum of Natural History (OUMNH).

Sampling and preparation of specimens for DNA sequencing

DNA for the polymerase chain reaction (PCR) was extracted from samples of 15 species and subspecies of *Danaus* and, as outgroups, two members of the sister genus (A & V-W), *Tirumala septentrionis* (Butler) and *T. limniace* (Cramer), and (nDNA only) two members of the sister subtribe Amaurina, *Amauris niavius* (L.) and *A. dannfelti* (Aurivillius) (Table 1). Butterflies were collected randomly in the field, boxed alive and later killed in ethyl ethanoate vapour, immediately before storage in 95% ethanol (and when in the laboratory at –20 °C). Two currently recognized *Danaus* species are missing from this study, *D. cleophile* (Godart) (Jamaica and Hispaniola) and *D. plexaure* (Godart) (South America south of the River Amazon drainage).

DNA amplification, sequencing and alignment

Laboratory methods for the extraction, amplification and sequencing of DNA for the 12S rRNA (12S) and cytochrome c oxidase I (COI) mitochondrial (mt) loci, and for the elongation factor one alpha (EF1-α) nuclear gene, are described in Lushai *et al*. (2003a, b, 2005b). Primers for the highly conserved region of the 18S rRNA (18S) nuclear gene, not hitherto described, were adapted from conserved sites in aphids (Black,

1991), 5′–GTAGTCATATGCTTGTCTC–3′ (forward) and 5′–GGCTGCTGGCACCAGACTTGC–3′ (reverse).

Every precaution was taken to ensure that anomalous sequence results were confirmed. For example COI genes of some individuals were sequenced twice using different primers (Lushai *et al*., 2003b, 2005b); in every case, the anomalous *dorippus-1* sequences (and others) were confirmed as correct. Sequences were submitted to GenBank by the National Center for Biotechnology Information's submission program *SEQUIN* 3.7.

Both sense and antisense fragments were sequenced. Manual sequences were read into text files by eye and autosequence files were screened by eye using CHROMAS 1.45, exported as text files and formatted as interleaved consensus sequences for each individual for multiple alignment by CLUSTAL X (1.5b) (Thompson, Higgins & Gibson, 1994). Sequences were also screened against the NCBI GenBank BLAST-NR database to compare them with known sequences: the results showed, for example, 89% homology with *Phycoides vesta* Edwards (Nymphalidae) for COI (GenBank ref. AY156686, Wahlberg, Oliveira & Scott, 2003a) and 94% with *Amauris ellioti* Butler (Nymphalidae) for EF1-α (AY218253, Wahlberg *et al*., 2003b).

Phylogenetic analysis

Four phylogenies are derived from different datasets as follows: (1) DNA sequences from the 12S and COI mitochondrial loci (Appendix 2); (2) morphological characters (Appendix 1A, B); (3) nuclear DNA sequences from the 18S and EF1-α genes (Appendix 2); (4) for a phylogeny based upon total evidence, datasets 1–3 are combined. The data were analysed by PAUP 4.0b (Swofford, 1998) for maximum parsimony (MP) and PHYLIP 3.573c (Felsenstein, 1993) for testing branch significance by maximum likelihood (ML). 1000 Bootstrap-Bremer support values are shown for MP each node (Bremer, 1994). Bremer support was calculated using AUTODECAY 5.03 (Eriksson, 1998). Trees were analysed for measures of branch lengths and pair-wise comparisons (PAUP: Tamura–Nei model) used to determine genetic distances. The topologies of trees used for illustration (Figs 2, 3) were constructed and tested by the heuristic and strict consensus algorithms (MP) and compared for congruence with ML and neighbour-joining (NJ) or minimum evolution analyses.

RESULTS

A phylogeny based upon mitochondrial DNA

The single most parsimonious tree obtained (Fig. 2A) is derived from sequences of the two mitochondrial loci 12S rRNA (344 bp) and COI (676 bp); the variable characters are shown in Appendix 2. The topology of this tree, derived from an heuristic analysis, is congruent with trees derived from strict consensus (MP), ML and NJ analyses (see figure legends for full tree statistics). *Danaus* is a monophyletic group and its member lineages remain unchanged in this analysis compared to A & V-W (Fig. 1). However, an unexpected and novel feature here is the strongly supported basal dichotomy which separates *dorippus-1* from the remainder of *Danaus* and of its subgenus *Anosia*. Since the common ancestor of *dorippus-1* and the other *Anosia* clades includes among its descendants all other *Danaus s.l*. species, *Anosia* is a paraphyletic group. In contrast to strong support for the basal *dorippus-1* dichotomy, neither of the subsequent nodes that underpin the A & V-W subgeneric clusters has adequate bootstrap/Bremer support; the two relevant weakly supported dichotomies are:

1. The node that separates the subgenera [*Danaus s.s.* = (*plexippus* + *erippus*)] from [*Salatura* = (*ismare* + *genutia* + *melanippus* + *affinis*)] + [*Anosia* = (*eresimus* + *gilippus* + *petilia* + *dorippus-2* + *bataviana* + *chrysippus s.s.* + *orientis* + *alcippus*)].
2. The node that divides *Salatura* from *Anosia*.

Consequently, the analysis based upon mitochondrial DNA shows that the A & V-W subgenera lack statistical significance. In contrast, the terminal sister-clades (Fig. 2A) that correspond to A & V-W species have strong bootstrap support.

A phylogeny based upon morphological and biochemical characters

The suite of species-level characters for *Danaus s.l.* available to A & V-W was drawn nearly exclusively from adult morphology. Here we take the opportunity to augment the morphological dataset by adding characters drawn from early stages of life histories (Kitching, 1985), allozymes (Kitching, 1986), pheromone chemistry (A & V-W) and nuclear colour genes (Smith, 1975, 1998; Smith & Owen, 1997) (Appendix 1A, B). The total number of variable characters is 67, of which 59 are parsimony informative. The single most parsimonious heuristic tree (Fig. 2B) is concordant with independent strict consensus (MP), ML and NJ trees.

The tree topology has weak bootstrap/Bremer support and the best supported nodes are uncontroversial terminal dichotomies. Weak support is pervasive for the more basal dichotomies that occurred early in the history of the genus and would validate the A & V-W subgeneric clusters, *Danaus s.s.*, *Salatura* and *Anosia*. The topology shows that the four *Salatura* species

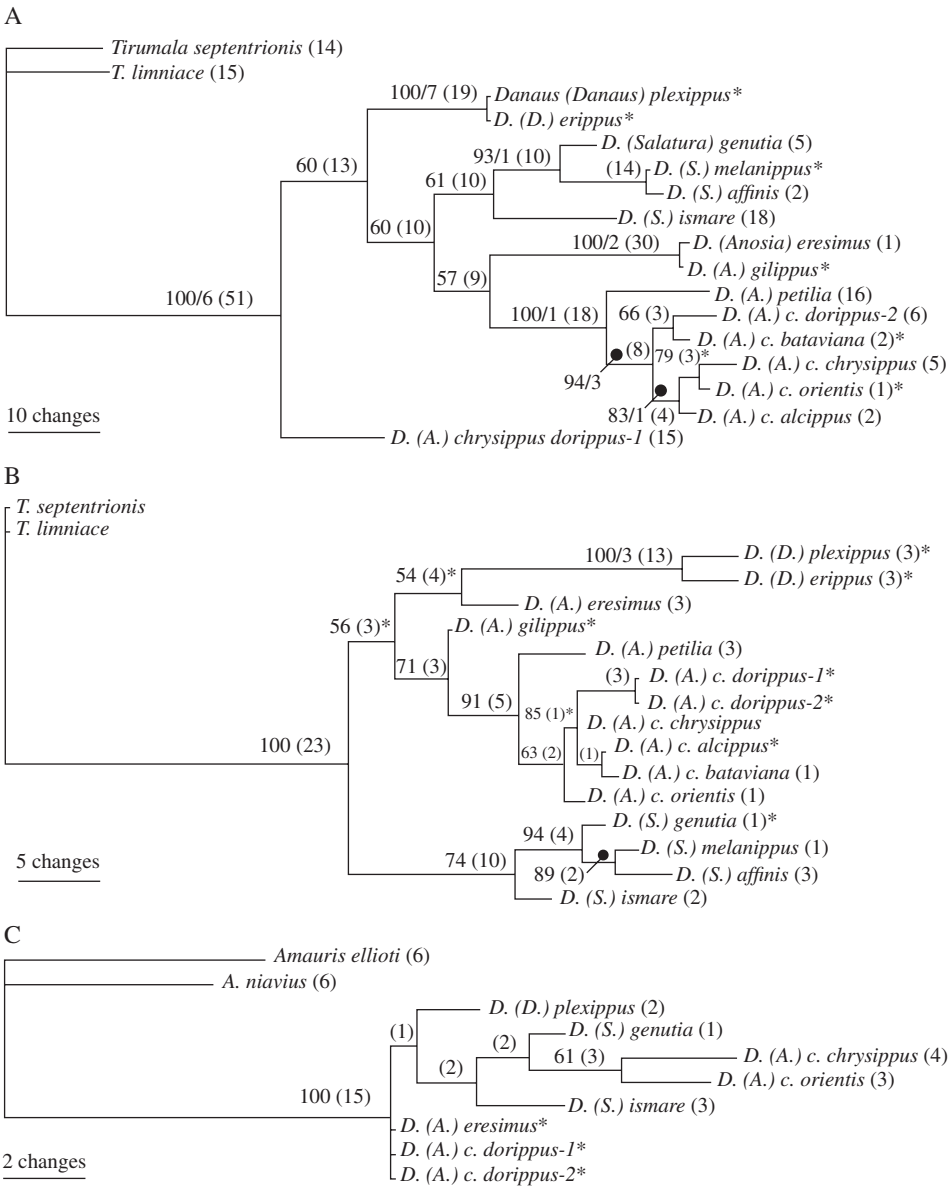

Figure 2. A, single most parsimonious tree based on amalgamated mtDNA 12S rRNA (344 bp†) and COI (676 bp†) genes (Appendix 2) for the genus *Danaus*, rooted by outgroups *Tirumala septentrionis* and *T. limniace* (†values before alignment). Tree statistics: characters in matrix 1025, variable informative characters 54, parsimony informative characters 139, heuristic search length 304, consistency index (CI) = 0.80, homoplasy index (HI) = 0.20, retention index (RI) = 0.81, rescaled consistency index (RCI) = 0.65. Bootstrap replicates/Bremer Support values are shown for each node where significant. Branch lengths are drawn proportional to nucleotide changes and indicated in parentheses. The MP topology depicted here is congruent with the MP strict consensus, NJ and ML algorithms not shown. ML branch significance is $P < 0.01$ unless marked *. See Table 1 for provenances and sample sizes.

