

Phylogenetics and divergence times of Papilioninae (Lepidoptera) with special reference to the enigmatic genera *Teinopalpus* and *Meandrusa*

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Abstract

Phylogenetic relationships of 18 genera of the swallowtail subfamily Papilioninae, four genera of Parnassiinae, and the monobasic Baroniinae are inferred based on 94 morphological characters and 5616 bp DNA from seven genes (*16S*, *COI*, *COII*, *ND1*, *ND5*, *EF-1 alpha* and *wingless*). Bayesian likelihood analyses show that Baroniinae are the sister of a clade comprising Parnassiinae and Papilioninae. Four Papilioninae tribes are recognized, Leptocircini, Teinopalpini, Papilionini and Troidini, with Leptocircini being the sister of the remaining tribes. *Meandrusa* and *Teinopalpus* are sister taxa and comprise the tribe Teinopalpini, which is the sister of a clade comprising Papilionini and Troidini. The tribe Troidini (pipevine swallowtails) comprises two subtribes: Battina (including only *Battus*) and Troidina. The endemic Madagascan genus *Pharmacophagus* is consistently placed as the sister to the remaining Troidina. The non-*Pharmacophagus* Troidina are tentatively divided into a Neotropical lineage and an Australasian lineage. Dispersal–vicariance analyses indicate that past dispersal events are most important for explaining current distribution patterns of Papilionidae. However, the division of the non-*Pharmacophagus* Troidina into a Neotropical lineage and an Australasian lineage is possibly due to the final break-up of southern Gondwana. A fossil-calibrated relaxed Bayesian molecular clock analysis confirms that the ages of the lineages fit this scenario. The basal lineages leading to the current subfamily-level diversity of Papilionidae probably arose around the K/T boundary. Analyses of larval host-plant relationships within Papilionidae show very little phylogenetic pattern. However, Aristolochiaceae-feeding apparently evolved independently in non-Parnassiini parnassiines and Troidini.

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Swallowtail butterflies are the best-known family of Lepidoptera and may be the most well-known group of invertebrate animals. Numerous studies have been made on speciation, polymorphism, mimicry, sexual selection, host relations, chemistry, physiology, anatomy and historical biogeography. These insects are a flagship group for invertebrate conservation, and several large

volumes have been devoted to their biology and evolution (e.g. Igarashi, 1979; Tyler et al., 1994; Scriber et al., 1995).

Many studies have also addressed their systematic and phylogenetic relationships. Vane-Wright (2003, p. 480) noted that “measured by effort per species, more work has gone into trying to understand the interrelationships of the 600 or so species of Papilionidae than any other family of Lepidoptera”, and yet “schemes abound, but we remain far from any consensus”.

One consensus widely agreed is the division of Papilionidae into three extant subfamilies: Baroniinae,

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Parnassiinae and Papilioninae. Parnassiinae and Papilioninae are generally considered sister taxa, with the monobasic Baroniinae representing the stem lineage (but see Nazari et al., 2007; Michel et al., 2008). Although placed in Papilioninae, the phylogenetic positions of *Meandrusa* and *Teinopalpus* have been particularly problematic.

Phylogenetic relationships

In the following account, few works pre-dating 1980 are dealt with; Hancock (1983), Igarashi (1984) and Miller (1987a) gave reviews of these sources. Munroe and Ehrlich (1960) and Munroe (1961) summarized previous systematic evidence and recognized two tribes [Parnassiini and Luehdorfiini (=Zerynthiini)] within

Parnassiinae, and four tribes (Leptocircini, Teinopalpini, Papilionini and Troidini) within Papilioninae. They considered *Meandrusa* to be a junior synonym of *Papilio*, placed *Cressida* and *Euryades* in Troidini, and followed Ford (1944a) in placing *Teinopalpus* in its own tribe, although a position within Leptocircini was also considered (Munroe and Ehrlich, 1960, p. 170; Munroe, 1961, p. 17).

Hancock (1983) presented the first modern (cladistic) attempt to resolve the classification and phylogeny of Papilioninae (Fig. 1a), although this work was methodologically mixed (Miller, 1987a). Within Papilioninae, Hancock recognized three tribes: Leptocircini, Papilionini and Troidini.

Igarashi (1984) presented a classification based on detailed studies of juvenile life stages. Although his phylogenetic reconstruction and classification were

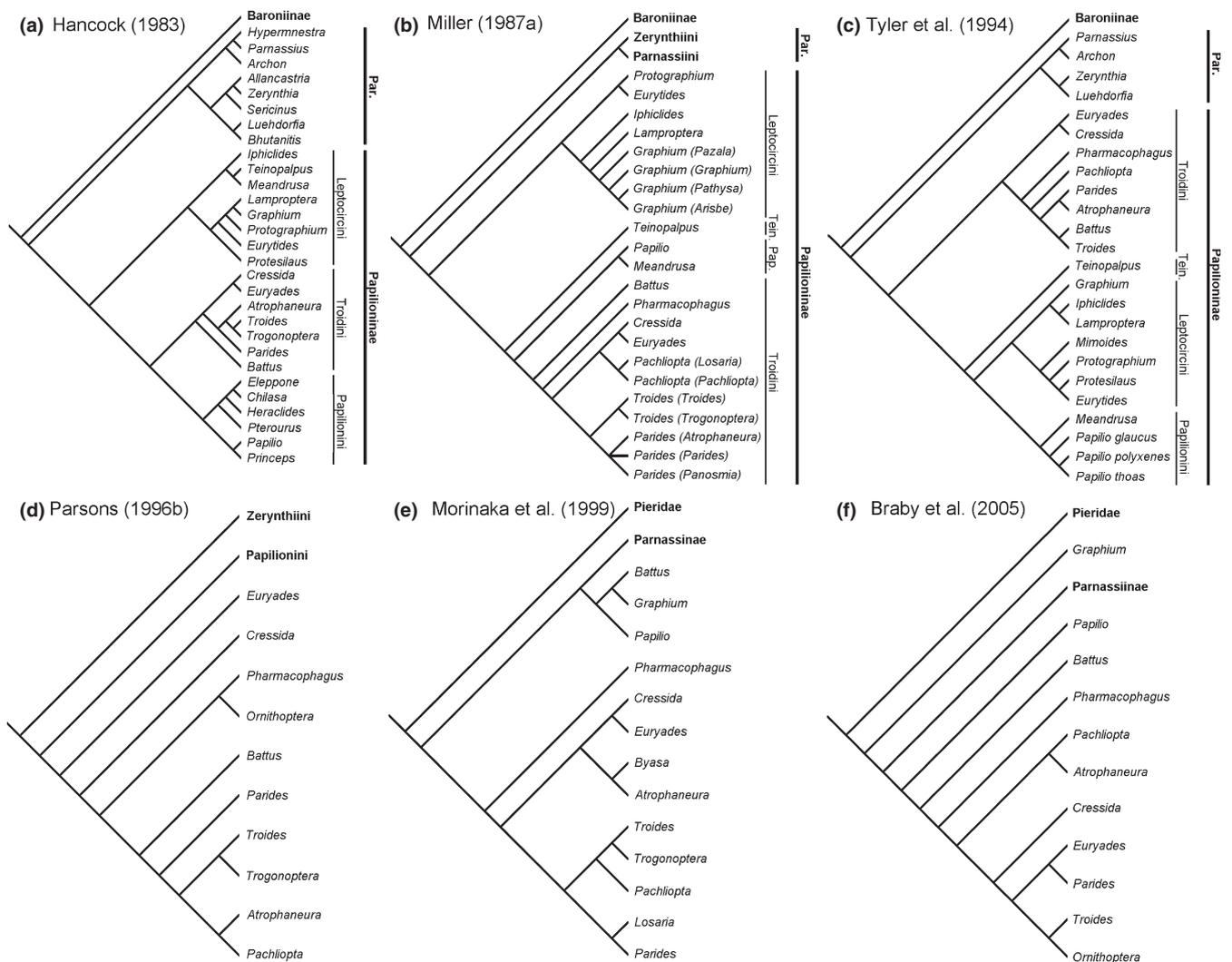


Fig. 1. Previous higher-level cladistic studies of focal groups of this paper. (a–c) Papilioninae. (a) Hancock (1983). (b) Miller (1987a). (c) Tyler et al. (1994). (d–f) Troidini. (d) Parsons (1996b). (e) Morinaka et al. (1999). (f) Braby et al. (2005).

hierarchical and not cladistic, he agreed with Hancock (1983) that *Teinopalpus* belonged to Leptocircini. Even so, Igarashi considered *Meandrusa* to be the “most derived” *Papilio* (p. 91). He also considered Papilionini and Leptocircini to be more closely related to each other than either was to the Troidini. Despite the non-cladistic approach, Igarashi’s various works (e.g. Igarashi, 1979, 1984, 1987; Igarashi and Fukuda, 1997, 2000) remain singularly important contributions to swallowtail systematics because of his detailed descriptions and superb illustrations of juvenile stages.

Miller (1987a) presented the first computer-implemented cladistic analyses of Papilionidae with a focus on the subfamily Papilioninae (Fig. 1b). The study was based on analyses of three separate datasets comprising 170 morphological characters from both juvenile and adult stages. The first analysis focused on the intertribal relationships within Papilioninae, with Baroniinae and the two parnassiine tribes Parnassiini and Luehdorfiini as outgroups. He concluded that the Leptocircini (= Graphiini) represented the sister group to the remaining Papilioninae. His second and third analyses focused on the Leptocircini and Troidini, respectively.

Tyler et al. (1994) presented the next comprehensive computer-based analyses of Papilionidae, based on 155 characters from morphology, ecology and behaviour of adults and juveniles (Fig. 1c). When their 85 adult characters were analysed separately, the result was essentially similar to that of Miller (1987a). In other analyses, Leptocircini and Papilionini were sister groups, with Troidini being sister to the remaining Papilioninae.

Higher-level papilionid phylogenetic research entered the molecular age with Yagi et al. (1999). Although focused on Japanese species, the study employed the nucleotide sequence of mitochondrial gene *ND5*, and included a broad range of papilionid genera. Yagi et al. found it difficult to recover Papilionidae as a monophyletic group, but the analyses consistently recovered Papilioninae and its tribes as monophyletic. Caterino et al. (2001) presented a more comprehensive molecular study of papilionid phylogeny, based on 21 species representing all major tribes of the family. Both parsimony and partitioned maximum-likelihood analyses were carried out based on DNA sequences of mitochondrial genes *COI* and *COII*, and nuclear gene *EF-1 alpha*. Maximum-likelihood resulted in a monophyletic Papilionidae, but failed to recover Parnassiinae as monophyletic. Inter-tribal relationships within Papilioninae were congruent with the traditional taxonomic arrangement. Wahlberg et al. (2005), in the first “total evidence” analyses of family-level butterfly phylogeny, did recover Papilionidae as monophyletic, but found it difficult to resolve basal relationships within the family.

Nazari et al. (2007) presented the first higher-level “total evidence” analyses of a papilionid group, based

on morphology and seven genes. Although mainly focused on the Parnassiinae, they reported several interesting results—most notably that Parnassiinae and *Baronia* formed a monophyletic group, albeit with low support, with the Papilioninae as their sister group. Michel et al. (2008), in another molecular study focused on Parnassiinae, found *Baronia* to be the sister group of the Parnassiini. They also failed to consistently recover Papilionidae. So far, no higher-level molecular study has included the two enigmatic genera *Teinopalpus* and *Meandrusa*, although Aubert et al. (1999) did include *Meandrusa* as an outgroup in a study focused on *Papilio*, and Makita et al. (2003) included *Teinopalpus* as part of a study of *Graphium*.

The tribe Troidini has probably received more attention than any other comparable group of insects. Ehrlich (1958), Munroe and Ehrlich (1960) and Munroe (1961) included Ford’s (1944a) tribe Cressidini, comprising *Cressida* and *Euryades*, in Troidini. Since then it has been widely agreed that the Troidini are divisible into two subtribes: *Battina*, which comprises only the primarily Neotropical genus *Battus* and the Troidina, comprising all remaining genera (but see Parsons, 1996a,b; Morinaka et al., 1999). Most agreements end here, however. Exactly how many genera Troidina includes and how they are interrelated is still strongly debated (Vane-Wright, 2003; for a full list of references see Braby et al., 2005). Miller (1987a) considered *Pharmacophagus* to be sister to the remaining Troidina (a relationship supported by others but disputed by Hancock, 1988, 2007), and that *Cressida* and *Euryades* formed a well-supported clade. These results were generally endorsed by Tyler et al. (1994). Parsons (1996a,b), in two studies based mainly on juvenile characters, came up with markedly different results from anything proposed before or since (Fig. 1d). Parson’s studies have since been critically assessed (de Jong, 2003, 2007; Vane-Wright, 2003; Braby et al., 2005). Morinaka et al.’s (1999) molecular study focused on Troidini using mitochondrial gene *ND5* (Fig. 1e). Notably, they did not recover Troidini as conventionally understood as monophyletic, but had *Battus* as sister to *Graphium*. Braby et al. (2005) presented a comprehensive study of Troidini based on analyses of three mitochondrial genes (*ND5*, *COI*, *COII*) and the nuclear gene *EF-1 alpha* (Fig. 1f). In their analyses of the combined datasets both Troidini and Troidina were consistently monophyletic. However, *Cressida* and *Euryades* were never found to be closely related; the latter was always sister to *Parides*.

Insect–plant interactions

Larval host-plant relationships within Papilionidae have been the subject of considerable interest.

Luehdorfiini and Troidini feed exclusively on members of the family Aristolochiaceae (Weintraub, 1995; Nazari et al., 2007). This close association has suggested a number of hypotheses on the evolution of host-plant associations within the family as very few other insects feed on Aristolochiaceae. Several authors have speculated that Aristolochiaceae-feeding represents the ancestral condition within Parnassiinae + Papilioninae. Based on cladistic analyses, this idea was rejected by Miller (1987b) and Weintraub (1995).

