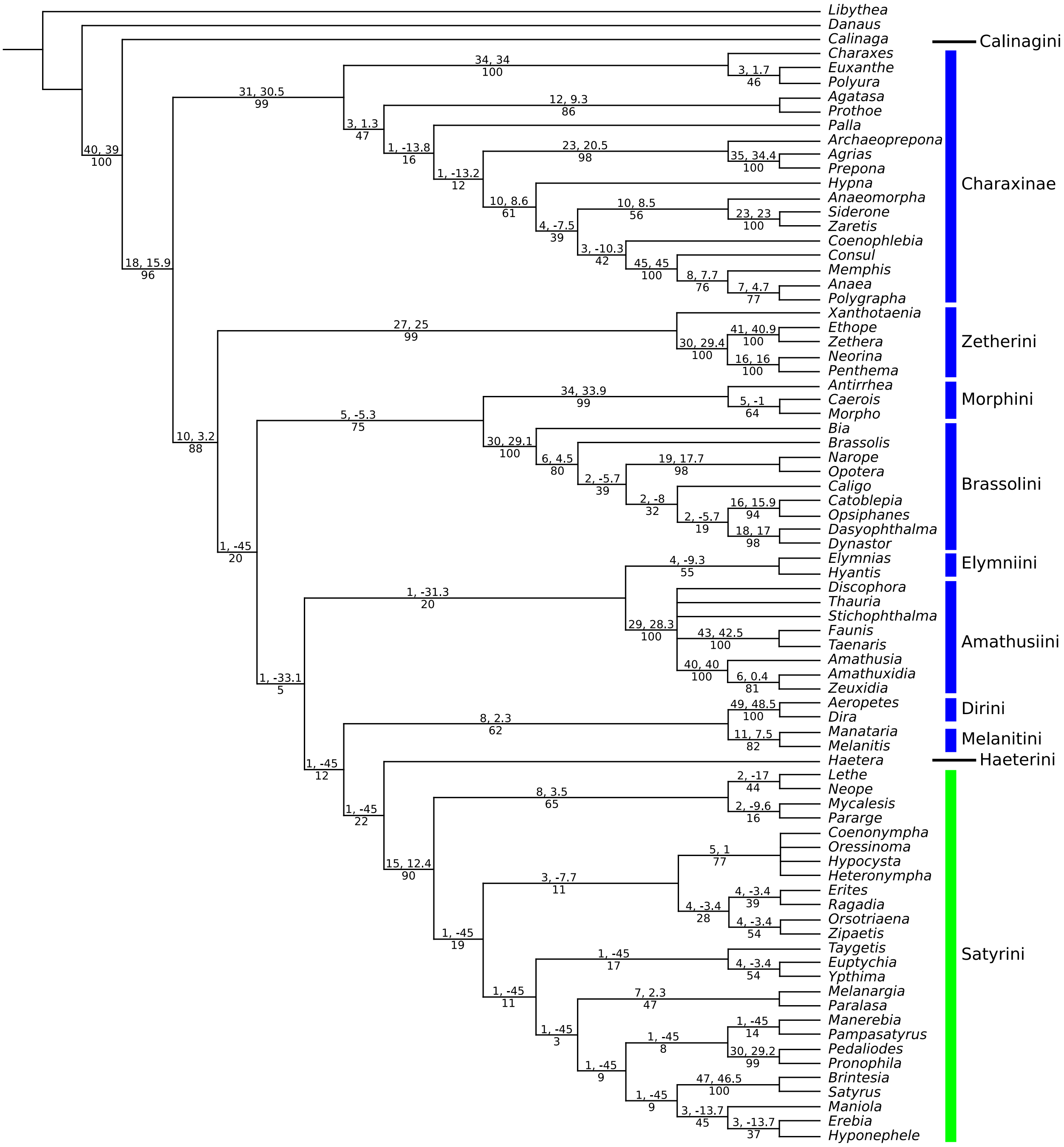
ELECTRONIC SUPPLEMENTARY MATERIAL

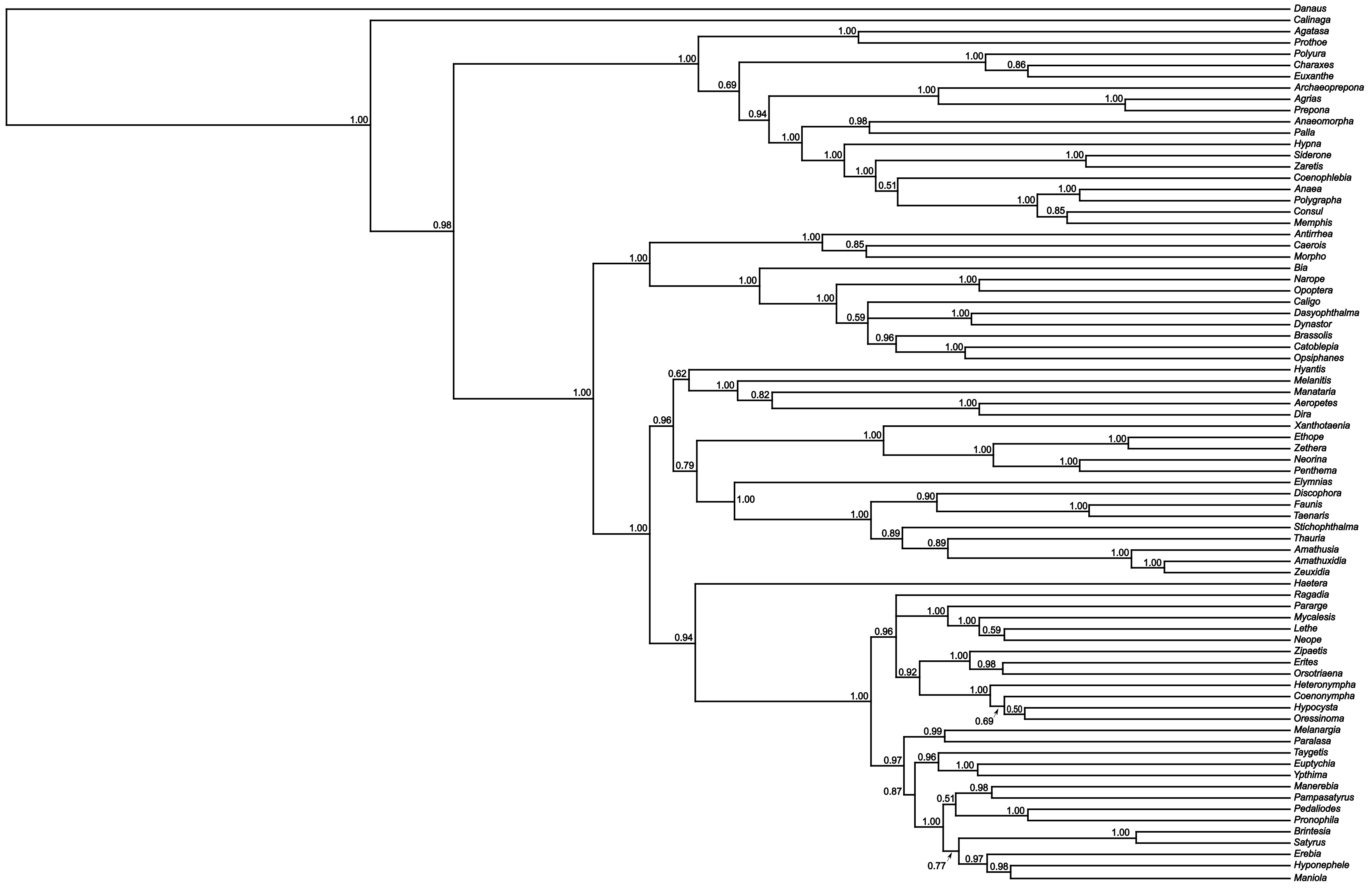
Prehistorical Climate Change Increased Diversification of a Group of Butterflies

