

Phylogenetic relationships and historical biogeography of tribes and genera in the subfamily Nymphalinae (Lepidoptera: Nymphalidae)

NIKLAS WAHLBERG^{1*}, ANDREW V. Z. BROWER² and SÖREN NYLIN¹

¹Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden

²Department of Zoology, Oregon State University, Corvallis, Oregon 97331–2907, USA

Received 10 January 2004; accepted for publication 12 November 2004

We infer for the first time the phylogenetic relationships of genera and tribes in the ecologically and evolutionarily well-studied subfamily Nymphalinae using DNA sequence data from three genes: 1450 bp of cytochrome oxidase subunit I (COI) (in the mitochondrial genome), 1077 bp of elongation factor 1-alpha (EF1- α) and 400–403 bp of *wingless* (both in the nuclear genome). We explore the influence of each gene region on the support given to each node of the most parsimonious tree derived from a combined analysis of all three genes using Partitioned Bremer Support. We also explore the influence of assuming equal weights for all characters in the combined analysis by investigating the stability of clades to different transition/transversion weighting schemes. We find many strongly supported and stable clades in the Nymphalinae. We are also able to identify 'rogue' taxa whose positions are weakly supported (the different gene regions are in conflict with each other) and unstable. Our main conclusions are: (1) the tribe Coeini as currently constituted is untenable, and *Smyrna*, *Colobura* and *Tigridia* are part of Nymphalini; (2) 'Kallimini' is paraphyletic with regard to Melitaeini and should be split into three tribes: Kallimini s.s., Junoniini and Victorinini; (3) Junoniini, Victorinini, Melitaeini and the newly circumscribed Nymphalini are strongly supported monophyletic groups, and (4) *Precis* and *Junonia* are not synonymous or even sister groups. The species *Junonia coenia*, a model system in developmental biology, clearly belongs in the genus *Junonia*. A dispersal-vicariance analysis suggests that dispersal has had a major effect on the distributions of extant species, and three biotic regions are identified as being centres of diversification of three major clades: the Palaearctic for the *Nymphalis*-group, the Afrotropics for Junoniini and the Nearctic for Melitaeini. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 86, 227–251.

ADDITIONAL KEYWORDS: DIVA – molecular phylogeny – Partitioned Bremer Support – sensitivity analysis.

INTRODUCTION

Butterflies belonging to the currently recognized subfamily Nymphalinae (Wahlberg, Weingartner & Nylin, 2003b) have contributed extensively to our knowledge of ecological and evolutionary processes, from hybrid zones and ring-species (Forbes, 1928; Silberglied, 1984; Dasmahaptra *et al.*, 2002; Austin *et al.*, 2003), metapopulation dynamics (Hanski, 1999) and evolutionary developmental biology (Carroll *et al.*, 1994; Brakefield *et al.*, 1996), to insect–plant interactions (Singer, 1971; Nylin, 1988; Janz, Nylin & Nyblom,

2001; Wahlberg, 2001). Despite the long-standing interest in these butterflies, the phylogenetic relationships among the various tribes and genera have remained remarkably obscure. Improving our understanding of the phylogenetic resolution of such scientifically popular taxa should be a high priority, so that this abundance of knowledge can be placed in an evolutionary framework.

Since Nymphalinae is the type subfamily of the diverse family Nymphalidae, its delineation has enjoyed a dynamic history, as various authors have considered diverse subsets of tribes and genera to represent 'typical nymphalids'. This confusion has led to several competing classification schemes based on different data sets (Ackery, 1988; Harvey, 1991; Kuznet-

*Corresponding author. E-mail: niklas.wahlberg@zoologi.su.se

zov & Stekolnikov, 2001; Wahlberg *et al.*, 2003b). The higher systematics of Nymphalidae is still in a state of flux and the delineation of Nymphalinae has not yet reached stability, though there is a growing consensus that the classification of Harvey (1991) (based on the classification of Müller, 1886), with the addition of the tribe Coeini (= Coloburini of authors), currently provides the most natural definition of the subfamily (Brower, 2000; Wahlberg *et al.*, 2003b; Freitas & Brown, 2004). This concept of Nymphalinae comprises a monophyletic group that includes the supposedly well-defined tribes Nymphalini, Coeini, Melitaeini and Kallimini. It is this hypothesis of nymphaline relationships that we take as our point of departure in our analysis and discussion.

Ehrlich's (1958) influential paper on the classification of butterflies included a much broader concept of Nymphalinae, which was based mainly upon symplesiomorphic and homoplastic characters and appeared to comprise those taxa that do not fall into any of his more restricted and homogeneous nymphalid subfamilies (Danainae, Ithomiinae, Satyrinae, Morphinae, Calinaginae, Charaxinae, Acraeinae). The taxa in Ehrlich's Nymphalinae are today considered to represent several different subfamilies, including Heliconiinae, Limenitidinae, Biblidinae and Apaturinae. Several subsequent authors have used much the same group of tribes and genera to represent the 'core nymphalids' (e.g. Ackery, 1984; Scott, 1985; Scott & Wright, 1990), though often splitting off some components as distinct subfamilies. At the other extreme, some authors have delineated Nymphalinae in a very narrow sense, to include only the tribes Nymphalini and Kallimini (Ackery, 1988) or Nymphalini and Melitaeini (Clark, 1948).

All of the above classifications have suffered from a lack of clearly described morphological synapomorphies that diagnose the various circumscriptions. Harvey (1991) proposed a classification of the family Nymphalidae based on a set of larval characters, that was accepted by many authors (e.g. Ackery *et al.*, 1999). He placed the tribes Nymphalini, Kallimini and Melitaeini together, based on the arrangement of spines on the larvae, but was unable to resolve the relationships among them due to character conflict within the subfamily. Harvey (1991) placed the genus *Amnosia* in the tribe Kallimini, but Wahlberg *et al.* (2003b) showed that *Amnosia* does not belong in Nymphalinae, but in Cyrestinae along with other members of Pseudergolini (with which *Amnosia* was affiliated prior to being moved to Kallimini (Ackery, 1988)).

Most historical classifications of the Nymphalidae have been intuitive rather than the product of rigorous phylogenetic analysis of character state distributions formalized in a data matrix. More recently,

however, several cladistic analyses have been published, based on either morphology (DeVries *et al.*, 1985; de Jong *et al.*, 1996; Penz & Peggie, 2003; Freitas & Brown, 2004) or DNA sequences (Weller *et al.*, 1996; Brower, 2000; Wahlberg *et al.*, 2003b). Three of these studies (Brower, 2000; Wahlberg *et al.*, 2003b; Freitas & Brown, 2004) sampled enough representatives of Nymphalidae to provide evidence bearing upon the monophyly and circumscription of the Nymphalinae. In all three, sampled taxa belonging to Nymphalini, Coeini, Kallimini and Melitaeini form a monophyletic group, with Coeini as the sister group to Nymphalini. Freitas & Brown (2004) and Wahlberg *et al.* (2003b) suggest an association of Kallimini with Melitaeini, while Brower's (2000) study has a basal, paraphyletic Kallimini, with regard to Melitaeini and Nymphalini + Coeini. The sister group to the Nymphalinae remains in doubt, with Freitas & Brown (2004) proposing Heliconiinae, Brower (2000) suggesting Biblidinae + Apaturinae and Wahlberg *et al.*'s (2003b) data implying Apaturinae.

Within Nymphalinae, several taxa have received attention from systematists in recent years. The relationships among genera and species groups have been investigated in Melitaeini (Kons, 2000; Wahlberg & Zimmermann, 2000) and Nymphalini (Nylin *et al.*, 2001; Wahlberg & Nylin, 2003) using both molecular and morphological data. In Melitaeini, it has become clear that Harvey's (1991) proposed division into three subtribes (Euphydryina, Melitaeina, Phyciodina) is not satisfactory. Wahlberg & Zimmermann (2000), based on mtDNA, found that the *Euphydryas*-, *Melitaea*-, *Chlosyne*- and *Phyciodes*-groups of species and genera were of equal status. Kons (2000), using morphology, also found these four major groups and additionally described a fifth group containing *Gnathotriche* species. However, the relationships of the four major groups are in conflict between these two studies. Wahlberg and Zimmermann inferred the *Melitaea*-group to be sister to the *Chlosyne*-group, whereas Kons found Phyciodina and *Gnathotriche* to be sister to the *Chlosyne*-group.

The two studies on Nymphalini (Nylin *et al.*, 2001; Wahlberg & Nylin, 2003) have established the monophyly of the the *Nymphalis*-group of genera (i.e. *Aglais*, *Nymphalis* and *Polygonia*) and the close relationship between *Araschnia*, *Mynes* and *Symbrenthia*, but otherwise the relationships of genera within Nymphalini remain unclear. A further recent morphological study of *Symbrenthia*, *Mynes* and *Araschnia* (Fric, Konvicka & Zrzavý, 2004) suggests that *Mynes* is nested within *Symbrenthia*.

In addition to these tribal-level studies, species-level phylogenetic studies have been done for the genera *Euphydryas* (Zimmermann, Wahlberg & Descimon, 2000), *Chlosyne* (Kons, 2000), *Hypanartia*

(Willmott, Hall & Lamas, 2001), *Anartia* (Blum, Birmingham & Dasmahapatra, 2003), *Phyciodes* (Wahlberg, Oliveira & Scott, 2003a) and *Symbrenthia*, *Mynes* and *Araschnia* (Fric *et al.*, 2004). Of these, only Kons (2000), Wahlberg *et al.* (2003a) and Fric *et al.* (2004) included sufficiently extensive outgroup sampling to test the monophyly of the genus being studied.

From this brief review of the current state of nymphaline systematics, it is clear that many questions remain unanswered. The relationships among genera in Kallimini and Coeini have never been investigated, and relationships within Melitaeini and Nymphalini are still contentious. In this study we test the monophyly of the most recent definition of Nymphalinae, comprising Nymphalini, Coeini, Kallimini and Melitaeini (Wahlberg *et al.*, 2003b), and endeavour to resolve the relationships among the tribes and genera within the subfamily. In the current circumscription, the subfamily Nymphalinae comprises about 496 species in 56 genera. We have studied the relationships of representative species belonging to the subfamily with DNA sequences from three gene regions. These are cytochrome oxidase subunit I (COI) from the mitochondrial genome, and elongation factor-1 α (EF1- α) and *wingless* from the nuclear genome. Based on our results, we investigate the biogeography of the group using dispersal-vicariance analysis (Ronquist, 1997). The investigation is intended to identify broad biogeographical patterns for closer inspection at a later date.

MATERIAL AND METHODS

We sampled as many species as possible from almost all of the genera belonging to Nymphalinae, a total of 161 species in 49 genera. Of the seven missing genera, six are in Melitaeini and one is in Coeini. In addition, we sampled 28 outgroup species, representing all subfamilies of the nymphaline clade (*sensu* Wahlberg *et al.*, 2003b), i.e. Cyrestinae, Biblidinae and Apaturinae, each of which may be the sister group to Nymphalinae (see Wahlberg *et al.*, 2003b). We also included a specimen of Heliconiinae and Limenitidinae, which belong to the putative sister clade to the nymphaline clade. The species sampled and their collection localities are listed in Appendix 1.

We extracted DNA mainly from one or two legs of freshly frozen or dried butterflies using QIAgen's DNEasy extraction kit, although some specimens were extracted following the protocol of Brower (1994). The spread voucher specimens can be viewed at <http://www.zoologi.su.se/research/wahlberg/>. For each specimen we sequenced 1450 bp of COI, 1077 bp of EF1- α and 400–403 bp of the *wingless* gene. Primers for COI were taken from Wahlberg & Zimmermann (2000), for EF1- α from Monteiro & Pierce (2001) and for *wingless* from Brower & DeSalle (1998). We performed all PCRs

in a 20 μ L reaction volume. The cycling profile for COI and *wingless* was 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 47 °C for 30 s, 72 °C for 1 min 30 s and a final extension period of 72 °C for 10 min. The cycling profile for EF1- α was 95 °C for 7 min, 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension period of 72 °C for 10 min. For all three genes, the PCR primers were also used for sequencing.

In addition, we developed two internal primers for sequencing: (EFmid 5'-CAA TAC CRC CRA TTT TGT-3') for EF1- α and (Patty 5'-ACW GTW GGW GGA TTA ACW GG-3') for COI. Sequencing was done with a Beckman-Coulter CEQ2000 or CEQ8000 capillary sequencer (Bromma, Sweden). We checked the resulting chromatograms using BioEdit (Hall, 1999) and aligned the sequences by eye. Clear heterozygous positions in the nuclear genes (chromatogram peaks almost or exactly equal) were coded according to the IUPAC ambiguity codes, but were treated as missing characters in further analyses. The sequences have been submitted to GenBank (Accession numbers in Appendix 1).

We searched for the most parsimonious cladograms from the equally weighted and unordered data matrix consisting of 189 taxa using a heuristic search algorithm in NONA 2.0 (Goloboff, 1998) via WINCLADA 1.00.08 (Nixon, 2002). The heuristic searches were conducted with 1000 random addition replicates using TBR branch swapping with ten trees held during each step and a final swapping to completion. We did this for each gene separately and for all three genes combined. Trees were rooted with *Heliconius* for display. For the separate analyses, we evaluated clade support using bootstrap with 100 pseudoreplicates. It is now widely recognized that assessing incongruence among data partitions is much more complex than measuring with a simple all-or-nothing significance test (Farris *et al.*, 1994; DeSalle & Brower, 1997; Miller *et al.*, 1997; Darlu & Lecointre, 2002). We have thus chosen to analyse the three gene regions as a single data set, and have assessed the impact of each gene region on the support values of each node using Partitioned Bremer Support (PBS) analyses (Baker & DeSalle, 1997; Baker *et al.*, 1998).

We evaluated the character support for the clades in the resulting cladograms using Bremer support (BS) (Bremer, 1988, 1994). We calculated BS and PBS values using anticonstraints in PAUP* 4.0b10 for Windows (Swofford, 2001). As in previous studies (Wahlberg & Nylin, 2003; Wahlberg *et al.*, 2003b), we refer to the support values as giving weak, moderate, good or strong support when discussing our results. We define 'weak support' as BS values of 1–2 (generally corresponding to bootstrap values <50%–63%), 'moderate support' as values between 3 and 5 (bootstrap values 64%–75%), 'good support' as values

between 6 and 10 (bootstrap values 76%–88%) and ‘strong support’ as values >10 (bootstrap values 89%–100%). We strongly endorse BS values over bootstrap values because they are a parameter of the data rather than an estimate based on manipulated subsamples of the data, and have no upper bound as support for a given clade increases.

