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Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data

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Abstract

We have inferred the first empirically supported hypothesis of relationships for the cosmopolitan butterfly subfamily Satyrinae. We used 3090 base pairs of DNA from the mitochondrial gene COI and the nuclear genes *EF-1α* and *wingless* for 165 Satyrinae taxa representing 4 tribes and 15 subtribes, and 26 outgroups, in order to test the monophyly of the subfamily and elucidate phylogenetic relationships of its major lineages. In a combined analysis, the three gene regions supported an almost fully resolved topology, which recovered Satyrinae as polyphyletic, and revealed that the current classification of suprageneric taxa within the subfamily is comprised almost completely of unnatural assemblages. The most noteworthy findings are that *Manataria* is closely related to Melanitini; *Palaeonympha* belongs to Euptychiina; *Oressinoma*, *Orsotriaena* and *Coenonympha* group with the Hypocystina; Miller's (1968). Parargina is polyphyletic and its components group with multiple distantly related lineages; and the subtribes Elymniina and Zetherina fall outside the Satyrinae. The three gene regions used in a combined analysis prove to be very effective in resolving relationships of Satyrinae at the subtribal and tribal levels. Further sampling of the taxa closely related to Satyrinae, as well as more extensive sampling of genera within the tribes and subtribes for this group will be critical to test the monophyly of the subfamily and establish a stronger basis for future biogeographical and evolutionary studies.

Keywords: Nymphalidae; Satyrinae; Molecular phylogeny; Partitioned Bremer support; Butterflies

1. Introduction

The butterflies are one of the most studied and best known groups of organisms. The vast amount of information gathered on this group spans a variety of topics in ecology, evolutionary biology and conservation biology (e.g. Boggs et al., 2003). However, the higher phylogenetic relationships of major groups of butterflies remain poorly known. This lack of knowledge is critical, since several disciplines in comparative biology (namely evolution of host plant preferences, mimicry, behavior, etc) depend on robust phylogenetic hypotheses to provide a framework for interpreting the evolution of putatively adaptive character systems.

Despite several recent important efforts to elucidate the higher level relationships of butterflies (Brower, 2000; Caterino et al., 2001; de Jong et al., 1996; Freitas and Brown, 2004; Wahlberg et al., 2003b, 2005), there is still only fragmentary knowledge about patterns of relationships among lineages within the six rhopaloceran families. This is particularly true in the nymphalid subfamily Satyrinae, one of the most diverse groups of butterflies.

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The cosmopolitan Satyrinae includes about 2400 species and occur on all continents except Antarctica (Ackery et al., 1999). Although the other major clades of Nymphalidae are comparatively well known, the subfamily Satyrinae remains poorly understood, with many undescribed genera and species, a higher classification rife with unnatural assemblages, and without any available comprehensive and empirically supported phylogeny (Freitas, 2003, 2004a; Lamas, 2004; Martin et al., 2000; Miller, 1968; Murray and Prowell, 2005; Peña and Lamas, 2005; Viloria and Pyrcz, 1994; Viloria and Camacho, 1999). The diversity of Satyrinae is not reflected by the number of studies on the systematics of the group. In fact, the most recent effort to encompass the whole group is Miller's (1968) important but now outdated work, which employed an orthogenetic criterion to develop a hypothesis of Satyrinae phylogeny.

The rank and position of Satyrinae among other nymphalid taxa has been a matter of confusion. The taxonomic rank, and even the taxa falling within the circumscription of Satyrinae has changed often in recent decades (Table 1). One of the first modern attempts to classify the butterflies is the work by Ehrlich (1958), who considered Satyrinae as a subfamily of Nymphalidae, being related to Morphinae and Calinaginae. Later, Ehrlich and Ehrlich (1967) used a quantitative phenetic approach to propose a scheme of classification retaining the same taxonomic status for Satyrinae. Following Clark (1947), Miller (1968) considered the group as having the family rank "Satyridae". Miller proposed additional new subfamily level groupings to classify the entire group, considering Biinae (including *Bia*, *Antirrhea*, *Caerois* and *Melanitis* therein) as members of his Satyridae. DeVries et al. (1985) used a cladistic analysis based on characters of mainly immature stages to show that Miller's Antirrhini (*sic*) should be moved into Morphinae, stated that Biini of Miller (*Bia*) is of uncertain position, and that Melanitini should remain in Satyrinae. Harvey's (1991) classification scheme, based on Miller's with the addition of features from immature stages, treated Satyrinae as a subfamily of Nymphalidae again, moved Brassolinae out of Miller's Satyridae to be a subfamily on its own, moved Miller's Antirrhini into Morphinae (as claimed by DeVries et al., 1985), and left *Bia* in Satyrinae. The status of *Bia* as a brassoline is no longer in any doubt: it was hypothesized based on morphological features of adults by DeVries et al. (1985), immatures by Freitas et al. (2002), and molecular data by Brower (2000), and is congruent with the successive weighting analysis tree of morphological data of Freitas and Brown (2004). Vane-Wright and Boppré's (2005) detailed description of wing patterns and androconial organs of *Bia* shows clear affinity with the brassolines. Hence, *Bia* is currently placed in Brassolini (Lamas, 2004; Vane-Wright and Boppré, 2005). For his classification of satyrine tribes and subtribes, Harvey (1991) largely followed Miller's scheme, but down-ranking his subfamilies and tribes to tribes and subtribes, respectively. The most recent global classification of butterflies is by Ackery et al. (1999), with minor changes to Harvey's (1991) classification but following entirely his conception of Satyrinae.

After these rearrangements, some level of consensus in placing the satyrine butterflies as a nymphalid subfamily was achieved. Studies by Brower (2000), Wahlberg and colleagues (2003b, 2005), and Freitas and Brown (2004) have shown that satyrine butterflies form a clade within the family Nymphalidae with the Morphinae, Charaxinae and Calinaginae being the closest relatives. These studies sampled only a few satyrine species and are not informative about relationships within Satyrinae. The resolution of these major lineages was the next logical step. The important study by Viloria (1998, 2003) was among the first efforts to address this subject. Viloria's (2003) cladistic and biogeographic study of satyrine butterflies from South America and New Zealand proposed that many of the genera considered to be in Pronophilina are instead more closely related to Erebiina and Hypocystina. Viloria's changes were adopted in the Checklist of Neotropical Butterflies edited by Lamas (2004). Recently, Murray and Prowell's (2005) molecular phylogenetic study of the subtribe Euptychiina found many of its genera to be para- or polyphyletic, recovering a non-monophyletic Euptychiina, with *Oressinoma* and *Euptychia* itself nested among the satyrine outgroups.

The remainder of recent works examining the relationships of satyrine butterflies are studies on species (Monteiro and Pierce, 2001; Nice and Shapiro, 2001) and genus level relationships (Martin et al., 2000; Torres et al., 2001). Martin et al. (2000) examined the phylogeny of some European satyrine genera, concluding that *Aphantopus hyperantus* should be transferred from Coenonymphina into Maniolina.

Except for Miller's (1968) foundation and the study of Viloria (2003), we have almost no knowledge about the phylogenetic relationships of the major lineages of Satyrinae. Since a robust phylogenetic hypothesis is crucial for integrating natural groups in our classification schemes, identifying the major lineages and resolving the relationships of the satyrine butterflies is a critical matter to accomplish. At the present time, the classification of Satyrinae remains based almost entirely on the work of Miller (1968).

For these reasons, the aims of this study are to test the monophyly of Satyrinae, to provide evidence that elucidates patterns of relationships among the major groups (tribes and subtribes) by using a cladistic analysis based on molecular data. The resulting phylogenetic hypothesis will be a first step towards understanding the diversification of this globally successful subfamily. In this study, we follow Ackery et al.'s (1999) classification for families and subfamilies, Miller's (1968) classification for the groups within Satyrinae as modified by Harvey (1991) and Lamas's (2004) checklist for nomenclature of Neotropical taxa (see Table 1).