Carlos Peña, Niklas Wahlberg

1. SUPPLEMENTARY FIGURES AND LEGENDS



Supplementary Figure 1. Strict consensus of three equally parsimonious trees from the combined dataset of all six genes and morphology



Supplementary Figure 2. Phylogenetic hypothesis based on Bayesian analysis of 6 genes, each modeled with a GTR + G + I model. Parameter values for models given in Table S2

2. SUPPLEMENTARY METHODS

1. Taxon Sampling.

We included 77 representative genera from the satyrine clade (*sensu* Wahlberg et al., 2003) as represented in Ackery et al. s (1999) classification for Charaxinae and Amathusiini, Lamas (2004) s for Morphini and Brassolini, including all major lineages in Satyrinae found in our previous paper (Peña et al., 2006), and two outgroup genera (*Libythea* and *Danaus*). All sequences have been deposited in GenBank. Appendix S1 shows the sampled species in their current taxonomic classification and GenBank accession numbers.

2. DNA isolation.

We extracted DNA from two butterfly legs, dried or freshly conserved in 96% alcohol and kept at -80C until DNA extraction. Total DNA was isolated using QIAGEN s DNeasy extraction kit (Hilden, Germany) following the manufacturer s instructions.

3. PCR amplification.

For each species, we amplified five nuclear genes and one mitochondrial gene by PCR using published primers (Table S1). Amplification was performed in 20 μL volume PCR reactions: 12.5 μL distilled water, 2.0 μL 10x buffer, 2.0 μL MgCl, 1.0 μL of each primer, 0.4 μL dNTP, 0.1 μL of AmpliTaq Gold polymerase and 1.0 μL of DNA extract. The reaction cycle profile consisted in a denaturation phase at 95C for 5 min, followed by 35 cycles of denaturation at 94C for 30s, annealing at 47 55C (depending on primers) for 30s, 72C for 1 min 30s, and a final extension period of 72C for 10 min.

4. Sequencing.

Sequencing was done using a Beckman-Coulter CEQ8000 eight-capillary sequencer using Dye CEQ Terminator Cycle Sequencing (DTCS) following instructions by the DTCS Quick Start Kit (California, USA). The PCR primers were also used for sequencing, and additional internal primers were used for this purpose (Table S1). All sequencing reactions were performed in a 20 μL volume: 13.5 μL distilled water, 2.0 μL DTCS Quick Start Master Mix, 1.5 μL CEQ Sequencing reaction buffer, 2.0 μL sequencing primer and 1.0 μL PCR product. Cycle sequencing reaction profile consisted in 30 cycles of a denaturation phase at 96°C for 20s, annealing phase at 50°C for 20s followed by 4 min at 60°C and a final extension period at 4°C.

5. Sequence Alignment.

All sequences are very conserved within genes, thus alignments were checked by eye using the program BioEdit (Hall, 1999). In total, we obtained 1450 bp of the cytochrome oxidase subunit I gene (COI) from the mitochondrial genome, 1240 bp of the *Elongation Factor-1α* gene (*EF-1α*), 400 bp of the *wingless* gene, 691 bp of the GAPDH gene, 733 bp of the MDH gene and 617 bp of the RPS5 gene from the nuclear genome. Primers and PCR protocols for GAPDH, MDH and RPS5 from Wahlberg and Weat (in press).

6. Morphological characters

We used Freitas and Brown Jr. (2004) s published morphological dataset and coded the same characters for our taxa from adult vouchers (Appendix S2). We also added four new characters to the matrix and coded them for our taxa (Appendix S3). In some cases, we coded characters from the literature (van Son, 1955; Vane-Wright and Smiles, 1975; Casagrande, 1979, 2002; Casagrande and Mielke, 1985; García-Barros, 1986; Igarashi and Fukuda, 1997, 2000) (see Appendix S4). It was not always possible to code the same species that were used for molecular characters. In such cases, a closely related species was coded instead (Appendix S4).

7. Phylogenetic analyses

The complete dataset consisted of 79 taxa and 5381 characters. We performed a maximum parsimony analysis treating all characters as unordered and equally weighted, doing a heuristic search using the program TNT 1.1 (Goloboff et al., 2003) with level of search 10, followed by branch-swapping of the resulting trees with up to 10000 trees held during each step. We evaluated clade robustness by using the Bremer support (Bremer, 1988) and the Partitioned Congruence Index (PCI) (Brower, 2006). The PCI was drawn from Partitioned Bremer Support (PBS) values (Gatesy et al., 1999) obtained by using the scripting feature of TNT (script pbsup.run taken from http://www.zmuc.dk/public/phylogeny/TNT/scripts/).

We also assessed clade stability by analyzing the complete dataset (morphology and molecules) with Bayesian inference using the program MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The evolution of sequences was modeled under the GTR + I + Γ model. The Bayesian analysis was performed on the combined dataset with parameter values estimated separately for each gene region (Table S2). The analysis was run twice for 38 million generations, with every 200th tree sampled and the first 89300 sampled generations discarded as burn-in (based on a visual inspection of when log likelihood values reached stationarity). We will refer to clades that are recovered under parsimony and Bayesian analyses as stable.

We rooted the resulting networks with *Libythea* because of the consensus in regarding this taxon as sister to the rest of Nymphalidae (Ackery et al., 1999; Brower, 2000; Ehrlich, 1958; Freitas and Brown Jr., 2004; Scott, 1985; Wahlberg et al., 2003).

