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The lesser wanderer butterfly, *Danaus petilia* (Stoll 1790) stat. rev. (Lepidoptera: Danainae), reinstated as a species

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Abstract The lesser wanderer butterfly, *Danaus* (*Anosia*) *chrysippus petilia* (Stoll) (Lepidoptera: Danainae), has been treated as a subspecies for the last 100 years. New mitochondrial DNA sequence data for *D. petilia*, in conjunction with allozyme, structural, morphometric and pattern characters, constitute a compelling case for its specific rank. The holotype of *D. petilia* has never been located and, as the type location is uncertain, a neotype is designated. Fresh material was collected and examined for this project. *Danaus petilia* and *D. chrysippus* have been separated at Lydekker's Line for an estimated 1.1 million years, and they remain interfertile. However, as an allopatric taxon, diagnosable from *D. chrysippus*, *D. petilia* merits specific status under the phylogenetic species concept. The following pairs of *D. chrysippus* subspecies are considered to be synonyms, the first member of each pair having priority: *chrysippus* + *aegyptius* (subspecies), *klugii* + *infumata* (hybrid phenotypes) and *orientis* + *liboria* (subspecies).

Key words allozymes, biological species concept, *Danaus chrysippus*, *Danaus petilia*, mitochondrial DNA, phylogenetic species concept, Systematics.

INTRODUCTION

The genus *Danaus* Kluk (monarchs and queens), subfamily Danainae (milkweed butterflies), family Nymphalidae, comprises 11 tropical and subtropical species (Ackery & Vane-Wright 1984). Three of the five Old World species occur in Australia; a further five have New World distributions, and one, *D.* (*Danaus*) *plexippus* (L.), is nearly cosmopolitan. *Danaus* is divided into three non-coordinate subgenera, *Danaus* (three species), *Salatura* Moore (four species) and *Anosia* Hübner (four species). The most widespread and ubiquitous member of the genus is the Old World *D.* (*Anosia*) *chrysippus* (L.), which is divided into 6–7 (varying with author) partially or absolutely vicariant subspecies (Fig. 1). Unlike other milkweed butterflies, which are predominantly forest dwellers, *Danaus* spp. generally frequent open country, ranging from semi-desert to light woodland and coastal mangrove swamp. The bold *Danaus* colour scheme of black, white and orange presents a memorable image that serves to remind potential predators of their distastefulness (Brower *et al*. 1968).

By the close of the 18th century four 'species' of '*Papilio*', which are now subsumed into the widespread and abundant Old World species *Danaus chrysippus* (L.), had already been named (Ackery & Vane-Wright 1984). Through the 19th century, European (mainly German) taxonomists added five further Old World 'species' under the various generic names *Danaida* Latreille, *Limnas* Hübner, *Euploea* Fabricius and *Danais* Latreille that would eventually be considered forms of *D. chrysippus*. Following Butler's naming of '*Limnas klugii*' in 1886, no less than nine 'species' would eventually be amalgamated as *D. chrysippus* (three new synonymies nominated here; see Table 1). The following pairs of *D. chrysippus* subspecies are considered to be synonyms, the first member of each pair having priority: *chrysippus* + *aegyptius* (subspecies, see Smith *et al*. 1997, 1998), *klugii* + *infumata* (hybrid phenotypes) and *orientis* + *liboria* (subspecies). After examination of Aurivillius' *infumata* and *orientis* type specimens in the Naturhistoriska Riksmuseet, Stockholm (NHRS), Smith *et al*. (1997, 1998) concluded that the former is a junior synonym for *klugii* while *orientis* is identical to and has priority over *liboria*; both synonyms are heterotypic since the nominated types in each pair of taxa have different though geographically adjacent type localities. Smith *et al*. (1997, 1998) argued the case for these changes but did not formally synonymise the names, and we do so here.

Meanwhile, three further species which, together with *D. chrysippus*, now comprise the subgenus *Anosia*, were recognised from the New World under the names '*Papilio*'