Table 1
Representative higher level classifications of satyrines

Miller (1968)	Harvey (1991)	Lamas (2004)	This paper
Satyridae	Satyrinae	Satyrinae	Satyrinae
Haeterinae	Haeterini	Haeterini	Elymniini
Haeterini	*Cithaerias*	*Cithaerias*	*Elymnias*
Cithaerias	*Haetera*	*Haetera*	Zetherini
Haetera	*Pierella*	*Pierella*	*Neorina*
Pierella	*Pseudohaetera*	*Pseudohaetera*	*Penthema*
Pseudohaetera	Biini	Elymniini	*Ethope*
Biinae	Melanititi	Parargina	*Zethera*
Melanitini	*Gnophodes*	*Manataria*	Melanitini
Gnophodes	*Melanitis*	Elymniina	*Aeropetes*
Melanitis	*Manataria* tribe uncertain	*Enodia*	*Paralethe*
Manataria tribe uncertain	Elymniini	Satyrini	*Manataria*
Elymniinae	Elymniiti	Hypocystina	*Gnophodes*
Elymniini	*Elymnias*	*Argyrophorus*	*Melanitis*
Elymnias	*Elymniopsis*	*Quilaphoetosus*	Haeterini
Elymniopsis	Lethiti	*Auca*	*Cithaerias*
Lethini	*Aeropetes*	*Chillanella*	*Haetera*
Aeropetes	*Paralethe*	*Cosmosatyrus*	*Pierella*
Paralethe	*Enodia*	*Elina*	*Pseudohaetera*
Enodia	*Lethe*	*Etcheverrius*	Satyrini
Lethe	*Neope*	*Nelia*	Parargina
Neope	*Satyrodes*	*Pampasatyrus*	*Kirinia*
Satyrodes	*Kirinia*	Euptychiina	*Lopinga*
Kirinia	*Lasiommata*	*Caeruleuptychia*	*Lasiommata*
Lasiommata	*Lopinga*	*Cepheuptychia*	*Pararge*
Lopinga	*Pararge*	*Chloreuptychia*	Lethina
Pararge	*Ethope*	*Cissia*	*Lethe*
Ethope	*Neorina*	*Cyllopsis*	*Enodia*
Neorina	Mycalesiti	*Magneuptychia*	*Satyrodes*
Mycalesini	*Bicyclus*	*Euptychia*	*Neope*
Bicyclus	*Hallelesis*	*Euptychoides*	Mycalesina
Hallelesis	*Henotesia*	*Forsterinaria*	*Bicyclus*
Henotesia	*Mycalesis*	*Godartiana*	*Hallelesis*
Mycalesis	*Orsotriaena*	*Harjesia*	*Henotesia*
Orsotriaena	Zetheriti	*Hermeuptychia*	*Mycalesis*
Zetherini	*Zethera*	*Magneuptychia*	Coenonymphina
Zethera	Satyrini	*Moneuptychia*	*Oreixenica*
Satyrinae	Hypocystiti	*Neonympha*	*Tisiphone*
Hypocystini	*Argyronympha*	*Pindis*	*Nesoxenica*
Argyronympha	*Dodonidia*	*Paramacera*	*Hypocysta*
Dodonidia	*Erebiola*	*Parataygetis*	*Lamprolenis*
Erebiola	*Geitoneura*	*Pareuptychia*	*Dodonidia*
Geitoneura	*Heteronympha*	*Paryphthimoides*	*Argyrophenga*
Heteronympha	*Hypocysta*	*Pharneuptychia*	*Erebiola*
Hypocysta	*Lamprolenis*	*Pindis*	*Percnodaimon*
Lamprolenis	*Nesoxenica*	*Posttaygetis*	*Heteronympha*
Nesoxenica	*Oreixenica*	*Rareuptychia*	*Geitoneura*
Oreixenica	*Percnodaimon*	*Splendeuptychia*	*Oressinoma*
Percnodaimon	*Tisiphone*	*Taygetis*	*Coenonympha*
Tisiphone	*Zipaetis*	*Yphthimoides*	*Orsotriaena*
Zipaetis	Ypthimiti	Coenonymphina	*Zipaetis*
Ypthimini	*Neocoenyra*	*Coenonympha*	*Argyronympha*
Neocoenyra	*Ypthima*	*Cercyonis*	Euptychiina
Ypthima	*Ypthimomorpha*	Erebiina	*Euptychia*
Ypthimomorpha	*Palaeonympha* tribe uncertain	*Erebia*	*Cyllopsis*
Palaeonympha tribe uncertain	Euptychiiti	*Ianussiusa*	*Paramacera*
Euptychiini	*Caeruleuptychia*	*Tamania*	*Palaeonympha*
Caeruleuptychia	*Cepheuptychia*	*Idioneurula*	*Pharneuptychia*
Cepheuptychia	*Chloreuptychia*	*Manerebia*	*Euptychoides*
Chloreuptychia	*Cissia*	Pronophilina	*Yphthimoides*
Cissia	*Cyllopsis*	*Apexacuta*	*Moneuptychia*
Cyllopsis	*Erichthodes*	*Corades*	*Paryphthimoides*
Erichthodes	*Euptychia*	*Daedalma*	*Amphidecta*

(*continued on next page*)

Table 1 (*continued*)

Miller (1968)	Harvey (1991)	Lamas (2004)	This paper
Euptychia	*Euptychoides*	*Eteona*	*Rareuptychia*
Euptychoides	*Forsterinaria*	*Foetterleia*	*Godartiana*
Forsterinaria	*Godartiana*	*Junea*	*Hermeuptychia*
Godartiana	*Harjesia*	*Lasiophila*	*Splendeuptychia*
Harjesia	*Hermeuptychia*	*Lymanopoda*	*Pindis*
Hermeuptychia	*Moneuptychia*	*Oxeoschistus*	*Cepheuptychia*
Magneuptychia	*Neonympha*	*Panyapedaliodes*	*Cissia*
Moneuptychia	*Oressinoma*	*Parapedaliodes*	*Caeruleuptychia*
Nenoympha	*Paramacera*	*Pedaliodes*	*Magneuptychia*
Oressinoma	*Parataygetis*	*Praepedaliodes*	*Chloreuptychia*
Paramacera	*Pareuptychia*	*Proboscis*	*Neonympha*
Parataygetis	*Paryphthimoides*	*Pronophila*	*Erichthodes*
Pareuptychia	*Pharneuptychia*	*Pseudomaniola*	*Pareuptychia*
Paryphthimoides	*Pindis*	*Punapedaliodes*	*Taygetis*
Pharneuptychia	*Posttaygetis*	*Steremnia*	*Harjesia*
Pindis	*Oressinoma*	*Steroma*	*Parataygetis*
Posttaygetis	*Rareuptychia*	Satyrina	*Posttaygetis*
Rareuptychia	*Splendeuptychia*	*Neominois*	*Forsterinaria*
Splendeuptychia	*Taygetis*	*Amphidecta* subtribe uncertain	*Cercyonis* subtribe uncertain
Taygetis	*Yphthimoides*		*Hyponephele* subtribe uncertain
Yphthimoides	Coenonymphiti		*Neocoenyra* subtribe uncertain
Coenonymphini	*Coenonympha*		Ypthimina
Coenonympha	*Aphantopus*		*Paralasa*
Aphantopus	Manioliti		*Ypthima*
Maniolini	*Cercyonis*		*Ypthimomorpha*
Cercyonis	*Hyponephele*		Melanargiina
Hyponephele	*Maniola*		*Melanargia*
Maniola	*Pyronia*		Maniolina
Pyronia	Erebiiti		*Pyronia*
Erebiini	*Erebia*		*Maniola*
Erebia	Pronophiliti		*Aphantopus*
Pronophilini	*Amphidecta*		Pronophilina
Amphidecta	*Corades*		*Nelia*
Corades	*Daedalma*		*Steremnia*
Daedalma	*Eteona*		*Steroma*
Eteona	*Junea*		*Manerebia*
Junea	*Lasiophila*		*Idioneurula*
Lasiophila	*Lymanopoda*		*Tamania*
Lymanopoda	*Oxeoschistus*		*Ianussiusa*
Oxeoschistus	*Panyapedaliodes*		*Lymanopoda*
Panyapedaliodes	*Parapedaliodes*		*Argyrophorus*
Parapedaliodes	*Pedaliodes*		*Etcheverrius*
Pedaliodes	*Praepedaliodes*		*Pampasatyrus*
Praepedaliodes	*Proboscis*		*Elina*
Proboscis	*Pronophila*		*Quilaphoetosus*
Pronophila	*Pseudomaniola*		*Cosmosatyrus*
Pseudomaniola	*Punapedaliodes*		*Chillanella*
Punapedaliodes	*Steremnia*		*Auca*
Steremnia	*Steroma*		*Panyapedaliodes*
Steroma	*Idioneurula*		*Pedaliodes*
Idioneurula	*Manerebia*		*Punapedaliodes*
Manerebia	*Argyrophorus*		*Praepedaliodes*
Argyrophorus	*Quilaphoetosus*		*Corades*
Quilaphoetosus	*Auca*		*Junea*
Auca	*Chillanella*		*Pronophila*
Chillanella	*Cosmosatyrus*		*Eteona*
Cosmosatyrus	*Elina*		*Foetterleia*
Elina	*Etcheverrius*		*Daedalma*
Etcheverrius	*Nelia*		*Oxeoschistus*
Nelia	*Pampasatyrus*		*Proboscis*
Pampasatyrus	Melanargiiti		*Lasiophila*
Melanargiini	*Melanargia*		*Apexacuta*
Melanargia	Satyriti		*Pseudomaniola*
Satyrini	*Arethusana*		Erebiina
Arethusana	*Berberia*		*Erebia*

Table 1 (*continued*)

Miller (1968)	Harvey (1991)	Lamas (2004)	This paper
Berberia	*Brintesia*		Satyrina
Brintesia	*Chazara*		*Berberia*
Chazara	*Hipparchia*		*Hipparchia*
Hipparchia	*Karanasa*		*Chazara*
Karanasa	*Neominois*		*Pseudochazara*
Neominois	*Oeneis*		*Satyrus*
Oeneis	*Paralasa*		*Arethusana*
Paralasa	*Pseudochazara*		*Brintesia*
Pseudochazara	*Satyrus*		*Karanasa*
Satyrus	*Penthema* tribe uncertain		*Neominois*
Penthema not mentioned			*Oeneis*

Last column shows the implied classification derived from our phylogenetic results. This classification is not to be considered as taxonomic act under ICZN article 8.3 (International Commission on Zoological Nomenclature, 1999).

