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Research Article

Phylogenetic relationships among genera of danaine butterflies (Lepidoptera: Nymphalidae) as implied by morphology and DNA sequences

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Cladistic relationships among genera and subtribes of Danaini (the milkweed butterflies) were inferred by analysis of data combined from five sources: morphology of adults and immature stages, and DNA sequences from three gene regions. The results corroborate and greatly increase support for prior hypotheses based on morphology alone. A new index summarizing incongruence among data partitions, the Partition Congruence Proportion (PCP), is introduced. The significance of the inferred pattern of phylogenetic relationships for comparative chemical ecology of milkweed butterflies is briefly discussed.

Key words: chemical ecology, cladistics, congruence, milkweed butterflies, simultaneous analysis

Introduction

The milkweed butterflies are a charismatic tribe of some 160 large, aposematically coloured species distributed in tropical and temperate habitats throughout the world, with a peak of diversity in the Indo-Australian region. Well-known members of the group include the Monarch (*Danaus plexippus* (L.)), the African Queen or Plain Tiger (*Danaus chrysippus* (L.)), the Tree-Nymph or Paper Kite (*Idea leuconoe* Erichson) and various Crows (*Euploea* spp.). The Danaini are united by the presence of paired eversible secondary sexual organs within the male abdomen referred to as hairpencils, and strongly clubbed, spinose, four-segmented foretarsi in females (Ackery & Vane-Wright, 1984). Both of these features are uniquely derived among Nymphalidae. Based on its membership in a well-supported clade comprising the Neotropical Ithomiini and Australasian Tellervini (Brower, 2000; Freitas & Brown, 2004; Wahlberg *et al.*, 2005b, 2009), we view Danaini here as a tribe and Danaini + Ithomiini + Tellervini as together comprising the nymphalid subfamily Danainae. However,

many authors past and present have considered the ‘danaids’ (Danaini) as a subfamily or even a family in their own right.

Historical efforts to circumscribe and arrange the danaines are described in detail in Ackery & Vane-Wright (1984). This comprehensive account of the Danaini included manually performed cladistic analyses of relationships among the tribe’s 11 genera (now 12: see Vane-Wright *et al.*, 2002) and some 157 species based on morphological characters derived mainly from adult specimens. As the authors noted, their results corroborated to a greater or lesser degree prior hypotheses of relationships suggested by Doubleday (1847), Bates (1862), Reuter (1896), D’Almeida (1939) and Forbes (1939). Kitching (1985) studied immature stages of a subset of Danaini and performed separate cladistic analyses of data matrices scored from egg, larval and pupal stages. His results implied relationships among danaine genera that are largely but not entirely concordant with those of Ackery & Vane-Wright (1984).

Here, we combine DNA sequence data from three gene regions with morphological characters from adults and immature stages derived from the literature, to yield a well-supported cladogram that provides yet another clear example of the general pattern of evidentiary synergy between

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molecules and morphology for phylogenetic inference (cf. Miller *et al.*, 1997; Wahlberg *et al.*, 2005a; Simonsen *et al.*, 2006; Warren *et al.*, 2009).

Methods

Taxon sampling

The taxa examined to generate the molecular data are listed in Table 1. We were able to obtain at least two representative species of all 12 of the currently recognized danaine genera except for *Miriamica*, and the very rare, poorly known and monobasic *Protoploea* (central New Guinea) and *Tiradelphe* (Guadalcanal, Solomon Is.). The last two genera are represented in the matrix only by characters from adult morphology.

Specimens for DNA extraction were netted in the field by the authors or collaborators, and preserved either in 100% ethanol with wings removed and stored separately, or as dried, papered specimens. Voucher specimens were prepared as per Brower (1996) or spread in the traditional manner after removal of two legs for DNA extraction, and are retained by the authors for eventual deposition in public institutions (Table 1).

Morphological characters

All morphological characters were obtained from the literature (see Table 2). Forty-four adult morphological characters and one larval host-plant association character were derived from Ackery & Vane-Wright's (1984) characters 1–53, with additional data added from Vane-Wright *et al.* (2002) for the recently described genus *Miriamica*. These characters represent those deemed informative at the generic level by Ackery & Vane-Wright. Eighty characters for immature stages that were originally coded or could be recoded with discrete states were derived from Kitching (1985). Kitching's sample of exemplar taxa was not comprehensive for all danaine genera. Corresponding character states for *Anetia* and *Lycorea* immature stages were scored from Ackery & Vane-Wright (1984), Brower *et al.* (1992), and Sourakov & Emmel (1996) (the last of these added character states for *Anetia* larvae to Kitching's larval matrix). Because Kitching's larval and pupal matrices used only a single ithomiine (*Methona*) for the outgroup and no outgroup for the egg data, the generality of ithomiine + tellervine outgroup character states was determined, where possible, from the data of Brown & Freitas (1994), which included *Tellervo*. Immature stages remain unknown for *Miriamica*, *Protoploea* and *Tiradelphe* (Vane-Wright & Boppré, 1990; Parsons, 1998): other than possible sightings, the last of these is still known only from the two female type specimens (Tennent, 2002).

Morphological character states for all exemplars of a given genus are coded identically. Intra-generic polymorphisms coded by the original authors are reported as such and treated as uncertain in the analysis, rather than attempting to determine specific character states for the exemplar species studied here. Some characters were recoded to reduce redundancy and/or improve synapomorphy content by consolidating multiple autapomorphies as more general synapomorphic conditions. Original and modified codings for individual morphological characters are described in Table 2.

Molecular characters

DNA was extracted from individual specimens, amplified and sequenced following standard protocols (Brower, 1994; Brower *et al.*, 2006; Wahlberg *et al.*, 2005b). We sequenced three gene regions: a 2335 bp region of mitochondrial DNA spanning the cytochrome oxidase subunit 1, tRNA leucine and cytochrome oxidase subunit 2 genes (COI-COII); a 1260 bp region of the nuclear gene elongation factor 1-alpha (Ef-1a); and a 382 bp region of the nuclear gene wingless (wg). These three regions have been widely employed in phylogenetic studies of various lepidopteran groups (e.g. Wahlberg *et al.*, 2005a, b, 2009; Brower *et al.*, 2006; Peña *et al.*, 2006; Warren *et al.*, 2008). As there are no implied insertions or deletions among the taxa examined, alignment was straightforward. Some published sequences (Brower, 2000; Wahlberg *et al.*, 2003, 2009; Brower & Jeansonne, 2004; Lushai *et al.*, 2005a; Whinnett *et al.*, 2005; Wahlberg & Wheat, 2008) were obtained from Genbank to enhance representation of taxa. Genbank accession codes for all new and previously published gene regions are given in Table 1. Some gene regions for certain taxa are represented by partial sequences.

Analysis

The data were concatenated into a single matrix (46 taxa × 4102 characters) and analysed in PAUP* (Swofford, 2000) under the parsimony criterion. The data matrix as analysed can be accessed as a NEXUS file at <http://mtsu.edu/~abrower/>. Characters were weighted equally and all character state transformations were treated as unordered. Trees were rooted with *Tellervo*. Ten thousand random addition sequences with TBR were performed. Implied branch supports for components for the dataset as a whole and partitioned branch support for the five 'partitions' (adult morphology, immature morphology, three gene regions) were calculated by the anticonstraint approach (see Brower, 2006) using a reduced dataset that did not include *Protoploea* and *Tiradelphe*, since these taxa lack data for four of the five partitions (the topology for the remaining taxa was the same). Anticonstraint searches were conducted with 100 random addition sequences and TBR. We report

Table 1. Specimens from which DNA was extracted for this study.