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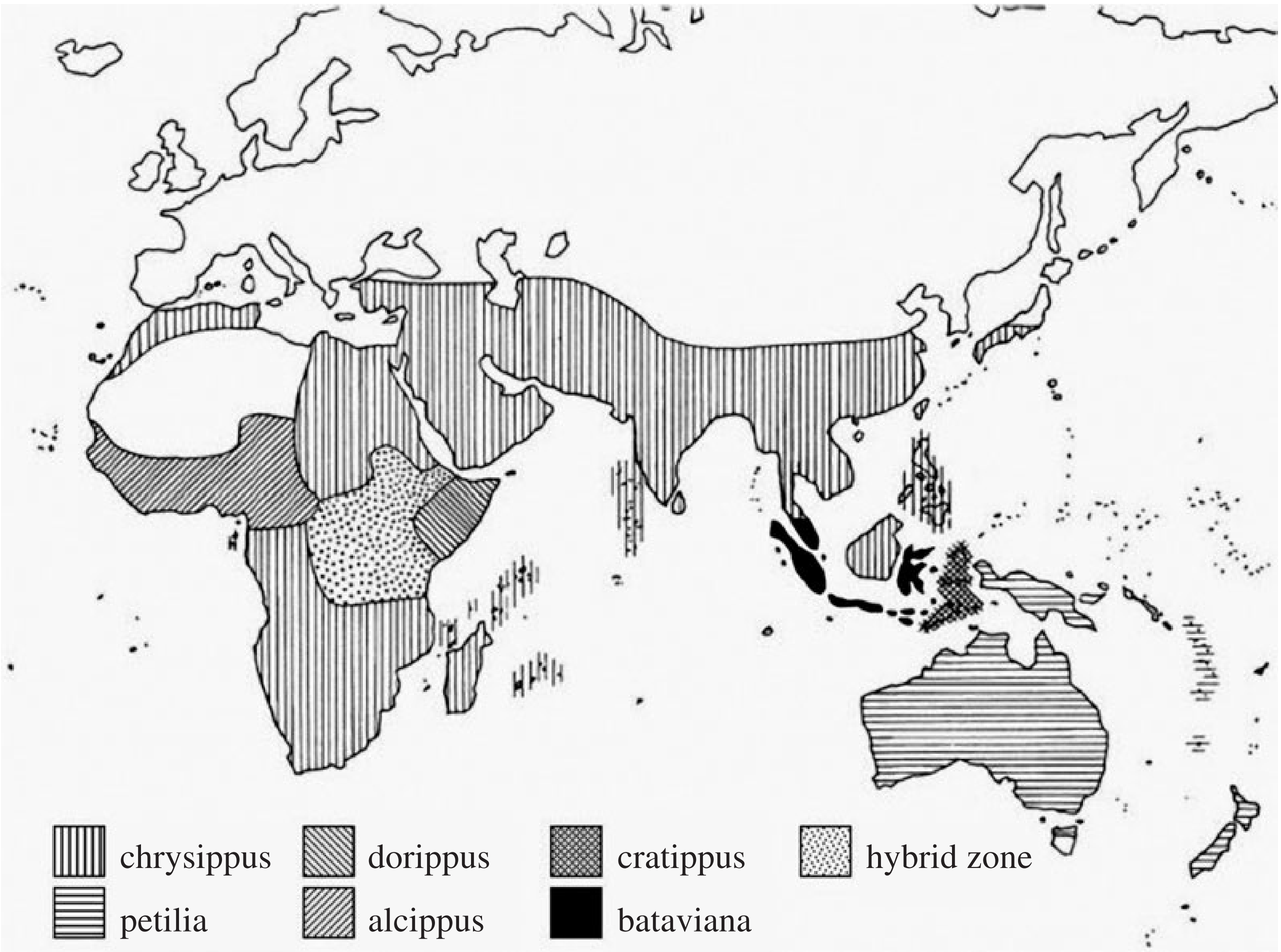

Fig. 1. Geographical distribution of the subspecies of *Danaus chrysippus*. The hybrid zone in Central and East Africa is an area where the ranges of two or more of the subspecies *chrysippus*, *alcippus* and *dorippus* overlap and interbreed (Smith *et al.* 1998).

gilippus Cramer, '*Papilio*' *eresimus* Cramer and '*Danais*' *plexaure* Godart. The New World *Anosia* species have stood the test of time relatively well. However, as the little-known *D. plexaure* lacks apomorphies with respect to *D. eresimus*, the former is probably best regarded as a replacing subspecies of the latter (G. Lamas in Ackery & Vane-Wright 1984).

About seven of the former 'species' that now comprise *D. chrysippus* are currently treated as polytypic forms, i.e., subspecies (Talbot 1943; Morishita 1981), while others have either changed status to morph, 'variety' or 'aberration' (Talbot 1943), or have sunk into synonomy (Smith *et al.* 1997, 1998; Table 1). Furthermore, genetical work has shown that two former 'species' are hybrid phenotypes (Smith *et al.* 1998) (Table 1). Nomenclature within the '*chrysippus*' complex has long been confused by failure to distinguish the essentially vicariant forms (that are subspecies or may even be species) from sympatric morphs and their hybrids (Smith *et al.* 1997).

Ever since Stoll (1790) described the lesser wanderer butterfly as a new species, *Papilio petilia*, distinct from the nominotypical *Papilio chrysippus*, the validity of the Australasian species seems to have been accepted without demur for at least 100 years. Although Stoll was aware of *Papilio chrysippus*, he may not have seen the holotype from Canton, China (Corbet 1949), which was among the collections purchased by Sir James Edward Smith from Linnaeus' estate after his death and, since 1788, kept at the Linnean Society's rooms in London. In his review of *Danaus*, Talbot (1943) listed *petilia* as one of seven subspecies of *D. chrysippus* (Table 1), and all subsequent authors have concurred (D'Abrera 1977; Gibbs 1980; Common & Waterhouse 1981; Ackery & Vane-Wright 1984; Parsons 1999; Braby 2000). However, Zalucki (1999), while observing that the taxonomic rank of *petilia* had never been seriously addressed, noted that an allozyme autapomorphy that distinguished *D. petilia*, not only from other *D. chrysippus*, but also from all the other seven *Danaus* species tested (Kitching 1986), could imply that *D. petilia* is a species. Kitching (1986) examined all *Danaus* species with the exception of *D.* (*Danaus*) *cleophile* (Godart), *D.* (*Salatura*) *ismare* (Cramer) and *D.* (*A.*) *eresimus*.