2. Material and methods

We obtained DNA sequences for three gene regions from 165 exemplar Satyrinae species representing 15 subtribes included in 4 tribes recognized by Harvey (1991), as well as some taxa of uncertain position (*Manataria*, *Amphidecta* and *Palaeonympha*). We have not yet obtained representatives from the remaining putative major satyrine lineages (tribes Eritini, Ragadiini and subtribe Dirina). Table 2 shows the sampled species in their current taxonomic classification and the GenBank accession numbers.

We extracted DNA from two butterfly legs, dried or freshly conserved in 96% alcohol, using QIAgen's DNEasy extraction kit. For each species, we sequenced 1450 bp of the cytochrome oxidase subunit I gene (COI) from the mitochondrial genome, 1240 bp of the *Elongation Factor-1α* gene (*EF-1α*), and 400 bp of the *wingless* gene, from the nuclear genome. Some sequences were drawn from matrices published by Wahlberg et al. (2003b, 2005) and Murray and Prowell (2005). The primers for COI were taken from Wahlberg and Zimmermann (2000), for *EF-1α* (primers ef51.9 and efrcM4) from Monteiro and Pierce (2001) and for *wingless* from Brower and DeSalle (1998). Additional primers from Cho et al. (1995) were used for *EF-1α* sequences, Starsky (sense: 5′-CAC ATY AAC ATT GTC GTS ATY GG-3′) and Luke (antisense: 5′-CAT RTT GTC KCC GTG CCA KCC-3′), another primer from Reed and Sperling (1999), Cho (sense: 5′-GTC ACC ATC ATY GAC GC-3′) and Verdi (courtesy of F. Sperling's lab) (antisense: 5′-GAT ACC AGT CTC AAC TCT TCC-3′). Voucher specimens will be deposited at the Department of Entomology, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Peru; the Department of Zoology, Stockholm University, Sweden; the African Butterfly Research Institute, Kenya; and the American Museum of Natural History, New York (Brower's material).

The PCR reactions were performed in a 20 μl volume. The reaction cycle profile for COI was 95 °C for 5 min, 34 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 reaction cycle profile for primers Starksy-Luke and Cho-Verdi was 95 °C for 7 min, 34 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, an extension period of 72 °C for 10 min and a final one of 20 °C for 10 s. The reaction cycle profile for primers ef51.9-efrcM4 and the *wingless* gene was 95 °C for 5 min, 39 cycles of 95 °C for 1 min, 51 °C for 1 min, 70 °C for 1 min 30 s and a final extension period of 72 °C for 7 min. The PCR primers were also used for sequencing of *EF-1α* and *wingless*, while in COI an internal primer designed by N. Wahlberg (Patty 5′-ACW GTW GGW GGA TTA ACW GG-3′) was used in addition to the PCR primers. Sequencing of the PCR products was done with a Beckman–Coulter CEQ8000 capillary sequencer. The resulting chromatograms were checked using the program BioEdit (Hall, 1999) and the sequences were aligned by eye. Some sequences were generated and processed according to the protocols described in Brower et al. (in press).

The complete data set consisted of 191 taxa (including 26 outgroups) and 3090 nucleotides. All characters were treated as unordered and equally weighted. The resulting data matrix was analyzed according to a cladistic framework by performing a heuristic search using the New Technology Search algorithms in the program TNT (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. This same procedure was applied for each gene separately and for all three genes combined. Some taxa with missing data were not included in the separate analysis of each gene, since we have been unable to obtain sequences for them to date (as indicated in Table 2).

We evaluated clade robustness by using the bootstrap (Felsenstein, 1985) and Bremer support (Bremer, 1988, 1994) in TNT (Goloboff et al., 2003). We assessed the contribution of each gene data set to total Bremer support in the combined analyses by using Partitioned Bremer Support (PBS) (Baker and DeSalle, 1997; Gatesy et al., 1999) using the scripting feature of the program TNT (Goloboff et al., 2003). In the results and discussion section, we will refer to weak Bremer support for values of 1–2 (bootstrap values 50–63%), moderate support for values between 3 and 5 (bootstrap values 64–75%), good support for values between 6 and 10 (bootstrap values