8. Timing of divergences

Divergence times were estimated using the rate-smoothing method of penalized likelihood (PL; Sanderson, 2002) as implemented in the program r8s 1.71 (Sanderson, 2003, http://ginger.ucdavis.edu/r8s/). PL is a semiparametric method that uses a penalty function against fast-rate DNA substitutions between a certain node and its descendant lineages by applying a smoothing parameter that controls the tradeoff between smoothness and goodness of fit of the data to the model of molecular evolution. We used the phylogenetic hypothesis obtained from the total evidence analysis in MrBayes (see above) retaining branch lengths as input data for the program r8s. We estimated the value of the smoothing parameter by a cross-validation procedure restarting each search 5 times. We obtained confidence intervals by doing 100 bootstrap replications of our combined molecular dataset in the package PHYLIP 3.66 (Felsenstein, 1989), which were used for estimating branch lengths in PAUP 4.0 beta (Swofford, 2002) using maximum likelihood and the GTR + I + Γ model for each replicate and then used as input for r8s, with the help of Perl scripts made available by T. Eriksson (Eriksson, 2006). We used an absolute calibration point from the fossil record to convert the estimates of relative ages from r8s into absolute dates. Nel et al. (1993) described a satyrine fossil from Late Oligocene (25 Mya) deposits in France, based on a well conserved fossil compression, which the authors placed in the extant genus *Lethe*. Therefore, ages of divergence were estimated for the bootstrap replications by fixing the age of the split between *Lethe* and its sister taxon, in this dataset, *Neope* at 25 Mya.

9. Patterns of butterfly/hostplant association

We used information from the literature on butterfly hostplants (Ackery, 1988; Ackery et al., 1999; DeVries, 1987; Igarashi and Fukuda, 1997, 2000) to code the use of plant families by each butterfly tribe (except for Charaxinae that was coded as a whole subfamily) as independent characters, except by Eudicot families and the lower plants, *Selaginella* (Lycopodiophyta) and the epiphytic moss *Neckeropsis undulata*, which were treated as two characters, Dicots and Lycopodiophyta and mosses respectively. We optimized the hostplant matrix using a reduced phylogeny at the tribal level derived from the Bayesian tree. We took into account all hostplant records for all species in each butterfly lineage. Thus, we studied the evolution of hostplant use not only for the species in our dataset, but for the satyrine clade as a whole.

Character list

Most of the characters were taken directly from Freitas and Brown's (Freitas and Brown Jr., 2004) matrix, although some characters were added and others suffered minor changes and/or corrections:

1–2. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

3. Egg ratio length/diameter: more than 1.0 (0), between 0.99 and 0.61 (round egg) (1), equal or less than 0.6 (hemispheric egg) (2). This character was recoded for all the species and Freitas and Brown's matrix was fixed to the right character states.

4. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

5. Egg longitudinal ridges: present (0), absent (1). Corrected this character state for *Dynastor* from absent to present.

6–12. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

13. Oviposition pattern: isolated eggs (0), grouped eggs (1). *Caligo beltrao* oviposits eggs in groups (see ref Casagrande (1979)). Changed from 0 to 1.

14–133. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

134. Pupal alar caps: not projecting (0), projecting laterally (1). *Caligo beltrao* pupa projects its alar caps (see ref Casagrande (1979)). Changed from 0 to 1.

135–136. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

137. Recoded as: Inflated subcostal vein: absent (0), present (1).

138–234. From Freitas and Brown's (ref (Freitas and Brown Jr., 2004)).

235. **New character.** Forewing: Inflated costal vein: absent (0), present (1).

236. **New character.** Forewing: Inflated Cu vein: absent (0), present (1).

237. **New character.** Forewing: Inflated vein 2A: absent (0), present (1).

238. **New character.** Genitalia: Male aedeagus with a scythe-like and very long ramification: absent (0), present (1).

Morphological matrix

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                   5    0    5    0    5    0    5    0    5    0    5    0
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Danaus         000000000000010000001100111100000?000011?1100010000??????000
Calinaga       00200000000000?1?1?00001010010000?001110000100???00??????001
Aeropetes      20101????1?00?0111??00???????1?00???1??000?100???00??????001
Agatasa        00101????1?10000????0000?10?10100?0011?1?01100???00??????000
Agrias         00?01????1?1?001????00?????????????????0?0?0?0???00??????001
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Anaea          ????????????????????????????????????????????????????????????
Anaeomorpha    ????????????????????????????????????????????????????????????
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Bia            0010001000000311111000001101000010101110000000???00??????001
Brassolis      0010000000001010011000011100000010100110100000???00??????001
Brintesia      ????????????????????????????????????????????????????????????
Caerois        00201????1?00111111000101101100110101110000100???00??????011
Caligo         0010001000001011111000011100100011101110001000???00??????101
Catoblepia     ????????????????????????????????????????????????????????????
Charaxes       20100000?1?1000111000000010100010?001110000100???00??????001
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Coenophlebia   ????????????????????????????????????????????????????????????
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Dasyophthalma  0010001000000011111000011100100011101110100000???00??????001
Dira           ????????????????????????????????????????????????????????????
Discophora     20101????1?0101????????????????????????010?100???00??????011
Dynastor       00100????1?00011111000011100100011101110000000???00??????001
Elymnias       201000000000000111000001110110010?00?110100000???00??????001
Erebia         ????????????????????????????????????????????????????????????
Erites         00101????1?0100??????????????????????1?000?100???00??????001
Ethope         ????????????????????????????????????????????????????????????
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3. SUPPLEMENTARY TABLES

Supplementary Table 1. Primer sequences for PCR and sequencing of genes used in this study.