In this paper, we present new taxonomic data derived from mitochondrial DNA (mtDNA) sequences and review the molecular and morphological characters that distinguish *D. petilia* from other *D. (Anosia)* species, including other

Table 1 Taxonomic history of the subtaxa that comprise *Danaus (Anosia) chrysippus*

Subtaxon	Present status	Original status (name)	Authority	Type locality
chrysippus	ssp. & m.†	sp. (*Papilio chrysippus*)	Linnaeus 1758	China: Canton§
aegyptius syn. n.	=*chrysippus*‡	sp. (*Papilio aegyptius*)	Schreber 1759	Egypt: 'Aegypto'¶
alcippus	ssp. & m.†	sp. (*Papilio alcippus*)	Cramer 1777	Sierra Leone: 'Cote de Guinée, Sierra Leona'¶
petilia	ssp.†	sp. (*Papilio petilia*)	Stoll (1790)	Coromandel Coast [India]
dorippus	m.†‡	sp. (*Euploea dorippus*)	Klug 1845	Sudan: 'Dongala, Ambukohl'¶
cratippus	ssp.†	sp. (*Danais cratippus*)	Felder 1860	Indonesia: 'Ambon'†
alcippoides	m. (Asia)† hp. (Africa)‡	sp. (*Limnas alcippoides*)	Moore (1883)	Nepal†
bataviana	ssp.†	sp. (*Limnas bataviana*)	Moore (1883)	Indonesia: 'Java'†
klugii	hp.‡	sp. (*Limnas klugii*)	Butler 1886	Somalia: 'South of Berbera'¶
infumata syn. n.	hp.‡ (=*klugii*)‡	ab. of *Danaida dorippus*	Aurivillius 1898	Sudan: 'Nubia'¶
transiens	hp.‡	ab. of *Danaus dorippus*	Suffert 1900	'Deutsche-Ostafrika bis Arabien'¶
albinus	hp.‡	ab. of *Danais dorippus*	Lanz 1896	Tanzania: 'Parumbira'¶
semialbinus	hp.‡	ab. of *Danaida dorippus*	Strand 1910	Tanzania: 'Amani'¶
orientis	ssp.†	var. of *Danaida chrysippus*	Aurivillius 1909	Comoro Is., Malagasy Republic, 'Aldabra'¶
liboria syn. n.	ssp. & m.† (=*orientis*)‡	ssp. of *Danaus chrysippus*	Hulstaert 1931	'Inde Continent, Afrique orientale'¶

†Talbot (1943); ‡Smith *et al.* (1997, 1998); §Corbet (1949); ¶Ackery *et al.* (1995).
ab., aberration; hp., heterozygous phenotype; m., morph; sp., species; ssp., subspecies; syn. n., new synonym; var., variety.

subspecies of *D. chrysippus*. The combined evidence leads us to reinstate *D. petilia* as a (phylogenetic) species (Cracraft 1983, 1989) more than two centuries after its original citation.

TAXONOMIC HISTORY

Papilio petilia Stoll (1790).
Danais petilia (Stoll): Godart (1819).
Danais chrysippus f. *petilia* (Stoll): Miskin (1874).
Danais chrysippus var. *petilia* (Stoll): Oberthür (1880)
Limnas petilia (Stoll): Moore (1883).
Danaus (*Limnas*) *petilia* (Stoll): Waterhouse (1903)
Danaida (*Limnas*) *chrysippus* ssp. *petilia* (Stoll): Fruhstorfer (1907) (Fruhstorfer's ssp. *petilia* comprises two forms, *petilia* and *cratippus* C Felder (as *Danais cratippus*): the latter form is now a separate subspecies (Talbot 1943; Morishita 1981)).
Danaida chrysippus petilia (Stoll): Waterhouse and Lyell (1914).
Danaus (Panlymnas) chrysippus petilia (Stoll): Bryk (1937).
Danaus (*Danaus*) *chrysippus* ssp. *petilia* (Stoll): Forbes (1939).
Danaus chrysippus ssp. *petilia* (Stoll): Talbot (1943).
Anosia chrysippus ssp. *petilia* (Stoll): Morishita (1981).
Danaus (*Anosia*) *chrysippus petilia* (Stoll): Ackery and Vane-Wright (1984).

The type locality given in Stoll's (1790) description of *P. petilia* has been previously misquoted variously as Java, China and Coromandel Coast (e.g. Godart 1819; Bryk 1937; Edwards *et al.* 2001). Stoll says 'I have often received this species from Samarang, on the north coast of Java, and from China, but this one is from the coast of Coromandel (J'ai souvent reçu cette espece de Samarang, sur la côte septentrionale de Java, & de la Chine mais celui-ci est de la côte de Coromandel . . .)' and implies that the description was based on a single specimen supposedly from the Coromandel Coast of India. This locality is obviously an error, as *D. chrysippus chrysippus* is the only subspecies recognised from India. Doubleday (1847) appears to have been the first to consider that the species did not occur in these countries, as he gave the distribution as 'Australia generally' without further discussion. Masters (1873) was the first Australian-based worker to confirm that the species was Australian.

Common and Waterhouse (1981) distinguish the Australian populations by the 'white central patch' on the hindwing underside. This patch varies in size, in some specimens it is almost absent, while in others it is very obvious; 37 specimens, Australia and Papua New Guinea in University of Queensland Insect Collection (UQIC). This white area, in its reduced form, occurs in other subspecies from south-east Asia. However, the broad black terminal band on the upperside of the hindwing is a character that appears to be unique to the Australasian population (see below). This broad band is obvious in Stoll's original figure (Fig. 2).

As the holotype of *P. petilia*, a female, has never been located (Parsons 1999) and there is confusion about the country of the type locality, a specimen collected with the material used for molecular studies is here proposed as a neotype. Collection data: Mount Crosby near Ipswich, Queensland 27°32′S 152°47′E, 14 September 1998, MP Zalucki and AR Clarke, deposited in the Australian Museum, Sydney.