We evaluated the stability (*sensu* Wheeler, 1995; Judd, 1998; Giribet, 2003) of clades inferred for the equally weighted data set to different character-state transformation weighting assumptions under parsimony searches using PAUP*. We weighted transversions 2, 3, 5, 7 and 10 times transitions for the combined data set. Such sensitivity analyses may help identify potential instances of long branch attraction (Giribet, 2003), and can provide a valuable heuristic tool to guide subsequent sampling strategies for refinement of the current hypothesis. We refer to clades that are recovered under all the tested weighting schemes as stable.

We investigated the historical biogeography of the subfamily using dispersal-vicariance analysis (Ronquist, 1997) as implemented in DIVA (Ronquist, 1996). Species distributions were recorded at the level of zoological biomes, i.e. Nearctic, Neotropical, Afrotropical, Palaearctic, Oriental and Australasian. Clades for which component species had identical distributions were collapsed into a single terminal. The maximum number of ancestral areas was either not constrained or restricted to two.

RESULTS

CHARACTERISTICS OF THE DATA SETS

The total combined data set consisted of 2935 nucleotides, of which 12 positions were coded as gaps in some taxa. All inferred gaps were in the *wingless* data set and represent indel events of whole codons. These include an inferred codon insertion in all Melitaeini sequences (as noted in Brower, 2000), an inferred codon insertion in *Tigridia acesta*, an inferred codon insertion in *Rhinopalpa polynice* and an inferred codon deletion in the three species of *Aglais* (as noted in Nylin *et al.*, 2001). All inferred indel events have

occurred between positions 91 and 137 in the *Colobura dirce* sequence (GenBank accession number AY090162) and were easily detected when aligning by eye (flanking regions were relatively conserved). Gaps were treated as a ‘fifth base pair’ in all phylogenetic analyses. The basic statistics for the three gene regions are given in Table 1. All three gene regions showed an ample amount of variation, though EF1- α was less variable than the other two genes. The two nuclear genes show about equal base frequencies, while COI has a high AT bias, in accordance with all previous publications on this gene in insects.

GENERAL PHYLOGENETIC PATTERNS

When analysed as separate data partitions, none of the three gene regions recovered the subfamily Nymphalinae as a monophyletic entity (data not shown). There are, however, several phylogenetic components common to the separate hypotheses: Melitaeini and Nymphalini (as delineated below) are monophyletic, the genera *Junonia*, *Precis*, *Hypolimnas*, *Yoma*, *Salamis* and *Protogoniomorpha* together form a monophyletic group, and the *Nymphalis*-group of species (see Wahlberg & Nylin, 2003) is monophyletic.

Combining the three data sets yields eight trees of length 17547 steps (CI = 0.13, RI = 0.58), the strict consensus of which gives a much clearer picture of the relationships among the various groups in Nymphalinae (Figs 1–4). The subfamily as currently delimited is inferred to be polyphyletic, with the genera *Historis* and *Baeotus* branching off close to the base of the larger nymphaline clade (*sensu* Wahlberg *et al.*, 2003b). The monophyly of Nymphalinae without *Historis* and *Baeotus* receives moderate support. Nymphalini and Melitaeini form monophyletic groups, while Coeini and Kallimini are poly- and paraphyletic, respectively. Three genera traditionally placed in Coeini (*Colobura*, *Tigridia* and *Smyrna*) are associated with Nymphalini with good support, while the two other sampled genera, *Historis* and *Baeotus*, are outside Nymphalinae with weak support. Kallimini + Melitaeini has moderate support, but the kallimine taxa form a paraphyletic

Table 1. The basic statistics for the three molecular data sets in 174 species of Nymphalidae

Gene	No. of sites	No. variable	No. informative	Empirical base frequencies (%)			
				T	C	A	G
COI	1450	775	614	39.9	14.7	31.9	13.6
EF1- α	1077	469	364	22.6	28.1	25.9	23.4
<i>wingless</i>	412	238	192	21.0	26.2	24.9	27.9

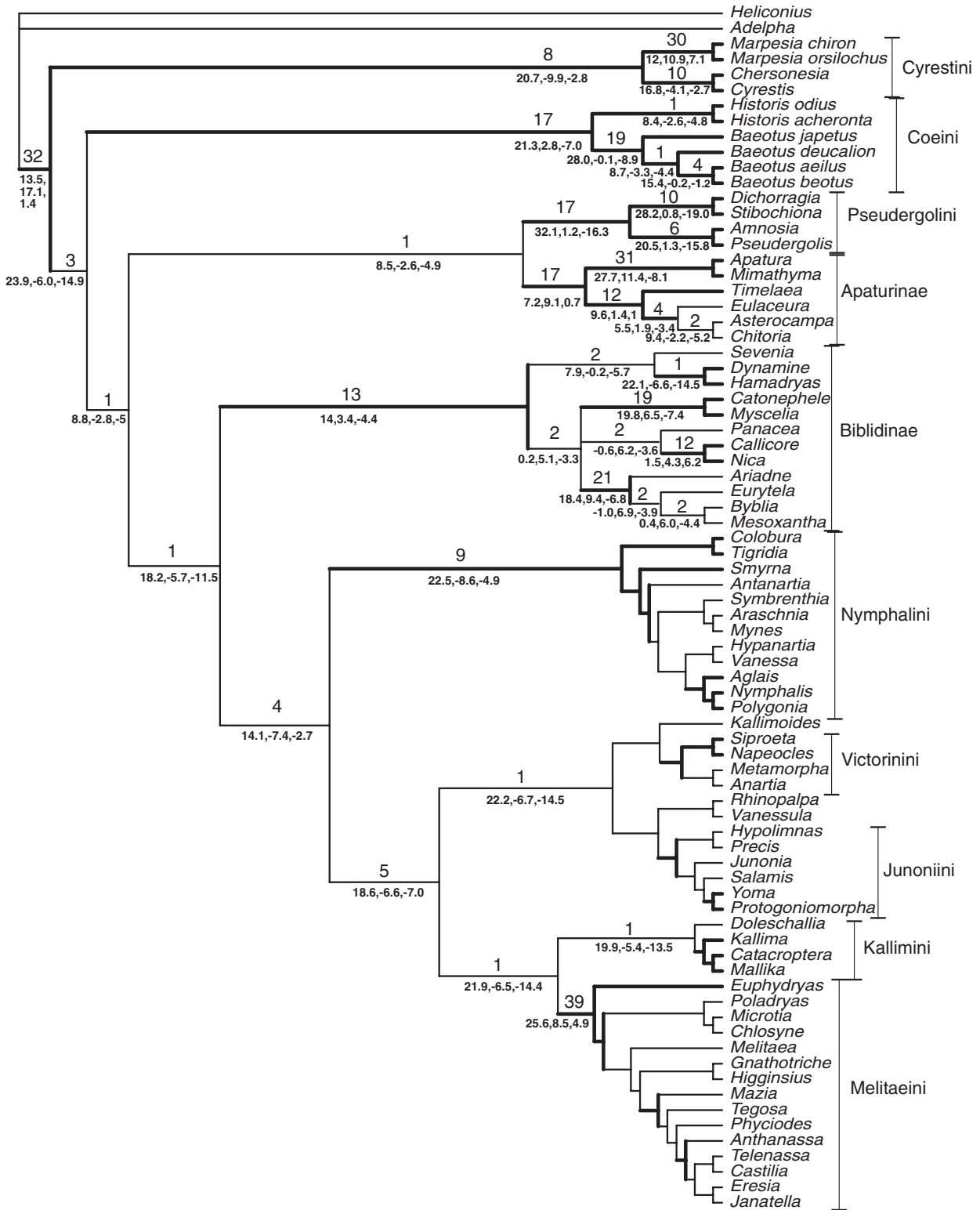


Figure 1. Summary of strict consensus of eight trees found for the combined data set when all changes weighted equally (length = 17547, CI = 0.13, RI = 0.58), pruned to show only genera. For unpruned trees, see Figs 2–4. Numbers above the branches are Bremer support values and numbers below are Partitioned Bremer Support values for the COI, EF1- α and *wingless* data partitions, respectively. Thickened branches are stable to changes in weighting schemes (transversions weighted 1, 2, 3, 5, 7 and 10 times transitions).

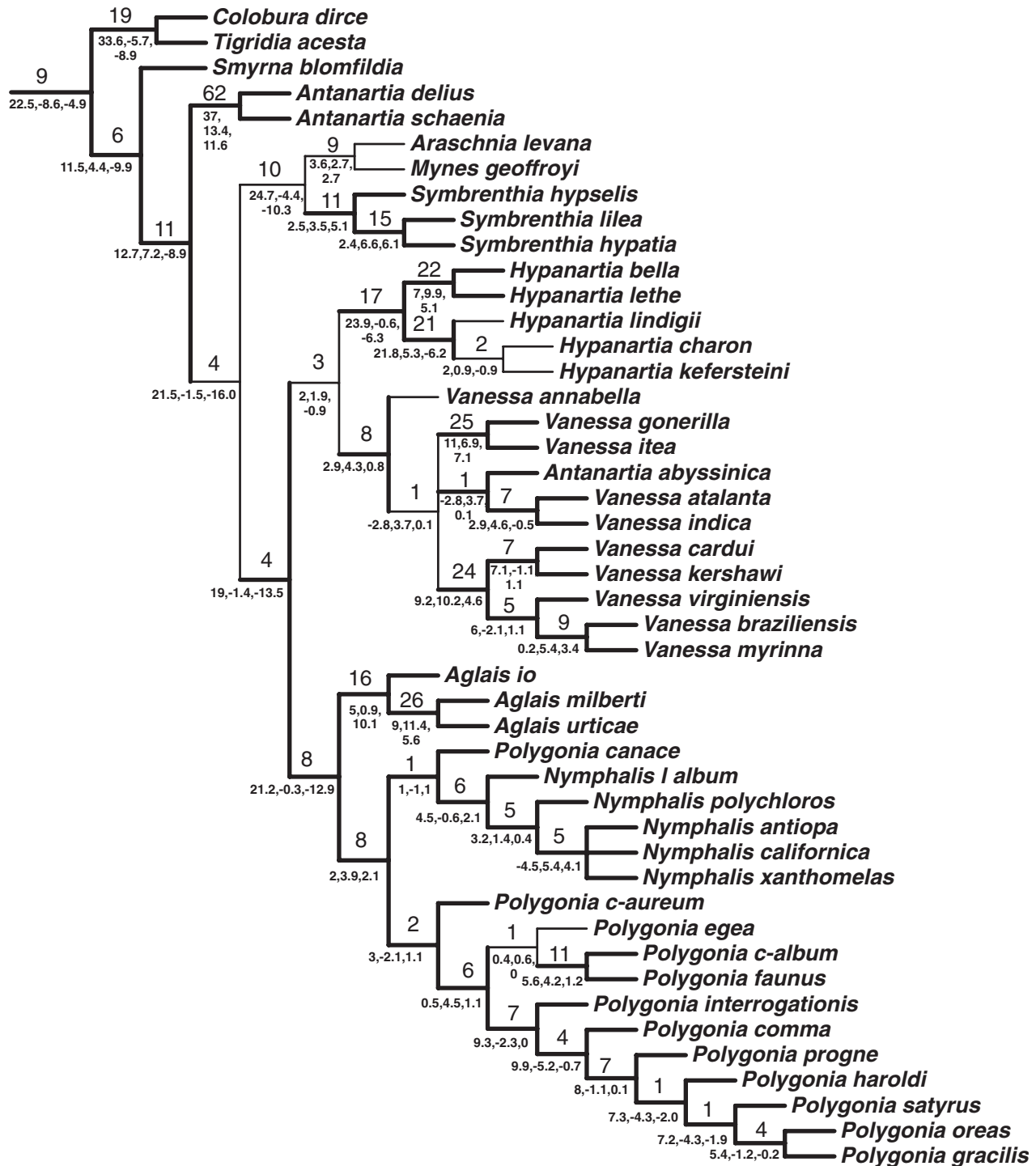


Figure 2. Relationships of sampled species from the tribe Nymphalini as delimited in Fig. 1. Tree statistics and branch thickness as in Fig. 1.

grade with respect to Melitaeini. The sister group of Nymphalinae (without *Historis* and *Baeotus*) is Biblidinae in the most parsimonious trees from the combined data set, although this clade has weak support (Fig. 1).