Historical biogeography

Historical biogeography and estimated divergence times for Papilionidae (chiefly Troidini) have experienced a recent rise in popularity due to the advent of DNA sequencing and reasonably credible molecular clocks (for reviews, see de Jong, 2007; de Jong and van Achterberg, 2007). Miller (1987a) noted that the sister-group relationship between *Cressida* and *Euryades* fitted a classical Gondwanan distribution, but otherwise concluded that the phylogeny of Papilioninae lacked biogeographical patterns associated with vicariance events. de Jong (2003) applied a molecular clock to the dataset of Morinaka et al. (1999) to calculate the ages of separation between *Cressida* and *Euryades*. The results indicated that this event was too recent to support a Gondwanan origin, a result contested by Braby et al. (2005). Based on zoogeographical calibration points, Zakharov et al. (2004a) found that the basal divergence within the genus *Papilio* occurred between 35 and 65 Ma. The split between *Papilio* and Troidini was estimated at 80–90 Ma. Basing their clock calibrations partly on the results of Zakharov et al. (2004a), Nazari et al. (2007) estimated that the initial diversification of Papilionidae took place in the Late Cretaceous (c. 90 Ma). In a review of butterfly and Hymenoptera biogeography, de Jong and van Achterberg (2007) found very little evidence for patterns caused by the break-up of Gondwana. Hancock (2007) revisited Troidini biogeography based on the results of Braby et al. (2005) and suggested that the Troidina overall comprise three evolutionary “Gondwanan lineages”.

Despite these more recent contributions, Miller (1987a) remains the most comprehensive morphological study, and forms the foundation for the morphological part of the present work. Here we present a phylogenetic study of the genus-level phylogeny of swallowtails, with a focus on Papilioninae (especially the relationships of *Teinopalpus* and *Meandrusa*), based on a detailed re-examination of Miller’s data as well as nucleotide sequences from seven genes representing at least two and probably three independent linkage groups. The new results are then used to re-assess the divergence times and historical zoogeography of the Papilioninae.

Materials and methods

Taxon sampling

Twenty-five species representing 18 genera of Papilioninae were sampled in the analysis, including all genera recognized by Miller (1987a). Of the genera listed by Häuser et al. (2005), only *Mimoides*, *Atrophaneura*, *Byasa*, *Pachliopta* and *Trogonoptera* are unrepresented. Miller (1987a) placed *Mimoides* in *Eurytides* (subgenus *Protesilaus*), *Trogonoptera* as a subgenus of *Troides*, *Byasa* (in subgenus *Panosmia*) and *Atrophaneura* in *Parides*, and placed the genus *Losaria* (included here) as a subgenus of *Pachliopta*. We attempted to include several of these genera in our analysis (*Pachliopta*, *Trogonoptera*, *Atrophaneura* and *Mimoides*), but the quality of available material did not allow us to amplify molecular markers. Seven species were included from the large genus *Papilio*, representing the six major lineages identified by Zakharov et al. (2004a,b) and the putative genus *Chilasa*. This last taxon was found to be nested within *Papilio sensu stricto* (s.s.) by Zakharov et al. (2004a), but was placed in a separate genus by Häuser et al. (2005). The classification initially follows the latter, but some changes are suggested based on our results. *Baronia brevicornis* from the monobasic Baroniinae was used as a near outgroup, as were four species (*Parnassius phoebus*, *Archon apollinus*, *Allancastris cerisy* and *Luehdorfia japonica*) representing four of the eight genera of the Parnassiinae. To evaluate the monophyly of Papilionidae and examine relationships among swallowtail subfamilies, the butterflies *Pyrgus communis* (Hesperiidae), *Pieris rapae*, *Colias eurytheme* (both Pieridae), *Coenonympha tullia* and *Vanessa cardui* (both Nymphalidae) were included as distant outgroups. A complete list of species included in our analyses is given in Table 1. Voucher images and label data are available at the E. H. Strickland Entomological Museum (UASM) website (<http://www.biology.ualberta.ca/facilities/strickland/Vouchers/>).

Morphological character sampling and preparation

The morphological character matrix was based on Miller’s (1987a) list, but adult characters from other studies were also considered (Tyler et al., 1994; de Jong et al., 1996; Parsons, 1996b). We refer the reader to the original publications for detailed character descriptions. The information content and practical utility of each character was reassessed, and adult characters were re-examined for nearly all species included here; the few exceptions are discussed below. Adults, prepared following Miller (1987a), were examined for both for the characters listed therein and potentially new features. Wing venation was examined by placing spread specimens upside down under a stereomicroscope and then

Table 1
Species included in this study, with GenBank accession numbers for each sequence

Species	<i>16S</i>	<i>COI</i>	<i>COII</i>	<i>ND1</i>	<i>ND5</i>	<i>EF1-a</i>	<i>WG</i>
<i>Pyrgus communis</i>	DQ351078	AF170857	AF170857	U25880	DQ351044	AF173396	AY569043
<i>Coenonympha tullia</i>	DQ351081	AF170860	AF170860	AF229952 ^a	DQ351047	AF173399	DQ351126
<i>Vanessa cardui</i>	EU334572	AY248782	GQ268357	AF412742	DQ028758	AY248807	AF412770
<i>Colias eurytheme</i>	AB194748	AF044024	AF044024	U32456	DQ351048	AF173400	AY569040
<i>Pieris rapae</i>	DQ351082 ^b	AY954581	GQ268364	DQ351061 ^b	AB013161	AY870550	AY954611
<i>Baronia brevicornis</i>	DQ351091	AF170866	AF170866	Unsequenceable	DQ351050	AF173405	AY569044
<i>Luehdorfia japonica</i>	AB186171	AF170867	AF170867	AB186205	AB095663	AF173407	DQ351138
<i>Allancastris cerisy</i>	AB186169	AF170869	AF170869	AB186203	AB095662	AF173409	DQ351146
<i>Archon apollinus</i>	AB186168	DQ351031	DQ351031	AB186202	AB095661	DQ351111	DQ351135
<i>Parnassius phoebus</i>	AB181639	AF170872	AF170872	AB186173	AB063354	AF173412	AY569045
<i>Lamproptera meges</i>	GQ268337	GQ268354	GQ268361	GQ268367	AB059542	GQ268396	GQ268402
<i>Iphiclides podalirius</i>	DQ351088	AF170873	AF170873	AJ224087	AB059546	AF173413	DQ351129
<i>Protographium marcellus</i>	GQ268338	AF044022	AF044022	GQ268368	GQ268380	AF044815	DQ351128
<i>Protesilaus glaucolus</i> ^{*,†}	GQ268339	GQ268355	GQ268362	GQ268369	GQ268381	GQ268397	GQ268403
<i>Eurytides serville</i> [*]	GQ268340	GQ268356	GQ268363	GQ268370	GQ268382	GQ268398	GQ268404
<i>Graphium agamemnon</i> [‡]	GQ268341	AF170874	AF170874	DQ351062	AB059510	AF173414 + GQ268389	AY569046
<i>Teinopalpus imperialis</i>	GQ268334	GQ268351	GQ268358	GQ268365	GQ268377	GQ268393	Unsequenceable
<i>Meandrusa sciron</i> [§]	GQ268335	GQ268352	GQ268359	GQ268366	GQ268378	GQ268394	GQ268400
<i>Meandrusa payeni</i> [§]	GQ268336	GQ268353	GQ268360	AJ224088	GQ268379	GQ268395	GQ268401
<i>Papilio machaon</i>	EF489473	AY457593	AY457593	EF485043	AB095666	EF485106	AY569124
<i>Papilio demoleus</i>	DQ673336	AY569092	AY569092	AJ224099	AB013159	AY569104	AY569115
<i>Papilio dardanus</i>	AF095451	AY457584	AY457584	AJ224097	GQ268383	AY457612	GQ268405
<i>Papilio alexanor</i>	GQ268343	AF044012	AF044012	AJ224090	GQ268385	AF044821 + GQ268390	GQ268407
<i>Papilio thoas</i>	GQ268344	AY457601	AY457601	GQ268372	GQ268386	AY457632	AY569126
<i>Papilio glaucus</i>	GQ268342	EF126474	EF126474	GQ268371	GQ268384	EU136675	GQ268406
<i>Chilasa clytia</i>	DQ673337	AY457594	AY457594	EU783951	GQ268387	AY457606	GQ268408
<i>Battus philenor</i>	DQ351083	AF170875	AF170875	AJ224086	AB027573	AF173415	DQ351130
<i>Battus crassus</i> [*]	GQ268345	AY804351	AY804387	GQ268373	AB027575	AY804423	GQ268409
<i>Pharmacophagus antenor</i>	GQ268346	AY919288	AY919288	GQ268374	AB027582	AY919293 + GQ268391	GQ268410
<i>Cressida cressida</i>	GQ268347	AY919289	AY919289	GQ268375	AB027577	GQ268399	GQ268411
<i>Euryades corethrus</i> [¶]	GQ268348	AY804356	AY804392	AM283072	AB027576	AY804428	GQ268412 ^c
<i>Losaria neptunus</i>	GQ268349	AF044023	AF044023	GQ268376	GQ268388	AF044829 + GQ268392	GQ268413
<i>Parides photinus</i>	DQ351085	AF170877	AF170877	DQ351064	AB027581 ^d	AF173417	DQ351124 ^d
<i>Ornithoptera priamus</i>	GQ268350	AY919291 ^c	AY919291 ^c	AM283071	AB044657	AY919296	GQ268414
<i>Troides helena</i>	DQ351084	AF170878	AF170878	DQ351063	AB084430	AF173418	AY569047
Species only included in the molecular clock							
<i>Bhutanitis mansfieldi</i>	DQ351097	DQ351036	DQ351036	AB071945	AB026727	DQ351116	DQ351140
<i>Hypermystris helios</i>	AB186166	DQ351025	DQ351025	AB186200	AB095659	DQ351106	DQ351131
<i>Sericinus montela</i>	DQ351100	AF170868	AF170868	DQ351071	AB095665	AF173408	DQ351143
<i>Zerynthia rumina</i>	AB186167	AF170870	AF170870	AB186201	AB095660	AF173410	DQ351144

Genes that could not be amplified are represented by replacement sequences (a–e): a = *C. dorus*, b = *P. napi*, c = *E. duponchelli*, d = *P. neophilus*, e = *O. euphorion*.

For morphological studies: *only males were available, †studied specimen was *Protesilaus protesilaus*, ‡female studied was *Graphium sarpedon*, §only males of *Meandrusa* were available, females characters were scored as identical for both species based on the drawings in Miller (1987a), ¶only males were available, but female characters were scored based on the drawings in Miller (1987a).

placing a droplet of xylene on the wing. Characters from skeletal morphology of all body regions were examined in 70% ethanol after the preparations had been stained lightly in Chlorazol Black. Ultrastructure of antennae and wing scales were examined with a JEOL JSM-6301FXV scanning electron microscope after being sputter-coated with gold. Juvenile characters were scored based on the literature. Females were not available for a few species, but in most cases female

characters could be scored from Miller's (1987a) illustrations (see Table 1 for full list). The fossil genera *Praepapilio* (Durden and Rose, 1978) and *Thaites* (Scudder, 1875) were used as calibration points for the relaxed Bayesian clock. However, neither was included in the phylogenetic analyses as we did not examine the fossils first hand and character scorings based on the literature were considered uncertain. Scanning electron microscopy (SEM) plates are available on MorphBank.

Molecular character sampling and preparation

For all taxa and genes not already available on GenBank we sequenced the mitochondrial genes *16S*, *COI*, *COII*, *ND1* and *ND5*, and the nuclear genes *EF1-alpha* and *wingless*. Genomic DNA was extracted from legs or thoracic tissue using the QIAGEN QIAamp DNA mini kit. PCRs were performed in either 50- or 20- μ l reactions. A complete list of primers is given in Table 2. The general cycling profile included an initial denaturation at 95 °C for 5–7 min followed by 35 cycles of 95 °C for 1 min, 45–52 °C for 1 min (depending on primer combination), and 72 °C for 1 min, with a final elongation step at 72 °C for 10 min. PCR products were purified using the QIAGEN QIAquick PCR Purification Kit. PCR primers were used for sequencing in both directions. PCR products were directly sequenced using ABI Big Dye terminator cycle sequencing (ABI, Foster City, CA, USA) following the manufacturer's recommendations. Sequencing products were purified with sodium acetate/ethanol precipitation and analysed with

an AB Hitachi 3730 DNA Analyzer capillary sequencer. Bidirectional sequence reads were assembled into contigs using Sequencer 4.1 (GeneCode Corp., Ann Arbor, MI, USA). Consensus sequences were aligned using Clustal X (Thompson et al., 1997) and visually inspected for alignment quality. Minor adjustments were done by eye in MacClade 4.0 (Maddison and Maddison, 2000) for all genes except for *16S*, which proved difficult to align. As a result, alignment for that gene was carried out using MAFFT v. 6 (<http://align.bmr.kyushu-u.ac.jp/mafft/online/server/index.html>) and the E-INS-i method (Katoh et al., 2005b; Katoh and Toh, 2008).

Phylogenetic analyses

The eight datasets with characters from each of the seven genes and morphology were combined in WinC-lada (Nixon, 2002). Five data combinations were analysed: total evidence (combined molecular and morphological data), combined molecular data, morphological data, mitochondrial DNA and nuclear DNA.