B, single most parsimonious tree based on morphological, biochemical and colour gene characters (Appendix 1A, B) for the genus *Danaus*, rooted by outgroups *T. septentrionis* and *T. limniace* (characters from *T. petiverana*, *T. hamata* and *T. ishmoides* were used to fill gaps); †character alignment was fixed to character). Tree statistics: characters in matrix 67†, variable uninformative characters 8, parsimony informative characters 59, heuristic search length 95, CI = 0.84, HI = 0.16, RI = 0.90, RCI = 0.75. Other notes as in (A).

C, one of 11 most parsimonious trees based on amalgamated nDNA 18S rRNA (525 bp†) and EF1-α (400 bp†) genes for the genus *Danaus*, rooted by outgroups *Amauris niavius* and *A. ellioti* (†values before alignment). Tree statistics: characters in matrix 926, variable uninformative characters 19, parsimony informative characters 24, heuristic search length 52, CI = 0.83, HI = 0.17, RI = 0.72, RCI = 0.60. Other notes as in (A).

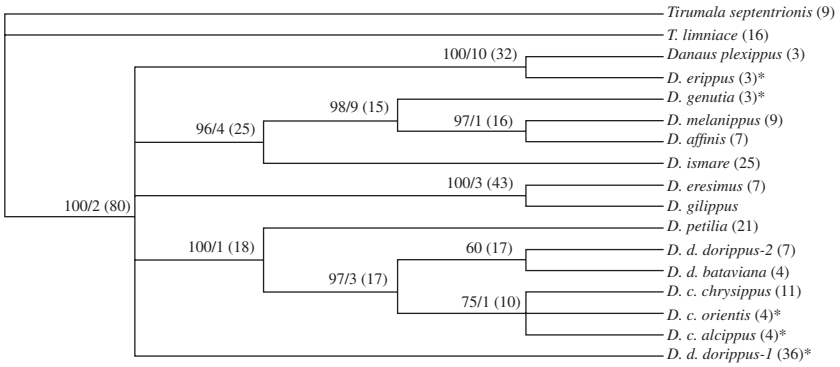

Figure 3. Single most parsimonious tree based on total evidence (amalgamated characters from Fig. 2) for the genus *Danaus*, rooted by outgroups *T. septentrionis* and *T. limniace*. Tree statistics: characters in matrix 2024, variable informative characters 76, parsimony informative characters 209, heuristic search length 445, CI = 0.79, HI = 0.21, RI = 0.80, RCI = 0.64. 1000 Bootstrap replicates/Bremer Support values are shown for each node where significant branch lengths are indicated in parentheses. This is the parsimony strict consensus tree and is congruent with trees from NJ and ML algorithms, not shown. ML branch significance is $P < 0.01$ unless marked *.

(*ismare*, *genutia*, *melanippus* and *affinis*) comprise a monophyletic basal cluster, with *ismare* at its base. The suggestion that *ismare* might be close to the ancestral *Danaus* is provocative, especially as its colour pattern is close to that found in most species of *Tirumala*, the sister genus of *Danaus* (A & V-W). However, a less basal position for *ismare* is suggested by the mtDNA analysis (Fig. 2A) and bootstrap support for its position in Figure 2B is weak.

The *Anosia* species form a loose-knit but monophyletic group, with the exception of *eresimus*, which clusters remotely with *Danaus s.s.* (*plexippus* + *erippus*). In the *Danaus s.s.* + *Anosia* cluster, the New World species (*plexippus*, *erippus*, *eresimus* and *gilippus*) are clearly basal to the Old World members; *eresimus* and *gilippus*, which are almost identical for mtDNA sequences, are well separated morphologically and even cluster, though weakly, with different subgenera, *eresimus* with *Danaus s.s.* and *gilippus* tentatively with *Anosia*; however, the relevant nodes all lack convincing statistical support.

A PHYLOGENY BASED UPON NUCLEAR DNA

We obtained sequences for 18S rRNA (525 bp) and a 400 bp fragment of the EF1-α genes (Lushai *et al.*, 2005b) for a limited number of *Danaus s.l.* species, with *Amauris niavius* and *A. ellioti* (Wahlberg *et al.*, 2003b) as outgroups (Table 1). Based on only 24 parsimony informative characters, 11 trees were identified, of which one MP heuristic tree, congruent with MP strict consensus, ML and NJ topologies, is described (Fig. 2C). Because the number of variable characters is small, node support is generally weak; however, while bearing in mind the taxa missing from this analysis, the salient findings are:

1. *Danaus s.l.* is a monophyletic genus divided into three noncoordinate clades, *eresimus* + *dorippus-1* + *dorippus-2*, *plexippus* and *ismare* + *genutia* + *chrysippus chrysippus* + *c. orientis*.
2. Within the latter cluster, *Salatura* is paraphyletic, thus casting doubt on its validity.
3. *eresimus* does not cluster, as expected, with *Anosia*, and is basal even to *Salatura*.
4. *dorippus-1* and subspecies *chrysippus s.s.* + *orientis* of *D. chrysippus s.l.* do not share most recent common ancestors and are therefore reciprocally monophyletic clusters.
5. *dorippus-2* clusters with (and is identical to) *dorippus-1*, as described for morphological and biochemical data (Fig. 2B), whereas for mtDNA (Fig. 2A) they are a paraphyletic grouping. Since (*dorippus-1* + *dorippus-2* = subspecies *dorippus*) is reciprocally monophyletic to (*chrysippus s.s.* + *orientis*), it follows that *D. chrysippus s.l.* is not a phylogenetic species.

A PHYLOGENY BASED UPON TOTAL EVIDENCE

A single strict consensus MP tree was obtained using combined data comprising all the characters described in Figure 2A–C, i.e. total evidence (Fig. 3). Note that the naming of taxa is here changed, compared to Figure 2A–C, in line with the taxonomic decisions made (see below). The salient features of the total evidence analysis are:

1. There is strong bootstrap/Bremer support for basal nodes that separate *Danaus s.l.* into five groups,

[*plexippus* + *erippus*], [*ismare* + *genutia* + *melanippus* + *affinis*], [*eresimus* + *gilippus*], [*petilia* + *dorippus-2* + *bataviana* + *chrysippus s.s.* + *orientis* + *alcippus*] and *dorippus-1.*

2. The paraphyly of *Anosia* has strong bootstrap support.
3. The other basal nodes that underpin the three A & V-W subgenera (Fig. 1) lack sound bootstrap support; indeed, an early radiation of *Danaus s.l.* into five nearly coordinate clusters has strong support. The implication of this virtual pentachotomy at the root of *Danaus* history is that the chronology of its four component cladogenetic events is so tightly compressed in time that their order of occurrence is uncertain. The biogeography of the five basal clusters that must have appeared nearly contemporaneously is as follows:
   (i) Old World (Afrotropics) *dorippus-1*
   (ii) New World *Danaus s.s.* (*plexippus* + *erippus*)
   (iii) Old World (Oriental) *Salatura* (*ismare* + *genutia* + *melanippus* + *affinis*)
   (iv) New World *Anosia* (*eresimus* + *gilippus*)
   (v) Old World (Afrotropics, Oriental and Australasian) *Anosia* (*petilia* + *dorippus-2* + *bataviana* + *chrysippus s.s.* + *orientis* + *alcippus*)
4. *Anosia* (less *eresimus*, *gilippus* and *dorippus-1*) is divided into three noncoordinate clusters, each representing a species. Each cluster has strong bootstrap support, thus emphasizing that subgenus *Anosia* is not well supported. In summary, a new subdivision of subgenus *Anosia* would comprise:
   (i) *petilia* (Lushai *et al*., 2005a)
   (ii) *dorippus* – *2* + *bataviana* = *dorippus* (in part),
   (iii) *chrysippus s.s.* + *orientis* + *alcippus* = *chrysippus s.l.* (stat. rev.).
5. All the terminal clades or clusters representing the 11 species *plexippus, erippus, genutia, ismare, melanippus, affinis, eresimus, gilippus, petilia*, *dorippus* and *chrysippus* have strong statistical support.

With the exception of the *dorippus-1* clade, the results based on combined evidence are remarkably congruent with the cladistic analysis of A & V-W.

DISCUSSION

THE SUBGENERA OF *DANAUS*

Two of three noncoordinate subgenera, *Danaus s.s.*, *Salatura* and *Anosia* (A & V-W), receive limited support as monophyletic groups. In particular, the four *Salatura* species (*ismare*, *genutia*, *melanippus* and *affinis*) and the two *Danaus* species (*plexippus* and *erippus*) are monophyletic clusters in analyses based on mtDNA (Fig. 2A), morphology (Fig. 2B) and total evidence (Fig. 3). However, the subgenera present the following problems:

1. The nodes that represent cladogenesis at subgeneric level, i.e. (*plexippus* + *erippus* = *Danaus s.s.*) (*genutia* + *melanippus* + *affinis* + *ismare* = *Salatura*) and (*eresimus* + *gilippus* + *petilia* + *dorippus-2* + *bataviana* + *chrysippus s.s.* + *orientis* + *alcippus* = *Anosia*) have weak support (Figs 2A, 3). In contrast, the terminal clades that represent A & V-W species are strongly supported in the total evidence tree and only some subspecies of *D. chrysippus s.l.* have inadequate support (Fig. 3).
2. *dorippus-1* is excluded from all three subgeneric clusters in the mtDNA (Fig. 2A) and total evidence (Fig. 3) analyses.
3. The subgenus *Anosia*, the species *D. chrysippus* and its former subspecies *dorippus* are all paraphyletic groupings.
4. The topology of the total evidence tree (Fig. 3) suggests that the A & V-W subgenus *Anosia* is a heterogeneous assemblage that comprises five strongly supported, noncoordinate clusters, namely *dorippus-1* (*chrysippus s.s.* + *orientis* + *alcippus*) (*dorippus-2* + *bataviana*), *petilia* and (*eresimus* + *gilippus*). As the nearest common ancestor of the *Anosia* assemblage is also ancestral to *Danaus s.s.* and *Salatura*, *Anosia* is a paraphyletic group. Relations of the terminal clades among these clusters include polyphyly, paraphyly and reciprocal monophyly.
5. The sister grouping of *ismare* on the one hand and the other three *Salatura* species on the other, and the paradoxical affinities of *eresimus* with *Danaus s.s.* for 'nuclear' characters (Fig. 2B, C) and with *gilippus* (i.e. *Anosia*) for mtDNA (Fig. 2A), are further problems with the subgenera.