Results of the PBS analysis show that all three gene regions are congruent at 52 of the 183 resolved nodes (Figs 1–4). These nodes generally have high BS values (range 3–63, mean 19) and tend to be concentrated in the clades describing Nymphalini and Melitaeini. At

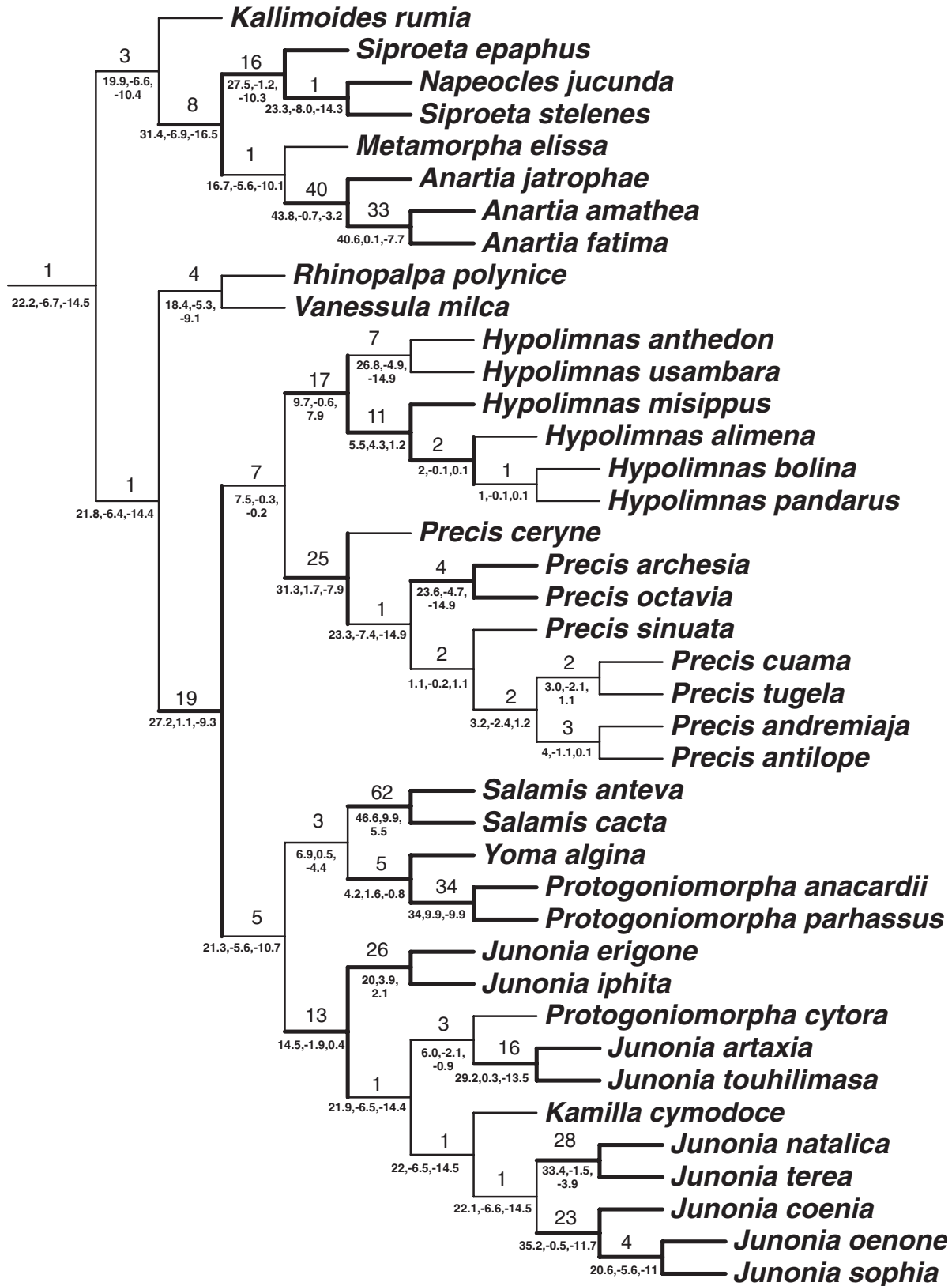


Figure 3. Relationships of sampled species from the tribes Junoniini and Victorinini, as delimited in Fig. 1, as well as species in the genera *Kallimoides*, *Vanessula* and *Rhinopalpa*. Tree statistics and branch thickness as in Fig. 1.

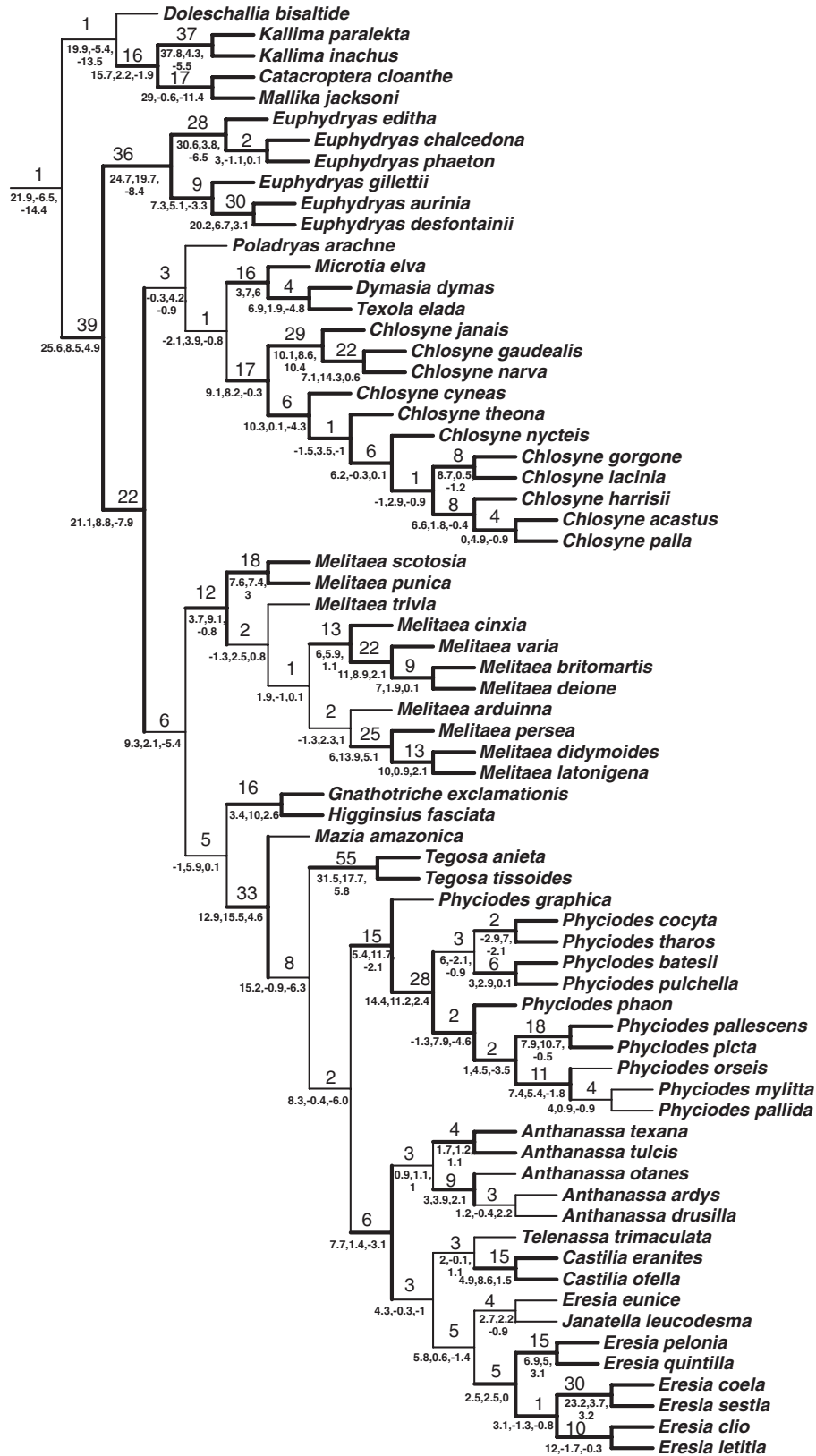


Figure 4. Relationships of sampled species from the tribe Melitaeini and Kallimini as delimited in Fig. 1. Tree statistics and branch thickness as in Fig. 1.

Table 2. Number of nodes in the ingroup at which different patterns of incongruence were observed. +, PBS score positive or 0; -, PBS score negative (magnitude not taken into account)

Number of nodes	COI	EF1- α	<i>wingless</i>
47	+	+	+
46	+	+	-
20	+	-	+
6	-	+	+
56	+	-	-
0	-	-	+
8	-	+	-

131 nodes, one or two gene regions provide signal that is incongruent with the weight of the combined evidence. The different patterns of incongruence are summarized in Table 2. Nodes which show incongruence tend to have lower BS values (range 1–19, mean 7.7). Most of the incongruent nodes lie in the basal branches of the cladogram (Fig. 1) and in the kalimene grade (Figs 3, 4).

The PBS results show that the data partitions are contributing unequally to the phylogenetic patterns inferred in this study. The COI data set conflicts at 17 nodes, of which one node has a PBS score of <-4 . The total PBS for the COI data partition is 2176.1 (mean 11.9). The EF1- α data partition is incongruent at 70 of the 183 resolved nodes, 23 of which have a PBS score of <-4 , and has a total PBS of 370.3 (mean 2.0). The *wingless* data alone have a negative PBS score at 106 of the 183 resolved nodes, 64 of which have a PBS score of <-4 , and the gene region has a total PBS of -533.5 (mean -2.9).

Many of the clades are very stable (*sensu* Giribet, 2003) and are present in a strict consensus of all trees found across the different weighting schemes from the equally weighted analysis to the analysis with 10 : 1 TV/TI weighting (Figs 1–4). These include the larger nymphaline clade (including Biblidinae, Cyrestinae, Apaturinae and Nymphalinae; see Wahlberg *et al.*, 2003b), Apaturinae and Biblidinae, Pseudergolini, Cyrestini, Nymphalini and Melitaeini (Fig. 1), and many of the well-defined genera (Figs 2–4). Weighting transversions 2 times transitions is the only analysis which gives a monophyletic Nymphalinae, with *Historis* + *Baeotus* coming out as sister to the rest of Nymphalinae.

PHYLOGENETIC PATTERNS IN COEINI

The tribe Coeini does not form a monophyletic group in any of the analyses (Figs 1, 2). In the combined analysis, *Smyrna* is sister to Nymphalini with good

support, *Colobura* and *Tigridia* are sister genera with strong support and they are sister to Nymphalini + *Smyrna*. The monophyly of *Smyrna*, *Tigridia*, *Colobura* and Nymphalini has good support and is stable, though there is conflict from the nuclear genes (Fig. 1). *Historis* and *Baeotus* form a monophyletic group with strong support and both genera are monophyletic (Fig. 1). *Historis odius* and *H. acheronta* (the latter sometimes placed in the genus *Coea*, from which the tribal name is derived) are sister species with only weak support and moderate conflict from the two nuclear genes. *Baeotus*, on the other hand, is a strongly supported monophyletic group with no conflict from the different data partitions. Constraining the five coeine genera to form a monophyletic group results in trees that are 19 steps longer than the most parsimonious trees found for the combined data sets. Constraining *Colobura*, *Tigridia* and *Smyrna* to form a monophyletic group results in trees seven steps longer than the most parsimonious trees.

The association of *Colobura*, *Tigridia* and *Smyrna* with Nymphalini is stable under all the different weighting schemes, as is the clade containing *Historis* and *Baeotus* species. However, the position of *Historis* + *Baeotus* changes as one increases the weight of transversions to transitions. When transversions are weighted 2 times transitions, this clade is sister to Nymphalinae. At higher tested weighting schemes (transversions weighted 3–10 times transitions), *Historis* + *Baeotus* appears as the sister clade to Apaturinae.

PHYLOGENETIC PATTERNS IN NYMPHALINI

Within the monophyletic Nymphalini, relationships within the *Nymphalis*-group of genera (i.e. *Nymphalis*, *Polygonia* and *Aglais*) are almost identical to those hypothesized in a previous study (Wahlberg & Nylin, 2003). The exception is *Polygonia canace*, which is sister to *Nymphalis* in the present study, whereas it was sister to the rest of *Polygonia* in the 2003 study. Wahlberg & Nylin (2003) was based on an additional gene sequence and a morphological data set and thus we prefer the hypothesis supported in that study. *Polygonia oreas* and *P. haroldi* were not included in previous phylogenetic studies of the *Nymphalis*-group, and our results suggest that the former is related to *P. gracilis* and the latter is sister to a clade containing *P. oreas*, *P. gracilis* and *P. satyrus*.

Other genera in Nymphalini are here sampled more broadly than in previous studies and we are now able to identify three additional clades within Nymphalini. Two species of *Antanartia* (*A. delius* and *A. schaenia*) form the sister group to the rest of Nymphalini. The next most basal clade is formed by the genera *Ara-schnia*, *Mynes* and *Symbrenthia*, which group together

with good support. The third major new clade comprises the genera *Vanessa* and *Hypanartia*, though this clade has only moderate support and is not stable. *Vanessa* + *Hypanartia* is sister to the *Nymphalis*-group clade. A surprising result is the position of *Antanartia abyssinica* within *Vanessa*; it is not at all closely related to the other *Antanartia* species. Within *Vanessa*, species often placed in the genus *Cynthia* do not form a monophyletic group, as one species, *V. annabella* (and presumably its putative sister species *V. carye*; Shapiro & Geiger, 1989), appears to be sister to the rest of *Vanessa*.

Sensitivity analyses suggest that the relationships within the *Nymphalis*-group are very stable; the only node which is unstable is the sister relationship between *Polygonia egea* and *P. c-album* + *P. faunus*. *Hypanartia* is stable, as are the relationships of species within the genus. Within *Vanessa*, the sister relationship of *A. abyssinica* to *V. atalanta* + *V. indica* is stable, as is the sister species relationship of *V. gonerilla* + *V. itea*, and the *V. cardui* clade (*V. cardui* to *V. myrinna* in Fig. 2). Other stable clades are *Antanartia delius* + *A. schaenia* and *Symbrenthia*.

PHYLOGENETIC PATTERNS IN KALLIMINI

The tribe Kallimini as currently circumscribed does not form a monophyletic group, but rather a paraphyletic grade with regard to Melitaeini. Constraining it to be monophyletic results in trees that are four steps longer than the most parsimonious trees found for the combined data set. The strict consensus of the most parsimonious trees shows two clades. The basal clade contains the following subclades: (1) a largely Neotropical subclade including *Anartia*, *Siproeta*, *Napeocles* and *Metamorphia* (termed the *Anartia*-clade), (2) the African *Kallimoides*, a subclade with *Vanessula* and *Rhinopalpa* (both monotypic genera that have not previously been associated with each other or indeed with any other kallimine genera) and (3) a subclade that contains the largely African and Asian genera *Junonia*, *Kamilla*, *Precis*, *Hypolimnas*, *Salamis*, *Protogoniomorpha* and *Yoma* (termed the *Junonia*-clade). The second clade in the Kallimini grade, which is sister to the tribe Melitaeini, includes the African *Catacroptera* and *Mallika*, the Asian *Kallima* and the Australasian *Doleschallia* (termed the *Kallima*-clade).

Weighting transversions more than transitions causes *Kallimoides* and *Vanessula* to become sister taxa. These two form the sister clade to *Rhinopalpa* and the *Anartia*- and *Junonia*-clades when transversions are weighted 2 or 3 times transitions. However, weighting schemes of 5 and above cause *Kallimoides*, *Vanessula*, *Rhinopalpa* and the *Anartia*-clade to become the most basal clades in the entire tree, after the outgroups *Heliconius* and *Adelpha*.

Within the *Anartia*-clade, *Napeocles* and *Siproeta* form a strongly supported, stable subclade, as do the three species of *Anartia*. The sister group of *Anartia* may be either *Siproeta/Napeocles* or *Siproeta/Napeocles* + *Metamorphia*. It is clear that *Metamorphia* is an entity distinct from *Siproeta* (as suggested by Fox & Forbes, 1971), though historically *Siproeta stelenes* has occasionally been placed in *Metamorphia*. The clade containing these neotropical genera is stable in sensitivity analyses. The position of *Kallimoides* as the most basal taxon of this clade has only weak support and the node is not stable, suggesting that its placement requires further investigation.