Table 2
Information of specimens used for molecular studies

Subfamily	Tribe	Subtribe	Species	Specimen ID	Source of specimen	COI	*EF-1α*	*Wingless*
Libytheinae			*Libythea celtis*	NW71-1	Spain: Barcelona	AY090198	AY090164	AY090131
Heliconiinae	Heliconiini		*Heliconius hecale*	NW70-6	UK, Stratford Butterfly Farm	AY090202	AY090168	AY090135
Danainae	Danaini	Danaina	*Danaus plexippus*	NW108-21	Portugal: Madeira, Monte	DQ018954	DQ018921	DQ018891
Calinaginae			*Calinaga buddha*	NW64-3	UK, Stratford Butterfly Farm	AY090208	AY090174	AY090141
Charaxinae	Charaxini		*Charaxes castor*	NW78-3	UK, Stratford Butterfly Farm	AY090219	AY090185	AY090152
Charaxinae	Anaeini		*Anaea troglodyta*	NW92-2	UK, Stratford Butterfly Farm	DQ338573	DQ338881	DQ338599
Charaxinae	Anaeini		*Hypna clytemnestra*	NW127-11	Brazil: São Paulo	DQ338574	DQ338882	DQ338600
Charaxinae	Anaeini		*Memphis appias*	NW127-6	Brazil: São Paulo	DQ338575	DQ338883	DQ338601
Charaxinae	Preponini		*Archaeoprepona demophon*	NW81-9	UK, Stratford Butterfly Farm	AY090220	AY090186	AY090153
Charaxinae	Pallini		*Palla decius*	NW124-7	Ghana	DQ338576	DQ338884	—
Morphinae	Morphini	Antirrheina	*Antirrhea philoctetes*	NW109-12	Costa Rica	DQ338577	DQ338885	DQ338602
Morphinae	Morphini	Morphina	*Morpho helenor*	NW66-5	UK, Stratford Butterfly Farm	AY090210	AY090176	AY090143
Morphinae	Amathusiini		*Amathusia phidippus*	NW114-17	Indonesia: Bali	DQ018956	DQ018923	DQ018894
Morphinae	Amathusiini		*Aemona lena*	DL-02-P687	Thailand: Chiang Mai	DQ338578	DQ338886	DQ338603
Morphinae	Amathusiini		*Discophora necho*	NW101-6	Indonesia: Palawan	DQ338747	DQ338887	DQ338604
Morphinae	Amathusiini		*Faunis menado*	NW118-19	Indonesia: Central Sulawesi	DQ338748	DQ338888	DQ338605
Morphinae	Amathusiini		*Stichophthalma howqua*	NW97-7	Taiwan: Taoyuan County	AY218250	AY218270	AY218288
Morphinae	Amathusiini		*Taenaris cyclops*	NW102-4	Indonesia: Sorong Island	DQ338749	DQ338889	DQ338606
Morphinae	Amathusiini		*Thaumantis klugius*	SA-3-2	Malaysia: Sabah, Luasong	DQ338750	DQ338890	DQ338607
Morphinae	Amathusiini		*Thauria aliris*	DL-02-B253	Thailand: Ranong	DQ338751	DQ338891	DQ338608
Morphinae	Amathusiini		*Zeuxidia dohrni*	NW101-2	Indonesia: Java	DQ338752	DQ338892	DQ338609
Morphinae	Brassolini	Biina	*Bia actorion*	EW11-3	Peru: Loreto	DQ338753	—	DQ338610
Morphinae	Brassolini	Biina	*Bia actorion*	99-004	Brazil: Rondonia	—	DQ338893	—
Morphinae	Brassolini	Brassolina	*Caligo telamonius*	NW70-10	UK, Stratford Butterfly Farm	AY090209	AY090175	AY090142
Morphinae	Brassolini	Brassolina	*Catoblepia orgetorix*	NW109-15	Costa Rica	DQ338754	DQ338894	DQ338611
Morphinae	Brassolini	Brassolina	*Opsiphanes quiteria*	NW109-10	Costa Rica	DQ018957	DQ018924	DQ018895
Morphinae	Brassolini	Naropina	*Narope* sp.	NW127-27	Brazil: São Paulo	DQ338755	DQ338895	DQ338612
Satyrinae	Haeterini		*Cithaerias pireta*	NW93-1	Peru: Loreto	DQ338756	DQ338896	DQ338613
Satyrinae	Haeterini		*Haetera piera*	CP01-84	Peru: Madre de Dios	DQ018959	DQ018926	DQ018897
Satyrinae	Haeterini		*Pierella lamia*	NW93-2	Peru: Loreto	DQ338757	DQ338897	DQ338614
Satyrinae	Haeterini		*Pseudohaetera hypaesia*	CP03-99	Peru: Junín	DQ338758	DQ338898	DQ338625
Satyrinae	Melanitini		*Gnophodes chelys*	NW102-13	Uganda: Kibale National Park	DQ338759	DQ338899	DQ338626
Satyrinae	Melanitini		*Melanitis leda*	NW66-6	Australia: Queensland, Cairns	AY090207	AY090173	AY090140
Satyrinae	Elymniini	Elymniina	*Elymnias casiphone*	NW121-20	Indonesia: Bali	DQ338760	DQ338900	DQ338627
Satyrinae	Elymniini	Elymniina	*Elymnias hypermnestra*	DL-02-P680	Thailand: Chiang Mai	DQ338761	DQ338901	DQ338628
Satyrinae	Elymniini	Elymniina	*Elymnias bammakoo*	NW117-20	Ghana	DQ338762	DQ338902	DQ338629
Satyrinae	Elymniini	Mycalesina	*Bicyclus anynana*	EW10-5	Zimbabwe: Harare	AY218238	AY218258	AY218276
Satyrinae	Elymniini	Mycalesina	*Hallelesis halyma*	CP10-05	Ghana	DQ338763	DQ338903	DQ338630
Satyrinae	Elymniini	Mycalesina	*Henotesia simonsii*	EW10-6	Zimbabwe: Harare	DQ338764	DQ338904	DQ338631
Satyrinae	Elymniini	Mycalesina	*Mycalesis* sp.	EW18-8	Australia: Queensland, Cairns	DQ338765	DQ338905	DQ338632
Satyrinae	Elymniini	Mycalesina	*Orsotriaena medus*	EW25-17	Bangladesh: Sylhet Div. Lowacherra Forest	DQ338766	DQ338906	DQ338633
Satyrinae	Elymniini	Parargina	*Aeropetes tulbaghia*	CP13-01	S. Africa	DQ338579	DQ338907	DQ338634
Satyrinae	Elymniini	Parargina	*Enodia portlandia*	DNA96-018	USA: Louisiana	AY508536	AY509062	—
Satyrinae	Elymniini	Parargina	*Kirinia roxelana*	CP10-09	Iran: Lorestan	DQ338767	DQ338908	DQ338615
Satyrinae	Elymniini	Parargina	*Lasiommata megera*	EW5-7	Sweden: Stockholm	AY090213	AY090179	AY090146
Satyrinae	Elymniini	Parargina	*Lethe minerva*	NW121-17	Indonesia: Bali	DQ338768	DQ338909	DQ338616
Satyrinae	Elymniini	Parargina	*Lopinga achine*	EW3-6	Sweden	DQ338769	DQ338910	DQ338617
Satyrinae	Elymniini	Parargina	*Manataria hercyna*	EW11-1	Costa Rica	AY218244	AY218264	AY218282

Table 2 (*continued*)