The case for abandoning the three A & V-W subgenera is overwhelming.

THE SPECIES OF *DANAUS*

Danaus is, in this study, a monophyletic genus with 15 terminal clades that comprise diverse stages in the species-subspecies continuum. To the 11 species recognized by A & V-W (Fig. 1) we add two new ones, *D. petilia* (Stoll) (Lushai *et al*., 2005a) and *D. dorippus* (Klug) (this paper), and remove *D. plexaure*. Therefore, with the addition of *D. cleophile*, which is missing from this analysis, and the exclusion of *D. plexaure* (see below), *Danaus* now comprises 12 species that probably satisfy most of the criteria for the biological species concept (BSC) (Dobzhansky, 1937; Mayr, 1942), though many are poorly known biologically. The three lineages (subspecies) of *D. chrysippus s.l.* included in this analysis are incipient species (Lushai *et al*., 2003a).

THE *ERIPPUS* ISSUE

From its first description as *Papilio erippus* Cramer, 1775, *erippus* has been conventionally separated from *plexippus*, which it replaces south of the Amazon. However, Forbes (1939), Clark (1941) and Urquhart (1960: 179) treated *erippus* as one of three subspecies of *plexippus* (the other two being the migratory *plexippus s.s.* and the nonmigratory *megalippe*). On the other hand, by the time he reached p. 182, Urquhart had changed his mind and *D. erippus* was once more a species! Whereas A & V-W grudgingly award *erippus* specific status, they say it is 'hardly distinct' and 'may be better regarded as conspecific [with *plexippus*]'.

The only distinguishing character observed in adult butterflies is the paler hindwing margin of *erippus* compared to *plexippus* and *megalippe* (character 60, Appendix 1A). However, Kitching (1985) found two pupal characters to distinguish *erippus* from *plexippus* and subsequently (Kitching, 1986) identified three diagnostic allozyme differences, though his sample of *erippus* was small ($N = 9$, plus 9 progeny). We have sequenced the mitochondrial 12S (344 bp) and nuclear 18S rRNA (525 bp) genes of both *plexippus* from Australia ($N = 8$) and north-eastern America ($N = 4$) and *erippus* ($N = 1$) and found no differences. Whereas both these genes are relatively conserved, the 12S locus is, nevertheless, otherwise 100% diagnostic for all the *Danaus* species, and even some subspecies of *D. chrysippus s.l.* (Lushai *et al.*, 2003a).

The *erippus* issue has, however, recently been addressed by Brower & Jeansonne (2004). Using mtDNA sequence data from the COI, $tRNA_{leu}$ and COII loci, they find an average genetic divergence (GD) of 4.8% between *erippus* (represented by only one specimen) and *plexippus* + *megalippe*, compared to a pairwise mean GD of only 0.3% that separates geographically diverse samples among the latter group. Thus, they claim that *D. erippus* is a distinct species, as originally described by Cramer, and not merely a southern race of *D. plexippus*, as suggested by Kitching, Ackery & Vane-Wright (1993). We concur with this decision.

Applying the 1.2% per million years mtDNA divergence rate hypothesized for closely related insect taxa (Brower, 1994), the separation of *D. erippus* and *D. plexippus* probably occurred some 2 million years ago (late Pliocene-early Pleistocene). We suggest the possibility that an ancestral *erippus-plexippus* population was initially divided during an interglacial period around this time, when successive raised sea-levels of +100 m and +60 m (compared to the present day) occurred. At such times Amazonia was inundated and became a series of huge brackish and freshwater lakes that extended west from the Atlantic to the foot of the Andes in eastern Peru (Haffer, 1987). A succession of Amazon embayments formed a geographical barrier that could have isolated *erippus* to the south from *megalippe* to the north for several thousand years at a stretch, ample time to trigger speciation.

THE *PLEXAURE* ISSUE

Morphological characters 36, 46, 54 and 57 (Appendix 1A, B) distinguish *eresimus* + *plexaure* from all other *Anosia* species. Whether or not these characters are considered to be *eresimus* autapomorphies depends on one's view of the status of *plexaure*. G. Lamas, in a personal comment (Ackery & Vane-Wright, 1984), maintained that the little known *plexaure* is merely a vicariant subspecies of *eresimus*, which it replaces south of the River Amazon in South America. A & V-W 'could not disagree' with this opinion. Although we have no new data, it seems to us bizarre that *plexaure*, first named by Godart in 1819, should, in the absence any distinguishing character, have survived 185 years and several major taxonomic revisions with its 'gestalt' status unsullied. Part of the explanation may be that *plexaure* is virtually unknown outside museum walls; nevertheless, parsimony requires that *plexaure* be reduced in status to a subspecies of *eresimus*. mtDNA analysis of *plexaure* is needed to confirm this status.

THE *ERESIMUS* ISSUE

The taxon *eresimus* + *plexaure* is sympatric with *gilippus* over 95% of their very extensive combined range, from Texas and Florida in the north to Uruguay and Argentina in the south. Where these two very similar species fly together, the feature that serves best to distinguish them on the wing is a series of pale patches (character 54, Appendix 1A, B) on the hindwing underside of *eresimus* + *plexaure*. This character, unique within *Danaus*, may aid species recognition and, thus, mate selection in sympatry. Because butterfly eyes are sensitive both to ultraviolet (Silberglied & Taylor, 1978) and polarized light (Sweeney, Jiggins & Johnsen, 2003) an investigation of the optical properties of these patches could be rewarding. Even to the human eye, the patches serve equally well to distinguish *plexaure* from *gilippus gilippus* in Argentina or *eresimus tethys* from *gilippus berenice* on Grand Cayman (DASS, unpublished).

The phylogenetic tree derived from mtDNA data (Fig. 2A) places the *chrysippus* + *petilia* clade as sister to *eresimus* + *gilippus*; with the proviso that *dorippus-1* is excluded from *Anosia*, the remaining species form a monophyletic group. The *Anosia* cluster, again excluding *dorippus-1*, is sister to *Salatura* and *Salatura* + *Anosia* is sister to *Danaus*. Comparing the

topology derived from mtDNA characters (Fig. 2A) with that based on morphological characters (Fig. 2B), it is clear that the relationships found for *eresimus* are discordant. Whereas the mitochondrial genomes of *eresimus* and *gilippus* are virtually identical (Lushai *et al*., 2003a), and the two New World species share a sister relationship with the Old World *Anosia* species, the nuclear data suggest otherwise. In the latter case, with the substantial caveat that the relevant nodes have weak support, *eresimus* does not cluster with *Anosia*, but rather with *plexippus* + *erippus*. The analysis of total evidence (Fig. 3) provides strong support for the *eresimus* + *gilippus* clade, but not for its inclusion in *Anosia*, nor indeed any other subgenus. These anomalies strengthen the case for abandoning the A & V-W subgenera of *Danaus s.l.* (Fig. 1).

THE *GILIPPUS* ISSUE

This problem is intertwined with the preceding one. A & V-W found no structural apomorphies to distinguish *gilippus* from *chrysippus s.l.* and suggested they might constitute a single cosmopolitan species. Smith *et al*. (2002) tested this hypothesis by crossing female *gilippus berenice* from Grand Cayman with male *chrysippus dorippus* (of unknown haplotype) from Kenya. They obtained an F_1 that comprised sterile males (when backcrossed to *dorippus* females), and nonviable females, thus demonstrating that the parents belonged to separate species. [A substantial caveat is that Smith *et al*. (2002) had not then recognized *dorippus* as a species distinct from *chrysippus*; it cannot therefore be assumed that *gilippus* × *chrysippus s.s.* crosses would produce the same result]. However, our analysis (Fig. 2A) shows that the *gilippus* matriline is very distinct from those comprising *D. chrysippus s.l.*, and is neither a sister cluster, nor even closely related (Lushai *et al*., 2005b).

Tamura–Nei GDs, based upon mtDNA (12S + COI loci), between *gilippus* and the *chrysippus s.l.* subtaxa are as follows: *dorippus-1*, 6.3%; *petilia*, 5.2%; *chrysippus s.s.*, 4.9%; these distances may be compared with a mere 0.1% that separates *gilippus* from *eresimus*. And yet, whereas *gilippus* and *eresimus* are reproductively isolated and morphologically distinct species that are sympatric over a huge geographical area, *gilippus* lacks observed structural apomorphies compared to the allopatric and genetically distant taxa that comprise *chrysippus s.l.* (A & V-W; Smith *et al*., 2002 for discussion). The similarity of *gilippus* and *eresimus* haplotypes implies that speciation has occurred only within the last 40 000 years or so (Lushai *et al*., 2003b). As the two species are now sympatric over most of their combined geographical range, cladogenesis too may have been sympatric, though allopatric or parapatric scenarios are at least equally plausible (Coyne & Orr, 2004).

If the *gilippus-eresimus* speciation was sympatric, prezygotic isolation through genitalic and species recognition markers (characters 36, 46, 54, Appendix 1A, B), may have been enhanced, either before speciation by reinforcement, or subsequently by reproductive character displacement (Butlin, 1989). Moreover, if cladogenesis occurred only millennia ago (Smith *et al*., 2002; Lushai *et al*., 2003b), the *gilippus* and *eresimus* clusters must have acquired prezygotic isolation within that short time.

Lushai *et al*. (2003b) applied molecular clocks for the COI gene in *Alphaeus* prawns (Knowlton *et al*., 1993) and the 12S locus in *Littorina* (gastropod molluscs), Reid, Rumbak & Thomas (1996) to the *gilippus* + *eresimus* and *chrysippus s.l.* haplotypes. The mean of the two correlated clock rates suggests that cladogenesis between these groups occurred ~2.8 million years ago (Mya), while divergence of the *gilippus* + *eresimus* and *dorippus-1* matrilines must have been even earlier, around 4.1 Mya. These calculations indicate that the divergence of *gilippus* + *eresimus* from all *chrysippus s.l.* taxa occurred in the Pliocene, 3–4 Myr before the *gilippus-eresimus* dichotomy.