Taxon	Collection locality	Voucher number*	Voucher site†	Genbank Accession Numbers		
				COI-COII	Ef-1a	Wingless
Tellervini						
<i>Tellervo zoilus</i> (Fabricius, 1775)	Australia: New South Wales	QL1	AB	DQ157549	DQ 177995	AF246563
Amaurina						
<i>Amauris ellioti</i> Butler, 1895	Tanzania: Mbeya Range	NW86-5	NW	AY218234	AY218523	AY218272
<i>Amauris niavius</i> (Linnaeus, 1758)	Tanzania	(Lushai)	OUM	AY256343	AY296132	—
<i>Amauris niavius</i> (Linnaeus, 1758)	Ghana: Bobiri Forest Preserve	GH-154	AB	GU365924	GU365947	GU365948
<i>Amauris tartarea</i> Mabilie, 1876	Ghana: Bobiri Forest Preserve	GH-058	AB	DQ071867	DQ071868	DQ071872
<i>Ideopsis gaura</i> (Horsfield, 1829)	Malaysia	NP95-Y318	MCZ	GU365916	GU365925	AF246592
<i>Ideopsis gaura</i> (Horsfield, 1829)	ex Malaysian dealer	N435	NW	GU365917	GU365926	GU365949
<i>Ideopsis vulgaris</i> (Butler, 1874)	Malaysia	NP95-Y252	MCZ	GU365918	GU365927	—
<i>Miriamica</i> sp.	Indonesia: Papua, Foja Mts	KSP 4486	MZB	GU365919	GU365928	GU365950
<i>Parantica agleoides</i> (C. & R. Felder, 1860)	Borneo: Sabah	SA-1-1	AB	GU356920	GU365929	AF246603
<i>Parantica aspasia</i> (Fabricius, 1787)	Malaysia: Cameron Highlands	NW112-8	NW	GQ864799	GQ864893	GQ864487
<i>Parantica</i> cf. <i>luzonensis</i> (C. & R. Felder, 1863)	Java: Bodogol	NW118-17	NW	GQ864779	GQ864873	GQ864467
<i>Parantica melusine</i> (Grose-Smith, 1894)	Papua New Guinea	PNG-02-03	AB	DQ175477	DQ071870	GU365951
Danaina						
<i>Danaus chrysippus</i> (Linnaeus, 1758)	Uganda	(Lushai)	OUM	AF394187	—	—
<i>Danaus chrysippus</i> (Linnaeus, 1758)	Ghana	(Lushai)	OUM	AF394191	—	—
<i>Danaus chrysippus</i> (Linnaeus, 1758)	Uganda	(Lushai)	OUM	AF426165	AY296142	—
<i>Danaus eresimus</i> (Cramer, 1777)	Cayman Is.	(Lushai)	OUM	AF394185	AY296135	—
<i>Danaus erippus</i> (Cramer, [1775])	Argentina: Buenos Aires	DE-1	AB	AY569158	GU365931	GU365966
<i>Danaus genutia</i> (Cramer, 1779)	Thailand: Chiangmai	DL02-P778	MCZ	GU365908	GU365930	GU365952
<i>Danaus gilippus</i> (Cramer, 1775)	USA: Arizona	AZ-1-3	AB	DQ071865	DQ071871	DQ 175476
<i>Danaus ismare</i> (Cramer, 1780)	Sulawesi	NW106-14	NW	AY855061	AY855062	—
<i>Danaus petilia</i> (Stoll, 1790)	Australia: Queensland	DL01-P178	MCZ	GU365907	GU365932	GU365953
<i>Danaus plexippus</i> (Linnaeus, 1758)	Colombia: Villavicencio	C-3-8	AB	AY569150	GU365933	AF246564
<i>Danaus plexippus</i> (Linnaeus, 1758)	Peru: Tingo Maria	PE-19-8	AB	AY569155	GU365934	GU365954
<i>Tirumala hamata</i> (MacLeay, 1827)	Irian Jaya: Biak Utara	IJ-1-1	AB	GU365921	GU365935	GU365955
<i>Tirumala hamata</i> (MacLeay, 1827)	Irian Jaya: Biak Utara	IJ-1-5	AB	GU365922	GU365936	GU365956
<i>Tirumala hamata</i> (MacLeay, 1827)	Australia: New South Wales	NSW1	AB	AY569159	—	—
<i>Tirumala septentrionis</i> (Butler, 1874)	Thailand: Chiangmai	DL02-P740	MCZ	GU365923	GU365937	GU365957

(Continued on next page)

Table 1. Specimens from which DNA was extracted for this study. (*Continued*)

Taxon	Collection locality	Voucher number*	Voucher site†	Genbank Accession Numbers		
				COI-COII	Ef-1a	Wingless
<i>Euploeina</i>						
<i>Euploea camaralzeman</i> Butler, 1866	captive reared	NW70-8	NW	AY090205	AY090171	AY090138
<i>Euploea corinna</i> [#] (Macleay, 1827)	Australia: Queensland	QL7	AB	GU365909	GU365938	AF246566
<i>Euploea eunice</i> (Godart, 1819)	Java: Bogor	JV2	AB	GU365910	GU365939	GU365958
<i>Euploea mulciber</i> (Cramer, 1777)	Sumatra: Benakat	SU1	AB	GU365911	GU365940	GU365959
<i>Euploea mulciber</i> (Cramer, 1777)	Sumatra: Benakat	SU2	AB	GU365912	GU365941	GU365960
<i>Idea leuconoe</i> (Erichson, 1834)	ex Malaysian dealer	BOP-1	AB	GU365913	GU365942	GU365961
<i>Idea leuconoe</i> (Erichson, 1834)	captive reared	NW84-14	NW	GQ864778	GQ864872	GQ864466
<i>Idea lynceus</i> (Drury, 1773)	Thailand: Ranong	DL02-B254	MCZ	GU365914	GU365943	GU365962
<i>Idea stollii</i> (Moore, 1883)	Borneo: Sabah	SA-3-4	AB	GU365915	GU365944	GU365963
<i>Anetia briarea</i> (Godart, 1819)	Dominican Republic: La Vega	NW152-6	NW	EU141366	EU141673	EU141247
<i>Anetia briarea</i> (Godart, 1819)	Cuba: Santiago de Cuba	CU1	AB	DQ071866	DQ071869	AF246579
<i>Anetia jaegeri</i> (Ménétriés, 1832)	Dominican Republic: La Vega	NW152-4	NW	GU365905	GU365945	GU365964
<i>Anetia pantheratus</i> (Martyn, 1797)	Dominican Republic: Pedernales	NW152-11	NW	GU365906	GU365946	GU365965
<i>Lycorea halia</i> (Hübner, 1816)	Brazil: Rondônia	RB241	AB	DQ157550	DQ177996	AF246567
<i>Lycorea halia</i> (Hübner, 1816)	Brazil: São Paulo	NW122-19	AF	GQ864788	GQ864882	GQ864476
<i>Lycorea ilione</i> (Cramer, 1775)	Brazil: São Paulo	B-17-37	AF	GQ864780	GQ864874	GQ864468

*Lushai *et al.* data were obtained from Genbank, and the individual voucher codes were not associated with the sequences there.

[#]The Australian region species *Euploea corinna* is often referred to as a subspecies of the Oriental region *E. core*, but is now considered distinct.

†Voucher site codes: AB = Middle Tennessee State University; AF = Universidade Estadual de Campinas, Brazil; MCZ = Harvard University, Cambridge; MZB = Museum Zoologicum Bogoriense, Java, Indonesia; NW = University of Turku; OUM = Oxford University.

the Partition Congruence Proportion (PCP) for all internal branches. $PCP = PCI/BS$, where PCI = Partition Congruence Index and BS = Branch Support (Brower, 2006). Expanded,

$$PCP = 1 - \left(\left(\sum_{i=1}^n |PBS_i| - BS \right) / BS^2 \right)$$

where n = the number of data partitions. PCP ranges from 1 for data partitions with no incongruence to

$$1 - \left(\sum_{i=1}^n |PBS_i| - BS \right)$$

for maximally incongruent data (the same minimum value as PCI). The latter is a negative number that grows more

negative as the support for incongruent alternative topologies implied by separate partitions increases. Brower (2006) introduced the PCI as a parameter summarizing the degree of congruence among data partitions, ranging from a maximum equal to the branch support value for a given node to the minimum resulting from the second formula above. The modification to PCI introduced here scales the maximum value to 1, allowing more straightforward comparison of relative degrees of incongruence among nodes. Negative PCI and PCP values are apparently rather rare in empirical data (Brower *et al.*, 2006; Warren *et al.*, 2009).

Results

A single most parsimonious cladogram was recovered in all random addition searches (Fig. 1). The tree implies that all danaine genera and subtribes are monophyletic.

Table 2. Morphological characters. Characters labelled A were derived from Ackery & Vane-Wright's (1984: "A&VW") matrix of adult characters for relationships among genera and coded identically, except as noted. All of these characters were originally coded as binary. They have been reordered to group related body parts together (the original sequence was organized to group synapomorphies for particular taxa). Characters labelled E, L and P were derived from Kitching's (1985) egg, larval and pupal data matrices. The states of characters that were originally or have been recoded as multistate are unordered in the current analysis. Characters included in the original publications that have been rejected here are listed at the end, with explanations for their rejection.