Subfamily	Tribe	Subtribe	Species	Specimen ID	Source of specimen	COI	*EF-1α*	*Wingless*
Satyrinae	Elymniini	Parargina	*Neope bremeri*	EW25-23	Taiwan: Pingtung County	DQ338770	DQ338911	DQ338618
Satyrinae	Elymniini	Parargina	*Paralethe dendrophilus*	CP13-03	S. Africa	DQ338771	DQ338912	DQ338619
Satyrinae	Elymniini	Parargina	*Pararge aegeria*	EW1-1	France: Carcassonne	DQ176379	DQ338913	DQ338620
Satyrinae	Elymniini	Parargina	*Satyrodes eurydice*	NEB-1-3	USA: Nebraska	DQ338772	DQ338914	DQ338621
Satyrinae	Elymniini	Parargina	*Ethope noirei*	NW121-7	Vietnam	DQ338773	DQ338915	DQ338622
Satyrinae	Elymniini	Parargina	*Neorina* sp.	NW118-14	Indonesia: West Java	DQ338774	DQ338916	DQ338623
Satyrinae	Elymniini	Zetherina	*Penthema darlisa*	CP-B02	Vietnam	DQ338775	DQ338917	DQ338624
Satyrinae	Elymniini	Zetherina	*Zethera incerta*	NW106-10	Indonesia: Sulawesi	DQ338776	DQ338918	DQ338635
Satyrinae	Satyrini	Coenonymphina	*Coenonympha hero*	CP-AC23-26	Russia: Spassk	DQ338580	DQ338919	DQ338636
Satyrinae	Satyrini	Coenonymphina	*Coenonympha pamphilus*	EW7-3	Sweden: Öland	DQ338777	DQ338920	DQ338637
Satyrinae	Satyrini	Erebiina	*Erebia epiphron*	EW24-3	France: Languedoc	DQ338778	DQ338921	DQ338638
Satyrinae	Satyrini	Erebiina	*Erebia ligea*	EW5-19	Sweden: Vallentuna	DQ338779	DQ338922	DQ338639
Satyrinae	Satyrini	Erebiina	*Erebia oeme*	EW24-7	France: Languedoc	DQ338780	DQ338923	DQ338640
Satyrinae	Satyrini	Erebiina	*Erebia palarica*	EW9-4	Spain: Galicia, Lugo	AY090212	AY090178	AY090145
Satyrinae	Satyrini	Erebiina	*Erebia sthennyo*	EW24-1	France: Languedoc	DQ338781	DQ338924	DQ338641
Satyrinae	Satyrini	Erebiina	*Erebia triaria*	EW9-1	Spain: Galicia, Lugo	DQ338782	DQ338925	DQ338642
Satyrinae	Satyrini	Erebiina	*Ianussiusa maso*	V35	Venezuela: Táchira	DQ338783	DQ338926	DQ338643
Satyrinae	Satyrini	Erebiina	*Idioneurula* sp.	V37	Venezuela: Táchira	DQ338784	DQ338927	DQ338644
Satyrinae	Satyrini	Erebiina	*Manerebia cyclopina*	CP03-63	Peru: Junín	DQ338785	DQ338928	—
Satyrinae	Satyrini	Erebiina	*Manerebia cyclopina*	CP04-80	Peru: Junín	—	—	DQ338645
Satyrinae	Satyrini	Erebiina	*Manerebia inderena*	E-39-09	Ecuador: Sucumbios	DQ338786	DQ338929	DQ338646
Satyrinae	Satyrini	Erebiina	*Tamania jacquelinae*	V29	Venezuela: Táchira	DQ338787	—	DQ338647
Satyrinae	Satyrini	Euptychiina	*Caeruleuptychia lobelia*	CP01-67	Peru: Madre de Dios	DQ338788	DQ338930	DQ338648
Satyrinae	Satyrini	Euptychiina	*Cepheuptychia* sp. n.	CP01-31	Peru: Madre de Dios	DQ338789	DQ338931	DQ338649
Satyrinae	Satyrini	Euptychiina	*Chloreuptychia* sp.	CP01-72	Peru: Madre de Dios	DQ338790	DQ338932	DQ338650
Satyrinae	Satyrini	Euptychiina	*Cissia* sp.	NW108-6	Brazil	DQ338581	DQ338933	DQ338651
Satyrinae	Satyrini	Euptychiina	*Cyllopsis pertepida*	AZ-1-6	USA: Arizona	DQ338791	DQ338934	DQ338652
Satyrinae	Satyrini	Euptychiina	*Erichthodes antonina*	CP02-24	Peru: Madre de Dios	DQ338792	DQ338935	DQ338653
Satyrinae	Satyrini	Euptychiina	*Euptychia ernestina*	NW136-14	Brazil: São Paulo	DQ338793	DQ338936	—
Satyrinae	Satyrini	Euptychiina	*Euptychia* sp.	DNA99-078	Ecuador: Pichincha	AY508541	AY509067	—
Satyrinae	Satyrini	Euptychiina	*Euptychia* sp. n. 2	CP01-33	Peru: Madre de Dios	DQ338794	DQ338937	DQ338654
Satyrinae	Satyrini	Euptychiina	*Euptychia* sp. n. 5	CP01-53	Peru: Madre de Dios	DQ338795	DQ338938	DQ338655
Satyrinae	Satyrini	Euptychiina	*Euptychia* sp. n. 6	CP04-55	Peru: Junín	DQ338796	DQ338939	DQ338656
Satyrinae	Satyrini	Euptychiina	*Euptychia* sp. n. 7	CP02-58	Peru: Junín	—	DQ338940	DQ338657
Satyrinae	Satyrini	Euptychiina	*Euptychia pronophila*	NW127-20	Brazil: Minas Gerais	DQ338797	DQ338941	DQ338658
Satyrinae	Satyrini	Euptychiina	*Euptychoides castrensis*	NW126-9	Brazil: São Paulo	DQ338798	DQ338942	DQ338659
Satyrinae	Satyrini	Euptychiina	*Forsterinaria boliviana*	CP04-88	Peru: Junín	DQ338799	DQ338943	DQ338660
Satyrinae	Satyrini	Euptychiina	*Godartiana muscosa*	NW127-8	Brazil: São Paulo	DQ338582	DQ338944	DQ338661
Satyrinae	Satyrini	Euptychiina	*Harjesia blanda*	CP01-13	Peru: Madre de Dios	DQ338800	DQ338945	DQ338662
Satyrinae	Satyrini	Euptychiina	*Hermeuptychia hermes*	NW127-16	Brazil: Minas Gerais	DQ338583	DQ338946	DQ338663
Satyrinae	Satyrini	Euptychiina	*Magneuptychia* sp. n. 4	CP01-91	Peru: Madre de Dios	DQ338584	DQ338947	DQ338664
Satyrinae	Satyrini	Euptychiina	*Moneuptychia paeon*	B-17-41	Brazil: São Paulo	DQ338801	DQ338948	DQ338665
Satyrinae	Satyrini	Euptychiina	*Neonympha areolata*	DNA96-019	USA: Louisiana	AY508564	AY509090	—
Satyrinae	Satyrini	Euptychiina	*Oressinoma sorata*	DNA99-065	Ecuador: Pichincha	AY508561	AY509087	—
Satyrinae	Satyrini	Euptychiina	*Oressinoma sorata*	PE-6-1	Peru: Cuzco	—	—	AF246602
Satyrinae	Satyrini	Euptychiina	*Oressinoma typhla*	CP07-71	Peru: Junín	DQ338802	DQ338949	DQ338666
Satyrinae	Satyrini	Euptychiina	*Paramacera allyni*	MEX-1-1	Mexico: D. F., Magdalena Contreras	DQ338803	—	DQ338667
Satyrinae	Satyrini	Euptychiina	*Parataygetis albinotata*	CP04-53	Peru: Junín	DQ338804	DQ338950	DQ338668
Satyrinae	Satyrini	Euptychiina	*Pareuptychia hesionides*	CP01-66	Peru: Madre de Dios	DQ338805	DQ338951	DQ338669
Satyrinae	Satyrini	Euptychiina	*Paryphthimoides grimon*	CP10-01	Brazil	DQ338806	DQ338952	DQ338670
Satyrinae	Satyrini	Euptychiina	*Paryphthimoides* sp.	NW126-7	Brazil	DQ338807	DQ338953	DQ338671
Satyrinae	Satyrini	Euptychiina	*Pharneuptychia innocentia*	CP12-06	Brazil: Minas Gerais	DQ338808	DQ338954	DQ338672
Satyrinae	Satyrini	Euptychiina	*Pharneuptychia* sp.	NW127-18	Brazil: Minas Gerais	DQ338809	DQ338955	—
Satyrinae	Satyrini	Euptychiina	*Pindis squamistriga*	MEX-3-1	Mexico: Guanajuato	AY508570	AY509096	—
Satyrinae	Satyrini	Euptychiina	*Posttaygetis penelea*	DNA97-009	Ecuador: Napo	AY508571	AY509097	—
Satyrinae	Satyrini	Euptychiina	*Posttaygetis penelea*	NW127-28	Brazil: São Paulo	—	—	DQ338673
Satyrinae	Satyrini	Euptychiina	*Rareuptychia clio*	CP01-23	Peru: Madre de Dios	DQ338810	DQ338956	—
Satyrinae	Satyrini	Euptychiina	*Rareuptychia clio*	NW126-23	Brazil: Acre	—	—	DQ338674
Satyrinae	Satyrini	Euptychiina	*Splendeuptychia itonis*	CP02-44	Peru: Madre de Dios	DQ338811	DQ338957	DQ338684
Satyrinae	Satyrini	Euptychiina	*Taygetis laches*	NW108-3	Brazil: São Paulo	DQ338812	DQ338958	DQ338683
Satyrinae	Satyrini	Euptychiina	*Taygetis rectifascia*	NW126-13	Brazil: São Paulo	DQ338813	DQ338959	DQ338682
Satyrinae	Satyrini	Euptychiina	*Yphthimoides borasta*	CP10-03	Brazil: São Paulo	DQ338585	DQ338960	DQ338680

(*continued on next page*)

Table 2 (*continued*)