Table 2
Primers used for PCR reactions

Gene	Location	Primer	Source	F/R	Sequence (5'–3')
<i>16S</i>	12 887	LR-J-12887	Aubert et al., 1999	F	CCGGTTTGAGCTCAGATC
<i>16S</i>	13 398	LR-N-13398	Aubert et al., 1999	R	CGCTGTTTATCAAAAACAT
<i>COI</i>	1460	K698	Sperling et al., 1994	F	TACAATTTATCGCCTAAACTTCAGCC
<i>COI</i>	1511	LCO	Folmer et al., 1994	F	AAACTTCAGGGTGACCAAAAAATCA
<i>COI</i>	2183	Jerry	Simon et al., 1994	F	CAACATTTATTTTGATTTTTTGG
<i>COI</i>	2325	Djernaes	New—this study	R	GCTATTATAGCATAAAATTATCC
<i>COI</i>	2329	K525	Simon et al., 1994	R	ACTGTAAATATATGATGAGCTCA
<i>COI</i>	2329	K525.2	Caterino et al., 2001	R	ACAGTAAATATATGATGAGCTCA
<i>COI</i>	3014	Pat2	Sperling et al., 1996	R	TCCATTACATATAATCTGCCATATTAG
<i>COII</i>	3038	Patrick	Caterino et al., 2001	F	CTAATATGGCAGATTATATGTAATGGA
<i>COII</i>	3782	Eva	Bogdanowicz et al., 1993	R	GAGACCATTACTGTTCAGTCATCT
<i>ND1</i>	12 075	[unnamed]	Aubert et al., 1999	F	ATCAAAAAGGAGCTCGATTAGTTTC
<i>ND1</i>	12 567	[unnamed]	Aubert et al., 1999	R	CGTAAAGTCTAGGTTATATTCAGATTCCG
<i>ND5</i>	6656	A1	Yagi et al., 1999	R	AATATDAGGTATAAATCATAT
<i>ND5</i>	7080	C2	Yagi et al., 1999	R	ATCYTTWGAATAAAAAYCCAGC
<i>ND5</i>	7095	A3	Yagi et al., 1999	F	TTCGAATTTAGCTTTATGTGG
<i>ND5</i>	7080	A4	New—this study	F	CAAGATATTCGHATATRGG
<i>ND5</i>	7490	V1	Yagi et al., 1999	F	CCTGTTTCTGCTTAAGTTCA
<i>EF1-a</i>	0	Starsky	Cho et al., 1995	F	CACATYAAATTGTCGTSATYGG
<i>EF1-a</i>	60	Ludwig	New—this study	F	ATYTACAAATGTGGTGGTATCG
<i>EF1-a</i>	237	Cho	Reed and Sperling, 1999	F	GTCACCATCATYGCACG
<i>EF1-a</i>	435	Wallace	New—this study	F	AACAAAATGGAYTCCACTGAGCC
<i>EF1-a</i>	479	Laverne	Cho et al., 1995	F	GAGGAAATYAARAAGGAAG
<i>EF1-a</i>	541	Luke	Cho et al., 1995	R	CAT RTT GTC KCC GTG CCA KCC
<i>EF1-a</i>	541	Luke5	New—this study	R	CATGTTTRTCTCCGTGCCAGCC
<i>EF1-a</i>	551	Petra	Caterino et al., 2001	R	TGGCTCCAGCATGTTGTCTCC
<i>EF1-a</i>	729	BJ	Cho et al., 1995	F	CARGACGTATACAAAATCGG
<i>EF1-a</i>	746	Verdi3	Nazari et al. 2007	R	GACACCAGTTTCAACTCTGCC
<i>EF1-a</i>	746	Verdi5	New—this study	R	GTACACCAGTTTTCYATCTGCC
<i>EF1-a</i>	880	Prachett	New—this study	R	TCYTGRAGRGTCTCGTGGTG
<i>EF1-a</i>	1134	Jordan	New—this study	R	ATTTACCRGTGACACGGTC
<i>EF1-a</i>	1241	Twecky	Cho et al., 1995	R	ACAGCVACKGTYTYCTCATR
<i>WG</i>	275	LepWG-1	Brower and De Salle, 1998	F	GARTGYAARTGYCAYGGYATGTCTGG
<i>WG</i>	679	modLepWG-2	Brower and De Salle, 1998	R	ACTICGCARCACCARTGGAATGTRCA

Positions for mtDNA are relative to *Drosophila yakuba* (Clary and Wolstenholme, 1985). *EF1-alpha* is relative to *Heliothodes diminutivus* (Cho et al., 1995), *wingless* is relative to *Junonia coenia* (Carroll et al., 1994).

All datasets were subjected to maximum-parsimony (MP), parsimony bootstrap (BS) and partitioned Bayesian likelihood analyses. MP and BS analyses were carried out in PAUP* 4.0b10 (Swofford, 2002). Heuristic MP analyses were carried out using TBR branch swapping and 1000 random replicates. BS analyses were carried out with 100 random replicates and 100 pseudo replicates for each analysis. Partitioned Bayesian likelihood analyses were carried out in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) with seven molecular partitions (*COI*, *COII*, *ND1*, *ND5*, *16S*, *EF-1 alpha* and *wingless*) analysed under the GTR + Γ + I model, with state frequencies and substitution rates estimated automatically by the program (default in MrBayes 3.1), and the morphological partition analysed under the JC model. Nucleotide frequencies (statefreq), substitution rates (revmat), shape of the gamma distribution of rate variation (shape), the proportion of invariable sites as well as the overall rate of evolution (ratepr) were allowed to vary between partitions. Four MCMC chains were run for one million generations with sampling every 100 generations for subanalyses, and two million generations with sampling every 100 generations for the analysis of the combined data. The first 25% of the sampled trees were discarded as burn in (as recommended in the program manual) and the lnL probability plot was checked for stationarity. An additional GTR + Γ + I Bayesian analysis of one million generations was run with the addition of sequences from the four parnassiine species—*Bhutanitis mansfieldi*, *Hypermnestra helios*, *Sericinus montela* and *Zerynthia rumina*. These species were included in the molecular clock analysis as explained below, and the Bayesian analysis was carried out to determine what effect this would have on the overall tree. The GTR + Γ + I model was used for the molecular partitions as all other models for molecular evolution are incorporated within it. The JC model was used for morphological data, being identical to the standard (parsimony) model in MrBayes 3.1, but allowing data to be in DNA format. This was necessary as the morphological data matrix had been converted from a numerical format to DNA format to be combined with the molecular data. Bremer support values (BR; Bremer, 1994) were calculated in TreeRot 2.0 (Sorenson, 1999) in conjunction with PAUP*. The combined molecular dataset with MrBayes partitions and the morphological dataset have been deposited at The Willi Hennig Society website (see Supporting Information). To evaluate character support for individual clades, morphological characters were traced on the tree from Bayesian analysis of the combined molecular and morphological dataset in MacClade.

Molecular clock analysis

Using the combined molecular dataset, a relaxed Bayesian clock analysis (Thorne and Kishino, 2002;

Sanderson et al., 2004; Drummond et al., 2006) was carried out in BEAST (Drummond and Rambaut, 2007) on an enforced topology of Papilioninae identical to the tree found by Bayesian analysis of the combined data. Four MCMC chains were run for ten million generations with sampling every 100 generations, and the first 25% discarded as burn in. The xml file for the BEAST analysis was created in BEAUti (part of the BEAST package) with the following non-default settings and priors: substitution model was set to GTR, site heterogeneity model was gamma + invariant sites, speciation tree prior was set to Yule Process, GTR base substitution parameters were all set to gamma distribution with default parameters. SiteModel.alpha and SiteModel.pInv were both set to gamma distributions with default parameters. The Yule Process was chosen based on Drummond and Rambaut (2007), and the remaining parameters were based on the model used in the original Bayesian analysis (GTR + Γ + I).

The root of the tree was set to 100 Ma \pm 75 Myr (normal distribution with a standard deviation of 45). This age and confidence limits were chosen based on Vane-Wright (2004), Wahlberg (2006) and de Jong (2007) to give a relaxed time span that, with reasonable certainty, would subtend most prior hypotheses on the possible age of origin of butterflies.

The minimum age of Parnassiinae + Papilioninae was set to 50 Myr (log normal distribution with zero-offset = 50, mean = 2.0, SD = 1.0) based on the 48-Myr-old fossil genus *Praepapilio* (Durden and Rose, 1978). Although this fossil clearly belongs to Papilionidae, it also lacks several of the apomorphies that unite Parnassiinae and Papilioninae (Durden and Rose, 1978; Kristensen and Skalski, 1999; Grimaldi and Engel, 2005; de Jong, 2007). It seems unlikely that the fossil represents a stem group for Parnassiinae + Papilioninae, and we do not know if the *Praepapilio* lineage and Parnassiinae + Papilioninae lineage coexisted for a significant period of time. Therefore, the age of *Praepapilio* (rounded to 50 Myr) can only provide a conservative estimate for the minimum age of the Parnassiinae + Papilioninae lineage.

The minimum age of the Parnassiini clade within Parnassiinae (Nazari et al., 2007) was set to 30 Myr (log normal distribution with zero-offset = 30, mean = 2.0, SD = 1.0) based on the fossil genus *Thaites* (Scudder, 1875). This genus is plausibly closely related to Parnassiini (Hancock, 1983). Although no precise ages have been given for the fossil, it has consistently been ascribed to the early Oligocene (Scudder, 1875; Grimaldi and Engel, 2005; de Jong, 2007), and hence the lower border of the Oligocene (30 Ma) was set as the minimum age for this clade. The reservations with respect to the minimum age of Parnassiinae + Papilioninae apply to this clade as well.

To obtain the best overall divergence estimate for Papilionidae, sequences from the four remaining parnassiine genera not included in the combined and morphological analyses (Nazari et al., 2007; : *Bhutanitis*, *Hypermnestrea*, *Sericinus* and *Zerynthia*; listed in Table 1, bottom) were included in the molecular clock analysis. By including these genera we were able to set the minimum age for Parnassiini as defined by Nazari et al. (2007), and thereby did not have to constrain the age of the genus *Parnassius* to 30 Myr.

Biogeographical analysis

A dispersal-vicariance analysis was carried out in DIVA 1.2 (Ronquist, 1997) based on the tree topology inferred by the Bayesian analysis of the combined dataset. Based on Enghoff (1995), Sanmartin et al. (2001), Sanmartín and Ronquist (2004) and Kodandaramaiah and Wahlberg (2009), ten areas were defined: Western Palearctic, Central Palearctic (extending from the Ural and Caucasus mountains to the Tibetan Plateau), Eastern Palearctic (extending from the Tibetan Plateau to the Pacific Ocean), Nearctic, African Region, Madagascar, Greater India (including Bangladesh and Pakistan), South East Asia, Australian Region and Neotropical Region. Analyses with constraints of a maximum of 2–10 ancestral areas for each node were run to find the optimization with the least number of dispersals. The DIVA dataset has been deposited at The Willi Hennig Society website.

Larval host-plant relationships

The evolution of larval host-plant relationships was analysed in MacClade by scoring host plant families for each genus in a nexus file and then analysing their distribution on the Bayesian tree under the ACCTRAN optimization. The result was compared with the most current angiosperm phylogeny available (APG (The Angiosperm Phylogeny Group), 2003). Larval host plant data are taken from Ehrlich and Raven (1964), Igarashi (1984), Scriber (1984), Igarashi and Fukuda (2000) and Smith and Vane-Wright (2001).

Results

Molecular character sampling

In all, up to 5616 bp were sequenced for one specimen of each species: *16S* (534 bp), *COI* (1538 bp), *COII* (638 bp), *ND1* (463 bp), *ND5* (802 bp) and the nuclear genes *EF-1 alpha* (1258 bp) and *wingless* (404 bp) (Table 1). In some cases, a gene could not be amplified for a particular species, and sequences (generally from GenBank) from closely related species had to be used as

a replacement (*Coenonympha dorus* for *C. tullia*, *Pieris napi* for *P. rapae*, *Euryades duponchelii* for *E. corethrus*, *Parides neophilus* for *P. photinus*, and *Ornithoptera euphorion* for *O. priamus*). In two cases, *Baronia brevicornis* (ND1) and *Teinopalpus imperialis* (*wingless*), where all amplification attempts were unsuccessful and no other specimens or sequences were available, the data had to be coded as missing. In a few cases, existing sequences of *EF-1 alpha* were augmented with an additional sequence of about 250 bp at the 3'-end. In all, approximately one-third of the sequences are new (published GenBank sequences were from Weller and Pashley, 1995; Weller et al., 1996; Aubert et al., 1999; Caterino and Sperling, 1999; Reed and Sperling, 1999; Vane-Wright et al., 1999; Morinaka et al., 1999; Yagi et al., 1999; Caterino et al., 2001; Nylin et al., 2001; Kondo et al., 2003; Wahlberg and Nylin, 2003; Omoto et al., 2004; Zakharov et al., 2004a; b; Braby et al., 2005; Katoh et al., 2005a,b; Silva-Brandão et al., 2005; Wahlberg et al., 2005; Chew and Watt, 2006; Nazari et al., 2007; Narita et al., 2006; Otaki et al., 2006; Putnam et al., 2007; Michel et al., 2008). A complete list of sequences including GenBank accession numbers is given in Table 1. Most new sequences were generated from DNA extracts already in the UASM. Fresh DNA extractions were required for the following 12 species: *Vanessa cardui* (Canada), *Graphium agamemnon* (Papua New Guinea), *Lamproptera meges* (China), *Protesilaus glaucolaus* (Brazil), *Meandrusa payeni* (Laos), *Meandrusa sciron* (China), *Teinopalpus imperialis* (Vietnam), *Papilio thoas* (Brazil), *Ornithoptera priamus* (Seram), *Euryades corethrus* (Argentina), *E. duponchelii* (Argentina) and *Losaria neptunus* (E. Simeulue Is.). Voucher images and label information are available online at the UASM website (<http://www.biology.ualberta.ca/facilities/strickland/Vouchers/>).

Morphological character sampling

In all, 94 morphological characters were scored. Some characters from Miller (1987a) were omitted as they were repeated in different subanalyses, or because they were uninformative for the taxa sampled in the current analyses—i.e. they were autapomorphies for terminal taxa, or were characters used by Miller to examine relationships between subgenera for which only one representative was included in the present taxon sample. Characters excluded for these reasons are (numbers from Miller's character lists): 28, 30, 53, 55, 57–59, 61, 62, 65–68, 73–76, 78, 80–98, 107, 112, 126–130, 132–142, 148, 152 and 158–170. Characters from Miller's dataset which were excluded from the analysis for other reasons are discussed in Appendix 1. Our character state scorings differ to some extent from Miller (1987a). In some cases this is due to the nature of the analyses (e.g. one combined analysis here versus three separate ones by

Miller). However, some deserve attention and are discussed in Appendix 1. One character from de Jong et al. (1996) and five new characters not previously used in swallowtail phylogenetics are listed and described in Appendix 1. Illustrations of important characters are given in Figs 2 and 3. A complete list of characters is given in Appendix 2.

Phylogenetic analyses

Results from parsimony analyses of data combinations and the Bayesian analysis of the combined molecular dataset are shown in Fig. 4. All analyses recovered Papilionidae, and the tribes Leptocircini, Papilionini, and Troidini as monophyletic. Only the analysis of morphology alone did not recover a sister-group relationship between *Teinopalpus* and *Meandrusa*. The major differences between the analyses were in the recovered inter-tribal relationships, the monophyly of Parnassiinae and the position of *Baronia*.

Bayesian and MP bootstrap analyses of the mitochondrial (mtDNA) and nuclear (ncDNA) datasets respectively (trees not shown) were made to calculate support values for the clades found in the combined molecular and morphological dataset Bayesian tree (Fig. 5). The Bayesian posterior probabilities, MP bootstrap and Bremer support values for each major clade in Fig. 5 based on the combined dataset and the various sub datasets are given in Table 3.