1 (A1).	Size of pulvilli and paronychial (features of the meso- and metathoracic tarsi): 0 = large; 1 = very small.
2 (A2).	Shape of tarsal claws: 0 = curved; 1 = straight.
3 (A34).	Condition of fifth tarsal segment of meso- and metathoracic legs: 0 = short, with small spines; 1 = long, armed with long spines. A synapomorphy for the species of <i>Danaus</i> s.s.
4 (A28).	Length of antennae: 0 = shorter than 1/2 length of forewing costa; 1 = longer than 1/2 length of forewing costa. This character was scored as an autapomorphy for <i>Tiradelphe</i> , but long antennae are present in both <i>Ithomiini</i> and <i>Tellervo</i> , suggesting that state 0 is derived.
5 (A9).	Hindwing precostal vein: forked or elbowed = 0; simple = 1. A&VW stated that <i>Tirumala</i> , <i>Danaus</i> (<i>Anosia</i>), <i>Protoploea</i> and <i>Euploea</i> were polymorphic for this character but apparently used a hypothetical groundplan state for these taxa. They are coded as polymorphic here.
6 (A39).	Site of origin of precostal vein: 0 = opposite or slightly before origin of Rs; 1 = well beyond origin of Rs.
7 (A43).	Precostal cell: 0 = present; 1 = absent. An autapomorphy for <i>Euploea</i> .
8 (A10).	Origin of forewing vein R ₂ : 0 = connate with R ₃₊₄₊₅ or arising from discal cell; 1 = stalked with R ₃₊₄₊₅ . A&VW described this character as polymorphic within many of the genera, but counted it as a synapomorphy for <i>Danaina</i> .
9 (A20).	Relationship of forewing veins R ₁ and Sc: 0 = simple; 1 = anastomosed for a short distance.
10 (A11).	Condition of ventral forewing cell membrane: 0 = hairy; 1 = bare.
11 (A12).	Condition of submarginal spot in forewing cell M ₃ : 0 = double, or single but offset; 1 = single, centrally located. This character appears to be subject to selective variation due to mimicry.
12 (A27).	Number of pattern elements in forewing cell M ₁ : 0 = three or fewer; 1 = four.
13 (A13).	Condition of scales in pale areas of dorsal hindwing cell: 0 = broad, appressed; 1 = narrow, upstanding.
14 (A3).	Condition of section of hindwing vein 1A+2A in males: 0 = unmodified; 1 = swollen.
15 (A19).	Distribution of androconia on male hindwing veins 1A and 2A: 0 = absent; 1 = in anal-cubital zone only; 2 = on distal half; 3 = along entire length. This character is related to character 14. A&VW intended this character as a synapomorphy for <i>Ideopsis</i> , which is the only taxon exhibiting state 3, and coded all other states as "0."
16 (A8).	Condition of pouched or pocketed cubital organ on male hindwing: 0 = absent; 1 = cell Cu1b underside with deep pouch containing particle-producing hairs; 2 = formed as a pocket in which the anterolateral 'roof' or flap originates as a dorsal outgrowth. This character is an amalgamation of A&VW characters 8, 21 and 24. Differences in structure and function of <i>Tirumala</i> and <i>Danaus</i> cubital organs suggest that they may be independently derived (Vane-Wright & Boppré, 1990).
17 (A44).	Condition of hairpencils: 0 = single; 1 = paired. An autapomorphy for <i>Idea</i> .
18 (A33).	Length of hairpencils: 0 = absent; 1 = long; 2 = short.
19 (A41).	Hairpencils with dorsal diverticulum: 0 = absent; 1 = present.
20 (A52).	Size of hairpencil diverticula: 0 = absent; 1 = large; 2 = small.
21 (A47).	Origin of hairpencils: 0 = from short pedicels; 1 = from long pedicels. An autapomorphy for <i>Protoploea</i> .
22 (A40).	Location of hairs on hairpencil hairs: 0 = confined to basal third; 1 = along almost entire sheath.
23 (A16).	Hairpencils with black contact hairs: 0 = absent; 1 = present. This character is an autapomorphy for <i>Amauris</i> (reversed in <i>A. vashti</i>).
24 (A4).	Condition of male 9 th tergite: 0 = large and sclerotized; 1 = membranous; 2 = reduced, forming a caudula; 3 = undetectable or absent. A&VW lumped the first two and last two states as composite binary alternatives. [The caudula, coded as State 2, may not be homologous in the <i>Itnina</i> and <i>Euploea</i> : P. Ackery, pers. comm. June 2009.]
25 (A6).	Posterior margin of male 8 th abdominal tergite armed with spines: 0 = no; 1 = yes. The character state for <i>Miriamica</i> is reported in Vane-Wright <i>et al.</i> (2002).
26 (A51).	Length of male eighth abdominal sternite: 0 = "normal"; 1 = long. An autapomorphy for <i>Lycorea</i> .
27 (A48).	Membranous emargination on male eighth abdominal tergite: 0 = absent; 1 = present. An autapomorphy for <i>Lycorea</i> .
28 (A15).	Condition of socii: 0 = "indistinct", "barely perceptible" or absent; 1 = small; 2 = large.
29 (A18).	Internal face of male clasper folded and produced into a downwardly-directed, pointed process: 0 = no; 1 = yes. The "0" state lumps together disparate conditions of the clasper, but the pointed process appears to be an autapomorphy for <i>Ideopsis</i> .
30 (A23).	Clasper with annularly-corrugated process: 0 = absent; 1 = present.
31 (A45).	Emargination of clasper: 0 = none; 1 = deeply emarginate on posterodorsal edge. An autapomorphy for <i>Protoploea</i> .
32 (A32).	Shape of saccus: 0 = short; 1 = long, swollen distally; 2 = long, tapering distally. This character combines A&VW's autapomorphic binary characters 32 and 50 into a single character.
33 (A42).	Harpe with large dorsal hook: 0 = absent; 1 = present.
34 (A25).	Male eighth sternite modified into pseudovalves: 0 = absent; 1 = present. A&VW note that <i>Danaus ismare</i> , <i>D. chrysippus</i> and <i>D. gilippus</i> lack this feature, but interpret these as homoplastic secondary losses. Rather than coding the states for these species as absent, we have chosen to subsume secondary loss within the "present" state. The character as coded here represents an autapomorphy for <i>Danaus</i> s. l.
35 (A36).	Juxta shape: 0 = broad; 1 = narrow. A synapomorphy for the species of <i>Danaus</i> (<i>Salatura</i>).
36 (A30).	Shape of aedeagus: 0 = short and wide; 1 = long and narrow. A synapomorphy for the species of <i>Danaus</i> s.s. (see Brower <i>et al.</i> , 2007).
37 (A37).	Position of incision on aedeagus: 0 = ventral; 1 = dorsal or dorsolateral.
38 (A38).	Well developed spines on aedeagus: 0 = absent; 1 = present. A synapomorphy for the species of <i>Danaus</i> (<i>Anosia</i>).
39 (A49).	Apical spines on aedeagus: 0 = absent; 1 = present. A synapomorphy for the species of <i>Lycorea</i> .
40 (A14).	Corpus bursae: 0 = single; 1 = double.
41 (A17).	Junction of hindwing cross-veins m ₁ -m ₂ and m ₂ -m ₃ : 0 = rectilinear; 1 = angled.
42 (A22).	Anteroventral lip of female eighth sternite with well-developed lateral, ventral-directed projections: 0 = absent; 1 = present. An autapomorphy for <i>Tirumala</i> .
43 (A29).	Shape of corpus bursae: 0 = bulbous; 1 = narrow and tubular. An autapomorphy for <i>Tiradelphe</i> .