Subfamily	Tribe	Subtribe	Species	Specimen ID	Source of specimen	COI	*EF-1α*	*Wingless*
Satyrinae	Satyrini	Euptychiina	*Yphthimoides cepoensis*	CP10-02	Brazil: Minas Gerais	DQ338814	DQ338961	DQ338681
Satyrinae	Satyrini	Euptychiina	*Yphthimoides* sp.	CP12-04	Brazil: Minas Gerais	DQ338815	DQ338962	DQ338675
Satyrinae	Satyrini	Hypocystina	*Argyronympha gracilipes*	NW136-1	Solomon Islands: Guadalcanal	DQ338816	—	DQ338676
Satyrinae	Satyrini	Hypocystina	*Argyronympha pulchra*	NW136-6	Solomon Islands: Choiseul	DQ338817	DQ338963	DQ338677
Satyrinae	Satyrini	Hypocystina	*Argyronympha rubianensis*	NW136-3	Solomon Islands: Kolombangara	DQ338818	DQ338964	—
Satyrinae	Satyrini	Hypocystina	*Argyronympha* sp.	NW136-7	Solomon Islands: Malaita	DQ338586	DQ338965	DQ338678
Satyrinae	Satyrini	Hypocystina	*Argyronympha ugiensis*	NW136-2	Solomon Islands: San Cristobal	DQ338819	DQ338966	DQ338679
Satyrinae	Satyrini	Hypocystina	*Argyronympha ulava*	NW136-5	Solomon Islands: Ulawa	DQ338820	DQ338967	DQ338685
Satyrinae	Satyrini	Hypocystina	*Argyrophenga antipodium*	NW123-18	New Zealand	DQ338821	DQ338968	DQ338686
Satyrinae	Satyrini	Hypocystina	*Dodonidia helmsi*	NW123-15	New Zealand	DQ338822	DQ338970	DQ338688
Satyrinae	Satyrini	Hypocystina	*Erebiola butleri*	NW123-16	New Zealand	DQ338823	DQ338971	DQ338689
Satyrinae	Satyrini	Hypocystina	*Geitoneura acantha*	NW124-22	Australia: Newcastle	DQ338824	DQ338972	DQ338690
Satyrinae	Satyrini	Hypocystina	*Geitoneura klugii*	NW123-10	Australia: Adelaide Hills	DQ338825	DQ338973	DQ338691
Satyrinae	Satyrini	Hypocystina	*Heteronympha merope*	EW10-4	Australia: Canberra	AY218243	AY218263	AY218281
Satyrinae	Satyrini	Hypocystina	*Hypocysta pseudirius*	NW123-5	Australia: Newcastle	DQ338826	DQ338974	—
Satyrinae	Satyrini	Hypocystina	*Lamprolenis nitida*	PNG-1-10	Papua New Guinea	DQ338827	DQ338975	—
Satyrinae	Satyrini	Hypocystina	*Nesoxenica leprea*	NW123-7	Australia: Collinsvale	DQ338587	DQ338976	DQ338692
Satyrinae	Satyrini	Hypocystina	*Oreixenica lathoniella*	NW124-23	Australia: Boreang Campground	DQ338828	DQ338977	DQ338693
Satyrinae	Satyrini	Hypocystina	*Percnodaimon merula*	NW123-17	New Zealand	DQ338829	DQ338978	DQ338694
Satyrinae	Satyrini	Hypocystina	*Tisiphone abeona*	NW124-21	Australia: Kulnura	DQ338830	DQ338980	DQ338695
Satyrinae	Satyrini	Hypocystina	*Zipaetis saitis*	D30	India	DQ338831	DQ338981	DQ338696
Satyrinae	Satyrini	Hypocystina	*Argyrophorus argenteus*	CP13-07	Chile	DQ338588	DQ338969	DQ338687
Satyrinae	Satyrini	Hypocystina	*Auca barrosi*	RV-03-V39	Chile: Céspedes	DQ338832	DQ338982	DQ338697
Satyrinae	Satyrini	Hypocystina	*Auca coctei*	RV-03-V13	Chile: Céspedes	DQ338833	DQ338983	DQ338698
Satyrinae	Satyrini	Hypocystina	*Chillanella stelligera*	CH-24A-1	Chile: Termas de Chillán	DQ338589	DQ338984	DQ338699
Satyrinae	Satyrini	Hypocystina	*Cosmosatyrus leptoneuroides*	CH-15-5	Chile: Cordillera Nahuelbuta	DQ338834	DQ338985	—
Satyrinae	Satyrini	Hypocystina	*Elina montrolii*	CH-25-1	Chile: Ñuble, Cueva Pincheira	DQ338835	DQ338986	—
Satyrinae	Satyrini	Hypocystina	*Etcheverrius chiliensis*	CH-30-4	Chile: Los Andes, Portillo	DQ338836	DQ338987	DQ338700
Satyrinae	Satyrini	Hypocystina	*Nelia nemyroides*	CH-8A-2	Chile: Los Lagos	AY508562	AY509088	—
Satyrinae	Satyrini	Hypocystina	*Pampasatyrus gyrtone*	NW126-12	Brazil: São Paulo	DQ338837	DQ338988	DQ338701
Satyrinae	Satyrini	Hypocystina	*Quilaphoetosus monachus*	CH-12-1	Chile: Valdivia	DQ338838	DQ338979	—
Satyrinae	Satyrini	Maniolina	*Aphantopus hyperanthus*	EW2-1	Sweden: Stockholm	AY090211	AY090177	AY090144
Satyrinae	Satyrini	Maniolina	*Cercyonis pegala*	EW8-2	USA: Oregon	AY218239	AY218259	AY218277
Satyrinae	Satyrini	Maniolina	*Hyponephele cadusia*	CP10-07	Iran: Hamadan	DQ338839	DQ338989	DQ338702
Satyrinae	Satyrini	Maniolina	*Hyponephele* sp.	CP10-13	Iran: Bakhtiari	DQ338840	DQ338990	DQ338703
Satyrinae	Satyrini	Maniolina	*Maniola jurtina*	EW4-5	Spain: Sant Ciment	AY090214	AY090180	AY090147
Satyrinae	Satyrini	Maniolina	*Pyronia bathseba*	RV-03-H546	Spain	DQ338841	DQ338991	DQ338704
Satyrinae	Satyrini	Maniolina	*Pyronia cecilia*	EW4-2	Spain: Sant Climent	DQ338842	DQ338992	DQ338705
Satyrinae	Satyrini	Melanargiina	*Melanargia galathea*	EW24-17	Francia: Languedoc	DQ338843	DQ338993	DQ338706
Satyrinae	Satyrini	Melanargiina	*Melanargia hylata*	CP10-10	Iran: Ardabil	DQ338844	DQ338994	DQ338707
Satyrinae	Satyrini	Melanargiina	*Melanargia russiae*	CP-AC23-83	Russia: Tuva	DQ338845	DQ338995	DQ338708
Satyrinae	Satyrini	Pronophilina	*Apexacuta astoreth*	CP09-78	Peru: Apurímac	DQ338846	DQ338996	DQ338709
Satyrinae	Satyrini	Pronophilina	*Corades cistene*	CP09-84	Peru: Apurímac	DQ338847	DQ338997	DQ338710
Satyrinae	Satyrini	Pronophilina	*Daedalma* sp.	CP13-05	Ecuador: Tungurahua	DQ338848	DQ338998	—
Satyrinae	Satyrini	Pronophilina	*Eteona tisiphone*	NW127-21	Brazil: Minas Gerais	DQ338849	DQ338999	DQ338711
Satyrinae	Satyrini	Pronophilina	*Foetterleia schreineri*	NW127-19	Brazil: Minas Gerais	DQ338590	DQ339000	DQ338712
Satyrinae	Satyrini	Pronophilina	*Junea dorinda*	CP06-94	Peru: Pasco	DQ338850	DQ339001	DQ338713
Satyrinae	Satyrini	Pronophilina	*Lasiophila cirta*	CP04-36	Peru: Junín	DQ338851	DQ339002	DQ338714
Satyrinae	Satyrini	Pronophilina	*Lasiophila piscina*	PE-5-5	Peru: Cuzco	DQ338852	DQ339003	—
Satyrinae	Satyrini	Pronophilina	*Lymanopoda rana*	CP03-33	Peru: Junín	DQ338853	DQ339004	DQ338715
Satyrinae	Satyrini	Pronophilina	*Oxeoschistus leucospilos*	CP04-67	Peru: Junín	DQ338854	DQ339005	DQ338716
Satyrinae	Satyrini	Pronophilina	*Panyapedaliodes drymaea*	CP09-53	Peru: Apurímac	DQ338855	DQ339006	DQ338717
Satyrinae	Satyrini	Pronophilina	*Parapedaliodes parepa*	CP07-51	Peru: Lima	DQ338591	DQ339007	DQ338718

Table 2 (*continued*)

Subfamily	Tribe	Subtribe	Species	Specimen ID	Source of specimen	COI	*EF-1α*	*Wingless*
Satyrinae	Satyrini	Pronophilina	*Pedaliodes* sp. n. 117	CP09-66	Peru: Apurímac	DQ338856	DQ339008	DQ338719
Satyrinae	Satyrini	Pronophilina	*Praepedaliodes phanias*	CP10-04	Brazil: São Paulo	DQ338592	DQ339009	DQ338720
Satyrinae	Satyrini	Pronophilina	*Praepedaliodes* sp.	CP12-01	Brazil: São Paulo	DQ338857	DQ339010	DQ338721
Satyrinae	Satyrini	Pronophilina	*Proboscis propylea*	CP07-15	Peru: Pasco	DQ338858	DQ339011	DQ338722
Satyrinae	Satyrini	Pronophilina	*Pronophila thelebe*	CP03-70	Peru: Junín	DQ338859	DQ339012	DQ338723
Satyrinae	Satyrini	Pronophilina	*Pseudomaniola loxo*	CP13-13	Colombia: Antioquia	DQ338860	DQ339013	—
Satyrinae	Satyrini	Pronophilina	*Pseudomaniola phaselis*	CP04-01	Peru: Junín	DQ338593	DQ339014	DQ338724
Satyrinae	Satyrini	Pronophilina	*Punapedaliodes flavopunctata*	CP07-87	Peru: Pasco	DQ338861	DQ339015	DQ338725
Satyrinae	Satyrini	Pronophilina	*Steremnia umbracina*	CP07-89	Peru: Huánuco	DQ338862	DQ339016	DQ338726
Satyrinae	Satyrini	Pronophilina	*Steroma modesta*	CP03-71	Peru: Junín	DQ338594	DQ339017	DQ338727
Satyrinae	Satyrini	Satyrina	*Arethusana arethusa*	CP11-06	Spain: Navarra	DQ338863	DQ339018	DQ338728
Satyrinae	Satyrini	Satyrina	*Berberia lambessanus*	EW26-29	Morocco: Moyen Atlas central	DQ338864	DQ339019	—
Satyrinae	Satyrini	Satyrina	*Brintesia circe*	CP-B01	France: Languedoc	DQ338865	DQ339020	DQ338729
Satyrinae	Satyrini	Satyrina	*Chazara briseis*	EW26-19	Morocco: Rif oriental	DQ338866	DQ339021	DQ338730
Satyrinae	Satyrini	Satyrina	*Hipparchia fidia*	RV-03-H920	Spain: San Masteu-Albociner	DQ338595	—	DQ338731
Satyrinae	Satyrini	Satyrina	*Hipparchia parisatis*	CP10-06	Iran: Isfahan	DQ338867	DQ339022	—
Satyrinae	Satyrini	Satyrina	*Hipparchia semele*	EW24-25	Sweden: Stockholm	DQ338868	DQ339023	DQ338732
Satyrinae	Satyrini	Satyrina	*Hipparchia statilinus*	EW25-24	Greece: Peloponnesos	DQ338596	DQ339024	DQ338733
Satyrinae	Satyrini	Satyrina	*Karanasa pamira*	CP-AC23-32	Russia: Vanch	DQ338869	DQ339025	DQ338734
Satyrinae	Satyrini	Satyrina	*Neominois ridingsii*	CD-1-1	USA: Colorado	DQ338870	DQ339026	DQ338735
Satyrinae	Satyrini	Satyrina	*Oeneis jutta*	EW4-1	Sweden	DQ018958	DQ018925	DQ018896
Satyrinae	Satyrini	Satyrina	*Paralasa jordana*	CP-AC23-35	Russia: Karasu	DQ338597	DQ339027	DQ338736
Satyrinae	Satyrini	Satyrina	*Pseudochazara mamurra*	CP10-11	Iran: Isfahan	DQ338598	DQ339028	DQ338737
Satyrinae	Satyrini	Satyrina	*Satyrus actaea*	EW20-12	France: Carcassonne	DQ338871	DQ339029	DQ338738
Satyrinae	Satyrini	Satyrina	*Satyrus ferula*	EW26-21	Morocco: Haut Atlas septentrional	DQ338872	DQ339030	DQ338739
Satyrinae	Satyrini	Satyrina	*Satyrus iranicus*	CP10-12	Iran: Hamadan	DQ338873	DQ339031	DQ338740
Satyrinae	Satyrini	Ypthimina	*Neocoenyra petersi*	NW91-5	Tanzania	DQ338874	DQ339032	DQ338741
Satyrinae	Satyrini	Ypthimina	*Ypthima baldus*	NW98-5	Indonesia: Central Sulawesi	DQ338875	DQ339033	DQ338742
Satyrinae	Satyrini	Ypthimina	*Ypthima confusa*	DL-01-N109	Thailand: Chiang Mai	DQ338876	DQ339034	DQ338743
Satyrinae	Satyrini	Ypthimina	*Ypthima fasciata*	NP-95-Y065	Malaysia	DQ338877	DQ339035	—
Satyrinae	Satyrini	Ypthimina	*Ypthimomorpha itonia*	NW117-23	Zambia: Ikelenge	DQ338878	DQ339036	DQ338744
Satyrinae	Satyrini	incertae sedis	*Amphidecta calliomma*	NW126-21	Brazil: Mato Grosso	DQ338879	DQ339037	DQ338745
Satyrinae	Satyrini	incertae sedis	*Palaeonympha opalina*	EW25-21	Taiwan: Pingtung County	DQ338880	DQ339038	DQ338746