The Bayesian analysis of the combined molecular and morphological dataset (Fig. 5) was chosen as this type of analysis allows evolutionary changes to be modelled more accurately, especially for the molecular part of the dataset, than via parsimony. Furthermore, parsimony is susceptible to long branch attraction (e.g. Felsenstein, 2004), and at least *Baronia* (e.g. Wahlberg et al., 2005; Nazari et al., 2007) is known to display long branch characteristics. The recovered relationships (including morphological support for individual clades) are discussed in detail in Appendix 3.

Relaxed Bayesian clock

Divergence time estimates for the last common ancestor of the numbered clades are given in Fig. 5. The mean rate of evolution across the tree was estimated to be 0.01909 substitutions per site/per million years, a value very similar to the long-used 0.02 (e.g. Brower, 1994).

Zoogeographical analysis

A ‘constraint’ of a maximum ten ancestral areas for each node in DIVA yielded the result with fewest dispersal events (39). Nevertheless, the result was highly homoplasious with almost every node having either

multiple possible combinations of possible ancestral areas, or the maximum number of allowed ancestral areas, which equals the total number of areas defined in the analysis (results not shown).

Larval host-plant evolution

A comparison between our swallowtail phylogeny and the phylogeny of major groups of larval host plants at the ordinal level (APG (The Angiosperm Phylogeny Group), 2003) is shown in Fig. 6. Although there is a general tendency towards feeding on basal angiosperm orders, there is very little correlation between the phylogeny of Papilionidae and the phylogeny of angiosperms. Assuming that Aristolochiaceae feeding evolved independently in non-Parnassiini parnassiines and Troidini is considerably more parsimonious than assuming that Aristolochiaceae feeding is basal to Parnassiinae + Papilioninae, and was subsequently lost in Parnassiini, Leptocircini, Teinopalpini and Papilionini (two versus five steps).

Discussion

Morphological character sampling and scorings

Our morphological dataset differs from Miller (1987a) in several important aspects: 82 of Miller’s characters (48% of that dataset) were excluded, scorings of seven characters differ in important ways, one character was added from de Jong et al. (1996) and five new characters were included.

Characters dealing with modified wing vestiture in males were found to be particularly difficult to use. Two characters (18 and 49) in Miller’s (1987a) dataset were related to the anal brush along the ventro-anal margin of the male hind wing. Another (118) was concerned with the presence of long, bristle-like scales along the anal vein on the dorsal surface of the male hind wing. Although definitely of phylogenetic importance, we find the anal brush difficult to employ as one or several meaningful characters. Miller (1987a) considered the structures to be present in the ground plan of Papilioninae despite acknowledging that several *Papilio* species lack them (including the species examined here).

Furthermore, the anal brushes show considerable variation throughout the groups treated here, including within the genus *Battus*. In some Troidini they are present as well-defined rows of specialized, thick and elongate scales, restricted to a narrow band along vein 1A + 2A. In others groups, such as many Leptocircini and *Teinopalpus*, they are present as a broad bed of apparently unspecialized, bristle-like scales in the anal region of the hind wing and are not restricted to the vein

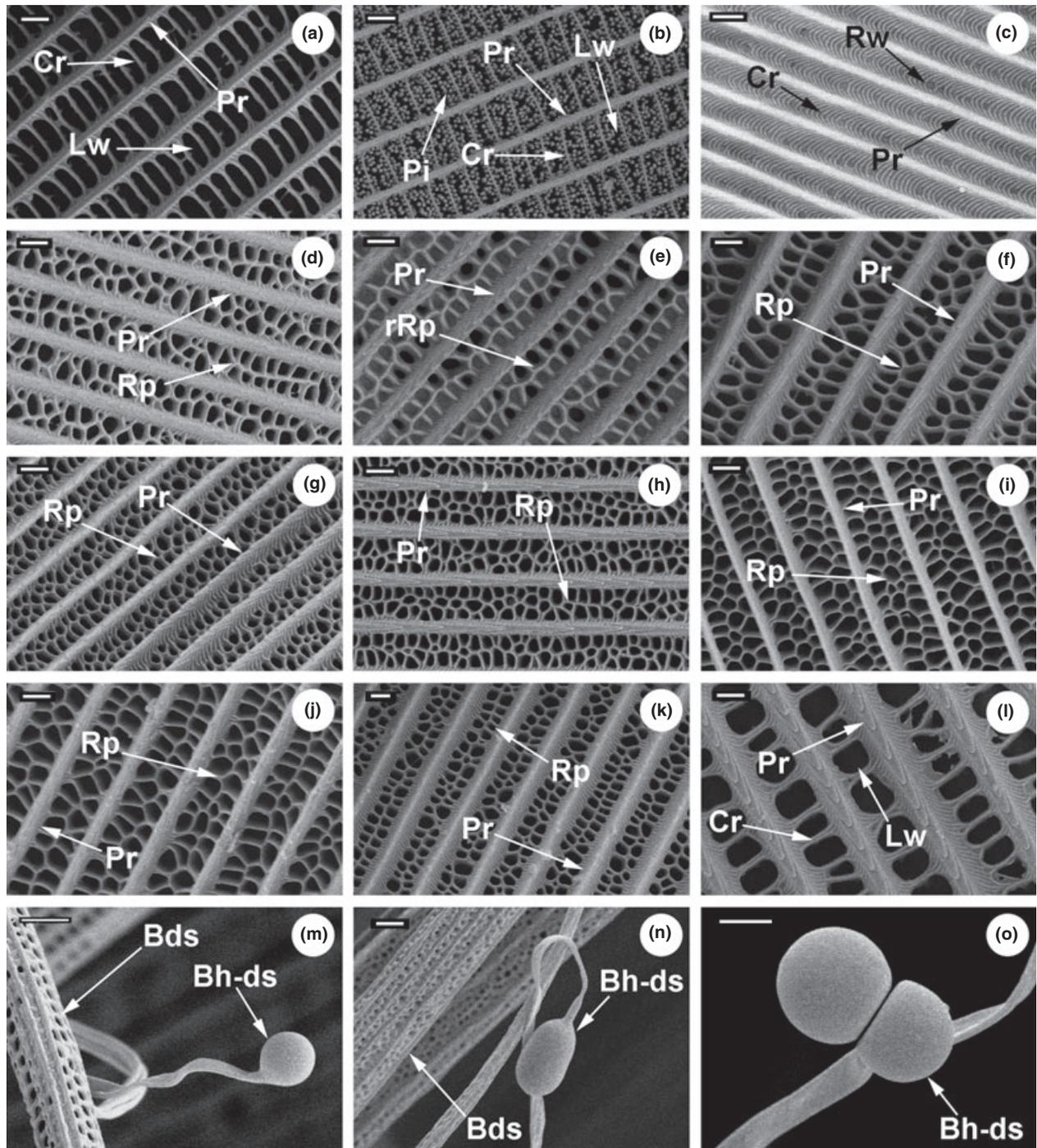


Fig. 2. Illustration of characters associated with wing scales. (a–l) Ultrastructure of dorsal wing scales, abwing surface. (a) *Coenonympha tullia*, “typical butterfly scale” with cross ribs (Cr), primary ridges (Pr) and large windows (Lw). (b) *Pieris rapae*; note the numerous pigment grains (Pi) between the cross ribs. (c) *Baronia brevicornis*; note the strongly reduced windows (Rw). (d) *Allancastris cerisy*; note the well-developed reticulate pattern (Rp) between the primary ridges. (e) *Luehdorfia japonica*; note the reduced reticulate pattern (rRp). (f) *Iphiclides podalirius*. (g) *Eurytides serville*. (h) *Teinopalpus imperialis*. (i) *Meandrusa payeni*. (j) *Papilio machaon*. (k) *Troides helena*. (l) *Euryades corethrus*; note the absence of reticulate patterning. (m–o) Details of deciduous sex scales in males. (m) *Graphium agamemnon*; note the thick body of deciduous scale (Bds) and the distinctive “ball-head” of the deciduous scale (Bh-ds). (n) *Protesilaus protesilaus*; note the slightly more elongate ball-head. (o) *Eurytides serville*. Scale bars = 1 μ m.

itself. In *Teinopalpus*, and to a lesser extent in other taxa, a less dense but otherwise similar scale arrangement is also found in the female. Bristle-like scales are also

found ventrally in the anal region of the hind wing in several outgroup taxa (e.g. *Parnassius*, *Luehdorfia* and *Vanessa*).

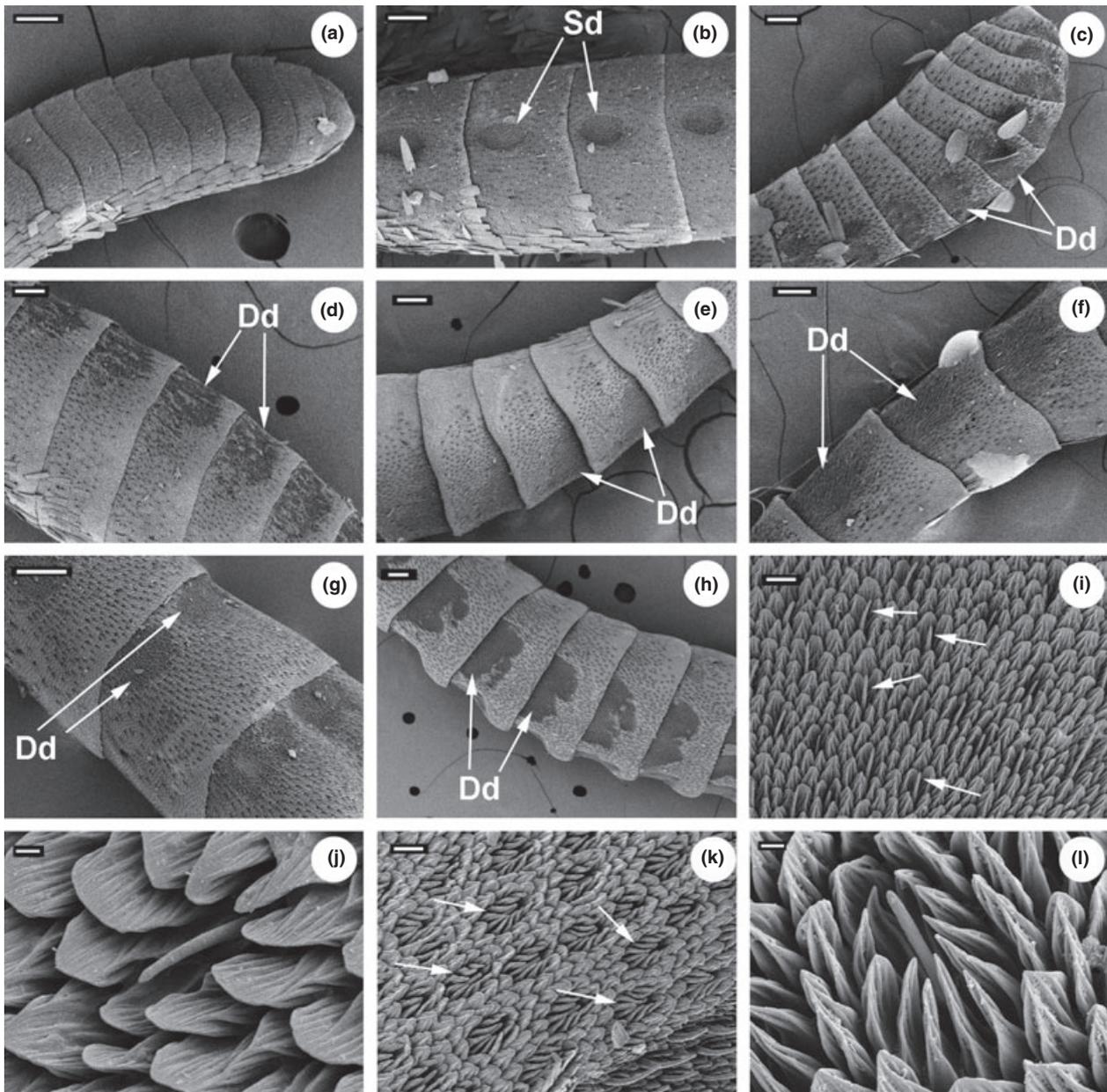


Fig. 3. Illustration of characters associated with antennae. (a–h) Antennal club. (a) *Pyrgus communis*; ventral view showing no pits or depressions with concentrated sensilla. (b) *Colias eurytheme*; ventral view showing a single, ventral depression per segment. (c) *Baronía brevicornis*; lateral view showing a row of latero-ventral depressions (Dd: double depressions as they are present on both sides of the antenna). (d) *Parnassius phoebus*; lateral view. (e) *Protographium marcellus*; lateral view. (f) *Teinopalpus imperialis*; lateral view. (g) *Papilio alexanor*; ventral view clearly displaying the double row. (h) *Parides photinus*; lateral view. (i–l) Detailed view of sensilla outside the depressions. (i) *Papilio thoas*; note the numerous sensilla basiconica (arrows). (j) *Papilio glaucus*; close up of sensilla basiconica. (k) *Ornithoptera priamus*; note the numerous sensilla coeloconica (arrows). (l) *Troides helena*; close up of sensilla coeloconica. Scale bars: a–h = 100 μm ; i, k = 10 μm ; j, l = 2 μm .

Dorsal bristle-like scales in the anal region of the hind wing appear to be widespread, even if scattered, in various groups of butterflies. A detailed study of the wing vestiture in the anal region of male hind wings throughout Papilionidae is thus much warranted, but outside the scope of the current study. The only characters regarding wing scales included in this study

are thus Miller's (1987a) characters 24, 40, 57, 105 and 143, and the character New3.