The *Junonia*-clade exhibits some of the greatest surprises of this study. The first is that *Junonia* and *Precis* are separate genera that are not even sister groups. The genus *Kamilla* is clearly within *Junonia*, as was concluded by Shirôzu & Nakanashi (1984) based on morphology (but see Larsen, 1991). In addition, the species *Protogoniomorpha cytora* is found within *Junonia*, rather than with other species of *Protogoniomorpha* or *Salamis*, with which it has always been associated. The sister group relation between *Precis* and *Hypolimnas* has good support and is stable, except when transversions are weighted 3 times transitions, when it is sister to the *Salamis* + *Yoma* + *Protogoniomorpha* + *Junonia* clade. Another surprise is that the African *Protogoniomorpha*, which has been usually considered to be a synonym of *Salamis*, is the sister group to the Australasian *Yoma*, and that *Salamis* is the sister group to *Protogoniomorpha* + *Yoma*. Vári (1979) has argued that species of *Protogoniomorpha* should not be considered to be congeneric with species of *Salamis* based on genitalic differences, a position corroborated by our data. The sister group relationship of *Yoma* and *Protogoniomorpha* has good support and is stable. To complete the surprise, *Salamis*, *Yoma* and *Protogoniomorpha*, usually considered to be related to *Hypolimnas*, are placed in our results as sister group to *Junonia*, though this clade disappears when transversions are weighted 5 or more times transitions.

The implied position of the *Kallima*-clade as sister to the Melitaeini is somewhat surprising given traditional classifications, but this grouping has weak support and may be due to long branch attraction. Indeed the sister group relationship is not stable and disappears when transversions are weighted 2 or more times transitions. The relationships between *Catacroptera*, *Mallika* and *Kallima* are strongly supported and stable. The strongly supported, stable sister relationship of *Catacroptera* and *Mallika* (both monotypic) has been suggested earlier by Shirôzu & Nakanishi (1984). *Catacroptera* and *Mallika* are usually associated with *Junonia* rather than *Kallima* (Larsen, 1991). The recently suggested (Parsons, 1999) associ-

ation of *Doleschallia* with *Kallima* is corroborated by our data, although support is not strong and there is strong conflict between the nuclear genes and the mitochondrial gene regions.

PHYLOGENETIC PATTERNS IN MELITAEINI

The monophyly of Melitaeini is very strongly supported and stable, as are *Euphydryas*, *Melitaea*, Phyciodina and the sister relationship between *Gnathotriche* and *Higginsius* (termed the *Gnathotriche*-group). The *Chlosyne*-group has moderate support but is unstable, with both the COI and *wingless* gene regions conflicting with the EF1- α data. The genus *Chlosyne* itself is a strongly supported, stable clade. The position of *Euphydryas* as the sister group to the rest of the melitaeines is strongly supported and stable, in agreement with previous studies (Kons, 2000; Wahlberg & Zimmermann, 2000). Relationships between the *Chlosyne*-, *Melitaea*-, *Gnathotriche*-groups and Phyciodina are not well-supported and are unstable. The most parsimonious trees from the equally weighted analysis suggests that the *Gnathotriche*-group is sister to Phyciodina with moderate support, and that the *Melitaea*-group is sister to these two with good support (Fig. 4). This arrangement is stable when transversions are weighted 2 and 3 times transitions, but breaks down at higher weights. The sister relationship of the *Gnathotriche*-group and Phyciodina is not stable and disappears after weights of 3 times. This study presents a third novel arrangement of these four clades, with Kons (2000) having the *Chlosyne*-group sister to Phyciodina + *Gnathotriche*-group and Wahlberg & Zimmermann (2000) having the *Chlosyne*-group sister to the *Melitaea*-group.

The positions of two genera (*Poladryas* and *Higginsius*) are not in agreement with previous studies. In contrast to Wahlberg & Zimmermann (2000) but in agreement with Kons (2000), *Poladryas* is here related to the *Chlosyne*-group. Morphological evidence that *Poladryas* is associated with *Higginsius* (Kons, 2000) is quite decisively contradicted by molecular evidence, which places *Higginsius* as sister to *Gnathotriche* with strong support and stability. Within the *Chlosyne*-group, the genera *Microtia*, *Texola* and *Dymasias* form a strongly supported, stable monophyletic group, as found by Kons (2000).

Relationships of the Neotropical Phyciodina (represented by the genera *Mazia*, *Tegosa*, *Eresia*, *Castilia*, *Telenassa*, *Janatella* and *Anthanassa*) are generally not well-supported or stable at the deeper nodes. *Eresia*, *Castilia*, *Telenassa*, *Janatella* and *Anthanassa* do form a stable monophyletic group to the exclusion of *Tegosa* and *Mazia*, even though the *wingless* data partitions is in conflict with the COI and EF1- α data at the node.

DISCUSSION

This is only the second comprehensive phylogenetic analysis of the relationships within a nymphalid subfamily, and the first to use molecular data. The sole previous attempt to infer relationships within a nymphalid subfamily is the recently published study by Penz & Pegg (2003) on Heliconiinae. This is not altogether surprising, given that the circumscriptions of the subfamilies have only recently stabilized (Harvey, 1991; Wahlberg *et al.*, 2003b), and because the high degree of variation in morphological characters among species in Nymphalidae has confounded previous attempts to delineate natural groups (de Jong *et al.*, 1996). In our study, we have been able to identify clades that are well-supported by the three gene regions and stable to varied character state transformation weights, as well as clades that are less robust and therefore likely to change with the addition of more data. Based on our results, we are proposing a new classification of the subfamily Nymphalinae, which is shown in Appendix 2.

THE CIRCUMSCRIPTION OF NYMPHALINAE

We have found that Nymphalinae as currently circumscribed is not monophyletic, although Nymphalini, Kallimini and Melitaeini do form a monophyletic group with the inclusion of three genera traditionally placed in Coeini (i.e. *Colobura*, *Tigridia* and *Smyrna*). Two genera also traditionally placed in Coeini (*Historis* and *Baeotus*) are clearly not related to these three genera and indeed do not appear to be closely related to Nymphalinae. Our results are not unprecedented, as Muysshondt & Muysshondt (1979) argued that based on larval morphology *Smyrna* should be placed in Nymphalini and that *Colobura* is closer to Nymphalini than *Historis* and *Baeotus*. However, the suggestion by Muysshondt & Muysshondt (1979) that Coeini is an unnatural group has not been followed by subsequent authors. On the other hand, Freitas & Brown (2004) found that, based on morphological data, *Historis*, *Colobura* and *Smyrna* formed a monophyletic group sister to *Hypanartia* and *Vanessa*. We were unable to sample the remaining ostensibly coeine genus, *Pycina*, which has morphological features in larvae and pupae that appear to be intermediate between those in *Historis/Baeotus* and *Colobura* (Muysshondt & Muysshondt, 1979). The unstable behaviour of the *Historis* + *Baeotus* clade in our study and its quite different position in the study by Freitas & Brown (2004) suggests that more data are needed to clarify its relationship within Nymphalidae.

THE CIRCUMSCRIPTION OF NYMPHALINI

The well-supported association of *Colobura*, *Tigridia* and *Smyrna* with Nymphalini suggests that they should be incorporated into the tribe, though the traditional delineation of the tribe is also well-supported and, in addition, is stable.

The placement of *Antanartia abyssinica* within *Vanessa* is clear. *Vanessa* (including *A. abyssinica*) is a well-supported clade and the sister relationship of *A. abyssinica* to *V. atalanta* + *V. indica* is stable. Indeed, larval morphology of *A. abyssinica* corroborates our evidence that it is unrelated to other *Antanartia* species and related to *Vanessa* (Nakanishi, 1989). *Antanartia* has been divided into two species-groups, the *delius*-group, comprising *A. delius*, *A. schaenia* and the unsampled *A. borbonica*, and the *hippomene*-group, to which *A. abyssinica*, and the unsampled *A. hippomene* and *A. dimorphica* belong (Howarth, 1966). Whether *A. hippomene* and *A. dimorphica* should also be placed in *Vanessa* is not clear; Nakanishi (1989) noted that the larvae of *A. abyssinica* differed greatly from those of *A. schaenia* and *A. hippomene*. Clearly, the missing species need to be sampled.

The divergent position of *Antanartia* with respect to *Hypanartia* is also surprising, as all species were included in *Hypanartia* prior to the description of *Antanartia* by Rothschild & Jordan (1903). *Antanartia* has always been assumed to be the sister group of *Hypanartia* (e.g. Willmott *et al.*, 2001). However, our results suggest that the sister group to *Hypanartia* is *Vanessa* and that *Antanartia* is sister to all the rest of the traditionally recognized Nymphalini. These positions are stable when transversions are weighted 2 and 3 times transitions, but they break down at higher weights. Weighting 5 and 7 times suggests that *Antanartia* is sister to the *Symbrenthia*/*Mynes*/*Araschnia* clade and that *Hypanartia* is sister to these. In no analysis does *Antanartia* appear as the sister to *Hypanartia*.

Vanessa is usually thought to be the sister group to the *Nymphalis*-group of genera (Wahlberg & Nylin, 2003). Our study suggests that *Vanessa* is sister to *Hypanartia* and that these two clades make up the sister group to the *Nymphalis*-group. This arrangement is stable when transversions are weighted 2 and 3 times transitions, while at higher weights only *Vanessa* is sister to the *Nymphalis*-group. Thus, it is likely that either *Vanessa* or *Vanessa* + *Hypanartia* is more related to the *Nymphalis*-group than to the other genera in Nymphalini, and further new characters (molecular and morphological) will help in refining the relationships further.

The relationships of *Symbrenthia*, *Mynes* and *Araschnia* are rather surprising, especially since the sis-

ter relationship between *Mynes* and *Araschnia* is stable up to a weighting of transversions 7 times transitions. A recent morphological study of this group found that *Mynes* is within *Symbrenthia* (Fric *et al.*, 2004). Normally, *Symbrenthia* has been associated with *Mynes* (Parsons, 1999; Nylin *et al.*, 2001; Wahlberg & Nylin, 2003). For our results to be congruent with those of Fric *et al.* (2004), we would have to find that *Mynes geoffroyi* is sister to *Symbrenthia hypselis*, which is clearly not the case. Obviously, more species of all three genera need to be sampled to resolve the conflicting results of the two studies.

This study contains a much more comprehensive sampling of species in Nymphalini than our two previous studies (Nylin *et al.*, 2001; Wahlberg & Nylin, 2003). The *Nymphalis*-group continues to be a well-supported and stable clade, though the stable position of *Polygonia canace* as sister to species in the genus *Nymphalis* is in contrast to earlier results. *Polygonia canace* is usually placed in its own genus *Kaniska*, and the uncertainty of its position may warrant the use of that genus name. However, our study confirms the stable relationship of *Aglais io* (usually placed in its own genus *Inachis*) with other species of *Aglais* and the stable relationship of *Nymphalis l-album* with other species of *Nymphalis*. Also stable and well-supported is the sister relationship of *Polygonia* and *Nymphalis*, with *Aglais* as sister to these two. This study includes two species of *Polygonia* that have not been included in previous phylogenetic studies, *P. oreas* and *P. haroldi*. The position of *P. oreas* as sister to *P. gracilis* is surprising as it is often considered to be a subspecies of *P. progne* (Scott, 1984). *Polygonia haroldi* is a little known species and morphologically it is somewhat intermediate between *P. progne* and *P. satyrus*. The relationships of species in *Polygonia* are being investigated in more detail currently (E. Weingartner, N. Wahlberg & S. Nylin, unpubl. data).

THE FATE OF KALLIMINI

The monophyly of Kallimini appears to be doubtful. The tribe, as previously circumscribed, never formed a monophyletic group in our analyses. Species belonging to Kallimini have occasionally been placed in higher taxa of their own, and our results show that some of these groups are strongly supported and stable. These well supported groups of genera should be recognized at the tribal level, unless one cares to resort to the (in our opinion, unacceptable) synonymization of Kallimini with Melitaeini. Names are available for two tribes: Victorinini Scudder, 1893 for the *Anartia*-clade and Junoniini Reuter, 1896 for the *Junonia*-clade. The *Kallima*-clade constitutes the newly circumscribed tribe Kallimini. The three remaining genera (*Kallimoides*, *Vanessula* and *Rhinopalpa*) have to remain

incertae sedis until their positions are stabilized by further investigation.

There are 11 species in four genera in the newly circumscribed tribe Victorinini. The close relationship of *Napeocles* and *Siproeta* has never been considered, though *Metamorph* has been considered to be a derived *Siproeta* (Fox & Forbes, 1971). Perhaps the superficial similarity of *Metamorph* to *Siproeta* and the highly distinctive wing patterns of *Napeocles* has led previous investigators to ignore the latter. However, weighting transversions higher than transitions causes *Napeocles* to become sister to *S. stelenes*, which is also recovered in some of the most parsimonious trees of the equally weighted analysis. All weighting schemes other than equal weighting also recover a sister relationship between *Metamorph* and *Anartia*, suggesting that *Metamorph* is not as closely related to *Siproeta* as previously thought. The relationships of the three species of *Anartia* included in this study are in concordance with a previous study on the genus (Blum *et al.*, 2003). It is clear from our study that Victorinini is a well-defined entity that deserves the rank of tribe in the Nymphalinae.

The Junoniini, as circumscribed here, is also a well-defined and stable group. The relationships of the genera in Junoniini found in the equally weighted analysis are stable under fairly severe weighting schemes (transversions weighted up to 5 times transitions). At higher weights, the *Yoma* + *Protogoniomorpha* clade becomes sister to the *Precis* + *Hypolimnas* clade, and *Salamis* is sister to the rest of Junoniini. The close relationships of *Protogoniomorpha* and *Yoma* and *Precis* and *Hypolimnas* are stable under all weighting schemes. The clean separation of *Precis* and *Junonia* in our study is both surprising and gratifying. These two genera have long been conflated in the literature, despite their biogeographical disjunction and the fact that de Lesse (1952) showed clear genitalic differences between them. de Lesse's (1952) delimitations of the two genera are corroborated here. For North American researchers it is important to emphasize that all New World species (including the well-studied *J. coenia*) belong to *Junonia* and that *Precis* is restricted to Africa.