(Continued on next page)

Table 2. (Continued)

44 (A31).	Shape of ductus bursae: 0 = short and wide; 1 = long and narrow. An autapomorphy for <i>Danaus</i> s.s. (A&VW noted that this character is likely to be correlated with character 36).
45 (A5).	Capacity to feed on Moraceae as larvae: 0 = no; 1 = yes. Brower <i>et al.</i> (1992) were able to rear <i>Anetia briarea</i> on <i>Cynanchum angustifolium</i> (Asclepiadaceae), although they suggest that another milkweed may be the preferred natural host. Sourakov & Emmel (1996) found and reared out an egg of <i>A. jaegeri</i> on <i>Asclepias nivea</i> .
46 (E1).	Arrangement of cells on egg surface: 0 = approximately square array; 1 = skewed hexagonal array. An autapomorphy for <i>Idea</i> .
47 (E3a).	Raised features on egg cell surface: 0 = absent; 1 = present.
48 (E3b).	Shape of lateral marginal elements of raised pattern on egg cell surface: 0 = absent; 1 = round; 2 = elongate. Kitching's character E3 has been recoded to increase synapomorphic content.
49 (E4).	Cell surface pitted: 0 = no; 1 = yes. Kitching coded this character with two autapomorphic states.
50 (E5).	Fusion of longitudinal ribs: 0 = common; 1 = rare.
51 (E6).	Transition of cell size approaching micropyle: 0 = gradual; 1 = abrupt.
52 (E7).	Form of cells of the annulus: 0 = irregular or incomplete; 1 = regular, concentric circles.
53 (E8).	Position of largest cells: 0 = not close to micropyle; 1 = 4 cells from micropyle. An autapomorphy in Kitching's analysis.
54 (E9).	Size and number of radial ridges around micropyle: 0 = few, small; 1 = many, large. Kitching coded this character with two autapomorphic states for different <i>Amauris</i> species.
55 (E10).	Pits on ribs: 0 = absent; 1 = present. An autapomorphy in Kitching's analysis.
56 (E11).	Aspect of cells in polar view: 0 = convex; 1 = concave.
57 (L6).	Number of type 2 sensilla on galea: 0 = 2; 1 = 3 or more. An autapomorphy for <i>Amauris</i> .
58 (L9).	Condition of cuticular ornamentation on body surface: 0 = attenuated; 1 = pointed; 2 = blunt.
59 (L10).	Pigmentation pattern on body: 0 = transversely striped, with or without spots; 1 = spotted only; 2 = uniformly pale; 3 = longitudinally striped. Kitching viewed these states as a transformation series, but we have treated them as unordered.
60 (L12).	Tubercle (filament) on larval segment 2 (mesothorax): 0 = absent; 1 = present. Kitching's outgroup, <i>Methona</i> , lacks tubercles, but other basal ithomine genera possess them (e.g., <i>Tithorea</i> , <i>Melinaea</i> ; Brown & Freitas, 1994).
61 (L13).	Tubercle on larval segment 3 (metathorax): 0 = absent; 1 = present.
62 (L14).	Tubercle on larval segment 5: 0 = absent; 1 = present.
63 (L15).	Tubercle on larval segment 10: 0 = absent; 1 = present.
64 (L16).	Tubercle on larval segment 11: 0 = absent; 1 = present.
65 (L17).	Pigmentation of head capsule: 0 = uniformly dark; 1 = black with white spots; 2 = black with white stripes.
66 (L19).	Distribution of setae on filaments: 0 = sparse; 1 = dense.
67 (L20).	Length of setae on filaments: 0 = long; 1 = short.
68 (L23).	Arrangement of ribs on filament cuticular ornaments: 0 = more-or-less linear and parallel; 1 = reticulate.
69 (L24).	Condition of labral secondary setae: 0 = absent; 1 = short and fine; 2 = long and stout.
70 (L25).	Position of labral seta M ₂ : 0 = closer to seta M ₁ ; 1 = closer to seta M ₃ (or equidistant).
71 (L28).	Position of labral seta L1 relative to widest point of labrum: 0 = distal; 1 = level.
72 (L29).	Condition of lateral spine plate on labrum: 0 = undifferentiated or at most with a few scattered spines; 1 = weakly developed, forming a single row of spines; 2 = well developed, with two or more rows of spines.
73 (L33).	Size of median sclerotized spot on labrum: 0 = small; 1 = large.
74 (L34).	Condition of area immediately surrounding labral campaniform sensillum: 0 = undifferentiated; 1 = distinctly pale and less-well sclerotized.
75 (L35).	Shape of base of labrum: 0 = irregular and often ill-defined; 1 = grossly and regularly undulate. Sourakov's interpretation of the character state for <i>Anetia</i> has been changed.
76 (L36).	Condition of basal and central epipharyngeal spines: 0 = single; 1 = combed.
77 (L37).	Length of basal and central epipharyngeal spines: 0 = long; 1 = short.
78 (L39).	Epipharyngeal spines between seta 1 and 2: 0 absent; 1 = present.
79 (L40).	Position of epipharyngeal seta 1 relative to seta 3: 0 = not distal; 1 = distal.
80 (L44).	Mandibular retinaculum: 0 = absent; 1 = present.
81 (L46).	Condition of mandibular knuckle: 0 = indistinct; 1 = pronounced.
82 (L48).	Number of mandibular pores: 0 = few; 1 = numerous. Kitching used a dummy state for <i>Amauris</i> and <i>Methona</i> , in which this character could not be observed. We have scored them as "missing."
83 (L50).	Condition of polygonal pattern on wrist of mandible: 0 = absent or very weak.; 1 = moderately well-developed; 2 = very distinct.
84 (L51).	Position of secondary mandibular setae relative to campaniform sensillum: 0 = most basal; 1 = most distal; 2 = all distal.
85 (L53).	Shape of galea: 0 = cylindrical; 1 = tapered distally.
86 (L54).	Shape of 1 st segment of galeal type 2 sensilla: 0 = long and curved; 1 = short and tapered; 2 = small and barrel-shaped.
87 (L56).	Number of campaniform sensilla on ventral surface of galea: 0 = 1; 1 = 2.
88 (L58).	Position of campaniform sensillum on ventral surface of maxillary palp segment 2: 0 = at about 3/4 of distance from base to tip; 1 = on distal margin.
89 (L61).	Position of seta on maxillary palp segment 1: 0 = distal; 1 = medial. The character description has been rephrased.
90 (L65).	Condition of stipital primary seta St1: 0 = undifferentiated from secondaries; 1 = present; 2 = absent.
91 (L66).	Size of cuticular ornamentation of unsclerotized part of stipes: 0 = undifferentiated from general body surface; 1 = much smaller.
92 (L70).	Sclerotization of submentum: 0 = absent; 1 = basally sclerotized.
93 (L71).	Cuticular ornamentation on submentum: 0 = absent; 1 = distinct.
94 (L72).	Distribution of submental cuticular ornamentation: 0 = not beyond primary setae; 1 = almost to edge of stipes.
95 (L73).	Shape of central submental cuticular ornamentation: 0 = blunt; 1 = pointed.
96 (L74).	Number of rows of major hypopharyngeal spines: 0 = 1; 1 = 2; 2 = many.
97 (L76).	Surface of hypopharynx: 0 = smooth; 1 = covered with spines.
98 (P1).	Degree of ventral anterior sclerotization of parallel bars beneath cremaster: 0 = none; 1 = two short bars; 2 = short bars and spots; 3 = bars and spots fused; 4 = bar, spot and exuvial holdfast tubercles fused. This character is recoded with one less state than Kitching's ordered transformation series.