Primer Location	Primer Name	Primer Utility	Sequence	Citation
mtDNA COI	LCO	PCR/Sequencing	5’ GGTCAACAAATCATAAAGATATTGG 3’	(Wahlberg and Zimmermann, 2000)
	HCO	PCR	5’ TAAACTTCAGGGTGACCAAAAAATCA 3’	(Wahlberg and Zimmermann, 2000)
	Jerry	PCR/Sequencing	5’ CAACAYTTATTTTGATTTTTTGG 3’	(Wahlberg and Zimmermann, 2000)
	Pat	PCR	5’ ATCCATTACATATAATCTGCCATA 3’	(Wahlberg and Zimmermann, 2000)
	Patty	PCR	5’ ACWGTWGGWGGATTAACWGG 3’	(Peña et al., 2006)
nDNA *EF1-*α	Starsky	PCR/Sequencing	5’ CACATYAACATTGTCGTSATYGG 3’	(Peña et al., 2006)
	Luke	PCR	5’ CATRTTGTCKCCGTGCCAKCC 3’	(Peña et al., 2006)
	Cho	PCR/Sequencing	5’ GTCACCATCATYGACGC 3’	(Peña et al., 2006)
	Verdi	PCR	5’ GATACCAGTCTCAACTCTTCC 3’	(Peña et al., 2006)
	EF51.9	PCR/Sequencing	5 CARGACGTATACAAAATCGG 3’	(Cho et al., 1995)
	EFrcM4	PRC	5 ACAGCVACKGTYTGYCTCATRTC 3’	(Cho et al., 1995)
nDNA *wingless*	LepWG1	PCR/Sequencing	5 GARTGYAARTGYCAYGGYATGTCTGG 3’	(Brower and DeSalle, 1998)
	LepWG2	PCR	5 ACTICGCARCACCARTGGAATGTRCA 3’	(Brower and DeSalle, 1998)
nDNA GAPDH	Frigga	PCR/Sequencing	5 AARGCTGGRGCTGAATATGT 3’	(Wahlberg and Wheat, 2008)
	Burre	PCR	5 GWTTGAATGTACTTGATRAGRTC 3’	(Wahlberg and Wheat, 2008)
nDNA RpS5	RpS5f	PCR/Sequencing	5 ATGGCNGARGARAAYTGGAAYGA 3’	(Wahlberg and Wheat, 2008)
	RpS5r	PCR	5 CGGTTRGAYTTRGCAACACG 3’	(Wahlberg and Wheat, 2008)
nDNA MDH	MDHf	PCR/Sequencing	5 GAYATNGCNCCNATGATGGGNGT 3’	(Wahlberg and Wheat, 2008)
	MDHr	PCR	5 AGNCCYTCNACDATYTTCCAYTT 3’	(Wahlberg and Wheat, 2008)

Supplementary Table 2. Parameter values estimated using Bayesian phylogenetic methods

Gene	TL (all)	r(A↔C)	r(A↔G)	r(A↔T)	r(C↔G)	r(C↔T)	r(G↔T)	pi(A)	pi(C)	pi(G)	pi(T)	alpha	pinvar
COI	8.46	0.058	0.098	0.052	0.020	0.765	0.006	0.415	0.084	0.074	0.427	0.457	0.628
EF-1α		0.055	0.379	0.097	0.047	0.394	0.039	0.284	0.244	0.199	0.273	0.800	0.482
GAPDH		0.088	0.244	0.155	0.043	0.427	0.043	0.250	0.244	0.196	0.310	1.128	0.510
MDH		0.088	0.323	0.124	0.061	0.369	0.035	0.301	0.172	0.196	0.331	1.116	0.440
RpS5		0.075	0.300	0.093	0.038	0.439	0.056	0.287	0.205	0.201	0.307	1.039	0.486
wgl		0.084	0.315	0.077	0.047	0.407	0.070	0.207	0.296	0.307	0.190	0.835	0.363

Values estimated separately for each gene region.

Supplementary Table 3. Larval food plants for the taxonomic groups used in this study.

Butterfly taxon	Plant group	Family	Reference
Libytheinae	Dicotyledonous	Ulmaceae	(1, 2)
Danainae	Dicotyledonous	Asclepiadaceae	(1)
Calinaginae	Dicotyledonous	Rosaceae	(3)
Charaxinae	Dicotyledonous	Fabaceae, Piperaceae, etc.	(3)
Morphini	Dicotyledonous	Fabaceae	(3)
	Monocotyledonous	Musaceae, Poaceae, Arecaceae	(3)
Brassolini	Dicotyledonous	Rubiaceae [dubious record]	(3, 4)
	Monocotyledonous	Arecaceae, Cannaceae, Heliconiaceae	(3)
		Marantaceae, Musaceae, Zingiberaceae	(3)
Melanitini	Monocotyledonous	Cyperaceae, Poaceae	(3)
Dirini	Monocotyledonous	Poaceae	(3)
Zetherini	Monocotyledonous	Arecaceae, Poaceae	(3)
Amathusiini	Monocotyledonous	Arecaceae, Smilaneaceae, Musaceae	(3)
		Agavaceae, Liliaceae, Orchidaceae	(3)
		Pandanaceae, Poaceae, Flagellariaceae	(3)
Elymniini	Monocotyledonous	Arecaceae	(3)
Haeterini	Monocotyledonous	Arecaceae, Heliconiaceae, Marantaceae	(3)
		Zingiberaceae, Cyperaceae, Poaceae	(3)
Satyrini	Monocotyledonous	Arecaceae, Marantaceae, Zingiberaceae	(3)
		Restionaceae, Xyridaceae, Cyperaceae	(3)
		Poaceae	(3)
	Lycopodiopsida	Selaginellaceae	(5)
	Bryopsida	Neckeraceae	(6)

(1) = Ackery et al. (1999); (2) = Kawahara (2003); (3) = Ackery (1988); (4) = Penz et al. (1999); (5) = Singer et al. (1971); (6) = Singer & Mallet (1986).

Supplementary Table 4. List of specimens and GenBank accession numbers for each gene used in the molecular studies.