MATERIALS AND METHODS

Collection of butterflies

The material of *D. petilia* for mtDNA sequencing comprised 13 adults (seven males, six females), netted on 14 September 1998 by MP Zalucki and AR Clarke at Mount Crosby (27°32′S 152°47′E), near Ipswich, South-eastern Queensland,

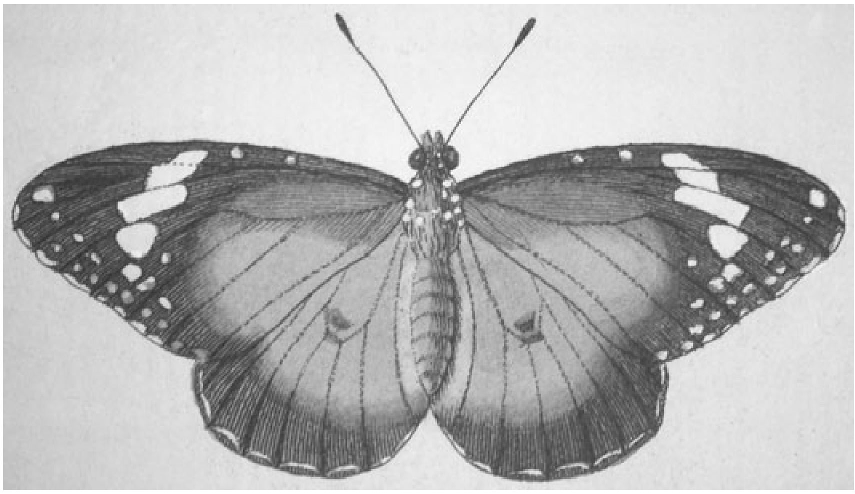

Fig. 2. Stoll's 1790 illustration of *Papilio petilia*.

Table 2 Samples of *Danaus* butterflies used to sequence the 12S rRNA and COI mitochondrial genes

Species and subspecies	Provenance	*12S* rRNA (347 bp)		*COI* (676 bp)	
		GenBank no.	*n*	GenBank no.	*n*
Tirumala septentrionis	Malaysia	AF389388	8	AF394182	8
Danaus (Danaus) plexippus	Australia, USA	AF389889	14	AF394183	9
D. (Anosia) chrysippus dorippus	Kenya	AF389890	13	AF394184	8
D. (Salatura) genutia	Malaysia, Thailand	AF389892	11	AY256344	2
D. (A.) eresimus tethys	Grand Cayman	AF389895	8	AF394185	8
D. (A.) gilippus berenice	Grand Cayman	AF389896	8	AF394186	8
D. (A.) petilia	Australia	AF389898	9	AF394188	8
D. (A.) c. bataviana†	Malaysia	AF389899	13	AF394189	8
D. (A.) c. chrysippus	Zambia, Tanzania	AF389903	23	AF394193	14
D. (A.) c. alcippus	Ghana, Uganda	AF389901	26	AF394191	10

†Form *alcippoides* Moore.

Australia. The habitat was a mixed stand of *Asclepias fruticosa* (=*Gomphocarpus fruticosus*) and *A. curassavica*. Butterflies were boxed in the field and later killed in ethyl ethanoate vapour. Wings were removed and retained in papers, while the body was immediately stored in 95% ethanol at −20°C. A further 15 males and 4 females from the same site provided material for morphological examination. For molecular phylogenetic analysis based upon mtDNA, samples of eight further *Danaus* lineages, including four subspecies of *D. chrysippus*, and *Tirumala septentrionis* (Butler) were also collected (Table 2). The collection of *D. petilia* in the Oxford University Museum of Natural History (OUMNH) ($n = 36$), which covers its entire geographical range, was examined to improve sample sizes for some morphological characters. The repository for all the new *D. petilia* material other than the neotype is the University of Queensland Insect Collection.

Extraction, amplification and sequencing of DNA

The new data consist of partial mtDNA sequences from the 12S rRNA-coding (*12S*) gene (347 bp) and the protein-coding cytochrome c oxidase, subunit I (*COI*) gene (676 bp). MtDNA was extracted using methods described for the *12S* gene by Lushai *et al*. (2003). Forward and reverse primers for the *COI* gene were, respectively, 5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′ and 5′-ATA CTT CTC TAG CAT ATA AAG-3′; for a later run for *D. (S) genutia* (Cramer), they were 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′ (forward) and 5′-TCCAATGCACTAATCTGCCATATTA-3′ (reverse). Polymerase chain reaction (PCR) amplifications were carried out in an Omnigene (Hybaid Ltd), using a hot start at 94°C for 2 min (1 cycle), denaturation at 92°C for 1 min, annealing at 49–55°C for 1 min (dependent on primer specificity), extension at 72°C for 2 min (30 cycles) and a final extension step of 72°C for 7 min (1-cycle). Sequencing for the *12S* gene is detailed in Lushai *et al*. (2003) and the same procedure was used for the *COI* gene. In all cases both sense and antisense fragments were sequenced, screened by eye with CHROMAS 1.45 or ALIGN-IR 1.2, and exported as text files. They were formatted as interleaved consensus sequences by sample, prior to multiple alignment using CLUSTAL X (1.5b) (Thompson *et al*. 1994).