Miller's (1987a) character 110 (Red colour [in red scales] composed of Pigment A) is also problematic. It was considered an autapomorphy for Troidina because *Battus* and *Papilio* [the sole outgroup in Miller's (1987a) analysis of Troidini] both have red Pigment B (Ford,

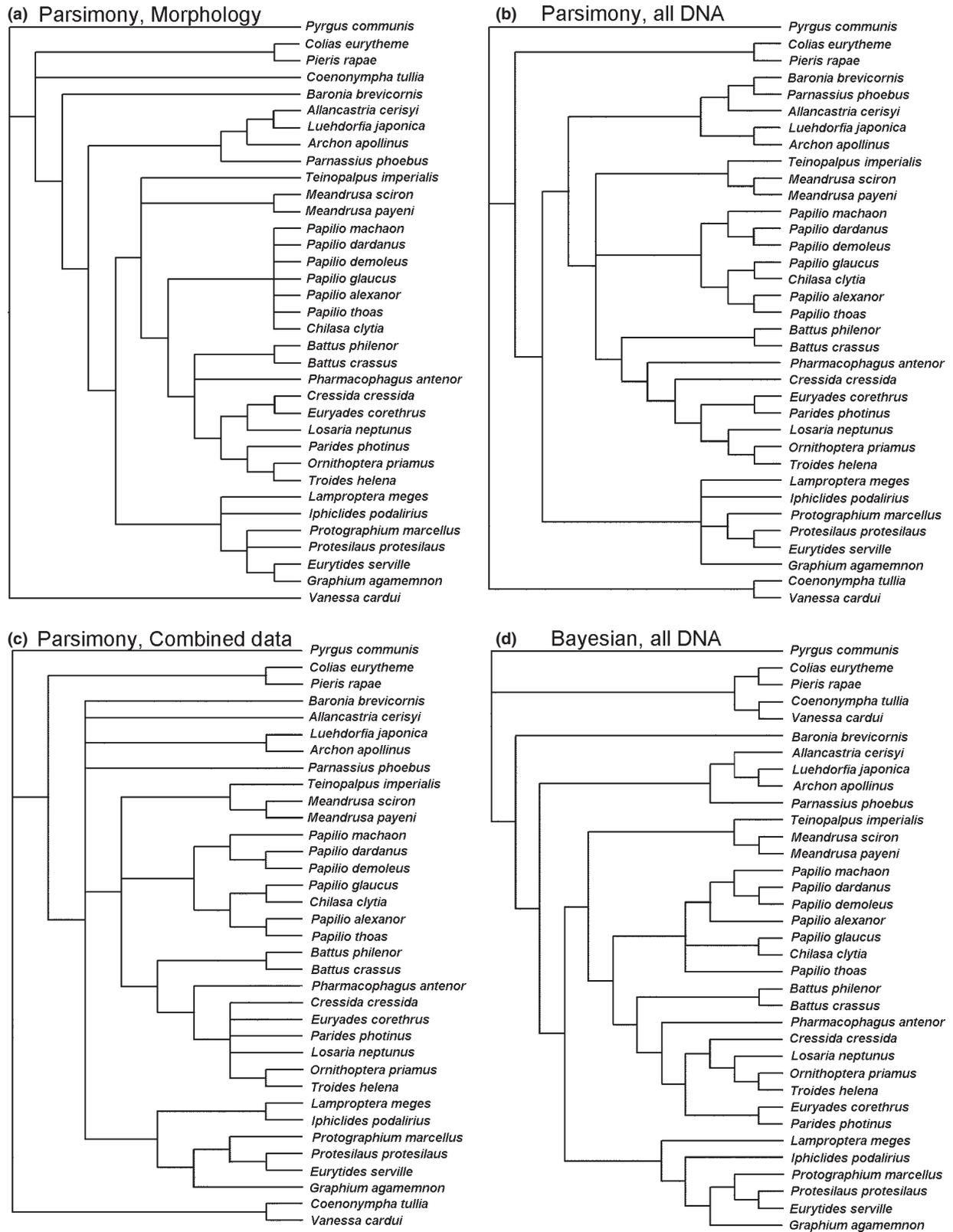


Fig. 4. Phylogenetic analyses of combinations of data. (a) Morphological dataset; strict consensus of 432 most-parsimonious trees, 189 steps long. (b) Combined molecular dataset; strict consensus of three most-parsimonious trees, 11 721 steps long. (c) Combined molecular and morphological dataset; strict consensus of six most-parsimonious trees, 11 943 steps long. (d) Combined molecular dataset; Bayesian inference. InL = -55 170.85.

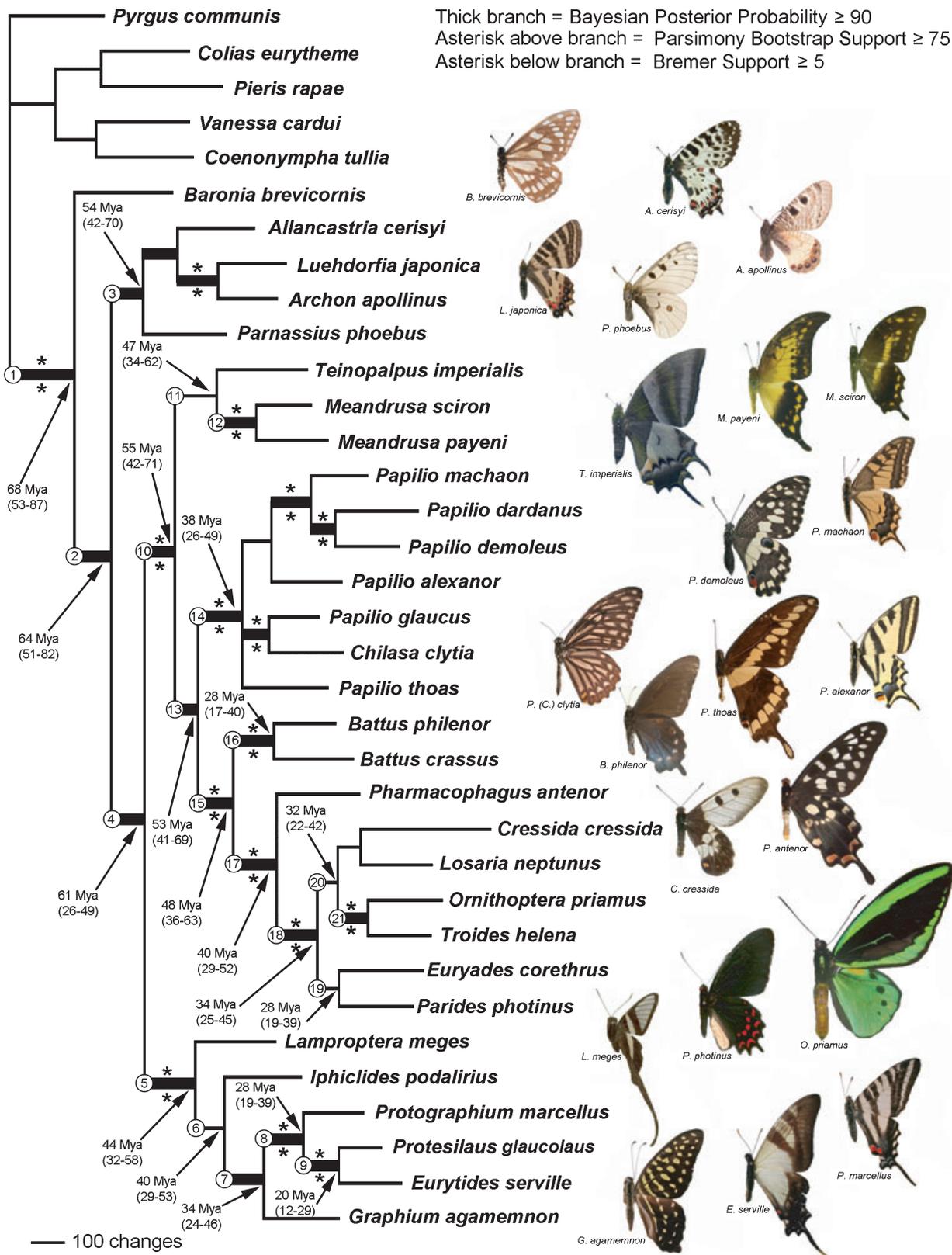


Fig. 5. Main tree. Combined molecular and morphology Bayesian phylogeny, with time estimates. Age estimates with 95% confidence intervals (in parentheses) from the relaxed Bayesian clock analysis are given for clades marked with an arrow. Clade numbers correspond to numbers in Table 3. InL = -56 453.51.

Table 3
Summary of support values for clades in the analyses of each major data partition

Dataset Clade	All data			All DNA			Morphology			mtDNA			ncDNA		
	BR	BS	BPP	BR	BS	BPP	BR	BS	BPP	BR	BS	BPP	BR	BS	BPP
1. Papilionidae	40	100	100	40	100	100	4	87	96	19	100	100	19	100	100
2. Parnassiinae + Papilioninae	0	< 50	100	0	< 50	98	3	94	99	0	< 50	93	0	< 50	< 50
3. Parnassiinae	0	< 50	100	0	< 50	100	2	82	57	0	< 50	92	3	75	< 50
4. Papilioninae	0	< 50	100	0	< 50	99	2	76	84	0	< 50	100	0	< 50	< 50
5. Leptocircini	30	100	100	22	100	100	5	97	100	18	98	100	13	100	100
6. <i>Iphiclides</i> Group	0	< 50	54	0	< 50	68	0	< 50	< 50	0	< 50	< 50	0	< 50	< 50
7. <i>Graphium</i> Group	1	< 50	100	0	< 50	100	2	69	98	0	< 50	58	2	72	100
8. Neotropical Leptocircini	7	85	100	8	77	100	0	< 50	< 50	2	60	79	6	84	100
9. <i>Eurytides</i> + <i>Protesilaus</i>	11	90	100	12	93	100	0	< 50	< 50	8	93	100	2	69	96
10. non-leptocircine Papilioninae	6	75	100	12	62	100	3	76	98	0	< 50	100	0	< 50	< 50
11. <i>Teinopalpini</i>	4	56	66	2	< 50	81	0	< 50	< 50	0	< 50	95	0	< 50	< 50
12. <i>Meandrusa</i>	40	100	100	34	100	100	7*	100	100	22	98	100	33	100	100
13. Papilionini + Troidini	0	< 50	93	0	< 50	73	1	67	91	0	< 50	96	0	< 50	63
14. Papilionini	56	100	100	55	100	100	1	69	64	15	99	100	12	99	100
15. Troidini	22	99	100	18	100	100	4	97	100	10	83	100	0	< 50	< 50
16. <i>Battus</i>	70	100	100	66	100	100	3	96	100	40	100	100	27	100	100
17. Troidina	37	100	100	35	100	100	0	< 50	< 50	28	100	100	0	< 50	< 50
18. non- <i>Pharmacophagus</i> Troidina	7	90	100	3	92	100	2	89	86	7	64	76	0	< 50	< 50
19. Neotropical Troidina	0	52	60	3	62	91	0	< 50	< 50	7	69	74	0	< 50	< 50
20. Australasian Troidina	0	< 50	58	0	< 50	91	0	100	100	0	< 50	< 50	0	< 50	< 50
21. <i>Troides</i> + <i>Ornithoptera</i>	49	100	100	38	100	100	9	100	100	30	100	100	6	90	100

BR, Bremer support; BS, parsimony bootstrap; BPP, Bayesian posterior probability. Clade numbers correspond to those in Fig. 5. Support values from data partitions are based on separate analyses of each partition.

*Female characters for both *Meandrusa* species were based on Miller's (1987a) drawings of *M. payeni*.

1944a,b). However, according to Ford (1944a,b) red Pigment A is widespread in Lepidoptera and occurs in all Papilionidae with red scales (on body or wings) except *Battus*, most *Papilio*, and *Iphiclides*, which all have red Pigment B. As red Pigment B occurs only in these three genera, it seems certain that the presence of Pigment A in Troidina and other Papilionidae is either a symplesiomorphy or highly homoplasious.

Phylogenetic relationships of *Teinopalpus* and *Meandrusa*

The two Southeast Asian genera *Teinopalpus* and *Meandrusa* have been notoriously difficult to place in a phylogenetic context. *Meandrusa* has been considered to be closely related to *Papilio* (e.g. Munroe and Ehrlich, 1960; Munroe, 1961; Igarashi, 1984; Miller, 1987a; Tyler et al., 1994; Aubert et al., 1999) or placed in the Leptocircini (Hancock, 1983). *Teinopalpus* has either been placed in or close to the Leptocircini (e.g. Hancock, 1983; Igarashi, 1984) or given tribal status (e.g. Munroe and Ehrlich, 1960; Munroe, 1961; Miller, 1987a; Tyler et al., 1994). In the three cladistic analyses of Papilioninae published to date (Fig. 1a–c), Hancock (1983) placed these two genera as sister taxa in the leptocircine subtribe *Teinopalpina* (as *Teinopalpiti*), Miller (1987a) placed *Teinopalpus* as the sister group

of Papilionini + Troidini, and Tyler et al. (1994) placed *Teinopalpus* as the sister group of Papilionini + Leptocircini. The latter two studies placed *Meandrusa* in the Papilionini, as the sister group of *Papilio sensu lato* (*s.l.*).