Subfamily	Tribe	Subtribe	Species	Specimen ID	Source of specimen	COI	*EF-1α*	*Wingless*	GAPDH	MDH	RPS5
Libytheinae			*Libythea celtis*	NW71-1	Spain: Barcelona	AY090198	AY090164	AY090131	EU141517	EU141641	EU141418
Danainae	Danaini	Danaina	*Danaus plexippus*	NW108-21	Portugal: Madeira, Monte	DQ018954	DQ018921	DQ018891	EU141486	EU141605	EU141382
Calinaginae			*Calinaga buddha*	NW64-3	UK: Stratford Butterfly farm	AY090208	AY090174	AY090141	EU141506	---	EU141406
Charaxinae	Charaxini		*Charaxes castor*	NW78-3	UK: Stratford Butterfly farm	AY090219	AY090185	AY090152	---	---	EU141422
Charaxinae	Charaxini		*Polyura maeri*	NW121-24	Indonesia: Bali	EU528325	EU528302	EU528282	---	EU528368	EU528459
Charaxinae	Euxanthini		*Euxanthe eurinome*	NW131-10	Ghana	EU141357	EU136664	EU141238	---	---	EU141390
Charaxinae	Pallini		*Palla decius*	NW124-7	Ghana	DQ338576	DQ338884	---	---	---	EU141389
Charaxinae	Prothoini		*Agatasa calydonia*	NW111-8	Malaysia	EU528310	EU528288	EU528266	---	EU528334	EU528420
Charaxinae	Prothoini		*Prothoe frank*	NW103-5		EU528327	EU528304	EU528284	---	EU528370	EU528462
Charaxinae	Preponini		*Agrias hewitsonius*	CP-M264	Peru: Poli	EU528311	EU528289	EU528267	---	---	EU528421
Charaxinae	Haeterini		*Prepona sp.*	CP-CI142	Peru: Madre de Dios, CICRA	EU528326	EU528303	EU528283	---	---	EU528460
Charaxinae	Preponini		*Archaeoprepona demophon*	NW81-9	UK: Stratford Butterfly farm	AY090220	AY090186	AY090153	---	---	EU141424
Charaxinae	Preponini		*Anaeomorpha splendida*	CP05-41	Peru: Loreto	EU528313	---	EU528269	---	---	EU528423
Charaxinae	Anaeini		*Coenophlebia archidona*	CP-M269	Peru: Poli	EU528316	EU528293	EU528272	---	EU528341	EU528429
Charaxinae	Anaeini		*Zaretis sp.*	CP05-05	Peru: Amazonas	EU528332	EU528309	---	---	EU528378	EU528470
Charaxinae	Anaeini		*Siderone marthesia*	NW124-6	Costa Rica	EU528329	EU528306	EU528285	---	EU528372	EU528464
Charaxinae	Anaeini		*Hypna clytemnestra*	NW127-11	Brazil: São Paulo	DQ338574	DQ338882	DQ338600	---	EU528352	EU528439
Charaxinae	Anaeini		*Anaea troglodyta*	NW92-2	UK: Stratford Butterfly farm	DQ338573	DQ338881	DQ338599	---	---	EU141428
Charaxinae	Anaeini		*Polygrapha tyrianthina*	CP06-88	Peru: Oxapampa	EU528324	EU528301	EU528281	---	EU528367	EU528458
Charaxinae	Anaeini		*Consul fabius*	NW109-16	Costa Rica	EU528317	EU528294	EU528273	---	EU528342	EU528430
Charaxinae	Anaeini		*Memphis appias*	NW127-6	Brazil: São Paulo	DQ338575	DQ338883	DQ338601	---	EU528355	EU528445
Morphinae	Morphini	Antirrheina	*Caerois sp.*	CP09-56	Peru: Madre de Dios, CICRA	EU528315	EU528292	EU528271	EU528384	EU528338	EU528426
Morphinae	Morphini	Antirrheina	*Antirrhea philoctetes*	NW109-12	Costa Rica	DQ338577	DQ338885	DQ338602	EU528383	EU528336	EU528424
Morphinae	Morphini	Morphina	*Morpho helenor*	NW66-5	UK: Stratford Butterfly farm	AY090210	AY090176	AY090143	EU141507	EU528356	EU141407
Morphinae	Brassolini	Biina	*Bia actorion*	99-004	Brazil: Rondonia	---	DQ338893	---	---	---	---
Morphinae	Brassolini	Biina	*Bia actorion*	EW11-3	Peru: Loreto	DQ338753	---	DQ338610	---	---	---
Morphinae	Brassolini	Biina	*Bia actorion*	CP01-78	Peru: Madre de Dios	---	---	---	EU532175	EU532180	EU532179
Morphinae	Brassolini	Brassolina	*Brassolis sophorae*	NW122-21	Brazil: São Paulo	EU528314	EU528291	EU528270	---	EU528337	EU528425
Morphinae	Brassolini	Brassolina	*Caligo telamonius*	NW70-10	UK: Stratford Butterfly farm	AY090209	AY090175	AY090142	---	EU141637	EU141414
Morphinae	Brassolini	Brassolina	*Catoblepia orgetorix*	NW109-15	Costa Rica	DQ338754	DQ338894	DQ338611	---	EU528339	EU528427
Morphinae	Brassolini	Brassolina	*Dasyophthalma creusa*	NW126-4	Brazil: São Paulo	EU528318	EU528295	EU528274	EU528387	EU528343	EU528431
Morphinae	Brassolini	Brassolina	*Dynastor darius*	NW109-11	Costa Rica	EU528320	EU528297	EU528276	EU528389	EU528346	EU528434
Morphinae	Brassolini	Brassolina	*Opoptera syme*	NW126-3	Brazil: São Paulo	EU528323	EU528300	EU528280	EU528403	EU528361	EU528450
Morphinae	Brassolini	Naropina	*Opsiphanes quiteria*	NW109-10	Costa Rica	DQ018957	DQ018924	DQ018895	---	EU528362	EU528451
Satyrinae	Brassolini	Naropina	*Narope sp.*	NW127-27	Brazil: Extrema, MG.	DQ338755	DQ338895	DQ338612	EU528401	EU528358	EU528447
Morphinae	Amathusiini		*Amathusia phidippus*	NW114-17	Indonesia: Bali	DQ018956	DQ018923	DQ018894	EU141488	EU141607	EU141384
Morphinae	Amathusiini		*Amathuxidia amythaon*	NW111-14	Malaysia	EU528312	EU528290	EU528268	EU528382	EU528335	EU528422