The once common concept of *Kallima* (i.e. containing *Kallimoides*, *Kamilla* and *Mallika*) is clearly untenable, as firmly concluded by Shirôzu & Nakanishi (1984). The Kallimini, as delimited here, contains only four genera: *Kallima*, *Catacroptera*, *Mallika* and *Dole-schallia*.

THE DELINEATION OF MELITAEINI

The monophyly of Melitaeini is beyond doubt. All three gene regions support the clade and it is stable in a variety of weighting schemes. The sister group to

Melitaeini appears to be the newly circumscribed Kallimini and/or Junoniini. Within Melitaeini, the sister relationship of *Euphydryas* to the rest of Melitaeini is in agreement with all previous studies and has a clear, well-supported, stable position in this study. The rest of Melitaeini appears to be divided up into four distinct groups: Phyciodina, Melitaeina (including only *Melitaea*), the *Gnathotriche*-group and the *Chlosyne*-group. Of these, only the *Chlosyne*-group is not stable, though *Poladryas* remains associated with *Chlosyne* with transversions weighted up to 7 times transitions. The *Microtia* + *Texola* + *Dymasia* clade becomes the sister to the rest of Melitaeini (excluding *Euphydryas*) when transversions are weighted 5 and 7 times transitions, though it returns to being sister to *Chlosyne* at the highest weighting scheme. The close relationship of *Microtia*, *Texola* and *Dymasia* was also found by Kons (2000), who suggested that *Texola* and *Dymasia* should be synonymized with *Microtia*.

The close relationship of *Higginsius* to *Gnathotriche* was suggested by Higgins (1981), but Kons (2000) found *Higginsius* to be associated with *Poladryas*. Our results strongly corroborate Higgins' (1981) hypothesis. The two genera are very curious members of the Andean fauna and appear to be always rare (Higgins, 1981). They comprise a total of six species and, with the unsampled Caribbean *Atlantea* and *Antillea* (also very species-poor and always rare), create something of a biogeographical mystery. We find that the sister relationship of *Gnathotriche* and *Higginsius* to Phyciodina is stable with transversions weighted up to 7 times transitions. When transversions are weighted 10 times transitions, the *Gnathotriche*-group becomes sister to *Melitaea*. Since Phyciodina is largely a Neotropical subtribe, its close relationship to the *Gnathotriche*-group is perhaps not surprising, but the possible origin of both groups in South America requires further investigation.

Of the six missing genera, four (*Tisona*, *Dagon*, *Ortilia* and *Phystis*) putatively belong to the subtribe Phyciodina (Higgins, 1981). The remaining two genera, *Antillea* and *Atlantea*, are restricted to the Greater Antilles in the Caribbean and have until recently not been associated with other melitaeine genera. Kons (2000) placed both in the *Chlosyne*-group, with *Atlantea* being sister to *Higginsius*. It is clear that these two genera need to be sampled for molecular data.

A NOTE ON PHYLOGENETIC PATTERNS IN THE OUTGROUPS

Although our study has concentrated on Nymphalinae, we have extensively sampled several potential sister groups to the subfamily. In particular, we have sampled all genera in the tribes Cyrestini and Pseud-

ergolini, which constitute the Cyrestinae in Wahlberg *et al.* (2003b). However, in the present study the two tribes do not form a monophyletic group; rather, Cyrestini appears as the most basal group in the nymphaline clade and Pseudergolini is sister to Apaturinae (Fig. 1). There are four clades in the outgroup appearing with good to strong support that correspond to the Apaturinae, Biblidinae, Cyrestini and Pseudergolini. However, the relationships among these four taxa and Nymphalinae are very poorly supported, are unstable and show much conflict among partitions. Clearly, the nymphaline clade (*sensu* Wahlberg *et al.*, 2003b) needs to be more extensively sampled and more data need to be added before any robust conclusions emerge about relationships of the major clades.

BIOGEOGRAPHICAL HISTORY OF NYMPHALINAE

Despite the limitations of our current phylogenetic hypothesis, some strong patterns emerge from our dispersal-vicariance analysis (Fig. 5). First of all, many dispersal events are required to explain the distribution patterns seen today: 44 when the maximum number of ancestral areas is not constrained and 46 when they are constrained to two. This should not be surprising, since Nymphalinae contains some of the most mobile butterflies known, such as *Vanessa cardui*, which is famous for being able to disperse over thousands of kilometers in one generation. Indeed, the genus *Vanessa*, which is present in all the major zoological biomes of the world, has a highly ambiguous reconstruction of its ancestral distribution. Perhaps the ancestor of *Vanessa* was a widespread species, much like *V. cardui* today, that subsequently speciated during intervals of isolation due to climatic or other factors.

The distribution of the most recent common ancestor of Nymphalinae (excluding *Historis* and *Baotus*) appears to be widespread (Fig. 5), though this may be an artefact of the program used and might be fixed by including outgroups with known ancestral distributions (Ronquist, 1996). We refrained from doing so, as our sampling of the outgroups is not sufficient to resolve their ancestral distributions; also, we are not confident about the current hypothesis of relationships among major groups in the nymphaline clade.

The tribe Nymphalini may have originated in South America, one of the seven possible ancestral areas in the unconstrained analysis (Fig. 5) and one of two in the constrained searches. Africa may have been colonized from South America by the common ancestor of *Smyrna* and the rest of Nymphalini. Once the lineage leading to *Smyrna* split off, it appears that the Palaearctic was colonized from Africa by the common ancestor of *Antanartia* and the rest of Nymphalini

(excluding *Colobura*, *Tigridia* and *Smyrna*). An alternative scenario would have a widespread common ancestor in South America, Africa and the Palaearctic that then speciated through a series of vicariance events (Fig. 5). The Palaearctic has, however, been a very important area for the diversification of the genera *Aglais*, *Nymphalis*, *Polygonia* and possibly *Vanessa*. There have clearly been several independent colonizations of the Nearctic from the Palaearctic by species in the the former three genera.

The clade that includes the tribes Junoniini and Victorinini, as well as *Kallimoides*, *Rhinopalpa* and *Vanessula*, appears to have originated in Africa (Fig. 5). There is a colonization of South America from Africa by the ancestor of *Kallimoides* and Victorinini. Also, the Oriental region appears to have been colonized independently several times. The patterns in this clade are obscured somewhat by the weak support for the current hypothesis of relationships; in addition, many missing species in the genera *Hypolimnna* and *Junonia* are likely to have had a decisive effect on the results due to their presence in the African, Oriental and Australasian areas. However, it is clear that the African region has been instrumental in the diversification of taxa in the clade.

The tribe Kallimini appears to have originated in the Oriental region, with a colonization of Africa by the common ancestor of *Kallima* and *Catacroptera/Mallika* (Fig. 5). This hypothesis needs to be tested by including the several species of *Doleschallia* found exclusively in the Australasian region.

The Nearctic region has been an important area for the diversification of the tribe Melitaeini (Fig. 5). One interpretation of the patterns recovered by DIVA is that the group originated in the Nearctic and subsequently colonized the Palaearctic (the ancestor of *Melitaea*) and the Neotropics (ancestor of the *Gnathotriche*-group and Phyciodina). Interestingly, the current phylogenetic hypothesis suggests a disjunct distribution for the ancestor of *Melitaea* + *Gnathotriche/Higginsius* + Phyciodina being found in the Palaearctic and the Neotropics. Sampling the two missing Caribbean genera will be important to understanding the historical biogeography of Melitaeini.

In summary, it is clear that several regions have been important areas of diversification of clades in Nymphalinae, viz. the Palaearctic for the *Nymphalis*-group of genera, Africa for Junoniini and related genera and the Nearctic for Melitaeini. Our DIVA analysis indicates that dispersal has had a major effect on the distributions of extant species in Nymphalinae. Whether dispersal or vicariance has been the more important process in generating the deeper divergences in Nymphalinae can be tested by dating divergences using molecular clocks calibrated with fossils. Such a study is in preparation and the implications of

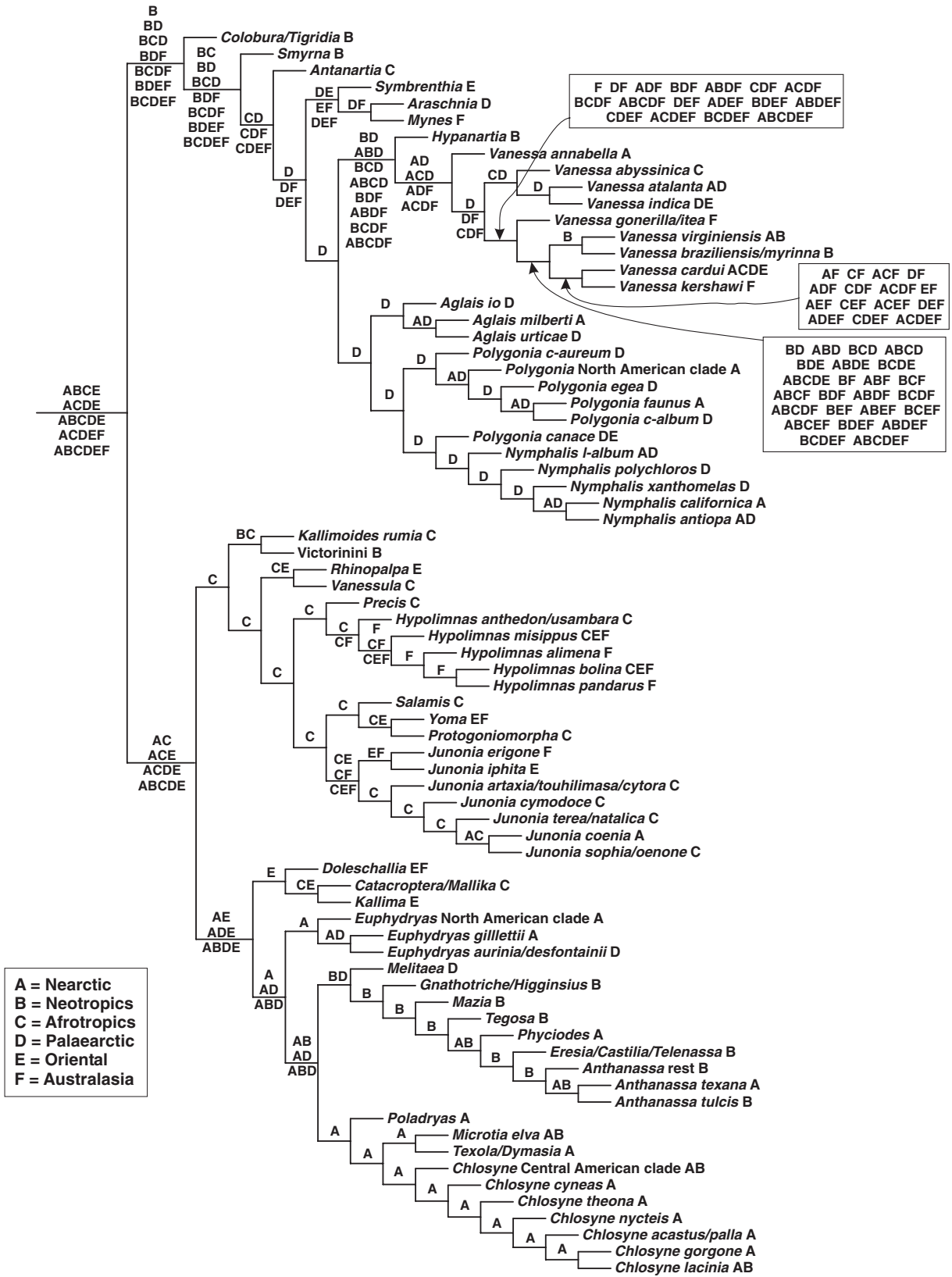


Figure 5. Reconstructed ancestral distributions according to a dispersal-vicariance analysis. Letters after the names of taxa give the current distribution of those taxa.

it will be discussed elsewhere (N. Wahlberg, unpubl. data).

PBS AND SENSITIVITY ANALYSIS

The ease with which molecular data can be generated has led to an ever-increasing number of published phylogenetic studies. Common to almost all these studies is the lack of sensitivity analyses and, in the case of multiple independent data sets, evaluation of congruence at given nodes (but see Reed & Sperling, 1999). An exception to the former are studies based on direct optimization of ribosomal DNA sequences (Wheeler, 1995; Giribet, 2003). Congruence among data partitions is usually evaluated at the level of entire data sets through tests such as the ILD (Farris *et al.*, 1994), rather than through evaluation at every resolved node. Again, a few exceptions are notable (e.g. Gatesy *et al.*, 1999; Cognato & Vogler, 2001; Lambkin *et al.*, 2002; Damgaard & Cognato, 2003; Wahlberg & Nylin, 2003). Both the PBS and sensitivity analysis allow detailed evaluation of which nodes are likely to be robust and stable to the addition of new data, as well as which nodes require further investigation. We emphasize that these are heuristic tools to assess the quality of the most parsimonious hypothesis and guide future sampling of characters and taxa, rather than alternate analytical approaches that challenge the philosophical basis of our preferred optimality criterion.

CONCLUSION

The main conclusions of this study are: (1) *Smyrna*, *Colobura* and *Tigridia* are associated with Nymphalini, (2) 'Kallimini' is more closely related to Melitaeini than to Nymphalini, (3) 'Kallimini' is not monophyletic, but is paraphyletic with regard to Melitaeini, (4) Melitaeini and Nymphalini plus the three coeine genera are strongly supported monophyletic groups, and (5) *Precis* and *Junonia* are not synonymous or even sister groups.

Major unanswered questions are: (1) the positions of *Historis* + *Baeotus* in the nymphaline clade, and (2) the sister group of Nymphalinae. Increased sampling of species in the subfamilies Biblidinae and Apaturinae, as well as increased character data (molecular and morphological), will help to resolve these questions.

ACKNOWLEDGEMENTS

As always, this study would not have been possible without the truly wonderful, altruistic help of both amateur and professional lepidopterists. We are eternally grateful to Jun-ichi Asahi, Alexei Belik, Anton

Chichvarkhin, Tim Davenport, Phil DeVries, André Freitas, Daniel Janzen, Chris Jiggins, Tjeerd Jongeling, Darrell Kemp, Norbert Kondla, Runar Krogen, Gerardo Lamas, Torben B. Larsen, Yi-Hsin Lee, Jorge León-Cortés, Mike Leski, Debra Murray, Naomi Pierce, James Scott, Mike Soukop, Constant' Stefanescu, Martin Steinbauer, Man-Wah Tan, Bruce Walsh, Andy Warren, and Keith Willmott for helping us to obtain specimens. We thank Tiina Berg and Simina Vintila for expert help in the lab. We also thank Andy Warren, Keith Willmott, Carla Penz and Torben B. Larsen for useful comments on a draft version of the manuscript.