Phylogenetic analysis

The amalgamated *12S* + *COI* distance tree is rooted by *T. septentrionis* as outgroup. The tree topology for terminal

Table 3 Variable sites in mtDNA sequences (1023 bp) of *D.* (*Anosia*) species and *D. (A.) chrysippus subspecies* (Table 2). *petilia* autapomorphies within *Anosia* are underlined

	12S gene		COI gene						
Variable	0011111222	33	0000001111	1111122222	2233333333	3334444444	4455555566	666
Site	5900399099	11	0123591123	7789946678	9902345667	8993466678	9922778801	366
Numbers	5324279049	12	3519191795	2703881218	4597984168	7695105843	3458395808	909
eresimus tethys	TACACC–TT	TT	TCGGGAACTC	TCATTTGCAC	TGTCTCTCTT	ATTCTTTAGT	TCTTCTTCCC	TAT
gilippus berenice	.....T............	..	....	....	....	....	....	.....
chrysippus petilia	CTTT-T..AC	CA	GTAAA.CAAA	CTGCACAT.T	CCAT.TCT..	TACTACACA.	AGGATACTAT	.TC
chrysippus bataviana	CTT.-TTAAC	.A	ATAAAGCACA	.T..ACATG.	.CATCTC.CC	TA.TA.AC.C	AGAATA.TAT	C..

clades, analysed using both PAUP 4.0b (Swofford 1998) and PHYLIP 3573c (Felsenstein 1993) (data not shown), is strongly supported by Parsimony and Maximum Likelihood models. Significant bootstrap support values (1000 replicates) are indicated for each node; distances described for the tree and in the text are based on the uncorrected-*p* model. *COI* sequences (Table 2), screened against the National Center for Biotechnology Information (NCBI) GenBank for homology with sequences in the BLAST-NR database, showed about 89% homology with the nymphalid butterfly *Phycoides vesta* Edwards, GenBank ref. AY156686 (Wahlberg *et al.*, 2003). Unpublished sequences were submitted to GenBank using the NCBI submission program SEQUIN 3.7.

RESULTS

Molecular characters

There is no variation within the *D. petilia* sample ($n = 8$) for either mitochondrial gene. Sequence comparisons (Table 3) show the sites in the *12S* and *COI* genes that vary among *D. petilia* and other subgenus *Anosia* spp. Within *D. (Anosia)*, *D. petilia* is distinguished by two site autapomorphies in the *12S* gene (347 bp) and 11 in the *COI* gene. Eighteen further base pair apomorphies, two *12S* and 16 *COI* (Table 3), separate *D. petilia* from all other subspecies of *D. chrysippus* (Lushai *et al*. 2003). Given the sample sizes (Table 2), each site difference between *D. petilia* and *D. chrysippus bataviana* (Moore) (West Malaysia, Sumatra, Java), geographically the closest subspecies of *chrysippus* we have sequenced, is very highly significant; for the *12S* characters, exact probabilities are 2.0×10^{-6} and, for the *COI* characters, 7.8×10^{-5}; *D. petilia* is separated from *D. c. bataviana* by an uncorrected-*p* genetic distance of 2.6% (Table 4). If a molecular clock conversion factor for mtDNA evolution in arthropod species of 2.3% change per million years (Brower 1994) is applied to the molecular data, the result suggests that *D. chrysippus* and *D. petilia* have been isolated for about 1.1 million years.

Kitching (1986) showed that *D. petilia* from Brisbane, Australia ($n = 25$) were monomorphic for allozyme band F of the acid phosphatase (ACP) enzyme, whereas nominotypical *D. (A.) chrysippus chrysippus* from Thailand ($n = 35$) were polymorphic for bands E and G (exact $P = 1.9 \times 10^{-17}$). The latter bands also occurred in *D. c. dorippus* (Klug) from Kenya ($n = 43$) and *D. c. alcippus* (Cramer) from Nigeria ($n = 6$) to the exclusion of band F. ACP/F is an autapomorphy for *D. petilia* within the eight *Danaus* species tested (*D. philene* (Stoll) and *D. affinis* (Fabricius) were treated by Kitching as two species, but are now considered conspecific (Ackery & Vane-Wright 1984)). Given the large samples, the difference for this character between *D. petilia* and all other *D. chrysippus* subspecies is very highly significant (exact $P = 3.6 \times 10^{-25}$).

Table 4 Genetic distances among the *Anosia* taxa based on *12S* and *COI* mtDNA sequences (Table 3): raw differences ($n = 1023$ sites) are above the diagonal and uncorrected-*p* genetic distances below

	1	2	3	4
1 *eresimus tethys*	–	1	56	51
2 *gilippus berenice*	0.001	–	55	50
3 *petilia*	0.055	0.054	–	26
4 *chrysippus bataviana*	0.050	0.049	0.025	–

Phylogenetic analysis

The neighbour-joining distance tree (Fig. 3) shows most subspecies of *D. chrysippus* comprising a monophyletic clade that is sister to *D. petilia*, with (*chrysippus* + *petilia*) sister to the New World clade (*eresimus* + *gilippus*). The paradoxical feature of the tree, the topology of which is fully endorsed by maximum parsimony and maximum likelihood analyses, is the exclusion from the (*chrysippus* + *petilia*) clade of the African subspecies *D. c. dorippus.* The relationship of (*chrysippus* + *petilia*) to *D. c. dorippus* is paraphyletic. This surprising result is supported by a preliminary analysis of sequences from the elongation factor 1 alpha (*EF1-′*) nuclear gene (data to be discussed elsewhere).