Our results endorse Hancock (1983) in placing *Teinopalpus* and *Meandrusa* as sister groups. Support for this arrangement mainly comes from the combined molecular dataset. Parsimony analysis of the morphological dataset does not recover the two genera as sister taxa, but when data are combined, three morphological characters are identified as tentative synapomorphies. Although independently developed in Parnassiinae and the pierid outgroup taxa, Miller's (1987a) character 13 (middle discocellular vein of forewing incurved) constitutes a unique synapomorphy for the two genera within Papilioninae. Miller's (1987a) character 26 (forewing discal cell less than half the wing length) was considered by Miller (1987a) to be independently developed in the two genera. Here it is identified as a putative synapomorphy that has been independently developed in the leptocircine genus *Lamproptera*. In the latter, however, the cell is only slightly shorter than half the length of the wing, whereas in *Teinopalpus* and *Meandrusa* it is considerably less than half. New4 (pupa with a large dorsal horn on basal margin of thorax) is problematic as its appearance differs considerably between the two

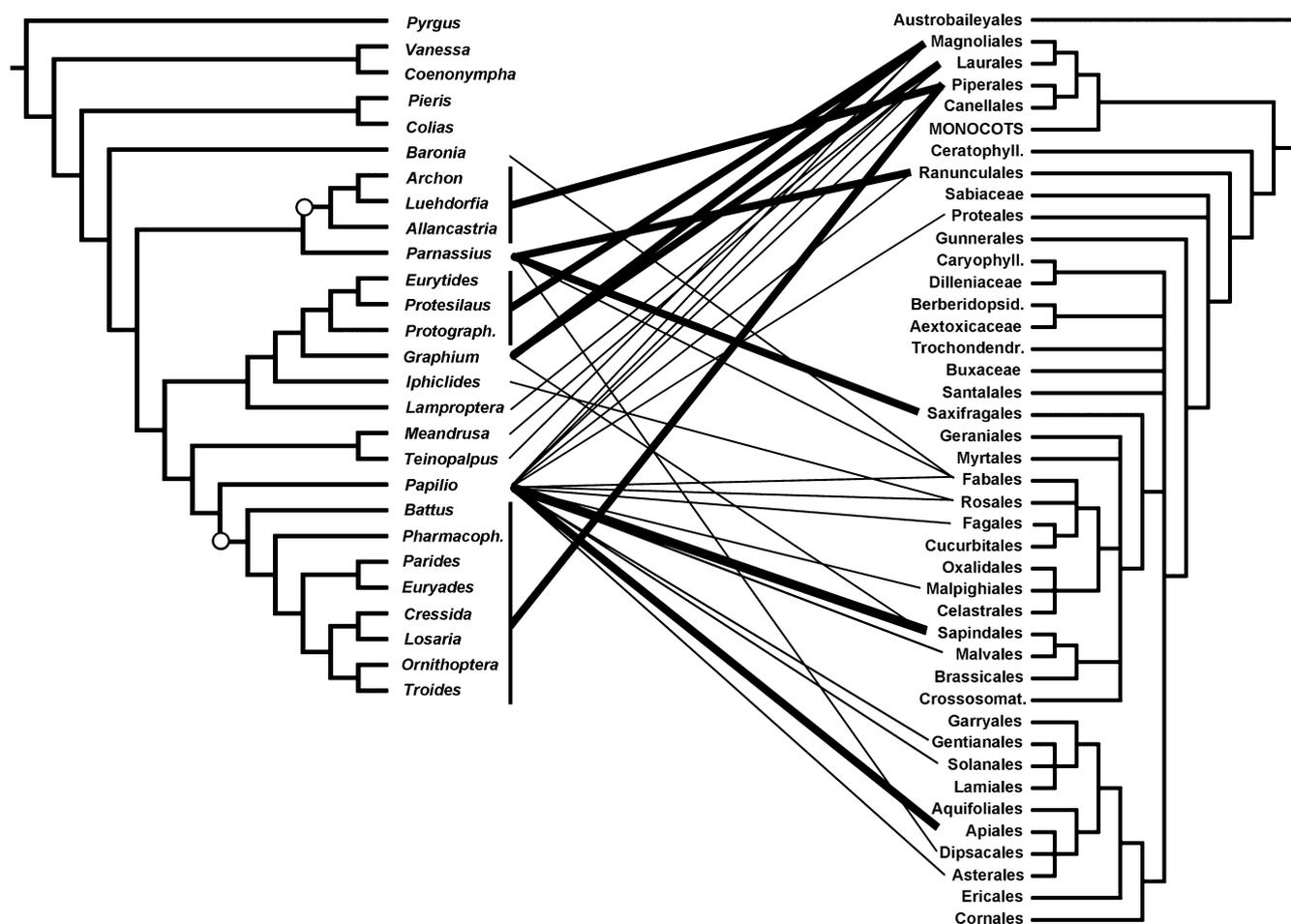


Fig. 6. Comparison between the proposed phylogeny of Papilionidae (Bayesian analysis of combined data) and phylogenetic relationships of host plant order based on APG (The Angiosperm Phylogeny Group) (2003). A thick line indicates a major host plant order for a diverse group. Open circles indicate proposed origin of Aristolochiaceae-feeding larvae.

(Igarashi and Fukuda, 2000; pls 67, 70)—but the position of the horn is similar in both, and different from in Leptocircini (e.g. Igarashi, 1984). However, some presumably subordinate species of *Papilio* have a pupal horn in a similar position (A.M.C., pers. obs.), and the appearance of the horn in *Teinopalpus* is more similar to the condition found in Leptocircini than *Meandrusa*. Nevertheless, in light of the molecular results, the horn could be considered a synapomorphy for the two genera.

Miller (1987a) grouped *Meandrusa* with *Papilio* and Troidini based on four putative synapomorphies not shared with *Teinopalpus* (Miller's characters 30–33). Although some of these characters are striking (i.e. char. 31: tegumen and 8th tergite fused), in the present study they are considered to have been reversed in *Teinopalpus*. These reversals are perhaps not as remarkable as they may seem: both chars 30 and 31 were found by Miller (1987a) to be reversed within the Troidini. Miller (1987a) identified three putative synapomorphies for *Meandrusa* and *Papilio* (Miller's

char29, 34 and 35). Characters 29 and 34 have been omitted for reasons described in Appendix 1, and char. 35 (basalfleck of labial palpus forming a large medial flap) was found to be present in *Teinopalpus* and otherwise homoplasious (i.e. absent in several *Papilio* examined).

Comparisons to earlier cladistic studies

Overall phylogenetic pattern. Apart from the position of *Teinopalpus* and *Meandrusa*, the overall phylogenetic pattern is similar to that found by Hancock (1983), Miller (1987a) and Caterino et al. (2001). The monobasic Baroniinae are the sister of a clade comprising the reciprocally monophyletic Parnassiinae and Papilioninae. Within Papilioninae, Leptocircini are the sister group of the remaining genera, and Papilionini and Troidini are sister groups. The major overall difference between the present results and those of Tyler et al. (1994) is in the position of Troidini.

Unlike Nazari et al. (2007) and Michel et al. (2008), our preferred analysis showed no evidence for a sister-group relationship between Baroniinae and Parnassiinae. Nazari et al. (2007) excluded several morphological characters from their dataset, including two that here support the monophyly of Parnassiinae + Papilioninae (cervical membrane with a lateral sclerite, and spina-sternum produced laterally at spina)—this may account for the position of Baroniinae that Nazari et al. found. Michel et al. (2008) did not include morphological characters, and used a smaller molecular dataset.

Phylogeny of Leptocircini. A major difference between our results and those of Hancock (1983), Miller (1987a) and Tyler et al. (1994) is the internal phylogeny of Leptocircini. In these three earlier studies *Graphium* consistently grouped with *Lamproptera*, and the sister group of these two was *Iphioides* (in the second and third studies). Our results indicate that *Graphium* instead forms the sister group of the Neotropical Leptocircini. The strongest morphological support for this relationship comes from the character New3 (deciduous scales of male hind wing very long and slender with a round ball-like end). Although the molecular analyses by Caterino et al. (2001) had more limited taxon sampling, they did find a sister-group relationship between *Graphium* and *Eurytides* relative to *Iphioides*.

Phylogeny of Troidini. The phylogenetic relationships of the Troidini (pipevine butterflies) have probably been subjected to more studies than most other groups of invertebrates except perhaps *Drosophila*—yet no consensus seems to have been reached (Vane-Wright, 2003; de Jong, 2007; Fig. 1d–f). Most studies (Hancock, 1983, 2007; Miller, 1987a; Tyler et al., 1994; Parsons, 1996b; Caterino et al., 2001; Braby et al., 2005; Michel et al., 2008) agree that the tribe is monophyletic. Only Morinaka et al. (1999) have challenged this by grouping *Battus* with *Papilio*, *Graphium* and Parnassiinae. Similarly, most studies have recovered the monophyly of the subtribe Troidina, comprising the non-*Battus* genera; only Parsons (1996b) placed *Battus* deep within the remaining genera. Our results strongly support the monophyly of both Troidini and Troidina.

Many authors have placed the monotypic *Pharmacophagus* as the sister group of the remaining Troidina. But Hancock (1983, 1988, 2007) considered *Pharmacophagus* to be a member of the *Atrophaneura* generic group (which includes *Cressida* and *Losaria*). Tyler et al. (1994) placed a clade comprising *Cressida* and *Euryades* as the sister of the remaining Troidina. Parsons (1996b) placed *Pharmacophagus* as sister of *Ornithoptera* and Michel et al. (2008) had *Cressida* as the sister of the remaining Troidina. Hancock's reasons for considering *Pharmacophagus* a member of the *Atrophaneura* group (summarized by Hancock, 2007) are mainly based on

overall similarities in the genitalia. Miller's (1987a) more convincing apomorphy for the remaining Troidina (char. 113: labial palpus 2-segmented) was considered to be reversed in *Pharmacophagus* by Hancock (2007). Although *Atrophaneura s.s.* was not included in the present study, the results here strongly support *Pharmacophagus* as the sister group of the remaining Troidina. The characters used by Hancock to place it in the *Atrophaneura* group are better explained as either sympleisomorphies or homoplasies.

Cressida and *Euryades* have often been considered sister taxa, although Parsons (1996b) and Hancock (2007) have argued that they are not closely related. Braby et al. (2005) suggested that the Neotropical *Euryades* is close to another Neotropical genus, *Parides*. Our results support this hypothesis and furthermore place the *Euryades* + *Parides* clade as sister group of a clade comprising the Australasian Troidina, a relationship not previously suggested. However, these relationships are only weakly supported here and more comprehensive sampling of taxa and characters will be needed to fully resolve these parts of the phylogeny.

Finally, most authors have considered the genera *Troides* and *Ornithoptera* to be closely related, often even congeneric. Indeed, Miller (1987a) did not even recognize *Ornithoptera* as a subgenus of *Troides*. Only Parsons (1996b) has placed the two groups far from each other. The results here strongly support the conventional view that *Troides* and *Ornithoptera* are closely related. But because the third putative genus of the group, *Trogonoptera*, was not included in the analyses, we cannot address the rank at which the *Troides* group genera should be recognized.

Divergence times and possible biogeographical patterns

Age of swallowtails. The age of the most recent common ancestor of butterflies has been subjected to a long and sometimes heated debate. Suggested ages for the divergence of the main lineages have ranged from the Jurassic (e.g. Shields, 1979; Miller and Miller, 1997) to the late Cretaceous/early Tertiary (e.g. de Jong, 2003; Vane-Wright, 2004) and virtually everything in between. As butterfly fossils are notoriously rare, age estimates have traditionally been based on presumed biogeographical patterns (i.e. particular vicariance events have led to the formation of major lineages). Recent years have, however, seen a rise in molecular clock estimates for various groups of butterflies (e.g. de Jong, 2003; Zakharov et al., 2004a; Braby et al., 2005; Wahlberg, 2006; Braby and Pierce, 2007; Kodandaramaiah and Wahlberg, 2007, 2009; Nazari et al., 2007; Michel et al., 2008; Wahlberg et al., 2009).

Our analysis (Fig. 5), which has been calibrated primarily on the basis of fossils rather than vicariance

events, indicates that Papilionidae started diverging around 68 Ma (53–87 Ma), just before the K/T boundary. The split between Parnassiinae and Papilioninae is estimated to have occurred 64 Ma (51–82 Ma). Divergence within the subfamilies Parnassiinae and Papilioninae began 54 Ma (42–70 Ma) and 61 Ma (48–78 Ma), respectively. The suggested divergence times for the major clades in Papilionidae thus correspond roughly to the divergence times for the major clades in the butterfly subfamily Nymphalinae, and are considerably younger than those for the family Nymphalidae suggested by Wahlberg (2006) and Wahlberg et al. (2009). At first it might seem counterintuitive that the diversification of the entire family Papilionidae occurred at roughly the same time as the diversification of the supposedly much younger clade Nymphalinae (Wahlberg et al., 2005). It is, however, important to keep in mind that the divergence time of living swallowtails does not necessarily correspond to the age of the swallowtail lineage. The poor fossil record does not allow us to say anything with confidence about the importance of extinction events in butterfly evolution. It is possible that most of the early diversification of the swallowtails has been lost due to extinction, e.g. at the K/T boundary. Other studies of groups with better fossil records have shown that the extinction event subsequently had little effect on terrestrial arthropods at the family level (e.g. Grimaldi and Engel, 2005, pp. 365–367; and references therein). However, Labandiera et al. (2002) also showed that phytophagous insects underwent a considerable decline in what is today North America at or just after the K/T boundary (but see Wappler et al., 2009; for different results from other regions), and Currano et al. (2008) demonstrated that the ecosystems did not recover fully for several million years. In addition, Wahlberg et al. (2009) recently demonstrated that Nymphalidae probably underwent a near-global extinction at the K/T boundary. If swallowtails went through an even more dramatic extinction bottleneck at the same time (with few lines or even only a single lineage surviving), this could be an explanation for the family's much lower number of species compared with Nymphalidae (e.g. Ackery et al., 1999). Moreover, it is not inconceivable that all of the major lineages of butterflies currently recognized diverged within a geologically short period of time, perhaps only a few million years.

The divergence times suggested here for various groups of Papilionidae differ from other molecular clock estimates for the family. Zakharov et al. (2004a) estimated Papilionini to be 35–65 Myr old. Braby et al. (2005) estimated that Troidini started diverging approximately 90 Ma, and that the split between *Troides* and *Ornithoptera* is at least 40 Myr old. Nazari et al. (2007) estimated that Papilionidae started diverging in the mid to late Cretaceous approximately 95 Ma, and that the split between Papilionini and Troidini occurred 82.5–

89.1 Ma. Parnassiinae were estimated to have started diverging in the early Palaeocene, c. 62 Ma. Michel et al. (2008) estimated that all Papilionidae tribes dated from before the K/T boundary. The split between *Papilio* and Troidini was estimated to have taken place 87 Ma, and the Parnassiinae (including *Baronia*) were estimated to be 86 Myr old. Our estimates are thus generally 25–30% more recent than the estimates of Zakharov et al. (2004a) and Nazari et al. (2007), and roughly 50% younger than the estimates of Braby et al. (2005) and Michel et al. (2008).

The main difference between our molecular clock analysis and those in the four previous studies is that we used fossil data for calibration, whereas the previous works mainly used biogeographical events. Nazari et al. (2007) and Michel et al. (2008) did so indirectly by using the age estimated from Zakharov et al. (2004a) and Braby et al. (2005) as calibration points. We agree with de Queiroz (2005), Yoder and Nowak (2006), de Jong (2007) and de Jong and van Achterberg (2007) that *a priori* acceptance of vicariance as the main (or only) explanation of current distribution patterns is problematic as it ignores the mounting evidence for long-distance dispersal events as important factors in biogeography (see de Queiroz, 2005; de Jong and van Achterberg, 2007; Kodandaramaiah and Wahlberg, 2009; and references therein). Furthermore, using vicariance events as calibration points for a molecular clock effectively excludes use of the results to test the importance of the same events in the biogeography of the group (de Jong, 2007; de Jong and van Achterberg, 2007).