Thus, the morphological features (characters 46, 54, 55, Appendix 1A, B) that distinguish the *gilippus* + *chrysippus s.l.* cluster from *eresimus* (Fig. 2B) are probably symplesiomorphic, not synapomorphies as believed by A & V-W. It follows that butterflies with *gilippus* + *chrysippus s.l.* structural morphology must have dispersed to the Americas from the Old World early in the history of the genus, whereas the distinctive morphological features of *eresimus*, in particular characters 46 and 54 (Appendix 1A, B), are apomorphic and of more recent Neotropical origin.

An alternative scenario is that *gilippus* and *eresimus* had originally evolved distinct mitochondrial genomes but subsequent hybridism, possibly a rare or localized event, has resulted in the introgression of cytoplasm from one species to the other (Lushai *et al*., 2003b) and thus erased the matrilineal history of the introgressed species. The possibility that such an event might be unique to Grand Cayman, where the samples of both species were collected, has to be acknowledged. However, it is unlikely that two relatively large and, moreover, highly vagile species would remain isolated on a far from remote island for long. This hypothesis could, however, be tested by sequencing mtDNA from sympatric sample pairs in other parts of their shared range.

Lushai *et al*. (2003a, 2005b) have produced evidence to suggest that hybridism in East Africa among partially isolated subspecies of the *D. chrysippus* complex is catalysed by female-biased sex ratios that result from male-killer *Spiroplasma* infections (Jiggins *et al*., 2000; see below). If similar events occurred in

the recent history of *gilippus* and *eresimus*, it could account for the mutual convergence and low diversity of their haplotypes (Hurst, Hurst & Majerus, 1997) in our samples ($N = 8$ for both taxa). Although Sperling (1993) has shown that mtDNA is unlikely to cross species boundaries in *Papilio* butterflies due to the Haldane effect in heterogametic females, he did find one apparent exception in crosses between *Papilio multicaudatus* and *P. rutulus*.

DANAUS PETILIA (STOLL)

The reinstated species *D. petilia* that inhabits Australia and Iryan Jaya/Papua New Guinea (IR/PNG) (Lushai *et al*., 2005a) is less controversial than *D. dorippus*. Although crossable with *D. chrysippus alcippus*, with both F_1 and F_2 progenies viable and fertile in laboratory conditions (Clarke, Sheppard & Smith, 1973), the two species are 100% diagnosable by morphological, mitochondrial and geographical criteria (Lushai *et al*., 2005a) and do not mix in nature. The reinstatement of *petilia*, formerly a subspecies of *D. chrysippus*, as a species by Lushai *et al*. (2005a) is supported by mitochondrial (Fig. 2A), morphological (Fig. 2B) and total evidence phylogenies (Fig. 3). In the mtDNA phylogeny (Fig. 2A), the *petilia* clade is sister to *eresimus* + *gilippus*, with the Old World *Anosia* cluster sister to *petilia*. This intriguing topology suggests the possibility of sequential speciation by westward dispersal from America (*eresimus* + *gilippus*) via Australasia (*petilia*) and then Asia (*dorippus-2* + *bataviana* and *chrysippus s.s.*) to Africa (*dorippus-2* and *chrysippus s.s.* + *orientis* + *alcippus*).

THE *DORIPPUS* ISSUE

The widespread hybridism in East Africa between *D. dorippus* and *D. chrysippus* is a strong deterrent to their acceptance as separate biological species. Whereas the two proposed species are substantially allopatric, their ranges overlap and, in sympatry, they interbreed throughout East Africa to produce viable and fertile offspring. There is, however, prezygotic isolation, albeit imperfect (Smith, 1984, 1998), linkage disequilibrium (Smith, 1980) and niche divergence (Smith & Owen, 1997) in sympatry. Moreover, Lushai *et al*. (2005b) suggest that interbreeding between *dorippus* and *chrysippus* may be enforced by pervasive sex ratio differences caused by *Spiroplasma* (see below), a matrilinearly transmitted, male-killer, bacterial parasite (Jiggins *et al*., 2000). Hybridism apart, the specific status of *D. dorippus* is further challenged because it fails the criterion of mitochondrial monophyly (albeit in line with almost one fourth of all animal species for which relevant information is to hand; Hebert *et al*., 2004).

However, a further twist in this case is that the two haplotypes, *dorippus-1* + *dorippus-2*, is a paraphyletic grouping and only the latter clusters with *D. chrysippus s.l.* or subgenus *Anosia* (Fig. 2A). Therefore, if *dorippus* is to be denied specific status, on the ground that it fails the test of mitochondrial monophyly, *D. chrysippus s.l.* is similarly compromised if *dorippus* remains one of its subspecies. On total evidence (Fig. 3), *dorippus-1*, *dorippus-2* + *bataviana* and *chrysippus s.s.* + *orientis* + *alcippus* = *chrysippus s.l.* are three well-differentiated clusters, each commanding solid bootstrap support. As *dorippus-1* and *dorippus-2* are morphologically identical and fly together in Kenya (Lushai *et al*., 2005b), they are clearly highly differentiated cytotypes of one species.

Because the 12S and COI sequences obtained are PCR-based and the *dorippus-1* sequence, in particular, does not 'fit', we considered the possibility that nuclear copies of mitochondrial genes (pseudogenes) were amplified (Lushai *et al*., 2005b). In summary, there are several reasons for confidence that pseudogenes are not implicated here (for details see Lushai *et al*., 2003a, b, 2005b):

1. Identical COI sequences (apart from fragment length) were obtained from each of the four individual *dorippus* butterflies sequenced twice with alternative primers (Lushai *et al*., 2005b).
2. COI sequences were easily read and aligned.
3. Six *dorippus-1* individuals were concordantly divergent from other *D. chrysippus* and *Anosia* clades for both COI (peptide-coding) and 12S (rRNA-coding) loci (Lushai *et al*., 2005b); however, pseudogenes descended from mitochondrial rRNA genes are unknown.
4. Some populations have only one *dorippus* haplotype, while others have two or more, but there is no evidence for heteroplasmy in the latter.

Hence the *dorippus* data present an array of challenges. The mitochondrial lineages *dorippus-2*, *dorippus-1* and *chrysippus s.l.* comprise a paraphyletic grouping (Figs 2A, 3) and independently so in gene trees for the 12S and COI mitochondrial loci (Lushai *et al*., 2003b, 2005b). Moreover, in the mitochondrial tree (Fig. 2A), *dorippus-1* is the lineage closest to the ancestral *Danaus*. On the other hand, although *chrysippus s.s.* is reciprocally monophyletic to *dorippus-1* for the nuclear DNA sequences (Fig. 2C), in this case, paradoxically, it is identically so compared to *dorippus-2* (Lushai *et al*., 2005b). Therefore, since *dorippus-1* and *dorippus-2* share morphology and nuclear DNA sequences, they comprise a single unorthodox paraphyletic species that is polymorphic for highly divergent haplotypes. As the two cytotypes also share structural morphology with all other clades of

chrysippus s.l., together they comprise a single cryptic species.

A plausible insight into the phylogenetic anomaly of *dorippus* is gained from a recently acquired understanding of the epidemiology of the male killer symbiont *Spiroplasma* (Jiggins *et al*., 2000). The periodic shortage of *chrysippus* males that channels the mate selection of *chrysippus s.s.* females towards plentiful *dorippus* males causes an excess of heterotypic matings (Smith *et al*., 1998). The consequent introgression of cytoplasm from *chrysippus s.s.* to *dorippus* matrilines occurs at high frequencies but, since *chrysippus s.s.* males are scarce and predominantly pair with homotypic females, reciprocal introgression is rare (Lushai *et al*., 2005b). Similar capture in the past of the *dorippus-2* haplotype by *dorippus-1* (or vice versa) may have originated the extraordinary haplotype polymorphism in the present *dorippus* population at Athi River, Kenya (Lushai *et al*., 2005b). To summarize the *dorippus* issue:

1. *dorippus-2* occurs throughout the (sampled) range of *dorippus*, i.e. in Tanzania, Kenya, Uganda and Oman, whereas *dorippus-1* may be more local. However, as samples sequenced from most sites are small, we cannot be sure, especially as *dorippus* is migratory (Williams, 1930; Smith & Owen, 1997; Lushai, Gordon & Smith, 2003c).
2. The *chrysippus s.l.* cluster and *dorippus-1* comprise a paraphyletic group.
3. *dorippus-1* is basal to all other *Danaus* clades; in the mitochondrial phylogeny the nearest common ancestor of *dorippus-1* and *dorippus-2* includes among its descendants all other *Danaus* species sampled.
4. Whereas the relationship of *dorippus-1* to *dorippus-2* is paraphyletic for two mitochondrial loci (Lushai *et al*., 2003b, 2005b) and on total evidence (Fig. 3), the two are identical for morphology (Fig. 2B) and nDNA (Fig. 2C). Since *Anosia* and *D. chrysippus s.l.* are paraphyletic groups for both mitochondrial and nuclear loci, they would generally be regarded as invalid taxa.
5. On the evidence of nDNA and shared morphology, *dorippus-1* and *dorippus-2* are two highly divergent haplotypes of a single biological species. We suggest, (a) that the haplotypes probably evolved in isolation and then came together through recombinational speciation and/or hybridism, (b) that natural selection has acted to conserve the mitochondrial polymorphism, and (c) that the catalyst for past reticulation in East Africa may have been *Spiroplasma*, then as now (see Lushai *et al*., 2003b, 2005b). The reclassification of *dorippus* as a cryptic species has implications for the affinity of some subspecies of *D. chrysippus*, in particular *bataviana* which clusters with *dorippus-2* for mtDNA (Fig. 2A) and total evidence (Fig. 3).

THE NATURE OF *DANAUS* SUBSPECIES

The subspecies of most *Danaus* species are diagnosable, polytypic forms for which we have virtually no biological information. Most subspecies of *ismare* (6–7), *genutia* (~16), *melanippus* (13–18) and *affinis* (~45) (Morishita, 1985) are allopatric island forms from South-east Asia, of which an unknown number may be species. Whereas the Neotropical species *gilippus* and *eresimus* both comprise numerous parapatric or vicariant subspecies (Talbot, 1943; A & V-W), whether or not any of them are reproductively isolated at their boundaries, and might thereby qualify for specific status, is unknown.

The diagnosable subspecies *plexippus s.s.* and *megalippe* of *D. plexippus* are not well known biologically. However, *megalippe* is said to be nonmigratory (Brown & Heineman, 1972) whereas *plexippus s.s.* is the most famous of all butterfly migrants. The extent to which *plexippus s.s.* and *megalippe* interbreed is seemingly unknown.