Morphology of adult *D. petilia*

Of the seven morphological characters that distinguish *D. petilia* from most other subspecies of *chrysippus* (Table 5), two (1–2) are metric, one (3) is structural, and four (4–7) are pattern characters.

1 Using forewing length as an index of body size *D. petilia* is significantly smaller than all other subspecies of *chrysippus*. Although *D. c. cratippus* (C

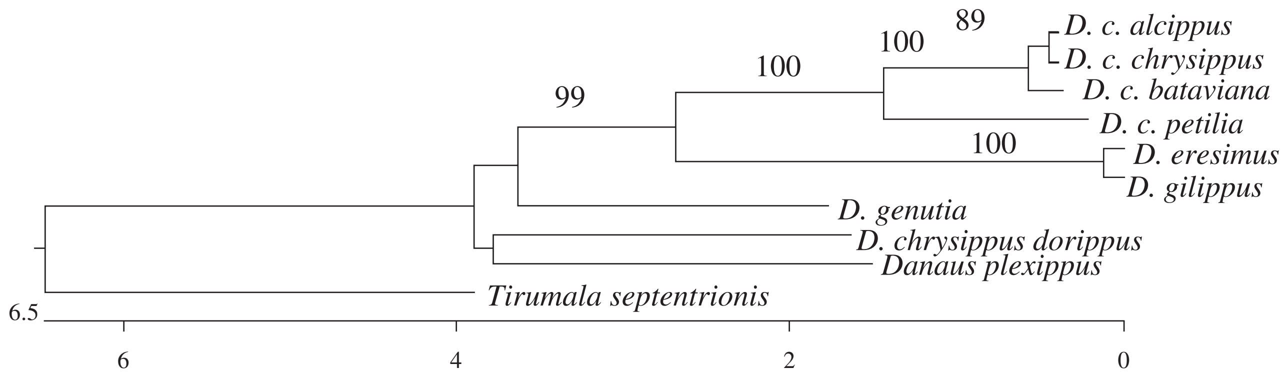

Fig. 3. Neighbour-joining distance tree for *12S* rRNA and *COI* mtDNA sequences (about 886 bp) for *Danaus*. The minimum evolution score is 0.18316; the tree is rooted using *Tirumala septentrionis* as outgroup. Significant support values shown for each node are based on 1000 bootstrap replicates. Negative branch lengths are allowed but set to zero for tree-score calculation. Distance measure = uncorrected-*p*. Genetic distance is drawn proportional to nucleotide changes ($x = 100$).

Table 5 Morphological characters that separate *Danaus petilia* from *D. chrysippus*

1. **Mean fw length (mm):**
   petilia, 32.2 ± 0.49 ($n = 71$) with no difference among sexes or locations (Fiji, Australia, New Hebrides, Papua New Guinea (PNG), Christmas Is.). Data for other subspecies (Smith 1980): *dorippus* ♂, 40.0 ($n = 20$); ♀, 40.0 ($n = 23$); *alcippus* ♂, 41.5 ($n = 15$); ♀, 39.0 ($n = 5$); *chrysippus* ♂, 41.3 ($n = 59$); ♀, 40.9 ($n = 113$). As all mean values for other subspecies lie well outside the 99.9% confidence interval (CI) (30.5–33.8) for *petilia*, the latter is significantly smaller than other *D. chrysippus* subspecies ($P < 0.001$).
2. **Mean width (mm) of black border on hw (d) at its intersection with vein Cu_1:**
   petilia, 5.6 ± 0.62 ($n = 51$) with no difference between sexes or locations; *chrysippus* (Kenya), 2.5 ($n = 26$); *D. gilippus berenice* (Grand Cayman), 4.5 ($n = 13$) (Smith *et al*. 2002). As mean values for all other *Anosia* taxa lie outside the 99.9% CI (4.6–6.7) for *petilia*, this character is defining ($P < 0.001$).
3. **Mean ratio (L : R) for numbers of spines in left and right bunches, viewed dorsally, at the posterior end of the aedaeagus: (fig. 2 in Lushai *et al*. 2003)**
   petilia = 0.96 ($n = 10$), *chrysippus* = 0.72 ($n = 20$) ($t_{28} = 2.09$; $0.05 > P > 0.02$).
4. **Double row of submarginal, white spots on hw (v):**
   petilia shares this character with *D. gilippus* and *D. eresimus*. The relevant spaces are $3A$ (1 pair), $1A + 2A$ (2 pairs), Cu_2 (2), Cu_1 (2), M_3 (2), M_2 (2), M_1 (2), R_5 (1), total 14 pairs: All other subspecies of *chrysippus* have the outer row of 14 spots and an inner row (if present) rarely exceeding 5 spots at the anal and/or costal end(s) of the wing margin.
5. **Double row of submarginal, white spots on fw (v) in spaces M_3, M_2 and M_1:**
   petilia shares this character with most individuals of its nearest neighbour subspecies *bataviana* (West Malaysia, Sumatra, Java) and *cratippus* (Moluccas), but all other *chrysippus* subspecies have only the outer row, i.e., 2 spots per space.
6. **Small white patch in space Cu_1 on hw (v):**
   An autapomorphy for *petilia* within *Danaus*.
7. **Ground colour tawny yellow or brownish buff on both surfaces of both wings:**
   An autapomorphy in *petilia* within *D. chrysippus*. Parsons (1999) describes ground colour as 'yellow' in PNG. However, 6 specimens from Fiji in the Oxford University Museum of Natural History and 9 from Vanuatu (New Hebrides) in the Muséum National d'Histoire Naturelle, Paris are pale brownish buff, a ground colour that is unique within *Danaus*.

d, dorsal; fw, forewing; hw, hindwing; L, left; R, right; v, ventral.