REFERENCES

- Ackery PR. 1984.** Systematic and faunistic studies on butterflies. In: Vane-Wright RI, Ackery PR, eds. *The biology of butterflies*. Princeton, NJ: Princeton University Press, 9–21.
- Ackery PR. 1988.** Hostplants and classification: a review of nymphalid butterflies. *Biological Journal of the Linnean Society* **33**: 95–203.
- Ackery PR, de Jong R, Vane-Wright RI. 1999.** The butterflies: Hedyloidea, Hesperoidea and Papilionoidea. In: Kristensen NP, ed. *Lepidoptera, moths and butterflies*. 1. *Evolution, systematics and biogeography. Handbook of Zoology* 4 (35), *Lepidoptera*. Berlin: de Gruyter, 263–300.
- Austin GT, Murphy DD, Baughman JF, Launer AE, Fleishman E. 2003.** Hybridization of checkerspot butterflies in the Great Basin. *Journal of the Lepidopterists' Society* **57**: 176–192.
- Baker RH, DeSalle R. 1997.** Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Systematic Biology* **46**: 654–673.
- Baker RH, Yu X, DeSalle R. 1998.** Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Molecular Phylogenetics and Evolution* **9**: 427–436.
- Blum MJ, Bermingham E, Dasmahapatra K. 2003.** A molecular phylogeny of the neotropical butterfly genus *Anartia* (Lepidoptera: Nymphalidae). *Molecular Phylogenetics and Evolution* **26**: 46–55.
- Brakefield PM, Gates J, Keys D, Kesbeke F, Wijngaarden PJ, Monteiro A, French V, Carroll SB. 1996.** Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**: 236–242.
- Bremer K. 1988.** The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.
- Bremer K. 1994.** Branch support and tree stability. *Cladistics* **10**: 295–304.
- Brower AVZ. 1994.** Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **3**: 159–174.
- Brower AVZ. 2000.** Phylogenetic relationships among the Nymphalidae (Lepidoptera), inferred from partial sequences

- of the *wingless* gene. *Proceedings of the Royal Society of London B* **267**: 1201–1211.
- Brower AVZ, DeSalle R. 1998.** Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. *Insect Molecular Biology* **7**: 73–82.
- Carroll SB, Gates J, Keys DN, Paddock SW, Panganiban GEF, Selegue JE, Williams JA. 1994.** Pattern-formation and eyespot determination in butterfly wings. *Science* **265**: 109–114.
- Clark AH. 1948.** Classification of the butterflies with the allocation of the genera occurring in America north of Mexico. *Proceedings of the Biological Society of Washington* **61**: 77–84.
- Cognato AI, Vogler AP. 2001.** Exploring data interaction and nucleotide alignment in a multiple gene analysis of *Ips* (Coleoptera: Scolytinae). *Systematic Biology* **50**: 758–780.
- Damgaard J, Cognato AI. 2003.** Sources of character conflict in a clade of water striders (Heteroptera: Gerridae). *Cladistics* **19**: 512–526.
- Darlu P, Lecointre G. 2002.** When does the Incongruence Length Difference test fail? *Molecular Biology and Evolution* **19**: 432–437.
- Dasmahaptra KK, Blum MJ, Aiello A, Hackwell S, Davies N, Bermingham EP, Mallet J. 2002.** Inferences from a rapidly moving hybrid zone. *Evolution* **56**: 741–753.
- DeSalle R, Brower AVZ. 1997.** Process partitions, congruence, and the independence of characters: inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. *Systematic Biology* **46**: 751–764.
- DeVries PJ, Kitching IJ, Vane-Wright RI. 1985.** The systematic position of *Antirrhea* and *Caerois*, with comments on the classification of the Nymphalidae (Lepidoptera). *Systematic Entomology* **10**: 11–32.
- Ehrlich PR. 1958.** The comparative morphology, phylogeny and higher classification of the butterflies (Lepidoptera: Papilionoidea). *University of Kansas Science Bulletin* **39**: 305–370.
- Farris JS, Källersjö M, Kluge AG, Bult C. 1994.** Testing the significance of incongruence. *Cladistics* **10**: 315–319.
- Forbes WTM. 1928.** Variation in *Junonia lavinia* (Lepidoptera: Nymphalidae). *Journal of the New York Entomological Society* **36**: 305–320.
- Fox RM, Forbes AC. 1971.** The butterflies of the genera *Siproeta* and *Metamorpha* (Lepidoptera: Nymphalidae). *Annals of the Carnegie Museum* **43**: 223–247.
- Freitas AVL, Brown KSJ. 2004.** Phylogeny of the Nymphalidae (Lepidoptera: Papilionoidea). *Systematic Biology* **53**: 363–383.
- Fric Z, Konvicka M, Zrzavý J. 2004.** Red & black or black & white? Phylogeny of the *Araschnia* butterflies (Lepidoptera: Nymphalidae) and evolution of seasonal polyphenism. *Journal of Evolutionary Biology* **17**: 265–278.
- Gatesy J, O'Grady P, Baker RH. 1999.** Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. *Cladistics* **15**: 271–313.
- Giribet G. 2003.** Stability in phylogenetic formulations and its relation to nodal support. *Systematic Biology* **52**: 554–564.
- Goloboff PA. 1998.** *NONA*. 2.0. Published by the author.
- Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment, editing and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- Hanski I. 1999.** *Metapopulation ecology*. Oxford: Oxford University Press.
- Harvey DJ. 1991.** Higher classification of the Nymphalidae, Appendix B. In: Nijhout HF, ed. *The development and evolution of butterfly wing patterns*. Washington, DC: Smithsonian Institution Press, 255–273.
- Higgins LG. 1981.** A revision of *Phyciodes* Hübner and related genera, with a review of the classification of the Melitaeinae (Lepidoptera: Nymphalidae). *Bulletin of the British Museum of Natural History* **43**: 77–243.
- Howarth TG. 1966.** Revisional notes on the genus *Antanartia* (Lepidoptera: Nymphalidae). *Bulletin of the British Museum of Natural History* **18**: 21–43.
- Janz N, Nylin S, Nyblom K. 2001.** Evolutionary dynamics of host plant specialization: a case study of the tribe Nymphalini. *Evolution* **55**: 783–796.
- de Jong R, Vane-Wright RI, Ackery PR. 1996.** The higher classification of butterflies (Lepidoptera): problems and prospects. *Entomologica Scandinavica* **27**: 65–101.
- Judd DD. 1998.** Exploring component stability using life-stage concordance in sabethine mosquitoes (Diptera: Culicidae). *Cladistics* **14**: 64–94.
- Kons HLJ. 2000.** *Phylogenetic studies of the Melitaeini (Lepidoptera: Nymphalidae: Nymphalinae) and a revision of the genus Chlosyne*. Gainesville: Butler Department of Entomology & Nematology, University of Florida, 799.
- Kuznetsov VI, Stekolnikov AA. 2001.** *New approaches to the system of Lepidoptera of world fauna*. St. Petersburg: Nauka.
- Lambkin CL, Lee MSY, Winterton SL, Yeates DK. 2002.** Partitioned Bremer support and multiple trees. *Cladistics* **18**: 436–444.
- Larsen TB. 1991.** *The butterflies of Kenya and their natural history*. Oxford: Oxford University Press.
- de Lesse H. 1952.** Note sur les genres *Precis* Hb. et *Junonia* Hb. (Lep. Nymphalidae). *Bulletin de la Société Entomologique de France* **57**: 74–77.
- Miller JS, Brower AVZ, DeSalle R. 1997.** Phylogeny of the neotropical moth tribe Josiini (Notodontidae: Dioprinae): comparing and combining evidence from DNA sequences and morphology. *Biological Journal of the Linnean Society* **60**: 297–316.
- Monteiro A, Pierce NE. 2001.** Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1alpha gene sequences. *Molecular Phylogenetics and Evolution* **18**: 264–281.
- Müller W. 1886.** Südamerikanische Nymphalidaeraupen: Versuch eines natürlichen Systems der Nymphaliden. *Zoologische Jahrbücher* **1**: 417–678.
- Muysshondt AJ, Muysshondt A. 1979.** Notes on the life cycle and natural history of butterflies of El Salvador. III C. *Historis odius* and *Coea acheronta* (Nymphalidae-Coloburinae). *Journal of the Lepidopterists' Society* **33**: 112–123.

- Nakanishi A. 1989.** Immature stages of *Antanartia abyssinica* (Felder) (Lepidoptera, Nymphalidae). *Japanese Journal of Entomology* **57**: 712–719.
- Nixon KC. 2002.** WINCLADA, 1.00.08. Published by the author.
- Nylin S. 1988.** Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). *Oikos* **53**: 381–386.
- Nylin S, Nyblom K, Ronquist F, Janz N, Belicek J, Källersjö M. 2001.** Phylogeny of *Polygonia*, *Nymphalis* and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis. *Zoological Journal of the Linnean Society* **132**: 441–468.
- Parsons M. 1999.** *The butterflies of Papua New Guinea: Their systematics and biology*. London: Academic Press.
- Penz C, Peggie D. 2003.** Phylogenetic relationships among Heliconiinae genera based on morphology (Lepidoptera: Nymphalidae). *Systematic Entomology* **28**: 451–479.
- Reed RD, Sperling FAH. 1999.** Interaction of process partitions in phylogenetic analysis: an example from the swallowtail butterfly genus *Papilio*. *Molecular Biology and Evolution* **16**: 286–297.
- Ronquist F. 1996.** DIVA, 1.1. Program and manual available from Uppsala University (ftp.uu.se or ftp.systbot.uu.se).
- Ronquist F. 1997.** Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**: 195–203.
- Rothschild W, Jordan K. 1903.** Lepidoptera collected by Oscar Neumann in north-east Africa. *Novitates Zoologica* **10**: 491–542.
- Scott JA. 1984.** A review of *Polygonia progne* (*oreas*) and *P. gracilis* (*zephyrus*) (Nymphalidae), including a new subspecies from the southern Rocky Mountains. *Journal of Research on the Lepidoptera* **23**: 197–210.
- Scott JA. 1985.** The phylogeny of butterflies (Papilionoidea and Hesperoidea). *Journal of Research on the Lepidoptera* **23**: 241–281.
- Scott JA, Wright DM. 1990.** Butterfly phylogeny and fossils. In: Kudrna O, ed. *Butterflies of Europe*. 2. Wiesbaden, 152–208.
- Shapiro AM, Geiger H. 1989.** Electrophoretic comparisons of vicariant *Vanessa*: genetic differentiation between *V. annabella* and *V. carye* (Nymphalidae) since the Great American Interchange. *Journal of the Lepidopterists' Society* **43**: 81–92.
- Shirôzu T, Nakanashi Nakanishi A. 1984.** A revision of the genus *Kallima* Doubleday (Lepidoptera, Nymphalidae). *Tyô to Ga* **34**: 97–110.
- Silberglied RE. 1984.** Visual communication and sexual selection among butterflies. In: Vane-Wright RI, Ackery PR, eds. *The biology of butterflies*. Princeton, NJ: Princeton University Press, 204–223.
- Singer MC. 1971.** Evolution of food-plant preference in the butterfly *Euphydryas editha*. *Evolution* **25**: 383–389.
- Swofford DL. 2001.** PAUP*: *phylogenetic analysis using parsimony (*and other methods)*, Version 4.0b10. Sunderland, MA: Sinauer Associates.
- Vári L. 1979.** *The butterflies of southern Africa. Part IV: Nymphalidae; Nymphalinae*. Pretoria, South Africa: Transvaal Museum.
- Wahlberg N. 2001.** The phylogenetics and biochemistry of host plant specialization in melitaeine butterflies (Lepidoptera: Nymphalidae). *Evolution* **55**: 522–537.
- Wahlberg N, Nylin S. 2003.** Morphology versus molecules: resolution of the positions of *Nymphalis*, *Polygonia* and related genera (Lepidoptera: Nymphalidae). *Cladistics* **19**: 213–223.
- Wahlberg N, Oliveira R, Scott JA. 2003a.** Phylogenetic relationships of *Phyciodes* butterfly species (Lepidoptera: Nymphalidae): complex mtDNA variation and species delimitations. *Systematic Entomology* **28**: 257–273.
- Wahlberg N, Weingartner E, Nylin S. 2003b.** Towards a better understanding of the higher systematics of Nymphalidae (Lepidoptera: Papilionoidea). *Molecular Phylogenetics and Evolution* **28**: 473–484.
- Wahlberg N, Zimmermann M. 2000.** Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mtDNA sequences. *Cladistics* **16**: 347–363.
- Weller SJ, Pashley DP, Martin JA. 1996.** Reassessment of butterfly family relationships using independent genes and morphology. *Annals of the Entomological Society of America* **89**: 184–192.
- Wheeler WC. 1995.** Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Systematic Biology* **44**: 321–331.
- Willmott KR, Hall JPW, Lamas G. 2001.** Systematics of *Hypanartia* (Lepidoptera: Nymphalidae: Nymphalinae), with a test for geographical speciation mechanisms in the Andes. *Systematic Entomology* **26**: 369–399.
- Zimmermann M, Wahlberg N, Descimon H. 2000.** Phylogeny of *Euphydryas* checkerspot butterflies (Lepidoptera: Nymphalidae) based on mitochondrial DNA sequence data. *Annals of the Entomological Society of America* **93**: 347–355.