The subspecies of *D. chrysippus* are better known biologically, especially in Africa, than those of any other *Danaus* species (Smith *et al*., 1997, 1998; Lushai *et al*., 2003a). We treat the African subspecies of *D. chrysippus s.l.* as allopatric or parapatric clusters between which there is, where investigated, linkage disequilibrium in contact zones (Smith, 1980; Lushai *et al*., 2003a, 2005b): where studied, parapatric subspecies are imperfectly isolated by ethology (assortative mating) (Smith, 1984) and allochrony (seasonally different migration patterns) (Smith & Owen, 1997). Therefore, in those cases where relevant data have been acquired, subspecies are incipient biological species that satisfy criteria for the phylogenetic species concept (PSC) (Cracraft, 1983; Panchen, 1992; Ridley, 1993) but, since they are crossable in the laboratory or interbreed in the wild, do not qualify as species under the BSC.

A REVISED CLASSIFICATION AND NOMENCLATURE FOR FORMS OF *D. CHRYSIPPUS* FROM WALLACEA

Corbet (1940) established the type locality of the nominotypical form of *D. chrysippus* (Linnaeus, 1758 as *Papilio chrysippus*) as Canton, China. In his species-level review of *Danaus*, Talbot (1943) classified all *D. chrysippus* from Arabia eastwards to the Malay Peninsula, Borneo, the Philippines and China as ssp. *chrysippus*. He distinguished three further Indo-Pacific subspecies, *bataviana* (Moore), *cratippus* (Felder) and *petilia* (Stoll), as replacing forms distrib-

uted eastwards and southwards across Wallacea (the Malay and Indonesian Archipelago) to IJ/PNG, Australia and Fiji (Lushai *et al.*, 2005a).

There are problems with the ambit, relationship, biogeography and correct name for *D. chrysippus* ssp. *bataviana*. Moore (1883: pl. XXX1, fig. 1) described '*Limnas alcippoides* n. sp.' from Nepal that had white on the hindwing, though rather less than in *alcippus* from West Africa, and, on the forewing, a broad subapical bar comprising [five] conjoined white spots [in spaces 4–6, 8–9], an enlarged spot below the bar [in space 3 (Cu_{1a})], and a bold submarginal white spot in the orange area [of space 2 (Cu_{1b})]. This description fits specimens from Penang south to the Singapore Strait in the Malay Peninsula, collected, bred or observed by us ($N = 172$, DASS unpublished).

Furthermore, the description embraces an OUMNH collection ($N = 35$) from Kuala Lumpur, West Malaysia, made by W. A. Lambourne in 1920–1. It should be noted that the latter is labelled '*Danaida chrysippus bataviana* Moore, subspecies *alcippus* Cramer'. In all these specimens, and similar ones from the same area, housed in the Natural History Museum, London (NHML) ($N = 7$) and the Muséum National d'Histoire Naturelle, Paris (MNHNP) ($N = 6$), the white area on the hindwing fully matches in extent that found in African *alcippus*. This suggests that Moore's *alcippoides* type from Nepal was heterozygous (*Aa*) at the A locus, which determines hindwing white (Clarke *et al.*, 1973; Smith, 1998). Moore's description also applies to specimens with full white hindwings in the NHML ($N = 29$) and MNHNP ($N = 3$) collected from Sumatra and Nias Island before 1935.

Moore (1883) also described but gave no figures for '*Limnas bataviana* n. sp.' from Java and '*Limnas bowringi* n. sp.' from Hong Kong. The former resembled *alcippoides* except that the hindwing was brown, whereas *bowringi* had an orange hindwing and 'subapical band composed of somewhat larger spots'. Both *bataviana* and *bowringi* had a second submarginal 'lower spot' [in space 1b (1 A + 2 A) of the forewing], as in 42% ($N = 105$) of *alcippoides* examined by us from the Malay Peninsula.

Having examined large collections from Malaysia and Indonesia in the OUMNH, NHML and MNHNP ($N = 478$), we now propose that four forms (including *gelderi* (Snellen) from Sulawezi), are minor variants of a single subspecies. Whereas *alcippoides* (white hindwing: Malay Peninsula, Sumatra and its neighbouring small islands), *bataviana* (brown hindwing: Java and the Greater Sunda Islands) and *gelderi* (white marks on hindwing: Sulawezi) have some geographical integrity, *bowringi* occurs as a morph throughout the Far East. Although the name *alcippoides* has priority by pagination over *bataviana* and *bowringi*, Moore's (1883) description of *bataviana* and the type locality (Java) best fits the material we have examined. The names *bowringi*, *alcippoides* and *gelderi* remain relevant for these distinct varieties.

In January 1996, one of us (DASS) undertook a *Danaus* transect, following the west coast of the Malay Peninsula from the Thai border in the north to the Singapore Strait in the south. Although not uncommon, *chrysippus s.s.* and *bataviana* are local in Malaysia, generally occurring in isolated but sometimes dense colonies where their food-plant, *Calotropis gigantea*, is established (Corbet & Pendlebury, 1992; DASS, unpublished). In the north-western province of Kedah (including Langkawi Island), all records ($N = 240$) were for *D. chrysippus chrysippus*. Only one hybrid individual with any white on the hindwing (genotype *Aa*) was taken. In contrast, from Penang southwards all sightings and captures ($N = 250$) were *bataviana* f. *alcippoides*, thus confirming Corbet & Pendlebury (1992). There was no sign of an anticipated hybrid zone.

These records may be compared with per cent frequencies in pre-1935 museum collections from the Malay Peninsula south of Kedah, Sumatra and Nias Island: orange hindwing (*A-*) 59.0, hybrid phenotype (*Aa*) 7.2, white hindwing (*aa*) 33.8 ($N = 237$). Pre-1904 percent frequencies are: *A-* 76.3, *Aa* 5.5, *aa* 18.2 ($N = 55$). These data suggest that heterozygotes were scarce pre-1935, indicating that *chrysippus chrysippus* and *bataviana* have always been substantially isolated, as they are today. It is clear, both from local records (Morishita, 1985) and analysis of museum material, that *bataviana* has largely displaced *chrysippus chrysippus* during the 20th century, both in peninsular Malaya north to Kedah and in Sumatra and Nias Island. The late D. F. Owen (pers. comm.), who visited Sumatra in 1986, saw only white hindwinged forms of both *bataviana* and its mimic *Hypolimnas misippus*. Thus we believe the geographical range of *bataviana* f. *alcippoides* (genotype *aa*) has expanded west and north through the twentieth century to displace *chrysippus chrysippus* (genotype *AA*) from Sumatra, its offshore islands and the Malay Peninsula north to Kedah.

Although *bataviana* has generally been considered a subspecies of *D. chrysippus* (Talbot, 1943; Morishita, 1985), it clusters unequivocally with *dorippus-2* (Figs 2A, 3) and is now best treated as a disjunct race of *D. dorippus*. Since *D. chrysippus* ssp. *cratippus* has a geographical range (Lesser Sunda Islands and Molluccas) parapatric to and sandwiched between those of *bataviana* and *petilia*, it too may be a subspecies of *dorippus* or possibly of *petilia*. The colour pattern of *cratippus* is intermediate between those of its two neighbours, indicating the possibility of east–west gene flow from the Sahul Shelf (Australia and IJ/PNG) across Lydekker's Line to Wallacea; however, in the

absence of DNA data, its status and relationships remain unclear.

A REVISED NOMENCLATURE FOR THE AFRICAN FORMS OF *D. CHRYSIPPUS*

Schreber (1759) described (as *Papilio aegyptius*) a butterfly from 'Aegypto' – Egypt – closely similar to ssp. *chrysippus*. A more recent treatment of *D. chrysippus* in the Indo-Australian region (Morishita, 1985) supported Talbot's arrangement of subspecies (distribution map in Lushai *et al*., 2005a). Ever since Linnaeus (1758) and Schreber, respectively, described them, the Asian *chrysippus* and African *aegyptius* races have been treated, either as species (e.g. Moore, 1883) or, more recently, as vicariant subspecies of *D. chrysippus s.l.* (e.g. Talbot, 1943).

The prevalent view of *D. chrysippus* in Africa (Owen & Chanter, 1968; Rothschild *et al*., 1975; Smith, 1975, 1980) has followed Talbot (1943) in recognizing *aegyptius* as the only African subspecies, with three main 'forms', *aegyptius s.s.*, *alcippus* (Cramer) and *dorippus* (Klug), and several 'minor forms', including *liboria* (Hulstaert). The crucial distinction between polytypic and polymorphic variation has been widely ignored (Smith *et al*., 1997). Other 'minor' African forms, merely listed by Talbot (1943), include (extra-limital) *alcippoides* (Moore), *transiens* (Suffert), *klugii* (Butler), *albinus* (Lanz) and *semialbinus* (Strand), all of which are now known to be F_1 or backcross phenotypes from crosses among the major colour forms, *aegyptius* × *alcippus* (*alcippoides*), *aegyptius* × *dorippus* (*transiens* and *klugii*) or *dorippus* × *alcippus* (*albinus* and *semialbinus*) (Owen & Chanter, 1968; Clarke *et al*., 1973; Smith, 1975, 1998; Smith *et al*., 1998).

The failure to distinguish polytypism and polymorphism (Talbot, 1943) has caused major confusion, since names such as *aegyptius*, *alcippus* and *dorippus* have been applied to both allopatric subspecies and sympatric morphs. The exceptional size of the hybrid zone in East Africa, where the ranges of semi-isolated subspecies and species overlap (Smith *et al*., 1997, 1998; Lushai *et al*., 2003a, 2005b), has frustrated the emergence of a biologically meaningful nomenclature.

Talbot (1943) describes the coloration of *aegyptius* as darker than that of *chrysippus*, but this is only generally true for butterflies from southern Africa. Whereas in southern Africa the ground colour is almost invariably nut-brown (genotype *B*-), the form that prevails from Kenya and Uganda northwards is tawny orange (genotype *bb*), as in the nominotypical Asian form. There is, however, a broad band of polymorphism in central and east Africa where both forms coexist and hybridize (Smith *et al*., 1993, 1997, 1998; Lushai *et al*., 2003a); furthermore, examination of museum material (Smith *et al*., 1998) shows that both orange and brown forms occur as rare outliers far beyond their heartlands. *chrysippus* (Asia) and orange *aegyptius* (North Africa) are probably synonymous as specimens from India ($N = 4$), Oman ($N = 8$) and Kenya ($N = 4$) were identical for both 12S (344 bp) and COI (676 bp) sequences (haplotype ST1 in Lushai *et al*., 2005b). We have therefore relegated the name *aegyptius*, as applied to *D. chrysippus* from North Africa, to the status of a junior heterotypic synonym of *chrysippus* (Lushai *et al*., 2005a).