Biogeographical patterns. The DIVA analysis produced homoplasious results with a large number of dispersals and an extremely ambiguous ancestral area pattern. This suggests that the historical biogeography of Papilionidae is, to a large extent, the result of dispersals and not the result of diversification following vicariance events. This is in agreement with Miller (1987a), who concluded that the phylogeny of the Papilioninae lacked overall biogeographical patterns that might be predicted by vicariance scenarios.

With their predominantly Southern Hemisphere distribution, the Troidini have been the focus of several biogeographical hypotheses over the past two decades. Two major points of interest have been: (i) the presence of the endemic genus *Pharmacophagus* on Madagascar and (ii) the possibility of a Gondwanan relationship between South American and Australasian genera.

The presence of the endemic and monobasic genus *Pharmacophagus* on Madagascar has been addressed by several authors (Hancock, 1988, 2007; Parsons, 1996b; Braby et al., 2005; de Jong and van Achterberg, 2007). Hancock (1988, 2007) concluded that *Pharmacophagus* probably dispersed to Madagascar from India. Parsons

(1996b) and Braby et al. (2005) concluded that the presence of *Pharmacophagus* in Madagascar today is due to a Cretaceous vicariance episode, and the separation of Madagascar from the greater Indian plate in the late to middle Cretaceous was hypothesized to be that event. On this basis, Parsons (1996b) argued that the split between *Pharmacophagus* and *Ornithoptera* found in his analysis was at least 90 Myr old. Based on their molecular clock analysis, Braby et al. (2005) agreed that the split between *Pharmacophagus* and the remaining Troidina could be old enough to support a vicariant explanation for the distribution pattern. However, as noted by de Jong and van Achterberg (2007), because Braby et al.'s (2005) molecular clock was calibrated by biogeographical data in the first place, this reasoning is circular. Furthermore, de Jong and van Achterberg (2007) concluded that vicariance is a highly unlikely explanation for the current distribution of *Pharmacophagus*. Our results support this conclusion. Even if Troidina first diversified in southern Gondwana (see below), possible land connections between Madagascar/Greater India and southern Gondwana were broken too early to have served as dispersal routes for the ancestral *Pharmacophagus*. Such connections are thought to have broken up *c.* 80 Ma (Yoder and Nowak, 2006). The result is in agreement with Yoder and Nowak (2006) who, based on studies of numerous Madagascan taxa, concluded that: "The inescapable inference therefore is that the living biota [of Madagascar] is predominantly comprised of neoendemics that have evolved from transoceanic dispersers."

The monotypic Australian genus *Cressida* and the southern South American genus *Euryades* have been considered closely related since Ford (1944a,b). As their distributions fit a classical southern vicariance scenario, they have been primary candidates for a possible southern Gondwana relationship within Troidini (de Jong, 2003, 2007; and references therein). However, the two most recent phylogenetic analyses of Troidini (Braby et al., 2005; present study) both failed to recover this sister-group relationship. Both studies have placed *Euryades* as the sister group of the only other Neotropical Troidina, the genus *Parides*, a relationship that was also recovered by Silva-Brandão et al. (2005) in a study focused on *Parides*. In the present study *Cressida* is grouped with *Losaria*, *Troides* and *Ornithoptera* in an Australasian clade. This clade then forms the sister of the Neotropical *Euryades* + *Parides* clade. Although support for these clades is weak, this relationship opens the intriguing possibility that Troidina originally evolved in southern Gondwana with *Pharmacophagus* dispersing from southern Gondwana to Madagascar. Under this scenario, the origins of the Neotropical and Australasian Troidina are the result of the final break up of southern Gondwana into southern South America, Antarctica and Australia (Sanmartín and Ronquist,

2004). The exact dates for the final break up of southern Gondwana and especially the climatic consequences are disputed. It is, however, generally accepted that Australia was fully separated from Antarctica 35 Ma. Southern South America separated subsequently (30 Ma), after which Antarctica quickly became glaciated (de Jong, 2003; Sanmartín and Ronquist, 2004; Braby et al., 2005, and references therein). We estimate the non-*Pharmacophagus* Troidina group to be 25–45 Myr old, which does fit the accepted dates for the final break up of southern Gondwana, especially if we again take into consideration that fossil based calibrations can only be minimum estimates. Although these conclusions should be considered preliminary, our results do support a Gondwanan influence on the biogeography of Troidini.

Larval host-plant relationships

Evolution of Papilionidae larval host-plant relationships has been the subject of considerable research and speculation over the past five decades. The exclusively Aristolochiaceae feeding habits of Troidini and the non-Parnassiini parnassiines have attracted special interest. Munroe and Ehrlich (1960) hypothesized that Aristolochiaceae feeding was the ancestral condition, at least for Parnassiinae + Papilioninae. Other feeding habits within the two subfamilies were hypothesized to represent derived conditions. Ehrlich and Raven (1964), in their pioneer study of Lepidoptera–host-plant relationships, supported this theory. They further suggested that transitions to other host families (chiefly Magnoliaceae) happened in areas where Aristolochiaceae were poorly represented. Based on his studies of juveniles, Igarashi (1984) agreed with these statements and concluded that *Aristolochia* species were among the food plants of ancestral Papilionidae. This was supported by Parsons (1996a) and Yagi et al. (1999). Reanalysing evolution of larval–host-plant relationships within Papilionidae in a cladistic framework, Miller (1987b) concluded that there was no evidence for parallel cladogenesis (Mitter and Brooks, 1983) or stepwise coevolution (Ehrlich and Raven, 1964). Weintraub (1995) critically reviewed host-plant relationships within Troidini, and found that there was little evidence for parallel cladogenesis within that lineage. Both Miller (1987b) and Weintraub (1995) suggested that the host association patterns within the tribe were a combination of successive specialization and colonization events. However, as acknowledged by both authors, their conclusions were hampered by the lack of a detailed and well-resolved phylogeny for the angiosperms.

Michel et al. (2008) examined the arguments for and against the single origin of Aristolochiaceae feeding and concluded that the monophagy (at host-plant family level) displayed by Aristolochiaceae-feeding swallowtails

was the best argument against a single origin of the feeding habit. Our mapping of larval host plants on the phylogeny of Papilionidae (Fig. 6) supports this contention from an explicitly cladistic viewpoint, as independent evolution of Aristolochiaceae-feeding non-Parnassiini parnassiines and Troidini requires only two steps, versus five steps for a single origin and four independent losses.

Our match of genus-level feeding habits of Papilionidae against the most current order-level phylogeny of the angiosperms (APG (The Angiosperm Phylogeny Group), 2003) supports the conclusions reached by Miller (1987b) and Weintraub (1995). Although there is a tendency towards feeding on the more basal angiosperm orders, there is little evidence for parallel cladogenesis. The very broad host-plant range of *Papilio* is also of interest. This ability to utilize a huge range of plant orders could well be a major reason for the relative evolutionary success of this genus. *Papilio* is not only by far the most species-rich genus in the family, it is also the only genus that occurs in all faunal regions and virtually all types of habitats, from lowland tropics to high alpine and the subarctic (Zakharov et al., 2004a).

Classification

Our results necessitate adjustments to the currently most widely accepted classification (Häuser et al., 2005). *Meandrusa* should be transferred from Papilionini to Teinopalpini, and *Chilasa* should be subsumed within *Papilio s.l.* Further taxonomic changes to *Papilio* should be based on a revision of the entire genus, utilizing morphology of all life stages as well as DNA data. The well-supported monophyly of *Graphium* with the Neotropical Leptocircini, and the close relationship between the Neotropical species suggest that the latter should be regarded as a subgenera of the single genus *Eurytides*, at least until more detailed analyses of Neotropical Leptocircini can resolve the relationships within this clade.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Nexus file containing the DIVA data set.

Data S2. Nexus file containing the molecular character matrix with Mr Bayes partitions.

Data S3. Nexus file containing the morphological character matrix.

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Appendix 1

Description and discussion of morphological characters: A, characters from Miller (1987a) that were excluded from our dataset; or B, were scored significantly different; C, character from de Jong et al. (1996); D, new characters identified in this study.

Characters excluded

Character 14 (Female with a sphragis). The presence of a sphragis varies even within genera (Orr, 1995) and data were not available for several genera.

Character 21 (Larvae with a saddle on abdominal segments). Despite its absence in most Leptocircini and several *Papilio*, Miller (1987b) considered this to be a ground plan apomorphy for Papilioninae. Omitted here because of insufficient data, but a species-level study of this character within the subfamily would be very useful.

Character 27 (Forewing vein R_3 short-stalked with $R_4 + 5$). Omitted as considerable individual variation was found, sometimes between the two sides of the same specimen.

Character 29 (Ductus bursae of female elbowed). Omitted as it was found to be preparation dependent.

Character 30 (Pseuduncus slender). Scoring of this character was considered to be problematic because the “broad” pseuduncus observed in some taxa (e.g. *Teinopalpus*) seems to have the same width at the base as a “slender” pseuduncus, the difference being that the latter form has a longer, slender tip. We have therefore treated this character as the reverse of character 111 and scored all taxa under that heading.

Character 34 (Fourth larva instar glossy). Omitted because of insufficient data, but a species-level study of this character within the subfamily would be very useful.

Character 37 (Vesica with a lateral process). Omitted due to technical difficulties, but this character should be examined for a much broader taxon sample of *Papilio*.

Character 70 (Long setose tubercles on thoracic segments 3 and 4, and on abdominal segment 10 of first instar larvae). Omitted as we have no data, and Miller (1987b) stated that it “requires more careful definition and more complete taxonomic delimitation”.

Significant changes in character scorings

Character 16 (Tibia and tarsus lacking scales). We found that *Protographium marcellus* lacks such scales, whereas they are present in *Papilio* subgenus *Chilasa*.

Character 17 (Pseuduncus present). We did not find a pseuduncus in all members of Papilioninae as the structure is absent in *Lamproptera meges* and *Iphiclides podalirius*.

Character 24 (Wing scales with reticulate pattern between longitudinal ridges). A close examination (Fig. 2a–l) revealed that such a pattern is present, not only in almost all Papilioninae including Leptocircini (contrary to Miller, 1987b), but also in some Parnassiinae. The extent of the pattern varies and, in certain species, some types of scales lack the pattern. It is also absent in several species of Troidina (e.g. *C. cressida*, *E. corethrus* and *O. priamus*).

Character 35 (Basalfleck of labial palpus forming a large median flap). A difficult character considered by Miller (1987b) to be a synapomorphy for *Meandrusa* and *Papilio* despite being absent from several species within the latter. We have scored it as absent in *P. machaon*, *P. dardanus*, *P. alexanor* and *P. (Chilasa) clytia*, but present in *Teinopalpus*, *Allancastris* and *Coenonympha*.

Character 63 (Phallus [aedeagus] pointed distally). Considered by Miller (1987b) to be a synapomorphy for *Iphiclides*, *Lamproptera* and *Graphium*. In his discussion of the character, Miller also mentioned that the phallus in these genera is heavily sclerotized disto-ventrally. A phallus with a distal point was found to be present in several of the taxa in this study, including *Eurytides serville*. However, the combination of a point and heavy disto-ventral sclerotization was only found in *Iphiclides podalirius*, *Graphium agememnon* and *Eurytides serville*, and not in *Lamproptera meges*.

Character 108 (Antennae with paired sense pits). Based on Jordan (1898) and later authors, Miller (1987b) considered these structures to be an autapomorphy for Troidina. However, SEM studies of the antennae (Fig. 3a–h) showed that all species of Papilionidae included here have paired shallow pits with numerous sensilla trichodea on the ventral surface of the antennae on at least the apical third of the flagellum. The pits may still have phylogenetic information at a lower taxonomic level and a new character illustrating this has been included (New5). The ventral surface of butterfly antennae displays considerable structural variation ranging from the unmodified surface found in *Pyrgus communis* (Fig. 3a), to the single row depressions found in *Colias eurytheme* (Fig. 3b), or even triple row depressions found in other pierids (R. I. Vane-Wright, unpublished observations), to the three well-developed longitudinal carinae with sensillae between found

in Nymphalidae (e.g. de Jong et al., 1996). It is unclear what (if any) the overall evolutionary pattern is, but a detailed comparative study of butterfly antennae is certainly much warranted.

Character 153 (Ostium of female with a medial process). Scored here for the examined species of *Papilio* as well, with the exception of *P. demoleus*. This structure was reported as present, but highly modified, by Smith and Vane-Wright (2008) in a study focused on the *demoleus* species-group. Smith and Vane-Wright (2001) reported a similar process (termed central ostial lobe) in most African *Graphium* (subgenus *Arisbe*). It is clear that this structure should be subjected to a closer examination at species level across the family.

Character from de Jong et al. (1996)

dJ 93 (Spinasternum produced laterally at spina). This character was included and scored as present for all Parnassiinae and Papilioninae.

New characters

Our morphological re-examination of adults and literature reports revealed five characters that were not used previously in swallowtail phylogenetics.

New1: Upper angle of forewing cell blunt. 0 = absent, 1 = present. Scored as present in *Teinopalpus*, *Meandrusa*, *Papilio* and Troidini, as well as some outgroup taxa.

New2: Club and distal half of antennae with numerous sensilla coeloconica. 0 = absent, 1 = present (Fig. 3k,l). Although sensilla coeloconica are generally few in number with two to four per antennal segment in other butterflies examined here (Fig. 3i,j), and phycitine Pyralidae (T. J. Simonsen, unpublished observations), all examined members of Troidini have large numbers of these sensilla (> 10) on each segment.

New3: Deciduous scales of male hind wing very long and slender with a round ball-like end. 0 = absent, 1 = present (Fig. 2m–o). The very long deciduous scales of *Protographium*, *Protesilaus*, *Eurytides* and *Graphium* are unique, terminating in a peculiar, rounded, ball-like structure.

New4: Pupa with a large dorsal horn on basal margin of thorax. 0 = absent, 1 = present. The pupae of *Meandrusa* and *Teinopalpus* are apparently unique in having a large horn on the dorso-basal margin of the thorax (illustrated by Igarashi and Fukuda, 2000) that is located more basally on the thorax than the distal horn in Leptocircini.

New5: Antennal sense pits deep, well developed, and clearly visible in a stereomicroscope. 0 = absent, 1 = present.