Felder) was not examined, it is of similar size to other *chrysippus* subspecies (Morishita 1981, plates 85–86).

2 Despite its smaller body size, the black border on the dorsal side of the hindwing is very significantly broader in *D. petilia* than in other *D. chrysippus* subspecies; the ratio yellow/orange : black along a line measured from wing insertion to margin, coincident with vein Cu_1, is 3 : 1 in the former compared to 7:1 in the latter. In *D. c. cratippus*, this character-state is intermediate (about 5 : 1).

3 The male genitalia of *D. chrysippus* and *D. gilippus* are inseparable, both structurally and morphometrically (Lushai *et al*. 2003). However, comparing *D. petilia* ($n = 10$) with *D. c. bataviana* ($n = 20$) from the Malay Peninsula, the distribution of spines on the aedeagi differs significantly (Table 5).

4 *D. petilia* has a complete double row of submarginal white spots on the hindwing underside, whereas other *chrysippus* subspecies have, essentially, a single row; the character-state in *D. petilia* is plesiomorphic (primitive) for *Danaus*.

5 The forewing spotting character separates *D. petilia* from most *chrysippus* subspecies, although it is shared with most individuals of neighbouring subspecies *D. c. bataviana* and *D. c. cratippus* (Morishita 1981; plates 85–86).

6 A white central patch on the hindwing underside of *D. petilia*, noted by Common and Waterhouse (1981), is a defining and relatively constant character.

7 The ground colour of *D. petilia* from Australia and Papua New Guines is generally described and/or illustrated as yellow (D'Abrera 1977; Common & Waterhouse 1981; Parsons 1999), compared to either tawny orange or nutbrown in other *chrysippus* (and *Danaus*) species. The Australian series in the Natural History Museum (London) (NHML) is 'very constant in colouring' (Talbot 1943). However, all specimens of *D. petilia* examined from the New Hebrides ($n = 9$) and Fiji ($n = 6$) are, uniquely within *Danaus*, brownish buff.

Geographical distribution of *D. petilia*

The distributions of the *D. chrysippus* subspecies are shown in Fig. 1. *Danaus petilia* occurs throughout Australia, including the interior, and Tasmania, where it is not regularly established (Common & Waterhouse 1981). It also inhabits Irian Jaya/Papua New Guinea, where it is generally rare and local (Parsons 1999). There are records for Norfolk Island, Lord Howe Island, Monte Bello Island, Cocos Island, Christmas Island and Thursday Island (Ackery & Vane-Wright 1984). *Danaus petilia* is an uncommon visitor to both North and South Islands of New Zealand (Gibbs 1980). Walker (1902) recorded *petilia* from Vanuatu (New Hebrides) and there are specimens from the Loyalty Islands and New Caledonia in the NHML and Muséum National d'Histoire Naturelle, Paris (MNHNP). Occasional records from eastern Indonesia, where *D. c. cratippus* is the resident subspecies and *D. petilia* a vagrant, include the Kai (Ké) Islands (de Nicéville & Kühn 1898), Tanimbar (Talbot 1943), Sumba (Morishita 1981; plate 86, no. 13), Seram (Ceram) (Miller & Miller 1978) and Java (single female collected by Aurivillius at Noesa Kembangan, 26.iv.1911 in NHRS). Ackery and Vane-Wright (1984) note a single record from Naitumba (Naituaba) Island, Fiji (Robinson 1975): there are, however, six specimens from Fiji in the OUMNH, collected by CG Griffiths in 1905, which were clearly unknown to them.

DISCUSSION

Compared to the other *Anosia* spp., *D. eresimus*, *D. gilippus* and all other subspecies of *D. chrysippus*, *D. petilia* has 19 invariable apomorphies. The varied range of characters includes 13 constant mtDNA site differences, in two different genes (Table 3), one allozyme character (Kitching 1986), one genitalia character (3, Table 5), two pattern characters (5–6, Table 5) and two metric characters (1–2, Table 5). An additional pattern character (4, Table 5), shared with *D. eresimus* and *D. gilippus*, distinguishes *D. petilia* from all other *D. chrysippus*. Whereas none of these characters could individually justify specific status for *D. petilia*, their concordance in large samples is decisive.

Furthermore, examination of the collections in the NHML, MNHNP and OUMNH show that *D. petilia* ($n = 164$) and its neighbour *D. c. cratippus* ($n = 129$) are vicariant taxa, separated by a deep sea barrier (200–2000 m), between the western rim of the Sahul Shelf and the Molluccas; this barrier, known as Lydekker's Line, must have remained intact throughout the Pleistocene (Heppner 1991; Lambeck *et al*. 2002). Though, on the one hand, there are scattered records for *D. c. cratippus* in the Northern Territory of Australia (Common & Waterhouse 1981) and Papua New Guinea (Hagen 1897) and, on the other, for *D. petilia* in the Molluccas (see above), they are so few that a territorial boundary at Lydekker's Line is probably crossed only rarely. With the exception of Christmas Island and Cocos Island, where it may have been introduced by humans, one record from Sumba illustrated by Morishita (1981), incorrectly labelled *D. c. cratippus*, and Aurivillius' single specimen from Java (this paper), *D. petilia* does not occur west of Lydekker's Line. The single OUMNH specimen from Christmas Island, collected in 1905, carries a note on the pin 'This is evidently a recent immigrant as previous collector failed to get it (cf. Andrews' Report on Xmas Is.)'.