Although Talbot (1943) noted that two *aegyptius*-like races occur in Africa, he did not distinguish them biogeographically. The two races are differentiated by three phenotypic characters (Talbot, 1943; Smith & Owen, 1997): (1) coloration (brown, or orange); (2) subapical band of five white spots on the forewing (spaces 4–6, 8–9), either broad and fused or narrow with some spots separated; (3) presence or absence of a submarginal white spot in the brown area of space 2 (Cu_{1b}) of the forewing. All these characters are under genetic control (Smith & Owen, 1997). The narrow-banded, orange form (genotype *bbll*), that we now describe as *chrysippus*, ranges from Kenya and Uganda northwards, whereas the brown, broad-banded form, with the spot in space 2 (genotype *BBLL*), has a southern distribution in Africa. We have named the latter form *orientis* (Lushai *et al*., 2005a): the argument for thus naming it is convoluted.

Pennington (1994: pl. 41, fig. 1aiv) lists and illustrates f. *liboria* Hulstaert, 1931 as one of seven forms of *D. chrysippus aegyptius* that have been recorded in South Africa, but fails to indicate that it is the predominant one. However, Hulstaert's type locality for *liboria*, 'Inde Continent, Afrique orientale', is unhelpful to say the least! Talbot (1943) adds further confusion by applying the name *liboria*, not only to a 'Malagassic race', occurring on most islands in the Indian Ocean, but also to a form of '*aegyptius*' that occurs on the African continent, distribution unstated! However, Aurivillius (1909) had earlier described *orientis* (as a variety of *Danaida chrysippus*), giving the type localities as 'Comoren' (Comoro Islands), 'Madagaskar' (Malagasy Republic) and 'Aldabra' (Seychelles).

Having ourselves collected and/or examined large samples of *D. chrysippus* from the Indian Ocean islands and southern Africa, and also inspected Aurivillius' type specimen of *orientis* in the Natur Historiska Riksmuseet, Stockholm (NHRS), we believe that *liboria* and *orientis* are heterotypic synonyms, the latter name having priority (Lushai *et al*., 2005a). Talbot himself recognized the synonymy but failed to give the due priority to *orientis*. Therefore, the subspecific epithet *orientis* should henceforth apply to all *D. chrysippus* populations from islands of the Indian Ocean and southern Africa. However, in

contrast to southern African populations, which are invariably brown, *orientis* from Indian Ocean islands ($N = 760$ examined) are polymorphic for orange (*bb*) or brown (*B-*).

In summary, our phylogenetic analysis identifies two forms of *chrysippus s.s.* in Africa: *chrysippus* (central and northern Africa) and *orientis* (central and southern Africa). The two forms are characterized, respectively, by a single adenine-guanine transition at site 330 in the mitochondrial COI locus (Lushai *et al*., 2005b), a character that is constant in the specimens examined ($N = 22$, 25, respectively, Table 1), and six base pair substitutions (1.5%) in the nuclear EF1-α gene (Lushai *et al*., 2005b). There is thus no doubt that the two forms are distinct clades that are separated by distribution, colour/pattern genes, nDNA and mtDNA characters. Although there is good evidence for assortative mating at the B locus in Tanzania and Ghana (Gordon, 1984; Smith, 1984), as the two forms interbreed readily in Uganda (Smith *et al*., 1993), we classify them as subspecies.

THE STATUS OF *ALCIPPUS*

Lushai *et al*. (2003a) have shown that *alcippus* and *chrysippus s.s.* are substantially vicariant taxa whose distributions overlap in Central and East Africa, where they interbreed. However, the maintenance of concordant nuclear and cytoplasmic genetic differences (linkage disequilibrium) in the face of hybridization in sympatry, is *prima facie* evidence for sexual isolation. Although there is no direct evidence for assortative mate choice between these taxa, a degree of sexual isolation, heterozygote deficiency at one site, differences in sex ratio and in deduced patterns of migration, suggest that *alcippus* is a nascent species (Lushai *et al*., 2003a). However, *alcippus* and *chrysippus s.s.* differ by only 0.3% at mtDNA sites. Therefore, in the absence of better evidence for pre- or postzygotic isolation in sympatry, the status of *alcippus* as a subspecies of *chrysippus s.l.* should remain unchanged. Application of mitochondrial clocks (Lushai *et al*., 2003b) suggests that *alcippus* and *chrysippus s.s.* diverged only within the last 0.5 million years.

A SPECIFIC CATALOGUE OF THE GENUS *DANAUS*

Synonymies are not given here for the genus *Danaus s.l.*, its erstwhile subgenera *Danaus s.s.*, *Salatura* and *Anosia*, or for species and subspecies which are unchanged from those set out by A & V-W. Furthermore, though we propose changes of status for some component taxa, the lineages that comprise *Danaus s.l.* are identical to those included by A & V-W. In cases where we have changed only the name or status of a taxon that is adequately described elsewhere, previous definitions are accepted without further comment. Moreover, subspecies of polytypic species are listed only if we have made changes, to their number or rank, compared to the works cited.

GENUS *DANAUS* KLUK, 1802 (NYMPHALIDAE, DANAINAE, DANAINI, DANAINA)

DANAUS CLEOPHILE (GODART, 1819)

Type locality: ?Haiti (Brown & Heineman, 1972). Monotypic species (A & V-W) (but see Brown & Heineman, 1972). *Range:* Jamaica, Haiti, Dominican Republic.

DANAUS PLEXIPPUS (LINNAEUS, 1758)

Polytypic species (A & V-W) with two subspecies (Forbes, 1939; Clark, 1941; Williams *et al*., 1942; Urquhart, 1960; fig. 43, for a map of subspecies ranges in the Americas; Smith, Miller & Miller, 1994).

ssp. *plexippus* (Linnaeus, 1758)
Type locality: USA, New York State. *Range:* N America to 50°N in summer, migrating south to California and Mexico to over-winter; also Canary Is., Madeira, Azores, Bermuda, many Pacific islands, including Galapagos Is., Hawaii, New Zealand, Australia, Moluccas, Philippines, Taiwan.

ssp. *megalippe* (Hübner, 1826)
Type locality: uncertain but probably USA, Georgia (Brown & Heineman, 1972). *Range:* USA (Georgia, Florida), West Indies, Central & South America north of the Amazon drainage; this subspecies is not migratory. We agree with Urquhart (1960), Brown & Heineman (1972) and Smith *et al*. (1994) that the following 'subspecies' are not geographical entities but mere colour varieties of *megalippe*: *leucogyne* (Butler, 1884), *portoricensis* (Clark, 1941) and *tobagi* (Clark, 1941).

DANAUS ERIPPUS (CRAMER, 1775)

Type locality: Brazil. *Range:* South America south of the Amazon drainage: E Brazil, Uruguay, Paraguay, Argentina, Bolivia, Chile & S Peru (A & V-W).

DANAUS ISMARE (CRAMER, 1780)

Type locality: S Moluccas, Ambon. Polytypic species (A & V-W) with 6–7 subspecies (see Morishita, 1985: 447 for a distribution map; D'Abrera, 1982). *Range:* Sulawezi, Molluccas.

DANAUS GENUTIA (CRAMER, 1779)

Type locality: China, Canton. Polytypic species (A & V-W) with ~16 subspecies (see Morishita, 1985: 451 for

a distribution map). *Range:* India from S Kashmir eastward to China, Taiwan, Philippines, Indo-China, Malaysia, Indonesia & N Australia.

DANAUS AFFINIS (FABRICIUS, 1775)

Type locality: Australia. Polytypic species (A & V-W) with *c*. 45 (see Morishita, 1985: 451 for a distribution map) or perhaps more realistically ~30 subspecies (D'Abrera, 1971, 1982). *Range:* Thailand, Malay Peninsula, Java eastward to Solomon Is., New Hebrides, New Caledonia, Philippines, N & E Australia.

DANAUS MELANIPPUS (CRAMER, 1777)

Type locality: Java. Polytypic species (A & V-W) with 13–18 subspecies, varying with author (see Morishita, 1985: 454 for a distribution map; d'Abrera, 1982). *Range:* Assam, Indo-China, Thailand, Malaysia, Indonesia, Sula Archipelago, Philippines and Taiwan.

DANAUS ERESIMUS (CRAMER, 1777) **COMB. NOV.**

Type locality: Surinam. Polytypic species (A & V-W) with a minimum of seven named subspecies; for the first time we include *plexaure* (Godart) stat. rev. as a subspecies of *eresimus*. For the range of the species, see A & V-W, table 29.

ssp. *plexaure* (Godart, 1819) **stat. rev.**
Danais plexaure Godart, 1819. *Type locality:* Brazil.
Danais plexaure (Godart); Haensch, 1909.
Danaus (Danaus) plexaure (Godart); Forbes, 1939.
Anosia plexaure (Godart); d'Almeida, 1939.
Danaus plexaure (Godart); Talbot, 1943.
Danaus (Anosia) plexaure (Godart); A & V-W, 1984; 208, pl. 18, fig. 109.
South America south of the Amazon drainage (E Brazil, Bolivia, Paraguay, Uruguay, Argentina).

DANAUS GILIPPUS (CRAMER, 1775)

Type locality: Brazil, Rio de Janeiro. Polytypic species (A & V-W) with some ten named subspecies (Clark, 1941; Talbot, 1943; Urquhart, 1960). For range, see A & V-W, table 29.

DANAUS PETILIA (STOLL, 1790)

Type locality: unknown; neotype Queensland, Australia (Lushai *et al*., 2005a). Monotypic species (Lushai *et al*., 2005a). *Range:* Australia, including Tasmania, New Zealand, Irian Jaya/Papua New Guinea, New Hebrides, Loyalty Is., New Caledonia, Fiji.

DANAUS DORIPPUS (KLUG, 1845) **STAT. REV.**

Polytypic species with two subspecies.
Euploea dorippus Klug, 1845.
Limnas dorippus (Klug); Moore, 1883.
Danaus chrysippus (L.) *aegyptius* (Schreber, 1759) f. *dorippus* (Klug); Talbot, 1943.
Danaus (Anosia) chrysippus (L.) f. *dorippus* (Klug); A & V-W, 1984.

ssp. *dorippus* (Linnaeus, 1758) **stat. nov.**
Type locality: Sudan, 'Dongala, Ambukohl'. *Range:* Somalia, Kenya, Tanzania, Uganda, Sudan, Ethiopia, Arabia, Iran, Pakistan (Baluchistan), India (Sind, Kutch).

ssp. *bataviana s.l.* (Moore, 1883) **stat. rev.**
Type locality: Java. (= *alcippoides* Moore, 1883; heterotypic synonym; = *bowringi* Moore, 1883; junior synonym by pagination).
Limnas bataviana; Moore, 1883.
Danaus chrysippus (L.) *bataviana* (Moore, 1883); Talbot, 1943; A & V-W,1984.