(Continued on next page)

Table 2. (Continued)

99 (P2).	Condition of sclerotized bars: 0 = absent; 1 = rounded; 2 = truncate.
100 (P3).	Degree of dorsal and lateral sclerotization of abdominal segments 8–11: 0 = reduced or absent; 1 = partial; 2 = nearly complete. This character has been simplified from Kitching's 8 states, several of which were autapomorphic, and one of which was not exhibited by any of the taxa in his matrix.
101 (P5).	Dorsal excrescence on cremaster crown: 0 = present; 1 = absent.
102 (P6).	Depth of stalk groove: 0 = shallow; 1 = deep.
103 (P7).	Dorsal/ventral grooves on stalk: 0 = absent; 1 = present.
104 (P8).	Lateral excrescences at base of stalk: 0 = absent; 1 = 1 pair, no subdivisions; 2 = 1 pair, multiple subdivisions; 3 = two pairs. This character has been simplified to eliminate intrageneric polymorphism.
105 (P10).	Grooved concavity on ventral surface of stalk: 0 = absent; 1 = present.
106 (P12).	Colour of anus: 0 = dark; 1 = pale.
107 (P13).	Colour of base of exuvial holdfast tubercles: 0 = uniformly dark; 1 = pale at base.
108 (P14).	Dorsal angle between stalk and abdomen: 0 = ~90°; 1 = ~60°.
109 (P15).	Texture of abdominal segments 4–7: 0 = smooth; 1 = rugose. This character has been simplified to eliminate intrageneric polymorphism.
110 (P18).	Contour of individual abdominal segments: 0 = convex; 1 = flat.
111 (P19).	Shape of curvature of dorsal abdominal segments 3–11: 0 = one radius of curvature; 1 = 2 radii of curvature.
112 (P20).	Transverse ridge on abdominal segment 3: 0 = absent; 1 = strongly present. This character has been recoded to eliminate autapomorphy.
113 (P21).	Angle between abdominal segments 1–3 and 3–8: 0 = obtuse; 1 = ~90°.
114 (P22).	Numerous small papulae in a transverse band around abdominal segment 3: 0 = absent; 1 = present.
115 (P23).	Transverse black stripe on abdominal segment 3: 0 = absent; 1 = interrupted; 2 = complete. This character has been recoded to eliminate autapomorphy.
116 (P24).	Transverse band of large black spots on abdominal segment 3: 0 = absent; 1 = present. This character has been recoded to eliminate autapomorphy and redundancy with P23.
117 (P25).	Transverse yellow (gold) band on abdominal segment 3: 0 = absent; 1 = present. Kitching scored this indirectly from exuviae, but it has been corroborated from photos of living pupae in A&VW and Brower <i>et al.</i> (1992).
118 (P26).	Dorsal gold spots: 0 = absent; 1 = present. This character has been simplified to eliminate autapomorphy.
119 (P28).	Pair of large dorsolateral papulae on abdominal segment 2: 0 = absent; 1 = present.
120 (P29).	Paired, weak brown spots: 0 = absent; 1 = present. This character has been simplified to eliminate polymorphism within <i>Euploea</i> .
121 (P30).	Pale brown patterning on exuviae: 0 = absent; 1 = present.
122 (P31).	Large, well-developed dorsal spots on abdomen: 0 = absent; 1 = present. This character has been recoded based on photos in A&VW. Kitching's coding does not appear to match patterns on photographs of living pupae.
123 (P32).	Pupal facies: 0 = elongate; 1 = truncate.
124 (P33).	Mesothorax definition: 0 = strongly hunched, forming saddle at base of abdomen; 1 = more or less continuous in outline with abdomen. This character has been recoded based on examination of images of pupae in A&VW.
125 (P34).	Large papulae at wing bases: 0 = absent; 1 = one pair; 2 = two pairs. This character has been simplified to improve synapomorphy.
Rejected characters	
A7.	Male ninth tergite membranous, not forming a caudula. This character is redundant with character 24 (A4) as recoded.
A21.	Male hindwing cell Cu1b underside with deep pouch containing particle-producing hairs. This character is redundant with character 16 (A8) as recoded.
A24.	Male hindwing alar organ formed as a pocket in which the anterolateral 'roof' or flap originates as a dorsal outgrowth. This character is redundant with character 16 (A8) as recoded.
A26.	Wings with bright orange pigment. A&VW listed this as a synapomorphy for <i>Danaus</i> , but some <i>Danaus</i> are not orange, while <i>Lycoreia</i> and (arguably) some <i>Anetia</i> are, as are many ithomiines. Until homology of the pigments is assessed, this character seems subjective.
A35.	Larval tubercle formula 2,5,11. This character is redundant with 60–64 (Kitching's characters L12–L16).
A46.	Length of hairpencil extremely short. This character is redundant with character 18 (A33).
A50.	Saccus long, tapering towards apex in dorsal aspect. This character is redundant with character 32 (A32) as coded here.
A53.	Hairpencils pale in colour. A&VW did not score this character for any taxa other than <i>Lycoreia</i> and <i>Anetia</i> .
E2.	Lateral walls of cells straight/uniformly curved or distinctly angled. Kitching noted that this character might be correlated with E1 (our 46). It seems to us to be not merely correlated, but logically the same.
E12–19.	These are size and meristic characters reported as mean values or quartiles.
L1–L5, L7, L8.	These characters are ratios or mean values relating to setae on the larval mouthparts and antennae. Kitching stated, "all these characters ... have large sample variances." We have chosen to exclude them as homology statements.
L11.	Larval tubercle formula is redundant with presence/absence of individual tubercles (characters 60–64 (L12–L16)).
L18.	Frontal suture converging rectilinearly onto adfrontal suture distally. This character is invariant among the danaines examined by Kitching.
L21.	Condition of cuticular ornamentation on filaments. Kitching states that this character is likely redundant with character 58 (L9).
L22.	Shape of base of cuticular ornaments on filaments. This character is invariant except for intraspecific polymorphism in Kitching's data matrix.
L26.	Labral campaniform sensilla basal to widest point of labrum. Kitching stated, "This character is variable and often difficult to score consistently, and so may be unreliable."
L27.	Position of secondary labral setae in relation to campaniform sensilla. Kitching stated, "This character is also unstable within samples ..."
L30.	Position of labral seta M ₂ in relation to M ₁ and L ₂ . This character is polymorphic among the <i>Amauris</i> species but otherwise invariant among Danaini scored by Kitching.
L31.	Position of subprimary labral seta. Kitching stated, "varies within samples, although not greatly."
L32.	Shape of labral lobes. This character is polymorphic among the <i>Amauris</i> species but otherwise invariant among Danaini scored by Kitching.
L38.	Position of outer epipharyngeal campaniform sensillum. This character is invariant among the danaines examined by Kitching.

(Continued on next page)

Table 2. (Continued)

L41.	Campaniform sensillum between epipharyngeal seta 2 and distal edge of labrum. This feature is present in one of two <i>Euploea</i> species examined by Kitching and absent elsewhere.
L42.	Position of labral seta L2. Kitching's character states overlapped to accommodate intraspecific polymorphism.
L43.	Condition of epipharyngeal setae. This character is invariant among the danaines examined by Kitching.
L45.	Shape of ventral outline of mandible. This character is invariant among the danaines examined by Kitching.
L47.	Presence of ventral mandibular teeth. This character is invariant among the danaines examined by Kitching.
L49.	Presence of pores on mandibular wrist. This feature is present in one of two <i>Euploea</i> species examined by Kitching and absent elsewhere.
L52.	Galea length/width ratio. This character is invariant among the danaines examined by Kitching.
L55.	Size of galeal type 1 sensilla. This character is invariant among the danaines examined by Kitching.
L57.	Position of type 6 sensillum on maxillary palp. This character is invariant among the danaines examined by Kitching.
L59, L60.	Presence of lateral and basal campaniform sensilla on maxillary palp segment 1. Kitching stated that he could not reliably score these characters.
L62.	Width of lateral arm of stipes. This character is invariant among the danaines examined by Kitching.
L63.	Presence of setae on posterolateral unsclerotized part of stipes. Kitching stated, "the separation of the two states may be due to sampling error."
L64.	Presence of stipital subprimary seta St3. This character is invariant among the danaines examined by Kitching.
L67.	Length of labial palp seta. This character is invariant among the danaines examined by Kitching.
L68.	Width of labial palp segment 1. This feature differs in one of two <i>Euploea</i> species relative to all remaining taxa examined by Kitching.
L69.	Gap between fusiger and mentum. This feature differs in one of two <i>Amauris</i> species relative to all remaining taxa examined by Kitching.
L75.	Size of lateral hypopharyngeal spines: 0 = small; 1 = large. This character is invariant among the danaines examined by Kitching.
L77.	Shape of hypopharynx. This character is invariant among the danaines examined by Kitching.
P4.	Angle of cremaster crown. This character is invariant among the danaines examined by Kitching.
P9.	Dorsal excrescences at base of cremaster. This character is polymorphic in <i>Idea</i> and invariant among other danaines examined by Kitching.
P11.	Anal area depressed. This character is polymorphic in <i>Idea</i> and invariant among other danaines examined by Kitching.
P16, P17.	Presence of median dorsal spots between abdominal segments 9 and 10 and on segment 9. These characters are polymorphic within <i>Danaus</i> and invariant among other taxa examined by Kitching.
P27.	Pair of small dorsolateral papulae on abdominal segment 1. This character is polymorphic within <i>Idea</i> and invariant among other taxa examined by Kitching.