As *D. chrysippus* is highly dispersive and a notorious migrant (Smith & Owen 1997), vagrancy is always possible. Since *D. c. cratippus* (Molluccas) and *D. c. bataviana* (Malay Peninsula south of Penang, Sumatra, Java and Lesser Sunda Islands) are intermediate between *D. petilia* and *D. chrysippus* from North Malaysia (Kedah) for characters 2 and 5 (Table 5), this suggests there has been some gene flow across the Malay Archipelago.

Whether the yellow and/or buff pigments of *D. petilia* are controlled by additional alleles at the B locus or result, respectively, from the action of modifier genes on *bb* (orange) and/or *BB* (brown) genotypes (Smith 1998), is unknown. Crosses between subspecies of the *D. chrysippus* complex have invariably produced fertile progenies in both F_1 and F_2 (Smith *et al*. 1998) and, in this respect, *D. petilia* is no exception. When Clarke *et al*. (1973) crossed *D. petilia* from Sydney, Australia with orange *D. c. alcippus* (there is also a brown form of *alcippus*) from Freetown, Sierra Leone, although they correctly observed that *alcippus* (*bb*) was 'more orange' than *petilia*, they did not describe the hue of the F_1 generation. Moreover, they did not note any segregation in F_2 or backcross progenies as expected if the, albeit similar, colours are controlled by different alleles.

Under the biological species concept (BSC) (Dobzhansky 1937), species are 'groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups' (Mayr 1963); therefore, as they hybridise readily *D. petilia* and *D. chrysippus* would be one species. However, the hybridisation test is rarely applied and the criterion could be applied in this case only because Clarke *et al*. (1973) happened to acquire stock (for genetical studies on pupal colouration) from widely allopatric populations that could never mix in nature. The hybridisation that occurred in their glasshouse in Liverpool was an unplanned event that provided serendipitous data for a paper on colour genetics (CA Clarke, pers. comm. to DAS Smith 1983). Moreover, mating in glasshouses is not an indication of potential mating in the wild, even in sympatry. The exclusive emphasis on isolation under the BSC, which in allopatric populations is rarely tested, is one objection among many that have been raised against it by, for example, Templeton (1989).

An example of conformity with the BSC in *Danaus* is the case of *D. gilippus* and *D. chrysippus*. The taxa have no structural apomorphies with respect to each other, leading Ackery and Vane-Wright (1984) to suggest they might constitute a single pantropical species. However, although their male genitalia are indistinguishable (Smith *et al*. 2002), the mtDNA genetic distance is 4.9% (Table 4). More relevant is that they hybridise only with extreme reluctance, to produce an F_1 that is sterile in both sexes (Smith *et al*. 2002). Consequently, there is no doubt that *D. gilippus* and *D. chrysippus* are allopatric species, that have been separated for an estimated 2.1 million years. In contrast, the male genitalia of *D. petilia* and *D. chrysippus* are similar though distinguishable (Table 5), and the former has 19 apomorphies with respect to the latter and a genetic distance of 2.6%. Although the taxa are allopatric (or parapatric), and have probably been separated for around 1.1 million years, they hybridise readily in captivity to produce F_1, F_2 and backcross progenies that are fertile in both sexes.

The different outcomes of hybridisation between *D. chrysippus* and *D. gilippus*, on the one hand, and *D. chrysippus* and *D. petilia*, on the other, may reflect the longer time in isolation of the former pair compared to the latter. If taxa, such as *D. petilia* and *D. chrysippus*, are allopatric, sexual isolation must be irrelevant to a *process* of speciation since it can have no influence on populations that never meet (Paterson 1985). Where sexual isolation has evolved in allopatry, as between *D. chrysippus* and *D. gilippus*, 'it is a pleiotropic consequence of independent genetic drift in long-separated populations rather than of selection' (Smith *et al*. 2002). Thus, the time that has elapsed since cladogenesis may be crucial to whether or not reproductive isolation has evolved (Smith *et al*. 2002) and may account for the isolation in experimental conditions of *D. gilippus* and *D. chrysippus* compared to its absence between *D. petilia* and *D. chrysippus*.

Hence, we concur with Cracraft's (1989) suggestion that 'populations are delineated as new taxa when they are, in principle, 100% diagnosable'. Under this phylogenetic species concept (PSC), a species is 'an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a pattern of ancestry and descent' (Cracraft 1983). It is in this sense, then, that *D. petilia* has species status. Morphological characters 2 and 4 in *D. petilia* (Table 5) are plesiomorphic (primitive) at both subgeneric (*Anosia*) and generic (*Danaus*) levels. *Danaus petilia*, *D. eresimus* and *D. gilippus* share both characters, though the black border on the hindwing (character 2) is significantly broader in *petilia* than in the Neotropical species and may be homoplasious. Therefore, two characters, confined within subgenus *Anosia* to *D. petilia*, *D. gilippus* and *D. eresimus*, suggest they might share a common ancestor and that the former could have originated by trans-Pacific dispersal from America. Whereas *Anosia* species are absent from all eastern Pacific islands between the Galapagos Islands and Fiji, in contrast for example to *D. plexippus*, which is a recent trans-Pacific invader of Australasia, the more ancient *D. petilia* ancestor may have become extinct in the eastern part of its former range. To test this hypothesis, molecular analysis of a geographically more comprehensive range of *Anosia* samples is essential.

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