APPENDIX 1

List of species sampled in this study along with the GenBank accession numbers of the three genes sequenced. For images of voucher specimens, see <http://www.zoologi.su.se/research/wahlberg>

Higher taxon	Species	Voucher code	Locality	COI	EF1- α	<i>wingless</i>
HELICONIINAE	<i>Heliconius hecale</i>	NW70-6	COSTA RICA: Butterfly farm supplier	AY090202	AY090168	AY090135
LIMENITIDINAE	<i>Adelpha bredowi</i>	NW107-16	USA: Oregon	AY788591	AY788693	AY788457
CYRESTINAE	<i>Cyrestis thyodamas</i>	NW100-11	BANGLADESH: Sylhet Division	AY218240	AY218260	AY218278
	<i>Chersonesia rahria</i>	NW111-3	INDONESIA: Sulawesi, Seko	AY788601	AY788703	AY788465
	<i>Marpesia orsilochus</i>	RB250	BRAZIL: Rondonia, Ariquemes	AY788604	AY788706	AF246532
	<i>Marpesia chiron</i>	RB227	BRAZIL: Rondonia, Ariquemes	AY788603	AY788705	AY788467
	<i>Amnosia decora</i>	NW101-1	INDONESIA: W. Sumatra, Brastagi	AY218235	AY218254	AY218273
	<i>Dichorragia nesimachus</i>	NW111-10	PHILIPPINES: S. Leyte, Hinunangan	AY788602	AY788704	AY788466
	<i>Pseudergolis wedah</i>	NW118-1	VIETNAM: Lao Cai	AY788605	AY788707	AY788468
	<i>Stibochiona nicea</i>	NW100-10	BANGLADESH: Sylhet Division	AY218249	AY218269	AY218287
BIBLIDINAE	<i>Ariadne enotrea</i>	NW82-10	UGANDA: Kibale Forest	AY218237	AY218256	AY218274
	<i>Byblia anvatara</i>	NW88-14	ZIMBABWE: Marondera	AY788595	AY788697	AY788460
	<i>Callicore pacifica</i>	NW119-3	COSTA RICA: AC Guanacaste, 03-srmp-11744	AY788596	AY788698	AY788461
	<i>Catonephele numilia</i>	NW62-5	COSTA RICA: Butterfly farm supplier	AY090215	AY090181	AY090148
	<i>Dynamine maeon</i>	RB249	BRAZIL: Rondonia, Ariquemes	AY788597	AY788699	AF246581
	<i>Eurytela dryope</i>	NW82-6	TANZANIA: Amani	AY218242	AY218262	AY218280
	<i>Hamadryas februa</i>	NW62-3	COSTA RICA: Butterfly farm supplier	AY090216	AY090182	AY090149
	<i>Mesoxantha esothea</i>	NW83-5	UGANDA: Kibale Forest	AY788598	AY788700	AY788462
	<i>Myscelia capensis</i>	NW109-4	ECUADOR	AY788599	AY788701	AY788463
	<i>Nica flavilla</i>	NW85-11	PERU: Road to Yurimaguas	AY218245	AY218265	AY218283
	<i>Panacea regina</i>	NW109-8	ECUADOR	AY788600	AY788702	AY788464
	<i>Sevenia boisduvali</i>	NW88-15	ZIMBABWE: Harare	AY218247	AY218267	AY218285
APATURINAE	<i>Apatura iris</i>	NW69-6	Butterfly farm supplier	AY090199	AY090165	AY090132
	<i>Asterocampa leilia</i>	NW82-15	USA: Arizona	AF187734	AY218257	AY218275
	<i>Eulaceura osteria</i>	NP95-Y227	MALAYSIA (MCZ voucher)	AY788593	AY788695	AF246588
	<i>Mimathyma schrenckii</i>	NW84-7	RUSSIA: Primorye	AY788594	AY788696	AY788459
	<i>Timelaea maculata</i>	NW97-8	TAIWAN: Taitung County	AY218251	AY218271	AY218289
	<i>Chitoria chrysolora</i>	NW97-11	TAIWAN: Taitung County	AY788592	AY788694	AY788458
NYMPHALINAE	<i>Baeotus japetus</i>	NW130-16	ECUADOR: Pastaza, Lorocachi	AY788613	AY788718	AY788479
'Coeini'	<i>Baeotus deucalion</i>	NW130-13	ECUADOR: Napo, Rio Yuturi	AY788616	AY788721	AY788482
	<i>Baeotus aeilus</i>	NW130-14	ECUADOR: Sucumbios, Cerro Lumbaqui	AY788614	AY788719	AY788480
	<i>Baeotus beotus</i>	NW130-15	ECUADOR: Imbabura, Rio Cachaco	AY788615	AY788720	AY788481
	<i>Historis odius</i>	NW81-7	COSTA RICA: Butterfly farm supplier	AY788632	AY788751	AY788512
	<i>Historis acheronta</i>	PE-18-16	PERU: Madre de Dios, Tambopata Pres.	AY788631	AY788750	AY788511
	<i>Colobura dirce</i>	NW68-11	COSTA RICA: Butterfly farm supplier	AY090228	AY090196	AY090162

APPENDIX 1 *Continued*

Higher taxon	Species	Voucher code	Locality	COI	EF1- α	wingless
Nymphalini	<i>Tigridia acesta</i>	RB349	BRAZIL: Rondonia, Ariquemes	AY788684	AY788822	AY788582
	<i>Smyrna blomfieldia</i>	NW85-2	BRAZIL: São Paulo	AY788678	AY788816	AY788576
	<i>Anantanartia abyssinica</i>	NW86-7	TANZANIA: Kitumbeine	AY788609	AY788711	AY788472
	<i>Anantanartia delius</i>	NW82-4	UGANDA: Kibale Forest	AY788610	AY788712	AY788473
	<i>Anantanartia schaenia</i>	NW65-5	CAMEROON	AY218236	AY218255	AF412780
	<i>Araschnia levana</i>	NW39-2	ESTONIA	AY248780	AY248805	AF412762
	<i>Mynes geoffroyi</i>	NW63-20	AUSTRALIA: Queensland	AY248778	AY248803	AF412760
	<i>Symbrenthia hypselis</i>	NW65-2	MALAYSIA: Selangor	AY248779	AY248804	AF412784
	<i>Symbrenthia hypselis</i>	NW97-2	TAIWAN: Kauhsiung County	AY788680	AY788818	AY788578
	<i>Symbrenthia lileia</i>	NW97-3	TAIWAN: Kauhsiung County	AY788679	AY788817	AY788577
	<i>Hypanartia bella</i>	PE-10-7	PERU: Cuzco, Quebrada Chapimayo	AY788638	AY788757	AF246590
	<i>Hypanartia charon</i>	NW89-11	ECUADOR: Sucumbios	AY788639	AY788758	AY788518
	<i>Hypanartia kefersteinii</i>	NW89-6	ECUADOR: Sucumbios	AY788640	AY788759	AY788519
	<i>Hypanartia lethe</i>	NW36-6	BRAZIL: Minas Gerais	AF187774	AY788760	AY788520
	<i>Hypanartia lindigii</i>	PE-05 A-15	PERU: Cuzco, Quebrada San Luis	AY248781	AY248806	AF412759
	<i>Vanessa annabella</i>	NW74-4	USA: Wyoming	AY788685	AY788823	AY788583
	<i>Vanessa atalanta</i>	NW63-21	SWEDEN: Stockholm	AY090221	AY090187	AF412772
	<i>Vanessa brazilensis</i>	NW89-4	ECUADOR: Esmeraldas	AY788686	AY788824	AY788584
	<i>Vanessa cardui</i>	NW63-3	USA: Missouri	AY248782	AY248807	AF412770
	<i>Vanessa gonerilla</i>	NW63-4	NEW ZEALAND	AY248784	AY248809	AF412782
	<i>Vanessa indica</i>	NW63-9	JAPAN	AY788687	AY788825	AY788585
	<i>Vanessa itea</i>	NW63-14	NEW ZEALAND	AY788688	AY788826	AY788586
	<i>Vanessa kershawi</i>	NW77-3	AUSTRALIA: South Australia	AY788689	AY788827	AY788587
	<i>Vanessa myrinna</i>	NW36-5	BRAZIL: Minas Gerais	AY788690	AY788828	AY788588
	<i>Vanessa virginiensis</i>	NW77-16	USA: Tennessee	AY248783	AY248808	AY248827
	<i>Aglais io</i>	NW63-16	SWEDEN: Stockholm	AY248785	AY248810	AF412766
	<i>Aglais milberti</i>	NW77-14	USA: Washington	AY248787	AY248812	AY248828
<i>Aglais urticae</i>	NW63-3	SWEDEN: Stockholm	AY248786	AY248811	AF412777	
<i>Nymphalis antiopa</i>	NW70-2	SWEDEN: Stockholm	AY218246	AY218266	AY218284	
<i>Nymphalis californica</i>	NW74-14	USA: Oregon	AY248789	AY248814	AY248830	
<i>Nymphalis l-album</i>	NW78-1	CANADA: British Columbia	AY248791	AY248816	AY248832	
<i>Nymphalis polychloros</i>	NW62-2	SWEDEN: Öland	AY248788	AY248813	AY248829	
<i>Nymphalis xanthomelas</i>	NW84-1	RUSSIA: Yakutia	AY248790	AY248815	AY248831	
<i>Polygonia canace</i>	EW19-11	JAPAN	AY248792	AY248817	AY248833	
<i>Polygonia c-album</i>	NW70-3	SWEDEN: Stockholm	AY090222	AY090188	AY090154	
<i>Polygonia c-aureum</i>	NW65-8	JAPAN	AY248799	AY248824	AF412786	
<i>Polygonia comma</i>	NW65-6	USA: Tennessee	AY248794	AY248819	AF412781	
<i>Polygonia egea</i>	NW77-15	GREECE	AY248800	AY248825	AY248838	
<i>Polygonia faunus</i>	NW74-12	USA: Oregon	AY248798	AY248823	AY248837	

<i>Polygona haroldi</i>	NW112-3	MEXICO: Sonora, Yecora	AY788662	AY788800	AY788560
<i>Polygona interrogattonis</i>	NW77-12	USA: Tennessee	AY248793	AY248818	AY248834
<i>Polygona oreas</i>	NW74-10	USA: Oregon	AY788663	AY788801	AY788561
<i>Polygona satyrus</i>	NW74-9	USA: Oregon	AY248796	AY248821	AY248835
<i>Polygona gracilis</i>	NW74-6	USA: Oregon	AY248797	AY248822	AY248836
<i>Anartia amathaea</i>	NW68-5	COSTA RICA: Butterfly farm supplier	AY788606	AY788708	AY788469
<i>Anartia fatima</i>	NW66-5	COSTA RICA: Butterfly farm supplier	AY788607	AY788709	AY788470
<i>Anartia jatrophae</i>	NW36-6	BRAZIL: Minas Gerais	AY788608	AY788710	AY788471
<i>Siproeta epaphus</i>	NW64-7	COSTA RICA: Butterfly farm supplier	AY788677	AY788815	AY788575
<i>Siproeta stelenes</i>	NW69-5	COSTA RICA: Butterfly farm supplier	AY218248	AY218268	AY218286
<i>Napeocles jucunda</i>	NW85-1	BRAZIL: Mato Grosso	AY788661	AY788788	AY788548
<i>Metamorpha elissa</i>	PE-10-10	PERU: Cuzco, Quebrada Chaupimayo	AY788658	AY788784	AY788544
<i>Hypolimnas alimena</i>	NW81-1	AUSTRALIA: Queensland	AY788633	AY788752	AY788513
<i>Hypolimnas anthedon</i>	NW68-8	KENYA: Butterfly farm supplier	AY788634	AY788753	AY788514
<i>Hypolimnas bolina</i>	NW62-6	AUSTRALIA: Queensland	AY090224	AY090190	AY090156
<i>Hypolimnas misippus</i>	NW68-3	KENYA: Butterfly farm supplier	AY788635	AY788754	AY788515
<i>Hypolimnas pandarus</i>	NW80-11	INDONESIA: Ceram Island	AY788636	AY788755	AY788516
<i>Hypolimnas usambara</i>	NW66-4	KENYA: Butterfly farm supplier	AY788637	AY788756	AY788517
<i>Precis andremiaja</i>	NW111-6	MADAGASCAR: Mandraka	AY788664	AY788802	AY788562
<i>Precis antilope</i>	NW88-4	ZIMBABWE: Marondera	AY788665	AY788803	AY788563
<i>Precis archesia</i>	NW88-6	ZIMBABWE: Marondera	AY788666	AY788804	AY788564
<i>Precis ceryne</i>	NW88-3	ZIMBABWE: Marondera	AY788667	AY788805	AY788565
<i>Precis cuama</i>	NW83-13	ZIMBABWE: Harare	AY788668	AY788806	AY788566
<i>Precis octavia</i>	NW68-9	KENYA: Butterfly farm supplier	AY788669	AY788807	AY788567
<i>Precis sinuata</i>	NW83-1	UGANDA: Kibale Forest	AY788670	AY788808	AY788568
<i>Precis tugela</i>	NW114-15	ZAMBIA: N of Mwinilunga, Lesombo R	AY788671	AY788809	AY788569
<i>Rhinopalpa polynice</i>	NW81-5	MALAYSIA	AY788674	AY788812	AY788572
<i>Salamis anteva</i>	NW111-9	MADAGASCAR: Mandraka	AY788675	AY788813	AY788573
<i>Salamis cacta</i>	NW82-3	UGANDA: Kibale Forest	AY788676	AY788814	AY788574
<i>Yoma algina</i>	NW80-13	PNG: Morobe Prov., Wau Valley	AY788692	AY788830	AY788590
<i>Protogoniomorpha anacardii</i>	NW73-15	KENYA: Butterfly farm supplier	AY090223	AY090189	AY090155
<i>Pr. parhassus</i>	NW82-7	UGANDA: Kibale Forest	AY788673	AY788811	AY788571
<i>Pr. cytora</i>	NW123-23	GHANA	AY788672	AY788810	AY788570
<i>Junonia artaxia</i>	NW114-11	ZAMBIA: S of Mwinilunga	AY788642	AY788762	AY788522
<i>Junonia coenia</i>	NW85-13	USA: Tennessee	AY788643	AY248801	AY248826
<i>Junonia iphita</i>	NW68-17	Butterfly farm supplier	AY090225	AY090191	AY090157
<i>Junonia erigone</i>	NW81-2	PNG: Morobe Province, Bulolo	AY788644	AY788763	AY788523
<i>Junonia natalica</i>	NW68-13	KENYA: Butterfly farm supplier	AY788645	AY788764	AY788524
<i>Junonia oenone</i>	NW68-1	KENYA: Butterfly farm supplier	AY788646	AY788765	AY788525
<i>Junonia sophia</i>	NW83-10	UGANDA: Kibale Forest	AY788647	AY788766	AY788526
<i>Junonia terca</i>	NW68-15	KENYA: Butterfly farm supplier	AY788648	AY788767	AY788527
<i>Junonia touhilimasa</i>	NW95-15	ZAMBIA: Kalene Hill	AY788649	AY788768	AY788528