There are four colour forms: (1) f. *alcippoides* (Moore), Malay Peninsula south of Penang, Sumatra and surrounding small islands; (2) f. *bataviana s.s.*, Java and Greater Sunda Islands east to Flores; (3) f. *gelderi* (Snellen) from Sulawezi, is now rare and largely replaced by *bataviana s.s.* (Morishita, 1985); (4) f. *bowringi* (Moore) stat. nov. occurs throughout the range of *bataviana s.l.* as a morph.

cratippus (C. & R. Felder, 1860), originally described as a species, is currently a subspecies of *D. chrysippus* (Talbot, 1943), but now of doubtful affinity; mtDNA data would be especially valuable (see above). With a colour pattern and geographical range intermediate between *D. dorippus bataviana* and *D. petilia*, it will probably be shown to be a subspecies of one of them, rather than of *D. chrysippus*.
Type locality: Indonesia, 'Ambon'.

Danais cratippus C. & R. Felder, 1860.
Limnas cratippus (Felder); Moore, 1883.
Danaus chrysippus (L.) *cratippus* (C. Felder); Talbot, 1943; A & V-W, 1984.
Range: Lesser Sunda Islands (east from Aru), Molluccas.

DANAUS CHRYSIPPUS (LINNAEUS, 1758) **COMB. NOV.**

Polytypic species (A & V-W) with three subspecies.

ssp. *chrysippus* (Linnaeus, 1758) **comb. nov.**
(= *aegyptius* Schreber, 1759; junior heterotypic synonym, Lushai *et al*., 2005a). *Type locality:* China, Canton. *Range:* China, Taiwan, Japan, Indo-China, Philippines, Borneo, Malay Peninsula (Kedah & Langkawi I), Thailand, Burma, India, Sri Lanka, Pakistan, Afghanistan, Iran, Iraq, Palestine, Lebanon, Turkey, Cyprus, Malta, Greece, Italy, Spain, Tunisia,

Algeria, Morocco, Canary Is., Arabia, Egypt, Sudan, Ethiopia, Kenya, Uganda, Congo Republic.

ssp. *alcippus* (Cramer, 1777)
Type locality: Sierra Leone, 'Côte de Guinée, Sierra Leona'. *Range:* Cape Verde Is., West Africa from Senegal east to Cameroun, Macias Nguema (Fernando Po), Central African Republic, Uganda, Sudan, Congo Republic, Ethiopia, Yemen, Oman, Kenya, Tanzania.

ssp. *orientis* (Aurivillius, 1909) **comb. nov.**
(= *liboria* Hulstaert, 1931, junior heterotypic synonym, Lushai *et al*., 2005a). *Type localities:* Comoro Is., Malagasy Republic, Seychelles including Aldabra. *Range:* Seychelles, Mauritius, Bourbon, Rodriguez, Nossé-Bé, La Réunion, Comoro Is., Madagascar, South Africa, St. Helena, Moçambique, Namibia, Botswana, Zimbabwe, Zambia, Malawi, Tanzania, Angola, Congo Republic, Gabon, Uganda.

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APPENDIX 1

A. MORPHOLOGICAL, BIOCHEMICAL AND COLOUR GENE CHARACTERS FOR *DANAUS* TAXA AND TWO *TIRUMALA* SPECIES (OUTGROUPS)

Bold Roman numerals indicate datasets (I-VI); character numbers (1–67) are column headings in the matrix (Appendix 1B))

I Allozymes[1]: 1 Acid phosphatase bands A/B, bands C/D, band F; (other); 2 Aldolase, band A (band C); 3 Hexokinase-1, bands F/G, band B; (other); 4 Esterase-D, polymorphic for bands B/C/D, C unique; polymorphic for bands A/B, A unique; (other); 5 Malic enzyme-2, band B; band D; band E; (other); 6 Superoxide dismutase-1, bands A/B; band G; (other); 7 Aspartate aminotransferase-1, bands F1/G; band C; (other); 8 Malate dehydrogenase, monomorphic for band S; polymorphic for bands F/S; polymorphic for bands B/S; 9 Phosphohexose isomerase, functional allele; (silent allele); 10 6-phosphogluconate dehydrogenase, band F; (other).

II Dihydropyrrolizines[2]: Hairpencil secretion contains 11 hydroxy-danaidol, 12 danaidal, 13 danaidone.

III Eggs[3]: 14 In polar view, ribs raised and narrow, cells concave (longitudinal ribs wide and flat, cells generally convex).

IV Larvae[3]: 15 Cuticular ornamentation on body surface pointed (blunt); 16 Filaments present on segment 5; 17 Subprimary labial seta lateral to seta *M*1 (mesial); 18 Epipharyngeal spines between seta 1 and 2 present distally; 19 Number of campaniform sensillae on ventral surface of galea, two (one); 20 Submentum, basally, at least as far as insertion of primary setae, sclerotized (unsclerotized); 21 Submental cuticular ornamentation not extending laterally of primary setae at the level of their insertion (extending almost to edge of stipes); 22 Major hypopharyngeal spines in one regular row (in one regular row and a slightly more distal irregular row); 23 Depressed area on hypopharynx, distal to major spines, smooth (covered in small spines).

V Pupae[3]: 24 Grooving on stalk deep (shallow); 25 Abdominal segments 4–7 smooth; slightly rugose; distinctly rugose; 26 Median dorsal spot on abdominal segment 9 absent (present); 27 Angle between abdominal segments 1–3 and 3–8 obtuse (approximately 90°); 28 Transverse yellow band on abdominal third segment of exuviae; 29 'Monarch gold spot array' only (additional median spots on abdominal segments 1 and 2, a lateral pair on segment 2 and a pair at the base of the hindwing); 30 Mesothorax continuous in outline with abdomen (slightly swollen dorsally).

VI Imagines[2,4–7]: 31 Male with a section of vein 1 *A* + 2 *A* swollen; 32 Deep pouch containing particle-producing hairs in male hindwing cell Cu_{1b}; 33 Well-developed, lateral, ventrally directed projections from anteroventral lip of female 8th sternite; 34 Clasper with an annularly corrugated process; 35 Male alar organ formed as a pocket (in which the anterolateral 'roof' or flap originates as a dorsal outgrowth); 36 Male terminalia with pseudovalves; 37 Forewing with four (three) pale pattern elements in forewing cell *M*1; 38 Aedeagus long and narrow; 39 Ductus long and narrow; 40 Saccus long, somewhat swollen in dorsal aspect; 41 Hairpencils long; short; vestigial; 42 Fifth tarsal segment of mid- and hind-legs long and armed with long spines; 43 Juxta narrow; 44 Aedeagus incised dorsally or dorso-laterally (ventrally); 45 Aedeagus with well-developed lateral spines mounted on processes; 46 Aedeagus with fewer, smaller, lateral spines[4] not mounted on processes; 47 Hindwing vein $Sc + R_1$ curved in an even, shallow arc; 48 Androconia distributed in cell Cu_{1b} about the entrance of the pouched alar organ; 49 Hindwing cell elongate, *c*. $^2/_3$ length of wing (*c.* $^1/_2$ length of wing); 50 Forewing cell *R*4 with single, large, marginal, white spot; single smaller spot; two small spots; 51 Clasper deeper than long, with one (two) curved (corrugated) processes; 52 Clasper very short, lacking postero-dorsal bulge; 53 Clasper extremely short, lacking postero-dorsal bulge; 54 Intracellular, postdiscal, pale markings in cells Cu_1, M_3, M_2, M_1 and *Rs* of hindwing underside; 55 Hindwing crossvein m_1-m_2 sharply angled; 56 Course of hindwing long veins beneath black and bordered with black (marked with pale, whitish scales); 57 Series of three black spots on *m-cu* crossveins of hindwing; 58[4] Marginal white spots on hindwing underside in a double row (single row); 59[4] Black margin of hindwing broad (narrow); 60 hindwing marginal area pale posteriorly; 61 Wings with blue (phycobilin) pigment; 62[6] Wings with extensive areas of yellow pigment; tawny orange pigment (*bb* genotype in *D. chrysippus*); nut-brown pigment (*B-* genotype in *D. chrysippus*); 63[5] The apical third (or more) of the forewing upperside black with white spotting (black pigment predominantly confined to wing margins; 64[6] Central, small white patch on hindwing underside; 65[5] Hindwing predominantly white (*aa* genotype in *D. chrysippus*); 66[5] Subapical pale spots in forewing cells $M3$, M_2, M_1 & R_5 (*cc* genotype in *D. chrysippus*); (spots absent, *C-* genotype in *D. chrysippus*); 67[7] Subapical pale spots in forewing cells $M3$, M_2, M_1 & R_5 (if present) large and fused. (*L-* genotype in *D. chrysippus*).

References: [1]Kitching (1986); [2]Ackery & Vane-Wright (1984); [3]Kitching (1985); [4]Smith *et al*. (2002); [5]Smith (1998); Lushai *et al*. (2003a-b, 2005a); [6]Common & Waterhouse (1972); [7]Smith & Owen (1997).