Figure 2 shows the corresponding phylogram from the reduced dataset (excluding *Tiradelphe* and *Protoploea*) used to calculate branch support values, which are > 25 for all genera and subtribes (except *Anetia*, = 6) (Table 3). The bulk of support is derived from the molecular data, although the data subsets are almost entirely congruent and only 2% of tree length is due to homoplasy among partitions (Table 3). Morphological characters analysed separately yield a largely similar topology, except for a basal position of *Amauris*, driven by apparently homoplastic immature characters (data not shown). All partitions have strongly positive

total support (sum of PBS over all nodes), and adult morphology provides the greatest proportional partitioned support (total support/number of informative characters; Table 3). There is proportionately twice as much incongruence between adult and larval morphology as there is among the three gene regions (probably due to conflict related to *Amauris*), and five to ten times as much homoplasy within individual partitions as among them. Only one node has a negative PCP value (*Euploea corinna* + *E. mulciber*), and this is due to weak support and incongruence among the molecular partitions.

Table 3. Parameters for data partitions. D is the additional steps implied by incongruence among the data partitions when they are combined. Total Support is the sum of branch support values for the entire tree for each partition.

Data partition	Number of characters	Number informative	Number of Trees	Tree Length	Intrinsic Homoplasy	D Homoplasy	Total Support	TS/#InfChar
Adult morphology	45	38	150	57	10 (17.6%)		39.92	1.05
Immature morphology	80	78	120	141	46 (32.6%)		51.42	0.66
Combined morphology	125	116	75	205		7 (3.4%)		
mtDNA	2335	681	1	2902	1252 (43.1%)		541.75	0.88
COI-COII								
Ef-1a [†]	1260	201	75	773	339 (43.9%)		106.42	0.53
Wingless [†]	382	113	1848	341	140 (41.1%)		88.5	0.78
Combined DNA	3977	995	2	4086		70 (1.7%)		
All data	4102	1111	1	4317		103 (2.4%)	828	0.75

[†]Partitioned analyses excluded taxa with missing data.

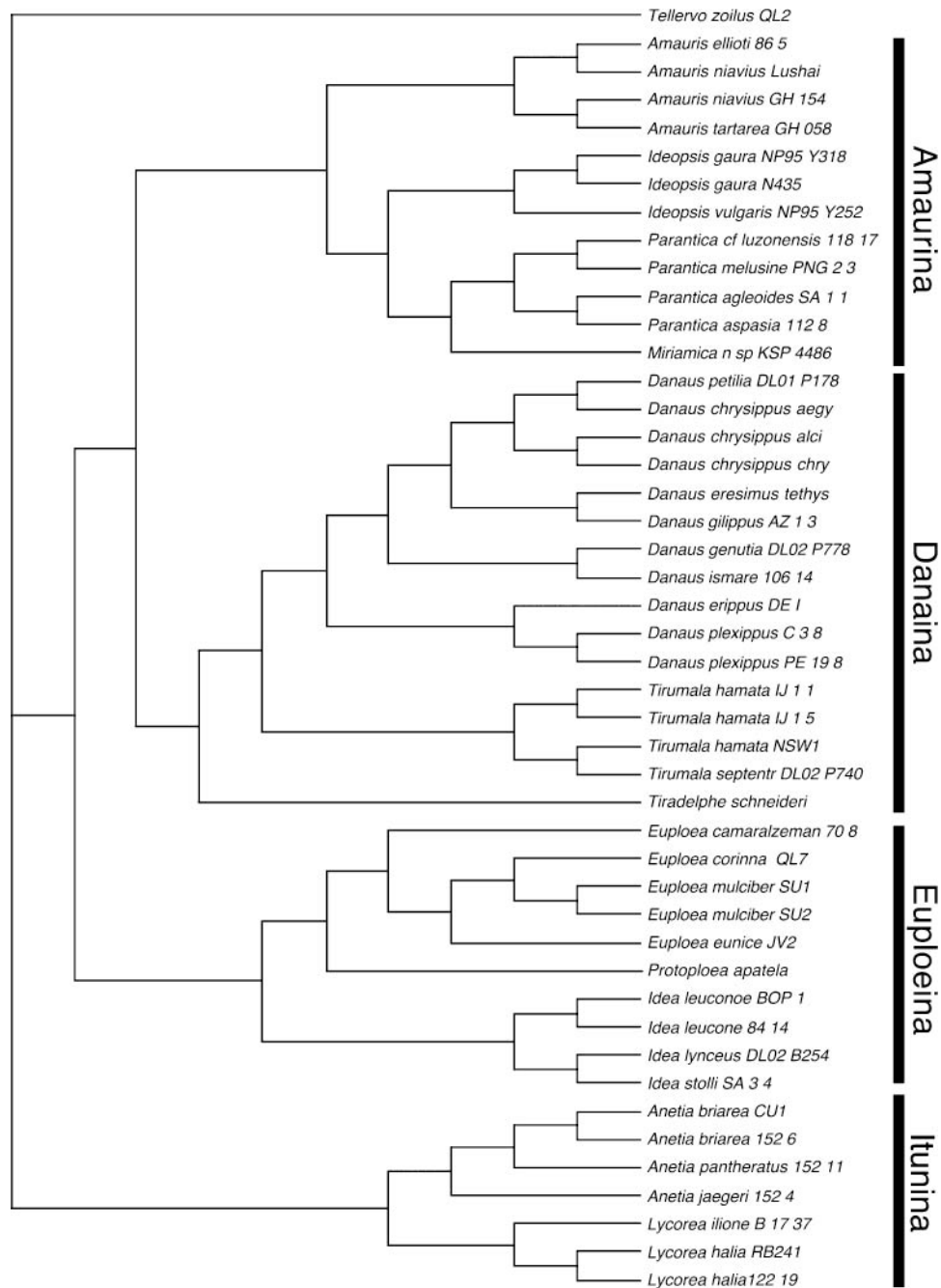


Fig. 1. Most parsimonious cladogram for all taxa and data included in the study. Tree length = 4323 steps.

Discussion

Phylogenetic relationships

The cladogram is one of the most uniformly well-supported hypotheses of relationships we have encountered in our research, and provides a robust phylogenetic framework for classification of danaine subtribes and genera. Note that with a single outgroup the monophyly of Danaini is not tested in this study, but it has been repeatedly corroborated

in prior morphological and molecular studies (see Introduction). Also, although the data provide strong resolution of subtribes and genera, relationships implied within genera should be considered tentative, given the relatively sparse sampling of species.

Figure 3 compares the generic relationships implied by our cladogram with the cladogram from Ackery & Vane-Wright (1984) and the pre-Hennigian proto-cladogram of Forbes (1939). The three are remarkably similar,