Appendix 2

List of characters used in the morphological and combined analyses. Numbers in parentheses refer to character numbers in Miller (1987b) (M), de Jong et al. (1996) (dJ), or new characters in this study (New).

Character 1 (M1): Larvae with osmeteria. 0 = absent, 1 = present.
Character 2 (M2): Pretarsal aroliar pads and pulvilli: 0 = normal, 1 = reduced.

Character 3 (M3): Vein 2A of forewing present as a free vein to wing margin. 0 = absent, 1 = present.

Character 4 (M4): Cervical sclerites joined ventromedially. 0 = absent, 1 = present.

Character 5 (M5): Male valve with lateral lobe. 0 = absent, 1 = present.

Character 6 (M6): Forewing vein R₄: 0 = vein present, 1 = vein absent.

Character 7 (M7): Third anal vein of hind wing: 0 = vein present, 1 = vein absent.

Character 8 (M8): Cervical membrane with ventral sclerite. 0 = absent, 1 = present.

Character 9 (M9): Uncus of male bifid. 0 = absent, 1 = present.

Character 10 (M10): Phallus thin and heavily sclerotized. 0 = absent, 1 = present.

Character 11 (M11): Ostial region of the female heavily sclerotized. 0 = absent, 1 = present.

Character 12 (M12): Third segment of labial palpus: 0 = normal, 1 = elongate.

Character 13 (M13): Middle disocellular vein of forewing: 0 = straight, 1 = incurved.

Character 14 (M15): Antennae with scales. 0 = absent, 1 = present.

Character 15 (M16): Scales on tibiae and tarsi: 0 = present, 1 = absent.

Character 16 (M17): Pseuduncus present. 0 = absent, 1 = present.

Character 17 (M19): Forewing with basal spur. 0 = absent, 1 = present.

Character 18 (M20): Metathorax with a distinct meral suture. 0 = absent, 1 = present.

Character 19 (M22): Patagia. 0 = sclerotized, 1 = membranous.

Character 20 (M23): Female with sclerotized invagination dorsal to opening of ductus bursa. 0 = absent, 1 = present.

Character 21 (M24): Wing scales with reticulate pattern between longitudinal ridges. 0 = absent, 1 = present.

Character 22 (M26): Forewing discal cell. 0 = more than half the length of the wing, 1 = less than half the length of the wing.

Character 23 (M31): Tegumen and 8th tergite. 0 = separate, 1 = fused.

Character 24 (M32): Prodiscrimen with a spine. 0 = absent, 1 = present.

Character 25 (M33): Lamella metadiscrimen connection high on furca. 0 = absent, 1 = present.

Character 26 (M35): Basalfleck of labial palpus forming a large medial flap. 0 = absent, 1 = present.

Character 27 (M36): Female with prominent ostial armature. 0 = absent, 1 = present.

Character 28 (M38): Signum zipper-like. 0 = absent, 1 = present.

Character 29 (M39): Tarsal claws. 0 = simple, 1 = bifid.

Character 30 (M40): Blue scales of underside of wing opalescent. 0 = absent, 1 = present.

Character 31 (M41): Uncus shoe-shaped. 0 = absent, 1 = present.

Character 32 (M42): Juxta with strong lateral flaps. 0 = absent, 1 = present.

Character 33 (M43): Phallus [= aedeagus] with a toothed flange on the distal portion. 0 = absent, 1 = present.

Character 34 (M44): Ductus bursae funnel-shaped at base. 0 = absent, 1 = present.

Character 35 (M45): Tentorium with high crests. 0 = absent, 1 = present.

Character 36 (M46): Cross vein between R_s and M₁ of hind wing. 0 = straight, 1 = sinuate.

Character 37 (M47): Phallus bell-shaped at base. 0 = absent, 1 = present.

Character 38 (M48): Dorso-lateral sclerite of valve articulate. 0 = absent, 1 = present.

Character 39 (M50): First instar larvae with bifid setae on thoracic and abdominal segments. 0 = absent, 1 = present.

Character 40 (M51): Pupa with lateral ridges extending from cremaster to head. 0 = absent, 1 = present.

Character 41 (M52): Papillae anales with peg-like setae. 0 = absent, 1 = present.

Character 42 (M54): Clasper triangular with serrate margin. 0 = absent, 1 = present.

Character 43 (M56): Dorso-lateral sclerite of valve triangular and fused to tegumen. 0 = absent, 1 = present.

Character 44 (M60): Clasper of male with a dorsal flange and a central process. 0 = absent, 1 = present.

Character 45 (M63): Phallus pointed distally. 0 = absent, 1 = present.

Character 46 (M64): Tegumen narrowed dorso-laterally. 0 = absent, 1 = present.

Character 47 (M69): Pupa with long dorsal horn. 0 = absent, 1 = present.

Character 48 (M71): Forewing upper discocellular vein longer than middle discocellular vein. 0 = absent, 1 = present.

Character 49 (M72): Tentorial crests sharply narrow. 0 = absent, 1 = present.

Character 50 (M77): Apophyses anterior: 0 = present, 1 = absent.

Character 51 (M79): Vein R₁ of forewing anastomosed with Sc. 0 = absent, 1 = present.

Character 52 (M99): Pleuron of female abdominal segment 8. 0 = membranous, 1 = sclerotized.

Character 53 (M100): Female with a row of closely spaced spines running the length of tibia. 0 = absent, 1 = present.

Character 54 (M101): Anal margin of male hind wing rolled dorsally. 0 = absent, 1 = present.

Character 55 (M102): Medial surface of papillae anales with four to seven long hooks per lobe. 0 = absent, 1 = present.

Character 56 (M103): Larvae with fleshy tubercles on thoracic and abdominal segments. 0 = absent, 1 = present.

Character 57 (M104): First anal vein on male hind wing with prominent groove. 0 = absent, 1 = present.

Character 58 (M105): Androconia in male hind wing fluted. 0 = absent, 1 = present.

Character 59 (M106): Vein A1 + A2 in male hind wing swollen. 0 = absent, 1 = present.

Character 60 (M108): Antennae with paired sense pits. 0 = absent, 1 = present.

Character 61 (M109): Collar, head, thoracic pleuron and abdomen with red scales. 0 = absent, 1 = present.

Character 62 (M111): Pseuduncus. 0 = normal size, 1 = small.

Character 63 (M113): Labial palpus. 0 = 3-segmented, 1 = 2-segmented.

Character 64 (M114): Pseuduncus hinged on 8th tergite. 0 = absent, 1 = present.

Character 65 (M115): Valve small, reduced dorsally. 0 = absent, 1 = present.

Character 66 (M116): Clasper elongate and thorn-like. 0 = absent, 1 = present.

Character 67 (M117): Hind wing vein A1 + A2 short. 0 = absent, 1 = present.

Character 68 (M119): Valve with a dorsal process located basally. 0 = absent, 1 = present.

Character 69 (M120): Signum absent. 0 = absent, 1 = present.

Character 70 (M121): Sphragis alate. 0 = absent, 1 = present.

Character 71 (M122): Juxta: 0 = broad, 1 = narrowed laterally.

Character 72 (M123): Dorsal lobe of valve. 0 = sclerotized, 1 = membranous.

Character 73 (M124): Wings transparent. 0 = absent, 1 = present.

Character 74 (M125): Spinasternum. 0 = narrow, 1 = broad.

Character 75 (M131): Male hind tibia swollen, covered with a mat of short spines. 0 = absent, 1 = present.

Character 76 (M143): Male hind wing margin with deciduous scales. 0 = absent, 1 = present.

Character 77 (M144): Male hind wing with anal cell enlarged. 0 = absent, 1 = present.

Character 78 (M145): Membrane between ostium and sternite 7 fluted. 0 = absent, 1 = present.

Character 79 (M146): R₁ in forewing arising opposite CuA₂. 0 = absent, 1 = present.

Character 80 (M147): Middle discocellular and upper discocellular in line. 0 = absent, 1 = present.

Character 81 (M149): Valve with a marginal process. 0 = absent, 1 = present.

Character 82 (M150): Signum with concentric folds. 0 = absent, 1 = present.

Character 83 (M151): Appendix bursae. 0 = absent, 1 = present.

Character 84 (M153): Ostium with a medial process. 0 = absent, 1 = present.

Character 85 (M154): Lobes of uncus bent ventrally. 0 = absent, 1 = present.

Character 86 (M155): Clasper with a basal hook. 0 = absent, 1 = present.

Character 87 (M156): Clasper with distal spines. 0 = absent, 1 = present.

Character 88 (M157): Male abdomen with androconia. 0 = absent, 1 = present.

Character 89 (dJ93): Spinasternum produced laterally at spina. 0 = absent, 1 = present.

Character 90 (New1): Upper angle of forewing cell blunt. 0 = absent, 1 = present.

Character 91 (New2): Sensilla coeloconica on club and distal half of antennae. 0 = few (1–4 per segment), 1 = numerous (10 or more per segment).

Character 92 (New3): Deciduous scales of male hind wing very long and slender with a round ball-like end. 0 = absent, 1 = present.

Character 93 (New4): Pupa with a large dorsal horn on basal margin of thorax. 0 = absent, 1 = present.

Character 94 (New5): Antennal sense pits deep, well developed, and clearly visible in a stereomicroscope. 0 = absent, 1 = present.

Appendix 3

Discussion of relationships recovered by the Bayesian analysis of the combined molecular and morphological dataset. Clade numbers refer to Fig. 5.

Clade 1: Papilionidae. The family is recovered as monophyletic with strong support from all data partitions. Important morphological characters that appear diagnostic are Miller's (1987a) characters 1–4 and 108. Character 1 (larva with osmeterium) is the time-honoured diagnostic character for the family (e.g. Jones, 1794; Munroe and Ehrlich, 1960; Miller, 1987a; Tyler et al., 1994). Character 108 (antennae with paired sense pits present) has long been considered an autapomorphy for *Troidina* (see Miller, 1987a). But our SEM studies suggest that it should be considered an autapomorphy for the entire family.

Clade 2: Parnassiinae + Papilioninae. The non-*Baronia* swallow-tails are recovered as monophyletic, but the clade is well supported only in analyses of the morphological dataset, and Bayesian analyses of most datasets (excluding ncDNA). Important morphological character supports are Miller's (1987a) characters 7 and 8, and de Jong et al. (1996) character 93.

Clade 3: Parnassiinae. The four genera from the subfamily included in our combined analyses form a monophyletic group, but it is well supported only in the Bayesian analyses of the two combined datasets. Important morphological character supports are Miller's (1987a) characters 10 and 11.

Clade 4: Papilioninae. The subfamily is monophyletic, but only well supported in the two Bayesian analyses of the combined datasets and the analyses of the morphological dataset. Important morphological character supports are Miller's (1987a) characters 19 and 20.

Clade 5: Leptocircini. The tribe appears as one of the most strongly supported clades in the cladogram, with high support from all analyses. Seemingly diagnostic morphological features are Miller's (1987a) characters 45, 46, 48, 50 and 51.

Clade 6: *Iphielides* Group. An uncertain group that is only weakly supported in the Bayesian analyses of the combined dataset, and by Miller's (1987a) character 52.

Clade 7: *Graphium* Group. A controversial group that contradicts earlier results (see below). But it receives strong support from most Bayesian analyses (except mtDNA), and from two morphological characters, Miller's (1987a) 143 (male hind wing with deciduous scales) and new1 (deciduous scales with a round ball-like end).

Clade 8: Neotropical Leptocircini. A well-supported group in all analyses except morphology. The monophyly of the neotropical Leptocircini seems undisputed even though no good apomorphies have been identified. The internal phylogeny and classification of portions of the group is uncertain and will require further revision.

Clade 9: *Protesilaus* + *Eurytides*. Although not supported by any morphological characters, this clade is well supported by all other data.

Clade 10: non-leptocircine Papilioninae. The non-leptocircine Papilioninae are recovered as monophyletic and well supported in the analyses of the combined dataset, morphology, and in the Bayesian analysis of mtDNA. Important morphological character supports are Miller's (1987a) characters 22 and 23 and New1 (upper angle of forewing cell blunt).

Clade 11: Teinopalpini. Although not particularly well supported, *Teinopalpus* + *Meandrusa* are recovered as sister taxa in the analyses of the combined datasets, and the Bayesian analysis of mtDNA. Putative apomorphies are Miller's (1987a) characters 13 and 26, and New4. The implications are dealt with in the discussion.

Clade 12: *Meandrusa*. The two representatives included here are recovered as monophyletic with strong support in all analyses.

Clade 13: Papilionini + Troidini. The sister-group relationship between the two tribes is well supported by the Bayesian analyses of the combined data, mtDNA and morphology. Putative morphological character support comes from Miller's (1987a) character 16, although it is subsequently reduced in *Chilasa*, and has been paralleled in *Luehdorfia*, *Allancastris* and *Protographium s.l.*

Clade 14: Papilionini. The seven representatives of the tribe included here are recovered as a monophyletic group strongly supported in all analyses, except for morphology where the support is only moderate. Potentially important morphological characters are Miller's (1987a) 36 and 38.

Clade 15: Troidini. The tribe is recovered as monophyletic and well supported in all analyses except ncDNA. Important morphological character supports are Miller's (1987a) characters 100, 103 and 104 and new2 (club and distal half of antennae with numerous sensilla coeloconica). This last is identified as a new autapomorphy for the tribe.

Clade 16: *Battus*. The two representatives included here are recovered as monophyletic with strong support in all analyses. Important morphological character supports are Miller's (1987a) characters 105 and 106.

Clade 17: Troidina. The subtribe is recovered as monophyletic and strongly supported in the analyses of the combined datasets and mtDNA. Important morphological character support comes from Miller's (1987a) character 109.

Clade 18: non-*Pharmacophagus* Troidina. The group is recovered as monophyletic and well supported in most analyses except ncDNA. Important morphological character supports are Miller's (1987a) characters 113 and 114.

Clade 19: Neotropical Troidina. An uncertain group only moderately supported in the analyses of the combined datasets and mtDNA. No good morphological character supports the recognition of such a clade.

Clade 20: Australasian Troidina. Another uncertain group only weakly supported by Bayesian analyses of the combined datasets. No good morphological character support.

Clade 21: *Troides* + *Ornithoptera*. The monophyly of *Troides s.l.* is strongly supported in all analyses. Important morphological character supports include Miller's (1987a) characters 146, 147, 149 and 154–157.