B. MORPHOLOGICAL, BIOCHEMICAL AND COLOUR GENE CHARACTER MATRIX FOR TWO *TIRUMALA* SPECIES (OUTGROUPS) AND 15 *DANAUS* TAXA

	Characters				
	I	**II**	**III–IV**	**V**	**VI**
	0000000001	111	1111112222	2222223	333333333444444444455555555556666666666
OTUs	1234567890	123	4567890123	4567890	12345678901234567890123456789012345678901234567
Ts†	0010100010	101	1101101111	1201000	11100000001000001100000001011010*0000
Tl†	0010100010	101	1101101111	1201000	11100000001000001100000001011010*0000
Dpx	1101010110	000	0000010101	0010111	00011111110100000010000001011002000000
Der	1101300011	000	0000010101	0100111	00011111110100000010000001011102000000
Dgn	2102021010	111	0010000110	1200011	00011110001011000012100001011002100010
Dm	2102021010	***	0*1*******	1200011	00011110001011000021100001011002101111
Da	2122022210	111	0010000110	1200011	00011110001011000021010001011002101111
Di	**********	***	**********	*******	00011010002011000001200001011010100010
Dem	0102200010	***	0*1*******	1200111	00011110001000010000200101011003000000
Dgl	0102000010	001	0010000011	1200111	00011010001000100000200011011003000000
Dpt	3102000010	001	0010000011	1200111	00011010001000100002200011011001110100
Dd1	0102000000	001	0010000011	1200111	00011010001000100002200010100002000000
Dd2	0102000000	001	0010000011	1200111	00011010001000100002200010100002000000
Ddb	**********	001	0010000011	1200111	00011010001000100002200010100002101111
Dcc	0102000010	001	0010000011	1200111	00011010001000100002200010100002100010
Dco	**********	001	0010000011	1200111	00011010001000100002200010100003100011
Dca	0102000010	001	0010000011	1200111	00011010001000100002200010100002101110

Character groups: I, allozymes; II dihydropyrrolizines; III, eggs; IV, larvae; V, pupae; VI, imagines.
Ts = *Tirumala septentrionis*, Tl = *T. limniace*, Dpx = *Danaus plexippus*, Der = *D. erippus*, Dgn = *D. genutia genutia*, Dm = *D. melanippus hegesippus*, Da = *D. affinis malayana*, Di = *D. ismare ismareola*, Dem = *D. eresimus tethys*, Dgl = *D. gilippus berenice*, Department = *D. petilia*, Dd1 = *D. dorippus-1*, Dd2 = *D. dorippus-2*, Ddb = *D. dorippus bataviana*. f. *alcippoides*, Dcc = *D. chrysippus chrysippus*, Dco = *D. chrysippus orientis*, Dca = *D. chrysippus alcippus*. Column numbers indicate variable sites. * = missing data or character unscorable. †As a complete dataset is unavailable for any *Tirumala* species, two outgroups are assembled primarily from *T. septentrionis* and *T. limniace* (to match the DNA datasets), using *T. petiverana*, *T. hamata* and *T. ishmoides* to fill character gaps.

APPENDIX 2

Character matrix for DNA sequences from fragments of the 18S rRNA (525 bp) and EF1α (400 bp) nuclear genes and the 12S rRNA (344 bp) and CO1 (676 bp) mitochondrial loci in *Tirumala septentrionis* and *T. limniace* (outgroups) and 15 taxa of *Danaus*

	Characters (variable sites)			
	18S	**EF1α**	**12S**	**CO1**
	11234	0001111112223333	00000000111111111111111111222233	00000000000000000111
	99043	1170022676891344	04566799002233334555566699909991 1	01223333444556789900 1
OTUs	34414	6900647455086767	75534223242625674456912757903491 2	35180169268129210925 1
Ts	ATT-A	**********	CTCTTT-TTAAATCTATT-TTTAAAT--AATTA	CTTTATTTTTAATTAATTTCT
Tl	*****	**********	**********	*TTTATCTTTAATCAATTTTT
Dpx	ATT-A	T-CTTCTAGATATCCT	*ATTTA-TTAAATTTTTA-TTTAATT--AATTA	CTACTTCAAATATCATAATCA
Der	ATT-A	**********	*ATTTA-TTAAATTTTTA-TTTAATT--AATTA	**********
Dgn	-AT-A	T-CTTCTCTAAACCTC	TTTTTA-TTAAATTATCAATATAATT--AATTA	*******AAACATTAAAAGCA
Dm	AT--A	**********	TTTAAA-TTAAATTATTAAAATAATT--AATTA	********AACATTAAAAGCA
Da	-TTGA	**********	*TTAAA-TTATATTATTAAAATATTT--AATTA	**********
Di	-TT-A	AGCTTTTCTAAATCCT	*TTTTATTTAAT-AGTTAAAATAATT--TATTG	TTACCTCAAACTTTTAAATCA
Dem	ATT-A	TGTTTCTCGAAATCCT	CTTTTATACAAACTTTTAAAATTATT--ATTTT	TCGCTTCGAAAGCTAAAATCA
Dgl	ATT-A	**********	CTTTTATATAAACTTTTAAAATTATT--ATTTT	TCGCTTCGAAAGCTAAAATCA
Dpt	ATT-A	**********	CTTTTATTTTAA-TTTTAAAATTATT--AACCA	GTACTTCAAAACTTAATATCC
Dd1	ATT-A	TGTTTCTCGAAATCCT	CACTTATTTAAATTTTTA-ACAAATT--AATTA	TTACTCCAAACATTATAATCA
Dd2	ATT-A	TGTTTCTCGAAATCCT	CTCTTATTTAAA-TTTTAAAATTATTTAAACTA	ATACTTCAAAACTTAATGTCC
Ddb	ATT-A	**********	CTCTTATTTAAA-TTTTAAAATTATTTAAACTA	ATACTTCAAAACTTAATGTCC
Dcc	ATT-A	TGCCCCCCTGAGCCCC	CTCTTATTTAAA-TTTTAAAATTATTTAAACTA	ATACTTCAAAACTTAATGTCC
Dco	ATT-A	TGCCTCTCGGAATTCC	CTCTTATTTAAA-TTTTAAAATTATTTAAACTA	ATACTTCAAAACTTAATGTCC
Dca	ATT--	**********	CTCTTATTTAAA-TTTTAAAATTATT--AACTA	ATACTTCAAAACTTAATGTCC

	Characters (variable sites)
	COI
	11111111111111111111111222222222222222233333333333333333333333333444444444444
	12223333444556667778899912344566777899990000011223334455667788899990112333456 6
OTUs	70692358147165681270635862478212139814580467928170396814162814703692173258100 5
Ts	CTTTTGATATTTATCTTTAATCTAACTTCTACGTTTTTCTACTTATTAATTTTAATTTTTTATATATACTTCTTTTTA
Tl	TTTTTAATAATTATCTTTAATCTAATTTCTACGCTTTTCTACTTATTAATTTTAATTTTTTATATGTATTTTTTTTTA
Dpx	AATTTAAATTACTTTATTTAATAATCAACTACGTATATCCTTACTTATAAATCTTTTTTTTTTTAACTTTTCTTTTTA
Der	**********
Dgn	ATTTTGAACAATTTTACTTATTATATATCTACGAATTTCCTTACTATTAAATCTGTTTATTTTTAATACCCTTTTTTA
Dm	ATTTTGAATAATTTTATTTATTATATATCTACGAATTTCCTCACTATTAAATCTTTTTATTTTTAATACCCTTCCTTG
Da	**********
Di	CTTTTAAACAATTATATTTATTAATTATCTACGACTATCCTTGCTATTAAATCTTTTTATTTCATATAATTTTCTTTC
Dem	CTTTTACATTTTTTTATTCATTATTTATTTGCATACATGCTTACTATTCAATCCTTCTATTTAAATTATTTTCTTTTT
Dgl	CTTTTACATTTTTTTATTCATTATTTATTTGCATACATGCTTACTATTCAATCCTTCTATTTAAATTATTTTCTTTTT
Dpt	ATTAAGAATTATTTTATCTGTCAATTATCTATATATACCTTTACAATTTAATCTTCTTATTTTAAACATTTTTTATCA
Dd1	AACACGAATATTCTTATTGATTAATTTTCCACGTATATCCTTACTTATAAAACTATTTTTCTTAAATTCTTTGCTCTA
Dd2	ATTCAGAATTATTTTATTTATTAATTATCTATGTACATCTTTACAATTTAACCTTCCCACTTTAAATATTTTTTATTA
Ddb	ATTCAGAATTATTTTATTTATTAATTATCTATGTACATCTTTACAATTTGACCTTCCCACTTTAAATATTTTTTATTA
Dcc	ATTCAGAATTATTTTATTTATTAATTATCTATGTATATCTTTACAATTTAACCTTCCTACTTTAAATATTTTTTATTA
Dco	ATTCAGAATTATTTTATTTATTAATTATCTATGTATATCTTTACAATTTGACCTTCCTACTTTAAATATTTTTTATTA
Dca	ATTCAGAATTATTTTATTTATTAATTATCTATGTATATCTTTACAATTTGACCTTCCTACTTTAAATATTTTTTATTA

APPENDIX 2 *Continued*

	Characters (variable sites)
	COI
	4444445555555555555555555566666666666666666666
	6789990111222466666777889000112333445556666
OTUs	8433457349589612457039586036581369250790369
Ts	ATTTCTTGGTTCTATTATAATTCTTATATTCTAAAATTGTTTT
Tl	ATTTCTTGGTTTTCTTATAATTTTCATACTCTAAAATTTTTCT
Dpx	AATTCTAAGCTTTTTCTCTATTTTTTTTTTTCTTATTTGTAAA
Der	AATTCTAAGCTTTTTCTCTATTTTTTTTTTTCTTATTTGTAAA
Dgn	AACTCTAAGTATTTATACTATT**********************
Dm	AATTCTAGATATTTATACTGTT**********************
Da	**
Di	AGTTCTAAGTATTTCCACTATATTTAATTTATTAATTTTTAAG
Dem	AGTTCTAAGTTTTAATATAACTTCTCTTTCTTATATTGTAAAT
Dgl	AGTTCTAAGTTTTAATATAACTTCTCTTTCTTATATTGTAAAT
Dpt	CATAGTAAGTGATAATATAATACTTATATTTTTTATTCTTAAC
Dd1	AGTTCAAGGTTTTTATATAATTCTTACATTCTATATTTTATAT
Dd2	CGCAGTAAGTAATAATATAATATTTATGTTTTTCATA**AAAG
Ddb	CGCAGTAAGTAATAATATAATATTTATGTTTTTCGTTTTAAAT
Dcc	CGCAGTAAGTAACAATATAATATTTATATCTTTCGTTTTAAAT
Dco	CGCAGTAAGTAACAATATAATATTTATATCTTTCGTTTTAAAT
Dca	CGCAGTAAGTAACAATATAATATTTATATCTTTCGTTTTAAAT

– = gap (18S and 12S only); A = adenine; T = thymine; C = cytosine; G = guanine. Other notation as in Appendix 1B. A** in Dd2 is a ten nucleotide sequence at sites 650–659 which shows eight base pair differences compared to all other taxa (Lushai *et al.,* 2005b); it is interpreted as a frameshift by a single position, caused by the downstream translocation of a single base pair from the 3′ to the 5′ end of the frame-shifted sequence; it is scored as a single character difference at site 650.