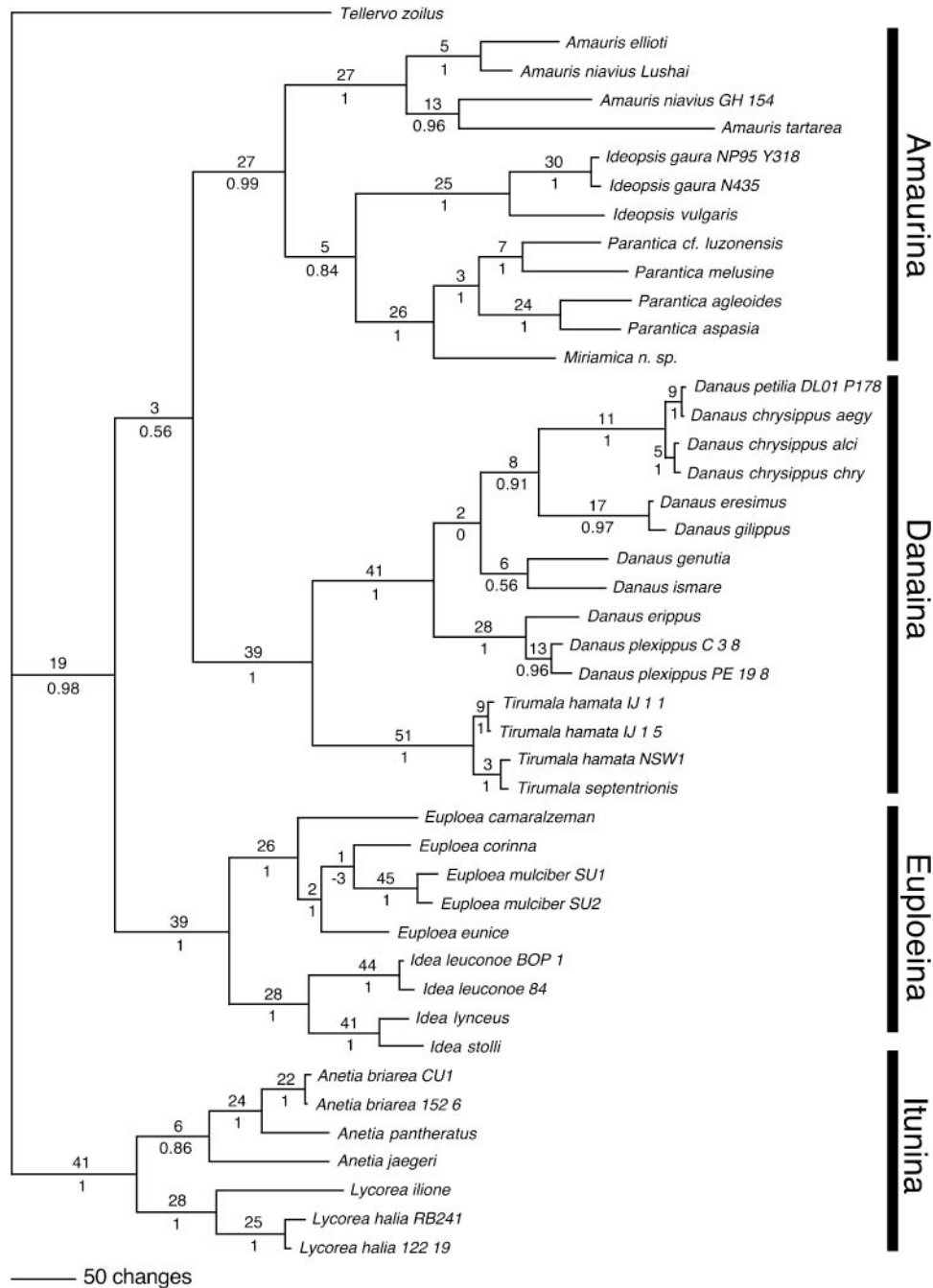


Fig. 2. Phylogram depicting relationships among exemplar Danaini implied by simultaneous cladistic analysis of all data, excluding *Protoploea* and *Tiradelphe*, which lack data for four of the five partitions and are not amenable to calculation of branch support measures. Topology identical to Fig. 1. Tree length = 4317 steps; CI = 0.4165; RI = 0.6995. Numbers above branches are branch support values, numbers below branches are Partition Congruence Proportions. Illustrated branch lengths are those implied by ACCTRAN optimization.

differing from one another only in the position of Itunina and *Amauris*. Ackery & Vane-Wright (1984) recognized two tribes and four subtribes within Danainae: Euploeini comprising Euploeina and Itunina, and Danaini comprising Danaina and Amaurina. Because we have reduced the rank of 'danaids' as a whole to a tribe and because in the current

hypothesis Itunina is sister to the remaining three subtribes, we have eliminated a name and rank for Danaina + Amaurina and instead represent them by phylogenetic sequencing (Nelson, 1974). Relationships among the subtribes are for the most part well-supported. The Itunina are sister to the remaining three subtribes, with Euploeina sister to

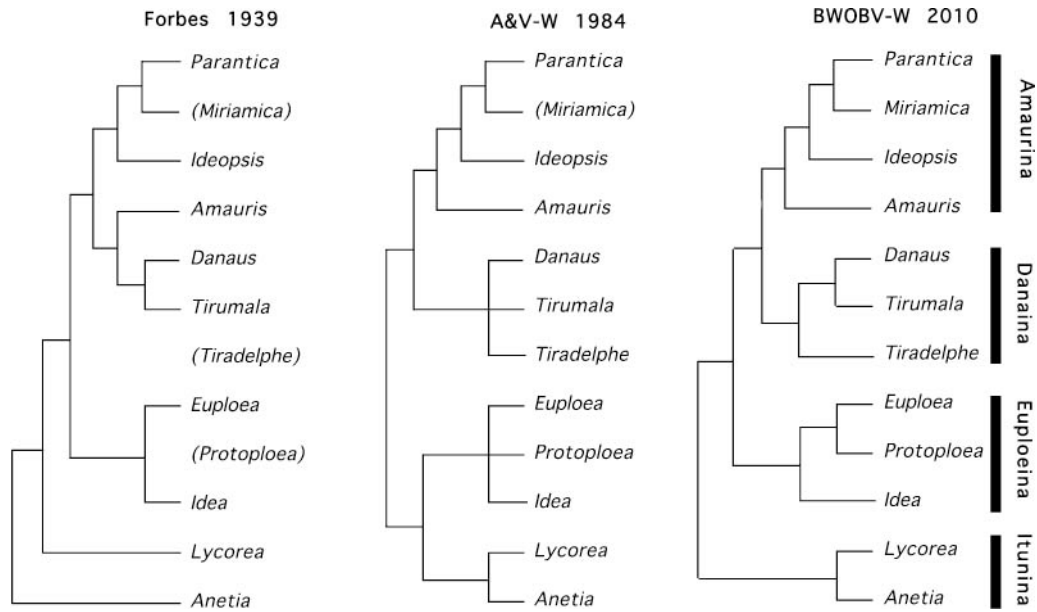


Fig. 3. Comparison of relationships of danaine genera as implied by Forbes (1939); Ackery & Vane-Wright (1984) and the current work. Subtribes are identified by the black bars.

(Amaurina + Danaina). The sister relationship of Amaurina and Danaina is only moderately supported (the more reason not to name it). The Amaurina and Danaina are the only Danainae that produce pheromone transfer particles – but these are not universal, and do not appear homologous (see Discussion). Boppré & Vane-Wright (1989: 125) suggested that the occurrence of double-lattice scales may link the Amaurina + Danaina, but similar structures are now known to occur in the alar organs of most Ithomiini (Willmott & Freitas, 2006: character 192, p. 343), and so this character is arguably another autapomorphy of the Danainae (or Danainae less *Tellervo*). However, if so, it has been lost in both of the ‘lower’ groups of the Danaini. The hairpencils of the Amaurina and Danaina are similar, and differ from those that occur in the Itunina and Euploeina, and may yet prove to be a good character linking the two subtribes.

The five species of *Anetia* bear a superficial resemblance to fritillaries (e.g. *Argynnis*), but have long been recognized as danaines on the basis of their male abdominal hairpencils. Forbes (1939) considered them to represent the danaine ancestral condition, implying a Neotropical origin for the group. If the sister group of Danaini is Ithomiini, then the hypothesis of a Neotropical origin is supported; however, relationships among Danaini, Ithomiini and the third danaine tribe, represented by the small Australasian genus *Tellervo* (Ackery, 1987), remain inconclusive (Brower, 2000; Freitas & Brown, 2004; Wahlberg *et al.*, 2009), rendering the biogeographical optimization of the basal danaine node ambiguous, as either Neotropical or Oriental/Australasian. The three other danaine subtribes apparently had an Ori-

ental/Australasian origin, with subsequent colonizations of the New World and Afrotropical regions by *Danaus*, of the Afrotropics by *Amauris* (endemic genus) and two *Tirumala*, and islands of the Indian Ocean by two *Euploea*.

There are three instances of species paraphyly implied by our analysis. *Tirumala septentrionis* is sister to a specimen of *T. hamata* from Australia, and those two together are sister to two more *T. hamata* from Papua (Indonesia). Ackery & Vane-Wright (1984) pointed out that these species are allopatric and have been considered conspecific, and more thorough sampling is necessary to determine relationships among these and the seven other species in the genus.

The Australian *Danaus petilia* (elevated from inclusion within *D. chrysippus* by Lushai *et al.*, 2005b) is nested among several African *D. chrysippus* in our tree. Lushai *et al.* (2005a, b) reported surprising results that implied the polyphyly of African *D. chrysippus* with respect to *D. eresimus*, *D. plexippus* and *D. genutia*, the last two of these species belonging to separate subgenera of *Danaus* according to the accepted classification. Our results corroborate Ackery & Vane-Wright’s (1984) hypothesis of relationships among subgenera of *Danaus*: (*Danaus* (*Salatura*, *Anosia*)), and a parsimonious ad hoc explanation of Lushai *et al.*’s unorthodox results is that perhaps samples were mixed up, which could explain not only the subgeneric incongruence they reported, but also the nesting of our Australian specimen among Lushai *et al.*’s African exemplars included in our analysis. Additional sampling will inform this problem.