†Kallimini†

APPENDIX 1 *Continued*

Higher taxon	Species	Voucher code	Locality	COI	EF1- α	<i>wingless</i>
	<i>Kamilla cymodoce</i>	NW114-8	ZAMBIA: N of Mwinilunga, Lesombo R	AY788652	AY788771	AY788531
	<i>Kallima inachus</i>	NW85-15	Butterfly farm supplier	AY788650	AY788769	AY788529
	<i>Kallima paralekta</i>	NW62-8	Butterfly farm supplier	AY090229	AY090197	AY090163
	<i>Kallimoides rumia</i>	NW96-8	GHANA: Ashanti Region	AY788651	AY788770	AY788530
	<i>Catacroptera cloanthe</i>	NW88-1	ZIMBABWE: Marondera	AY788619	AY788724	AY788485
	<i>Mallika jacksoni</i>	NW122-6	TANZANIA	AY788653	AY788772	AY788532
	<i>Doleschallia bisaltide</i>	NW64-5	Butterfly farm supplier	AY788621	AY788735	AY788496
	<i>Vanessula milca</i>	NW96-5	GHANA: Ashanti Region	AY788691	AY788829	AY788589
Melitaeini	<i>Euphydryas aurinia</i>	NW6-4	FRANCE: Cerveières	AF187746	AY788743	AY788504
	<i>Euphydryas chalcedona</i>	NW14-4	USA: California	AF187752	AY788744	AY788505
	<i>Euphydryas desfontainii</i>	NW70-4	SPAIN: El Guix	AY090226	AY090193	AY090159
	<i>Euphydryas editha</i>	NW5-8	USA: California	AF187765	AY788745	AY788506
	<i>Euphydryas gillettii</i>	NW24-6	USA: Montana	AF187771	AY788746	AY788507
	<i>Euphydryas phaeton</i>	NW13-3	USA: Maryland	AF187797	AY788747	AY788508
	<i>Chlosyne acastus</i>	NW35-15	USA: Colorado	AF187735	AY788725	AY788486
	<i>Chlosyne cyneas</i>	NW38-17	USA: Arizona	AF187757	AY788726	AY788487
	<i>Chlosyne gaudedalis</i>	NW37-2	COSTA RICA: La Selva	AF187770	AY788727	AY788488
	<i>Chlosyne gorgone</i>	NW34-4	USA: Colorado	AF187772	AY788728	AY788489
	<i>Chlosyne harrisii</i>	NW35-10	USA: New York	AF187773	AY788729	AY788490
	<i>Chlosyne janais</i>	NW62-1	COSTA RICA: Butterfly farm supplier	AY788620	AY788730	AY788491
	<i>Chlosyne lacinia</i>	NW62-4	COSTA RICA: Butterfly farm supplier	AY090227	AY090195	AY090161
	<i>Chlosyne narva</i>	NW37-3	COSTA RICA: La Selva	AF187786	AY788731	AY788492
	<i>Chlosyne nycteis</i>	NW34-5	USA: Colorado	AF187788	AY788732	AY788493
	<i>Chlosyne palla</i>	NW20-4	USA: California	AF187791	AY788733	AY788494
	<i>Chlosyne theona</i>	NW27-6	USA: Arizona	AF187808	AY788734	AY788495
	<i>Dymasia dymas</i>	NW27-7	USA: Arizona	AF187764	AY788785	AY788545
	<i>Microtia elva</i>	NW61-1	MEXICO: Chiapas	AY788660	AY788787	AY788547
	<i>Texola elada</i>	NW7-1	USA: Texas	AY788659	AY788786	AY788546
	<i>Poladryas arachne</i>	NW27-4	USA: California	AF187740	AY788799	AY788559
	<i>Melitaea arduinna</i>	NW23-5	GREECE: Pissoderoi	AF187742	AY788774	AY788534
	<i>Melitaea britomartis</i>	NW69-8	SWEDEN	AY788655	AY788775	AY788535
	<i>Melitaea cinxia</i>	NW73-14	SWEDEN: Stockholm	AY788656	AY788776	AY788536
	<i>Melitaea deione</i>	NW95-5	FRANCE: Aude	AY788657	AY788777	AY788537
	<i>Melitaea didymoides</i>	NW26-1	RUSSIA: Buryatia	AF187762	AY090194	AY090160
	<i>Melitaea latonigena</i>	NW25-3	RUSSIA: Buryatia	AF187780	AY788778	AY788538

<i>Melitaea perseae</i>	NW34-10	LEBANON: Mohafazat Beharré	AF187796	AY788779	AY788539
<i>Melitaea punica</i>	NW34-11	LEBANON: Mohafazat Kesronan	AF187803	AY788781	AY788541
<i>Melitaea scotostia</i>	NW27-11	CHINA: Hebei Province	AF187804	AY788780	AY788540
<i>Melitaea trivita</i>	NW23-6	GREECE: Pissoderi	AF187810	AY788782	AY788542
<i>Melitaea varia</i>	NW24-13	FRANCE: Laus de Cervières	AF187812	AY788783	AY788543
<i>Gnathotriche exclamatoris</i>	NW89-9	ECUADOR: Sucumbios	AY788629	AY788748	AY788509
<i>Higginsius fasciatus</i>	PE-10-20	PERU: Cuzco, Quebrada Chaupimayo	AY788630	AY788749	AY788510
<i>Anthanassa drusilla</i>	NW76-6	ECUADOR: Esmeraldas	AY788611	AY788714	AY788475
<i>Anthanassa texana</i>	NW12-6	USA: Texas	AF187806	AY788716	AY788477
<i>Anthanassa ardyi</i>	NW22-4	COSTA RICA: Monteverde	AF187743	AY788713	AY788474
<i>Anthanassa otanes</i>	NW24-4	COSTA RICA: Monteverde	AF187790	AY788715	AY788476
<i>Anthanassa tulcis</i>	NW104-12	PANAMA: Gamboa	AY788612	AY788717	AY788478
<i>Castilia erantites</i>	NW76-2	ECUADOR: Pichincha	AY788617	AY788722	AY788483
<i>Castilia ofella</i>	NW105-3	PANAMA: Achioté Road	AY788618	AY788723	AY788484
<i>Eresia clio</i>	NW76-5	ECUADOR: Esmeraldas	AY788622	AY788736	AY788497
<i>Eresia coela</i>	NW104-3	PANAMA: Path to Gloria Alta	AY788623	AY788737	AY788498
<i>Eresia eunice</i>	NW92-5	BRAZIL: São Paulo	AY788624	AY788738	AY788499
<i>Eresia letitia</i>	NW91-9	ECUADOR: Sucumbios	AY788625	AY788739	AY788500
<i>Eresia quintilla</i>	NW76-3	ECUADOR: Esmeraldas	AY788627	AY788741	AY788502
<i>Eresia pelonia</i>	NW108-11	PERU	AY788626	AY788740	AY788501
<i>Eresia sestia</i>	NW76-8	ECUADOR: Esmeraldas	AY788628	AY788742	AY788503
<i>Janatella leucodesma</i>	NW85-16	PANAMA: Gamboa	AY788641	AY788761	AY788521
<i>Mazia amazonica</i>	NW76-6	ECUADOR	AY788654	AY788773	AY788533
<i>Phyciodes batesii</i>	NW72-4	CANADA: Ontario	AF187747	AY788789	AY788549
<i>Phyciodes cocyta</i>	NW11-4	CANADA: British Columbia	AF187755	AY090192	AY090158
<i>Phyciodes mylitta</i>	NW11-10	CANADA: British Columbia	AF187785	AY788791	AY788551
<i>Phyciodes orseis</i>	NW67-3	USA: California	AY156631	AY788792	AY788552
<i>Phyciodes pallescens</i>	NW64-2	MEXICO: Michoacán	AY156640	AY788793	AY788553
<i>Phyciodes pallida</i>	NW34-6	USA: Colorado	AF187792	AY788794	AY788554
<i>Phyciodes phaon</i>	NW35-11	MEXICO: Mazatlan	AF187798	AY788795	AY788555
<i>Phyciodes picta</i>	NW34-7	USA: Colorado	AF187800	AY788796	AY788556
<i>Phyciodes pulchella</i>	NW67-14	USA: Oregon	AY156662	AY788797	AY788557
<i>Phyciodes tharos</i>	NW34-2	USA: Minnesota	AF187807	AY788798	AY788558
<i>Phyciodes graphica</i>	NW67-9	MEXICO: Mexico State	AY156684	AY788790	AY788550
<i>Telenassa trimaculata</i>	NW91-6	ECUADOR: Sucumbios	AY788683	AY788821	AY788581
<i>Tegosa anieta</i>	NW91-11	ECUADOR: Sucumbios	AY788681	AY788819	AY788579
<i>Tegosa tissooides</i>	NW76-4	ECUADOR: Esmeraldas	AY788682	AY788820	AY788580

APPENDIX 2

Proposed higher classification of the subfamily Nymphalinae based on results presented in this paper. Genera marked with asterisks were not sampled for this study. For a full synonymic list of the species please see <http://www.zoologi.su.se/research/wahlberg>

Subfamily NYMPHALINAE Rafinesque, 1815

incertae sedis

- Pycina** Doubleday, 1849
- Kallimoides* Shirôzu & Nakanishi, 1984
- Vanessula* Dewitz, 1887
- Rhinopalpa* Felder & Felder, 1860

Tribe Coeini Scudder, 1893

- Historis* Hübner, 1819
- = *Coea* Hübner, 1819
- = *Aganisthos* Boisduval & Le Conte, 1835
- = *Megistanis* Doubleday, 1845
- = *Megistanis* Boisduval, 1870

Baeotus Hemming, 1939

Tribe Nymphalini Rafinesque, 1815

- Colobura* Billberg, 1820
- = *Gynoecia* Doubleday, 1845
- Tigridia* Hübner, 1819
- = *Callizona* Doubleday, 1848
- Smyrna* Hübner, 1823
- Antanartia* Rothschild & Jordan, 1903
- Araschnia* Hübner, 1819

- Symbrenthia* Hübner, 1819
- = *Laogona* Boisduval, 1836
- = *Brensymthia* Huang 2001

Mynes Boisduval, 1832

- Hypanartia* Hübner, 1821
- = *Eurema* Doubleday, 1845
- Vanessa* Fabricius, 1807
- = *Nymphalis* Latreille, 1804
- = *Cynthia* Fabricius, 1807
- = *Pyrameis* Hübner, 1819
- = *Bassaris* Hübner, 1821
- = *Ammiralis* Rennie, 1832
- = *Neopyrameis* Scudder, 1889
- = *Fieldia* Niculescu, 1979

Aglais Dalman, 1816

- = *Ichnusa* Reuss, 1939
- = *Inachis* Hübner, 1819
- = *Hamadryas* Hübner, 1806

Nymphalis Kluk, 1780

- = *Scudderia* Grote, 1873
- = *Eu Vanessa* Scudder, 1889
- = *Roddia* Korshunov, 1996

Polygonia Hübner, 1819

- = *Kaniska* Moore, 1899
- = *Eugonia* Hübner, 1819
- = *Comma* Rennie, 1832
- = *Grapta* Kirby, 1837

Tribe Victorinini Scudder, 1893

- Anartia* Hübner, 1819
- = *Celaena* Doubleday, 1849
- = *Celoena* Boisduval, 1870
- = *Anartiella* Fruhstorfer, 1907
- Siproeta* Hübner, 1823
- = *Victorina* Blanchard, 1840
- = *Amphirene* Doubleday, 1845
- = *Amphirene* Boisduval, 1870
- = *Aphnaea* Capronnier, 1881

Napeocles Bates, 1864*Metamorphia* Hübner, 1819

Tribe Junoniini Reuter, 1896

- Junonia* Hübner, 1819
- = *Alcyoneis* Hübner, 1819
- = *Aresta* Billberg, 1820
- = *Kamilla* Collins & Larsen, 1991

Salamis Boisduval, 1833

- Yoma* Doherty, 1886
- = *Yoma* de Nicéville, 1886

Protogoniomorpha Wallengren, 1857

- Precis* Hübner, 1819
- = *Coryphaeola* Butler, 1878
- = *Kallimula* Holland, 1920

Hypolimnas Hübner, 1819

- = *Esoptria* Hübner, 1819
- = *Diadema* Boisduval, 1832
- = *Euralia* Westwood, 1850
- = *Eucalia* Felder, 1861

Tribe Kallimini Doherty, 1886

- Kallima* Doubleday, 1849
- Doleschallia* Felder & Felder, 1860
- = *Apatura* Hübner, 1819
- Catacroptera* Karsch, 1894
- Mallika* Collins & Larsen, 1991

Tribe Melitaeini Newman, 1870

incertae sedis

- Antillea** Higgins, 1959
- Atlantea** Higgins, 1959
- Gnathotriche* Felder & Felder, 1862
- = *Gnathotrusia* Higgins, 1981
- Higginsius* Hemming, 1964
- = *Fulvia* Higgins, 1959
- Chlosyne* Butler, 1870
- = *Morpheis* Geyer, 1833
- = *Synchloe* Doubleday, 1845
- = *Anemeca* Kirby, 1871
- = *Coatlantona* Kirby, 1871
- = *Limnaecia* Scudder, 1872
- = *Charidryas* Scudder, 1872
- = *Thessalia* Scudder, 1875

Microtia Bates, 1864

- Texola* Higgins, 1959
- Dymasia* Higgins, 1960
- Poladryas* Bauer, 1961

Subtribe Euphydryina Higgins, 1978

- Euphydryas* Scudder, 1872
- = *Lemonias* Hübner, 1806
- = *Occidryas* Higgins, 1978
- = *Eurodryas* Higgins, 1978
- = *Hypodryas* Higgins, 1978

Subtribe Melitaeina Newman, 1870

- Melitaea* Fabricius, 1807
- = *Lucina* Rafinesque, 1815
- = *Schoenis* Hübner, 1819
- = *Cinclidia* Hübner, 1819
- = *Mellicta* Billberg, 1820
- = *Didymaeformia* Verity, 1950
- = *Athaliaeformia* Verity, 1950

Subtribe Phyciodina Higgins, 1981

- Phyciodes* Hübner, 1819
- Phystis** Higgins, 1981
- Anthanassa* Scudder, 1875
- = *Tritanassa* Forbes, 1945
- Dagon** Higgins, 1981
- Telenassa* Higgins, 1981
- Ortilia** Higgins, 1981
- Tisona** Higgins, 1981
- Tegosa* Higgins, 1981
- Eresia* Boisduval, 1836
- Castilia* Higgins, 1981
- Janatella* Higgins, 1981
- Mazia* Higgins, 1981