For images of voucher specimens, see http://www.zoologi.su.se/research/wahlberg.

76–88%), and strong support for values >10 (bootstrap values 89–100%). We have also assessed clade stability by analyzing a subset of the data with Bayesian inference using the program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). We chose only taxa for which all three genes were successfully sequenced for a total of 124 taxa. The evolution of the sequences was modeled under the GTR + G + I model. The Bayesian analysis was performed on the combined data set with parameter values estimated separately for each gene region (Table 3). The analysis was run twice for 1 million generations, with every 100th tree sampled and the first 1000 sampled generations discarded as burn-in (based on a visual inspection of when log likelihood values reached stationarity). The purpose of this analysis was to investigate the effects on the results under a different tree-building method. 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 will refer to clades that are recovered under parsimony and Bayesian analyses as stable.

Table 3
Parameter values estimated using Bayesian phylogenetic methods

Gene	TL (all)	$r(A \leftrightarrow C)$	$r(A \leftrightarrow G)$	$r(A \leftrightarrow T)$	$r(C \leftrightarrow G)$	$r(C \leftrightarrow T)$	$r(G \leftrightarrow T)$	pi(A)	pi(C)	pi(G)	pi(T)	alpha	pinvar	m
COI	19.77	0.034	0.357	0.019	0.069	0.483	0.038	0.422	0.092	0.025	0.461	0.357	0.358	1.735
EF-1α		0.055	0.282	0.1	0.054	0.455	0.054	0.297	0.217	0.209	0.276	0.825	0.468	0.312
wgl		0.083	0.304	0.099	0.039	0.388	0.086	0.178	0.324	0.325	0.173	0.761	0.308	0.468

Values estimated separately for each gene region.

We rooted the resulting networks with *Libythea* because of the widely held belief that this taxon is the sister group to the rest of Nymphalidae (e.g. Ackery et al., 1999; Brower, 2000; Ehrlich, 1958; Freitas and Brown, 2004; Scott, 1985; Wahlberg et al., 2003b). Additional outgroups, including taxa from the "satyroid" subfamilies (sensu Freitas and Brown, 2004), were included to test the monophyly of Satyrinae.

3. Results and discussion

3.1. General properties of sequences

The full data set consisted of 3090 aligned nucleotide sites with no indels. We were not able to amplify the COI gene for one taxon, the *EF-1α* for four taxa, and the *wingless* gene for 20 taxa (Table 2). Of the 1450 bp sequenced for COI, 848 sites were variable and of these 680 were parsimony informative. The respective numbers for *EF-1α* are 1240 bp, 657 variable and 468 parsimony informative, and for *wingless*, 400 bp, 264 variable and 198 parsimony informative sites.

3.2. Phylogenetic analyses

In the separate analyses of each gene region, the partitions produced partially resolved strict consensus trees (Figs. 1–3), recovering only Melanitini as monophyletic group. Each of the three partitions implies relationships that are broadly incongruent with traditionally recognized groupings (i.e., COI recovered *Antirrhea* sister to *Pierella*) (Fig. 1); *EF-1α* recovered many outgroups in spurious derived positions (e.g. Morphinae) (Fig. 2), and *wingless* shows some derived taxa at basal positions (e.g. *Orsotriaena* as sister to *Libythea*) (Fig. 3).

Analysis of the combined data set produced 16 equally parsimonious cladograms. The strict consensus (Figs. 4–6) shows relationships among the major clades of Satyrinae as well as relationships of Satyrinae relative to the outgroups. Our data imply that the Morphinae (sensu Ackery et al., 1999) and Satyrinae are both polyphyletic, grouping together in a clade with strong Bremer and good bootstrap support, appearing as sister to Charaxinae. These subfamilies together with the more basal Calinaginae form part of the "satyroid" (sensu Freitas and Brown, 2004) butterfly subfamilies. Individual clades are discussed in detail below.

Bayesian analysis produced a tree which is broadly congruent with the most parsimonious trees from the combined analysis (Fig. 7). Parameter values for the models used in the analysis are given in Table 3. The major difference is in the position of *Zipaetis* + *Orsotriaena*, which in the parsimony trees is within the subtribe Hypocystina, but in the Bayesian tree is sister to the "advanced satyrines" (as defined below). Polyphyly of Morphinae and Satyrinae is implied by both analytical methods, as is the non-monophyly of many other groups previously hypothesized to be natural (see below for discussion of these).

3.3. Support

Examination of the contributions to the support of various clades by the three gene regions employed in the simultaneous analysis reveals that the major source of conflict is the COI partition. In the combined analysis, the COI data set conflicts in 68 of the 182 nodes of the strict consensus tree, while the conflicting nodes are 32 and 52 for *EF-1α* and *wingless*, respectively (Figs. 4–6). The COI partition conflicts in both deep and shallow nodes. The *EF-1α* partition provides the majority of support at almost all the deeper nodes in the combined analysis, conflicting in only four nodes (Figs. 4–6). Apparently, the partition values for *EF-1α* and *wingless* are high enough to overcome the conflicting signal of the COI.

The COI gene has been very useful for uncovering relationships at the generic and specific level (Caterino and Sperling, 1999; Wahlberg et al., 2003a) due to its hypothesized rapid evolutionary rate. In this study, the COI gene carries much of the phylogenetic signal, although the main source of support in the combined analysis comes from the *EF-1α* and *wingless* data sets. *EF-1α* has traditionally been considered to be more informative for resolving deeper divergences and more inclusive categories (Mitchell et al., 1997). Here, the *EF-1α* gene is responsible for recovering some subtribal relationships (Fig. 2), and in the combined analysis it contributes positively to shallow relationships. Thus, the *EF-1α* data set contains some degree of phylogenetic information, contributing positively to several nodes in both deeper and shallow relationships when used in combination with the two other genes. The good resolution and support in our combined tree, despite a high degree of homoplasy within the data partitions, agrees with the Källersjö et al. (1998) statement of the possibility to recover phylogenetic information from such genes, provided that extensive taxonomic sampling is undertaken.

3.4. Implied relationships of Satyrinae

3.4.1. Is Satyrinae monophyletic?

In our combined analyses (Figs. 4–7), the traditional "satyrid" groups (Satyrinae, Morphini, Amathusiini, Brassolini) form a well-supported clade with respect to their sister taxon, Charaxinae, and the other outgroups. Satyrinae, as circumscribed in recent classifications (Ackery et al., 1999; Harvey, 1991), appears as a polyphyletic assemblage, with some representatives, traditionally considered to be "primitive" satyrines (Miller, 1968), grouping with tribes of the Morphinae.