The third instance of implied species paraphyly occurs between the Lushai *et al.* *Amauris niavius*, which appears as sister to *A. ellioti*, and ours, which is sister to *A. tartarea*.

The latter grouping corroborates the relationships inferred by Ackery & Vane-Wright's (1984) morphological analysis of *Amauris*, the shared possession of a unique mode of PTP production in *niavius* and *tartarea* (Boppré & Vane-Wright, 1989), and the analysis of male pheromone components carried out by Schulz *et al.* (1993). The two 'niavius' specimens are quite strongly supported in their respective sister-relations, and we again suspect that samples may have been mixed up or misidentified. They are mimetic butterflies, after all.

Aside from these minor incongruities, our results corroborate and greatly increase support for almost all clades in the phylogenetic hypothesis of Ackery & Vane-Wright (1984). Only the position of Itunina as sister to all remaining danaines instead of to Euploeina differs between these hypotheses. Interestingly, both Forbes (1939) and Kitching (1985, pupal data) both inferred the basal position for Itunina recovered here. Indeed, Ackery and Vane-Wright's adult morphology data by themselves imply that the Euploeina + Itunina hypothesis is only a single step shorter than the current hypothesis (data not shown). Important areas for future work include corroborating and resolving patterns of relationship among species of the relatively diverse *Parantica* (41 species, including an undescribed species noted by Vane-Wright & de Jong, 2003) and *Euploea* (59 species), rediscovery and improved characterization of *Protoploea apatela* and *Tiradelphe schneideri* (if they are not extinct), and the elucidation of biogeographical patterns among the many geographically polytypic danaine species ranging across the archipelagos of the southwestern Pacific.

Biology

Chemical ecology

Chemical ecology is a key aspect of milkweed butterfly biology, responsible for many conspicuous elements of their behaviour – notably intra- and interspecific communication (including mimicry), use of elaborate androconial organs, and the direct and indirect utilization of secondary chemicals, notably cardenolides (CGs) and pyrrolizidine alkaloids (PAs), derived from larval and/or adult host plants.

Larval host plants. The great majority of larval host plant records for the Danaini fall within in the Asclepiadaceae and Apocynaceae (Ackery, 1988); however, only species belonging to a few, widely separated tribes belonging to this large family (Apocynaceae *s.l.*, cf. Endress *et al.*, 2007) are actually used by milkweed butterflies (Boppré & Liede-Schumann, unpubl.). Due to under-recording (records available for less than 50% of species), plant misidentifications, and changes in plant taxonomy (cf. Boppré & Liede-Schumann, unpubl.), although there is within-tribe differentiation in larval host use, currently only four general statements can be made: larval host plants for many species

appear surprisingly unspecific; feeding on CG-containing species is relatively rare, and sequestration of CGs is limited to *Danaus* (Ackery & Vane-Wright, 1985); larval feeding on PA-containing plants is restricted to *Euploea* and *Idea*; and feeding on non-apocynaceous families is only well established for some *Euploea* and *Lycorea*, which use *Ficus* (Moraceae), and some *Lycorea*, which also feed on Caricaceae. Far more intensive sampling of larval hosts is required and, in particular, accurate identifications are imperative (cf. Boppré & Liede-Schumann, unpubl.).

Adult host plants. In addition to plants providing nectar, adult Danaini and many Ithomiini also forage and sequester PAs for defence, and for pheromone precursors, from withered or dry plant parts of Apocynaceae (Apocynoideae: Echiteae), Asteraceae (Eupatorieae), Boraginaceae and Fabaceae (Crotalarieae)—of these, only the Echiteae are related to larval hostplants. This pharmacophagous uptake of PAs (Boppré, 1984) is exhibited by all adult Danaini investigated (except *Idea*, which obtain PAs from their larval host plants), and is usually restricted to males; however, adult females of *Euploea* also forage for PAs (Boppré, unpubl.).

Male courtship pheromones

There are species-specific blends of volatiles (e.g. Schulz *et al.*, 1993), but our best general knowledge concerns pheromone components derived from PAs – which are unspecific, but nonetheless significant signals in close-range communication. Unfortunately, our knowledge is still too incomplete to relate different pheromones made from plant PAs (necine-base derived dihydrorrrolizines of various oxidation status, unmodified necic acid derivatives, and uniquely modified necic acid derivatives), together with their associated biochemical pathways, to phylogeny. However, as shown for *Amauris* (Vane-Wright *et al.*, 1992), the range of chemicals making up the androconial bouquets may be sufficiently complex to offer valuable chemical data for phylogenetics, to analyse alongside adult and early stage morphological, and DNA sequence data partitions.

Androconial organs

The androconial organs of the Danaini that secrete, store and transmit PA-derived and other volatiles, and, we presume, various non-volatile components, differ widely in details of their macroscopic and microscopic structures. Although the full data set used to generate the cladogram includes many of the features discussed below, we have run the analysis to exclude all adult morphological characters, and the topology remains identical. It is thus legitimate to make these comparisons by reference to the total evidence tree, despite the potential adaptive nature of some of its supporting characters (Schuh & Brower, 2009).

Abdominal hairpencils

With respect to the universal abdominal hairpencils (unique to the Danaini), the Itunina and Euploea have the 'bottle brush' type (Boppré & Vane-Wright, 1989), with hairs inserted the whole length of the eversible tube. The species of *Lycorea* (Itunina) differ, however, insofar as the full-length insertions are limited to a narrow band or strip. In the Danaina and Amaurina, the hairs are only inserted towards the distal end of the tube.

Pheromone-transfer-particles (PTPs)

Pheromone-transfer-particles are only produced by members of the Danaina and Amaurina, but not all species belonging to these two groups do so. Among the majority that do produce PTPs, different taxa do so in several different ways, with *Tirumala* notable as the only milkweed butterflies to produce PTPs on their alar organs rather than in the hairpencils. However, there is a possibility that the unknown males of *Tiradelphe* do the same, while the alar organs of *Miriamica* produce what could be, but most probably are not, PTPs (Vane-Wright *et al.*, 2002).

Alar organs

No alar organs occur in the Itunina, *Idea* and *Protoploea*, while those of *Euploea* are diverse, but never comparable to those found in the Danaina and Amaurina. The alar organs of the Danaina occur in the cubital area of the hindwing, whereas those of the Amaurina occur in the anal area of the hindwing. Many *Euploea* have forewing androconia, unknown in the Danaina or Amaurina.

Mimicry and courtship behaviour

The Danaini are all involved in mimicry complexes, and larvae as well as adults are aposematic in colour as well as in behaviour. Because they represent the most widespread source of chemical defence among members of the tribe, PAs appear to be fundamental to understanding danaine mimicry relationships. However, the intra-individual and temporal variations in the amount and variety of PAs present, intrinsically associated with the pharmacophagous gathering of these chemicals, generate many questions (Boppré, 1997). It would not be surprising if a third group of compounds, either sequestered or synthesized *de novo* by the larvae and perhaps also the adult butterflies, were found to 'fill the gap'; phenanthroindolizidine alkaloids (cf. Abe *et al.*, 2001) might plausibly be a candidate.

Differences in courtship behaviour are partly related to the presence or absence of PTPs, but remain poorly understood.

Conclusions

Because there has been no up-to-date review of the chemoeology of Danaini since Ackery & Vane-Wright (1984), a complete discussion would require citation of numerous references – and much has been achieved in this last quarter-century. This brief account has glossed over a number of supposed exceptions (many of them doubtful), and related problems due to under-sampling, insufficient chemical analyses, and unreliable identifications. Many species escape proper study because of the difficulty of obtaining living specimens. However, it is clear that there is great diversity within the Danaini among almost all factors relating to chemical ecology, and there is much potential for interesting correlations. The emergence of a stable generic and subtribal phylogenetic system should give new impetus to gathering better and more focused ecological data, and give confidence that the results can be interpreted meaningfully, against a robust phylogenetic framework.

Lastly, we would like to draw the reader's attention to another recent molecular study of relationships of Danaini by Shinkawa *et al.* (2007), who examined the mitochondrial ND5 gene for exemplars of five danaine genera. Their results are entirely compatible with ours.

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