Morphinae	Amathusiini		*Discophora necho*	NW101-6	Indonesia: Palawan	DQ338747	DQ338887	DQ338604	---	EU528345	EU528433
Morphinae	Amathusiini		*Faunis menado*	NW118-19	Indonesia: Central Sulawesi	DQ338748	DQ338888	DQ338605	EU528393	EU528350	EU528438
Morphinae	Amathusiini		*Hyantis hodeva*	NW102-5		EU528322	EU528299	EU528278	EU528394	EU528351	---
Morphinae	Amathusiini		*Stichophthalma howqua*	NW97-7	Taiwan: Taoyuan County	AY218250	AY218270	AY218288	EU528413	EU528373	EU528465
Morphinae	Amathusiini		*Taenaris cyclops*	NW102-4	Indonesia: Sorong Island	DQ338749	DQ338889	DQ338606	EU528414	EU528374	EU528466
Morphinae	Amathusiini		*Thauria aliris*	NW111-15	Malaysia	EU528330	EU528307	EU528286	---	EU528375	EU528467
Morphinae	Amathusiini		*Zeuxidia dohrni*	NW101-2	Indonesia: Java	DQ338752	DQ338892	DQ338609	EU528417	EU528379	EU528471
Morphinae	Amathusiini		*Xanthotaenia busiris*	NW142-8	Indonesia: Kalimantan	EU528331	EU528308	EU528287	EU528415	EU528376	EU528468
Satyrinae	Haeterini		*Haetera piera*	CP01-84	Peru: Madre de Dios	DQ018959	DQ018926	DQ018897	EU141475	EU141593	EU141371
Satyrinae	Melanitini		*Melanitis leda*	NW66-6	Australia: Queensland Carins	AY090207	AY090173	AY090140	EU141508	EU141631	EU141408
Satyrinae	Elymniini	Elymniina	*Elymnias casiphone*	NW121-20	Indonesia: Bali	DQ338760	DQ338900	DQ338627	---	---	EU141388
Satyrinae	Elymniini	Mycalesina	*Mycalesis sp.*	EW18-8	Australia: Queensland Carins	DQ338765	DQ338905	DQ338632	EU528400	EU528357	EU528446
Satyrinae	Elymniini	Mycalesina	*Orsotriaena medus*	EW25-17	Bangladesh: Sylhet Div. Lowacherra Forest	DQ338766	DQ338906	DQ338633	EU528405	EU528363	EU528453
Satyrinae	Elymniini	Parargina	*Aeropetes tulbaghia*	CP13-01	South Africa	DQ338579	DQ338907	DQ338634	EU528381	EU528333	EU528419
Satyrinae	Elymniini	Parargina	*Lethe minerva*	NW121-17	Indonesia: Bali	DQ338768	DQ338909	DQ338616	EU141492	EU141611	EU141387
Satyrinae	Elymniini	Parargina	*Manataria hercyna*	EW11-1	Costa Rica	AY218244	AY218264	AY218282	EU528396	EU528353	EU528442
Satyrinae	Elymniini	Parargina	*Neope bremeri*	EW25-23	Taiwan: Pingtung County	DQ338770	DQ338911	DQ338618	EU528402	EU528359	EU528448
Satyrinae	Elymniini	Parargina	*Pararge aegeria*	EW1-1	France: Carcassonne	DQ176379	DQ338913	DQ338620	EU141476	EU141594	EU141372
Satyrinae	Elymniini	Parargina	*Ethope noirei*	NW121-7	Vietnam	DQ338773	DQ338915	DQ338622	EU528391	EU528348	EU528436
Satyrinae	Elymniini	Parargina	*Neorina sp.*	NW118-14	Indonesia: West Java	DQ338774	DQ338916	DQ338623	---	EU528360	EU528449
Satyrinae	Elymniini	Zetherina	*Penthema darlisa*	CP-B02	Vietnam	DQ338775	DQ338917	DQ338624	EU528408	EU528366	EU528457
Satyrinae	Elymniini	Zetherina	*Zethera incerta*	NW106-10	Indonesia: Sulawesi	DQ338776	DQ338918	DQ338635	EU141483	EU141602	EU141379
Satyrinae	Satyrini	Coenonymphina	*Coenonympha pamphilus*	EW7-3	Sweden: Öland	DQ338777	DQ338920	DQ338637	EU528385	EU528340	EU528428
Satyrinae	Satyrini	Erebiina	*Erebia oeme*	EW24-7	France: Languedoc	DQ338780	DQ338923	DQ338640	EU141479	EU141597	EU141375
Satyrinae	Satyrini	Erebiina	*Manerebia cyclopina*	CP04-80	Peru: Junín	---	---	DQ338645	---	---	---
Satyrinae	Satyrini	Erebiina	*Manerebia cyclopina*	CP03-63	Peru: Junín	DQ338785	DQ338928	---	EU528397	EU528354	EU528443
Satyrinae	Satyrini	Pronophilina	*Pedaliodes sp. n. 117*	CP09-66	Peru: Apurímac	DQ338856	DQ339008	DQ338719	EU528407	EU528365	EU528456
Satyrinae	Satyrini	Pronophilina	*Pronophila thelebe*	CP03-70	Peru: Junín	DQ338859	DQ339012	DQ338723	EU528410	EU528369	EU528461
Satyrinae	Satyrini	Euptychiina	*Taygetis laches*	NW108-3	Brazil: São Paulo	DQ338812	DQ338958	DQ338683	EU141487	---	EU141383
Satyrinae	Satyrini	Euptychiina	*Euptychia sp. n. 2*	CP01-33	Peru: Madre de Dios	DQ338794	DQ338937	DQ338654	EU528392	EU528349	EU528437
Satyrinae	Satyrini	Euptychiina	*Oressinoma typhla*	CP07-71	Peru: Junín	DQ338802	DQ338949	DQ338666	---	---	EU528452
Satyrinae	Satyrini	Hypocystina	*Heteronympha merope*	EW10-4	Australia: Canberra	AY218243	AY218063	AY218281	EU141477	EU141595	EU141373
Satyrinae	Satyrini	Hypocystina	*Hypocysta pseudirius*	NW123-5	Australia: Newcastle	DQ338826	DQ338974	---	---	---	EU528440
Satyrinae	Satyrini	Hypocystina	*Zipaetis saitis*	D30	India	DQ338831	DQ338981	DQ338696	EU528418	EU528380	EU528472
Satyrinae	Satyrini	Hypocystina	*Pampasatyrus gyrtone*	NW126-12	Brazil: São Paulo	DQ338837	DQ338988	DQ338701	EU528406	EU528364	EU528454
Satyrinae	Satyrini	Maniolina	*Hyponephele cadusia*	CP10-07	Iran: Hamadan	DQ338839	DQ338989	DQ338702	EU528395	---	EU528441
Satyrinae	Satyrini	Maniolina	*Maniola jurtina*	EW4-5	Spain: Sant Ciment	AY090214	AY090180	AY090147	EU141481	---	EU141376
Satyrinae	Satyrini	Melanargiina	*Melanargia galathea*	EW24-17	France: Languedoc	DQ338843	DQ338993	DQ338706	EU528398	---	EU528444
Satyrinae	Satyrini	Satyrina	*Brintesia circe*	CP-B01	France: Languedoc	DQ338865	DQ339020	DQ338729	EU141474	EU141592	EU141370
Satyrinae	Satyrini	Satyrina	*Paralasa jordana*	CP-AC23-35	Russia: Karasu	DQ338597	DQ339027	DQ338736	EU532176	---	EU528455
Satyrinae	Satyrini	Satyrina	*Satyrus actaea*	EW20-12	France: Carcassonne	DQ338871	DQ339029	DQ338738	EU528412	EU528371	EU528463

Satyrinae	Satyrini	Ypthimina	*Ypthima baldus*	NW98-5	Indonesia: Central Sulawesi	DQ338875	DQ339033	DQ338742	EU528416	EU528377	EU528469
Satyrinae	Ragadiini		*Ragadia makuta*	CP16-10	Indonesia: Kalimantan	EU528328	EU528305	---	---	---	---
Satyrinae	Ragadiini		*Ragadia makuta*	CP16-09	Indonesia: Kalimantan	---	---	---	EU532177	---	EU532178
Satyrinae	Eritini		*Erites argentina*	CP16-13	Indonesia: Kalimantan	EU528321	EU528298	EU528277	EU528390	EU528347	EU528435
Satyrinae	Dirini		*Dira clytus*	CP15-04	South Africa	EU528319	EU528296	EU528275	EU528388	EU528344	EU528432

Supplementary Table 5. Information sources for species used for the morphological matrix

Higher taxon	Species	Adult stage sources	Immature stages sources
Calinagini	*Calinaga buddha*	M: SU NW64-4	(1)
Anaeini	*Consul fabius*	(4)	(4)
Anaeini	*Hypna clytemnestra*	(4)	(4)
Anaeini	*Memphis ryphea*	(4)	(4)
Anaeini	*Coenophlebia archidona*	M: SU CP-M269, genitalia CP-10, legs CP-4	
Anaeini	*Siderone marthesia*	(4)	(4)
Charaxini	*Polyura maeri*	M: SU NW121-24, genitalia CP-33, legs CP-24	
Charaxini	*Polyura delphis*		(2)
Charaxini	*Charaxes bupalus*	-	(1)
Euxanthini	*Euxanthe eurinome*	F: SU NW131-10, genitalia CP-36, legs CP-27	
Pallini	*Palla decius*	M: SU NW124-7, genitalia CP-31, legs CP-22	
Preponini	*Agrias claudina*	M: SU CP-M278, genitalia CP-9, legs CP-1	(3)
Preponini	*Archaeoprepona chalciope*	(4)	(4)
Prothoini	*Agatasa calydonia*	M: SU NW111-8, genitalia CP-34, legs CP-28	(2)
Prothoini	*Prothoe frank*	(2)	(2)
Antirrheina	*Caerois chorineaus*	(4)	(4)
Antirrheina	*Antirrhea archaea*	(4)	(4)
Morphina	*Morpho achilles*	(4)	(4)
Biina	*Bia actorion*	(4)	(4)
Brassolina	*Brassolis sophorae*	(4)	(4)
Brassolina	*Caligo beltrao*	(4)	(4,7)
Brassolina	*Dasyophthalma creusa*	(4)	(4)
Brassolina	*Dynastor darius*	(4)	(4)
Brassolina	*Opsiphanes invirae*	(4)	(4)
Naropina	*Narope cyllene*	(8)	(8)
Amathusiini	*Amathusia phidippus*	M: SU NW114-17, genitalia CP-5, legs CP-17	(2)
Amathusiini	*Amathuxidia amythaon*	M: SU NW111-14, genitalia CP-4, legs CP-15	
Amathusiini	*Discophora necho*	M: SU NW101-6, genitalia CP-8, legs CP-10	
Amathusiini	*Discophora timora*		(2)
Amathusiini	*Faunis menado*	(1)	(1)
Amathusiini	*Stichophthalma howqua*	F: SU NW97-7, genitalia CP-21, legs CP-14	(1)
Amathusiini	*Taenaris cyclops*	M: SU NW102-4, genitalia CP-16, legs CP-3	
Amathusiini	*Taenaris onolaus*		(4)
Amathusiini	*Thauria aliris*	F: SU NW111-15, genitalia CP-7	(2)
Amathusiini	*Zeuxidia dohrni*	M: SU NW101-2, genitalia CP-6	
Amathusiini	*Zeuxidia aurelius*		(2)
Amathusiini	*Xanthotaenia busiris*	(1)	(1)
Haeterini	*Haetera diaphana*	(1)	(1)
Melanitini	*Melanitis leda*	M: SU NW66-6, genitalia CP-18, legs CP-11	(4)
Elymniina	*Elymnias casiphone*	M: SU NW112-9, genitalia CP-14, legs CP-7	
Elymniina	*Elymnias hypermnestra*		(2)
Mycalesina	*Orsotriaena medus*	F: SU EW25-17, genitalia CP-26	(2)
Mycalesina	*Mycalesis terminus*	M: SU EW18-8, genitalia CP-32, legs CP-26	
Mycalesina	*Mycalesis perseus*		(2)
Parargina	*Aeropetes tulbaghia*	M: SU CP13-01, genitalia CP-12, legs CP-9	(5)
Parargina	*Ethope noirei*	M: SU NW121-7, genitalia CP-35, legs CP-23	
Parargina	*Lethe minerva*	M: SU NW121-17, genitalia CP-30, legs CP-25	
Parargina	*Lethe verma*		(2)
Parargina	*Manataria hercyna*	F: SU EW11-1, genitalia CP-20, legs CP-13; M: MUSM, genitalia CP-84	
Parargina	*Neope bremeri*		(1)
Parargina	*Neorina* sp.	(2)	(2)
Parargina	*Pararge aegeria*	M: SU EW1-3, legs CP-18	
Parargina	*Pararge aegeria*	M: SU EW1-1, genitalia CP-23	
Zetherina	*Penthema darlisa*	M: SU CP-B02, genitalia CP-15, legs CP-8	

Zetherina	*Penthema formosanum*		(2)
Zetherina	*Zethera pimplea*	(2)	(2,9)
Coenonymphina	*Coenonympha pamphilus*	M: SU EW24-16, genitalia CP-13, legs CP-5	
Erebiina	*Erebia oeme*	M: SU EW24-9, genitalia CP-24	
Euptychiina	*Oressinoma typhla*	F: SU, genitalia CP-29, legs CP-21	
Euptychiina	*Taygetis laches*	(4)	(4)
Hypocystina	*Heteronympha merope*	M: SU EW10-4, genitalia CP-28, legs CP-20	
Hypocystina	*Hypocysta aroa*	(1)	(1)
Maniolina	*Maniola jurtina*	M: SU EW4-5, genitalia CP-27, legs CP-19	
Maniolina	*Hyponephele lupina*	M: SU EW20-10, genitalia CP-39, legs CP-31	
Melanargiina	*Melanargia galathea*	M: SU EW24-17, genitalia CP-25	
Melanargiina	*Melanargia montana*		(1)
Satyrina	*Paralasa hades*	M: SU NW139-13, genitalia CP-38, legs CP-30	
Satyrina	*Brintesia circe*	M: SU CP-B01, genitalia CP-11, legs CP-6	(6)
Satyrina	*Satyrus actaea*	M: SU EW20-12, genitalia CP-37, legs CP-29	
Ypthimina	*Ypthima sempera*	(1)	(1)
Ragadiini	*Ragadia luzonia*	(2)	(2)
Eritini	*Erites angularis*	(1)	(1)
Dirini	*Dira clytus*	M: SU NW144-8, genitalia CP-17, legs CP-16	

M = male; F = female; SU = Department of Zoology, Stockholm University, Stockholm; MUSM = Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru. (1) = Igarashi and Fukuda (2000); (2) = Igarashi and Fukuda (1997); (3) = Casagrande and Mielke (1985); (4) = Freitas and Brown (2004); (5) = van Son (1955); (6) = García (1986); (7) = Casagrande (1979a); (8) = Casagrande (2002); (9) = Vane-Wright and Smiles (1975).

4 SUPPLEMENTARY DISCUSSION

1. Cladistic approach

Analysis of the combined dataset in the cladistic approach produced 3 equally parsimonious cladograms. The strict consensus (Fig. S1) shows 6 well supported clades: Charaxinae, Zetherina (including *Ethope*, *Neorina* and *Xanthotaenia*), Morphini, Amathusiini (including *Elymnias*), Melanitini (including *Aeropetes*, *Dira* and *Manataria*) and Satyrini (including Mycalesina, Parargina, Ragadiini and Eritini). These clades were consistently recovered in previous analyses with different amounts of data (not shown). The PCI values show strong conflicting signals from the different partitions (PBS values) for the Amathusiini clade and some basal nodes (Fig. S1) that were recovered in different relationships in the Bayesian approach (see below).

2. Bayesian approach

The Bayesian analysis produced a tree that is congruent with the strict consensus from the cladistic analysis (Fig. S2). Parameter values for the models used in the analysis are given in Table S2. The major differences were (1) the position of *Hyantis* appearing in the Melanitini clade, that appears as sister to Zetherina + Amathusiini, and (2) the relationships of the major clades indentified by the cladistic analysis (see above): Melanitini is not sister to *Haetera* + Satyrini, Zetherina is sister to Amathusiini, while Morphini branched off after the Charaxinae (Fig. S2). These differences in topology are reflected by the low Bremer support values and PCI obtained in the cladistic analysis.

3. Hostplant use in the satyrine clade

After mapping the use of hostplants by the satyrine clade onto the phylogenetic hypothesis inferred by the Bayesian approach, we identified 5 major colonization events (Fig. 2). The Charaxinae feeds entirely on several dicotyledonous families. The Morphini shifted onto monocotyledons (mainly family Arecaceae) with the exception of the genus *Morpho* that feeds on dicots. The Melanitini innovated by feeding on grasses (family Poaceae) while the putative sister Amathusiini feeds on Poaceae and Arecaceae. The Haeterini retained the monocot-feeding trait but do not use the Poaceae, while its sister Satyrini feeds mainly on Poaceae (Fig. 2).

4. Ages estimates for the satyrine clade butterflies and their hostplants

The estimated ages of origin for butterflies inferred by the program r8s are shown in Fig. 1. Our analyses place the time of divergence of the satyrine clade in the Late Cretaceous (80.5 Mya), significantly after the estimated origin for angiosperms (Early Cretaceous, around 140 Mya). We believe that the use of Penalized likelihood provides reliable time estimates as compared to other methods, such as Bayesian relaxed clock method. Wheat *et al.* 2007 found a correspondence among the estimated obtained by both PL as implemented in the program r8s and the Bayesian relaxed clock method. The 5 major plant colonization events by the major lineages of butterflies in the satyrine clade took place considerably after the main diversification and radiation of angiosperms (around 100 Mya), an average delay of 48 ± 11 My. Several lineages of this group of butterflies diversified almost simultaneously between 56 and 48 Mya (Melanitini, Amathusiini, Satyrini + Haeterini, Morphini and Charaxinae). However, the lineages Zetherina and Satyrini are somewhat younger (35 36 My old). The Charaxinae feed entirely on dicotyledonous plants while its sister group colonized several families of monocot plants. Within the monocot feeders, the more species-rich groups feed extensively on Poaceae (grasses, bamboos and sedges), some Amathusiini feed on Poaceae but have retained the ancestral character of feeding on Arecaceae, Musaceae, Heliconiaceae, etc. It seems that Poaceae was colonized early in the evolution of Satyrinae, however the Amathusiini + Zetherina + Melanitini clades expanded their hosts ranges to basal monocots and early Poales, while the Satyrini is more restricted to graminid Poales.

5. Phylogenetic relationships within the satyrine clade

Taking into account both the cladistic and Bayesian analyses, we found evidence for three major lineages: Calinaginae, Charaxinae and Satyrinae *sensu lato*. We also identified 6 major lineages in the Satyrinae s.l. that received good support by both phylogenetic approaches (see results). However, both analyses recovered different relationships among these clades, and the position of *Hyantis* is ambiguous. These clades we identified reflect the need for a reassessment of the current taxonomic classification of Nymphalidae butterflies, since the Satyrinae subfamily is a polyphyletic group as found by Peña et al. (2006). In order to have a natural classification, it is necessary to broaden the scope of Satyrinae, to include all the groups but the Charaxinae. Thus, the other lineages should belong to Satyrinae, having the status of tribes: Morphini, Melanitini, Amathusiini, Zetherini, Haeterini and Satyrini.

6. Pattern of evolution of hostplant use

At the level of subfamilies, Satyrinae Charaxinae and Calinaginae appeared and radiated much later than their plant counterparts. With our evidence, we can rule out a possible phenomenon of cospeciation as hypothesized by Ehrlich and Raven (1964). Our results provide strong evidence for a sequential colonization and diversification of butterflies on their much older and already diversified hostplants. Our age estimates for butterflies are consistent with a recent study on the Nymphalinae butterflies (Wahlberg, 2006), where this subfamily diversified soon after the K/T event (around 65 Mya), putting back the origin of butterflies potentially older than the currently acknowledged 70 My old (Vane-Wright, 2004). This pattern of delayed colonization seems to be the most common pattern of insect/plant relationships (Lopez-Vaamonde et al., 2006). However, the simultaneous spread of grasses and diversification of Satyrini members evidences that the latter underwent an adaptive radiation once the hosts became abundant and widespread.

The similar ages of diversification for these lineages of butterflies (Fig. 2), as reflected by the short branches in the Bayesian tree, imply rapid speciation, probably following colonization events on different groups of hosts (dicots, monocots grass-like groups and rest). The relatively long delay for diversification of Satyrini, may be due to the necessity for the right enviromental conditions that permitted the spread of grasses. It is likely that ancestral Satyrini developed adaptations to inhabit open, dry grasslands which preadapted them for colonization of new habitats made available by the advance of grasses.

5. SUPPLEMENTARY NOTES

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