In the "amathusiine" clade, the traditional Amathusiini is grouped with representatives of Zetherina and Parargina (*Neorina* and *Ethope* in this study). *Neorina* and *Ethope* form a clade with Zetherina having strong Bremer and bootstrap support values (>30 steps; 100%), but Zetherina itself (*Penthema* and *Zethera* in this study) is recovered as polyphyletic since *Penthema* and *Zethera* group with *Neorina* and *Ethope*, respectively. It is likely that sampling additional taxa will not change this grouping since it is strongly supported. Amathusiini is sister to the Zetherina + *Neorina* + *Ethope* clade, though

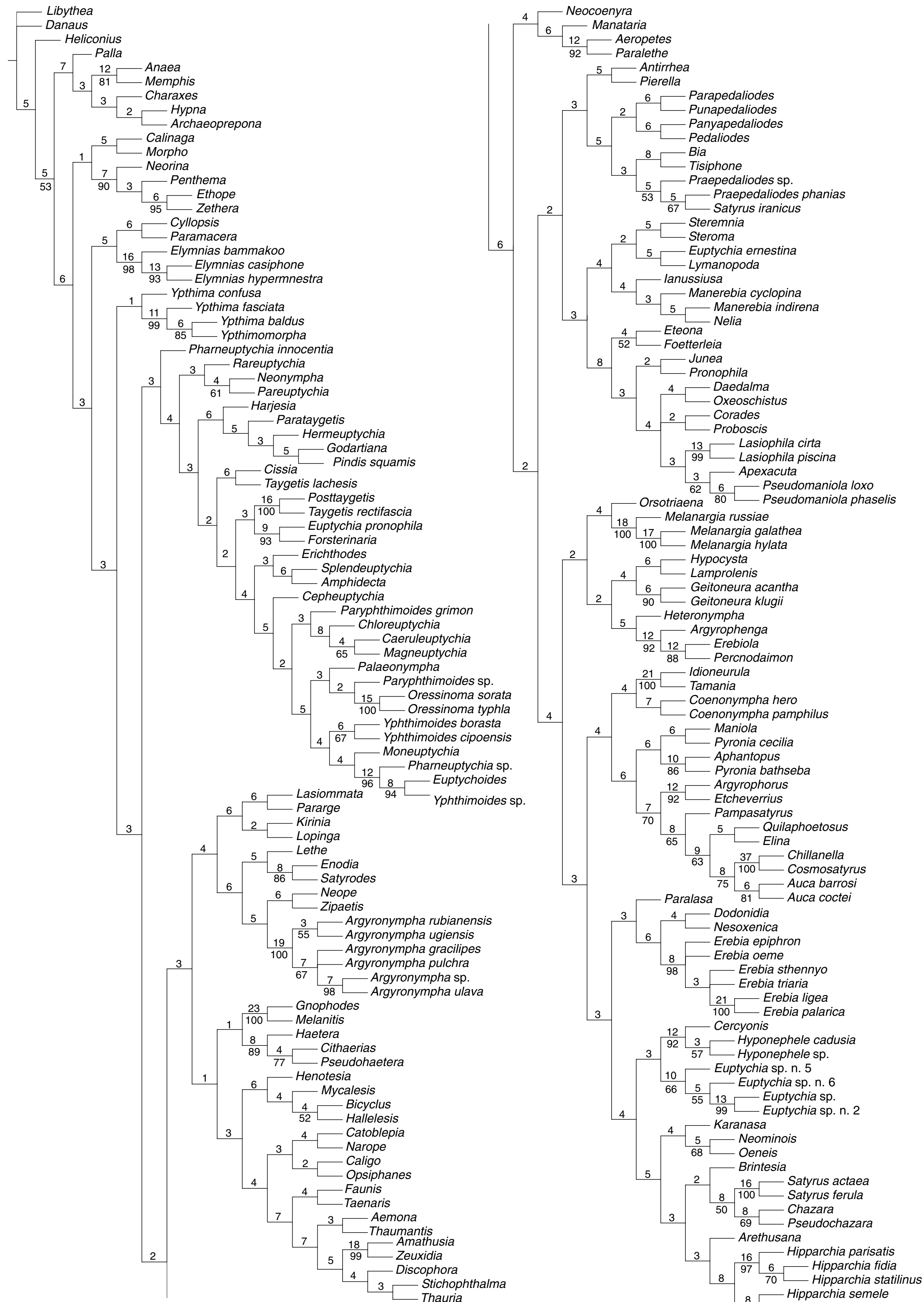

Fig. 1. Strict consensus of 5 equally parsimonious trees from the cladistic analysis of the COI gene data set (length 14376, CI 0.10, and RI 0.33). Numbers given above branches are Bremer support values and numbers below the branch are bootstrap values for the node to the right of the number.

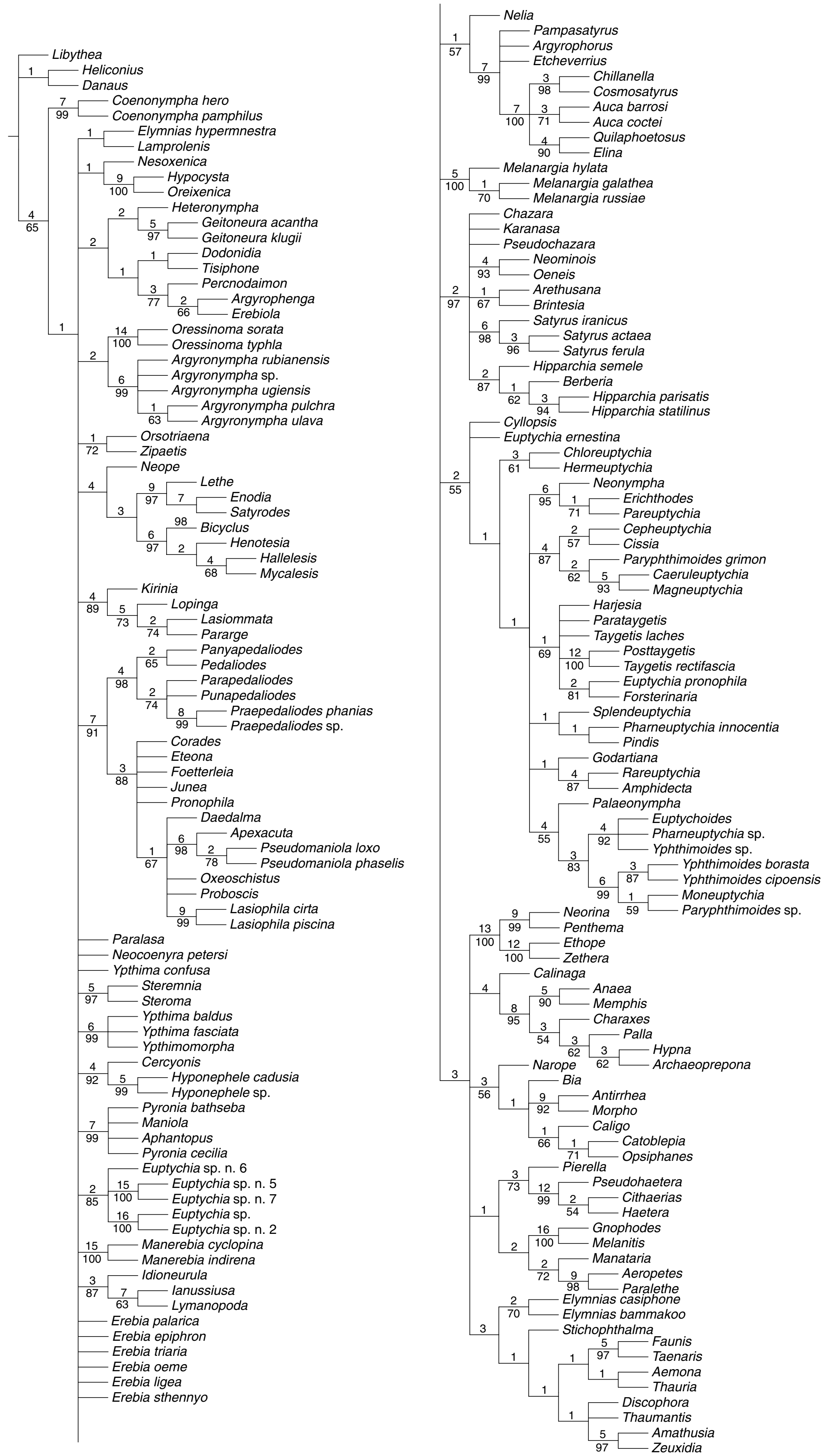

Fig. 2. Strict consensus of 10 equally parsimonious trees from the cladistic analysis of the *EF-1α* gene data set (length 7231, CI 0.15, and RI 0.50). Numbers given above branches are Bremer support values and numbers below the branch are bootstrap values for the node to the right of the number.

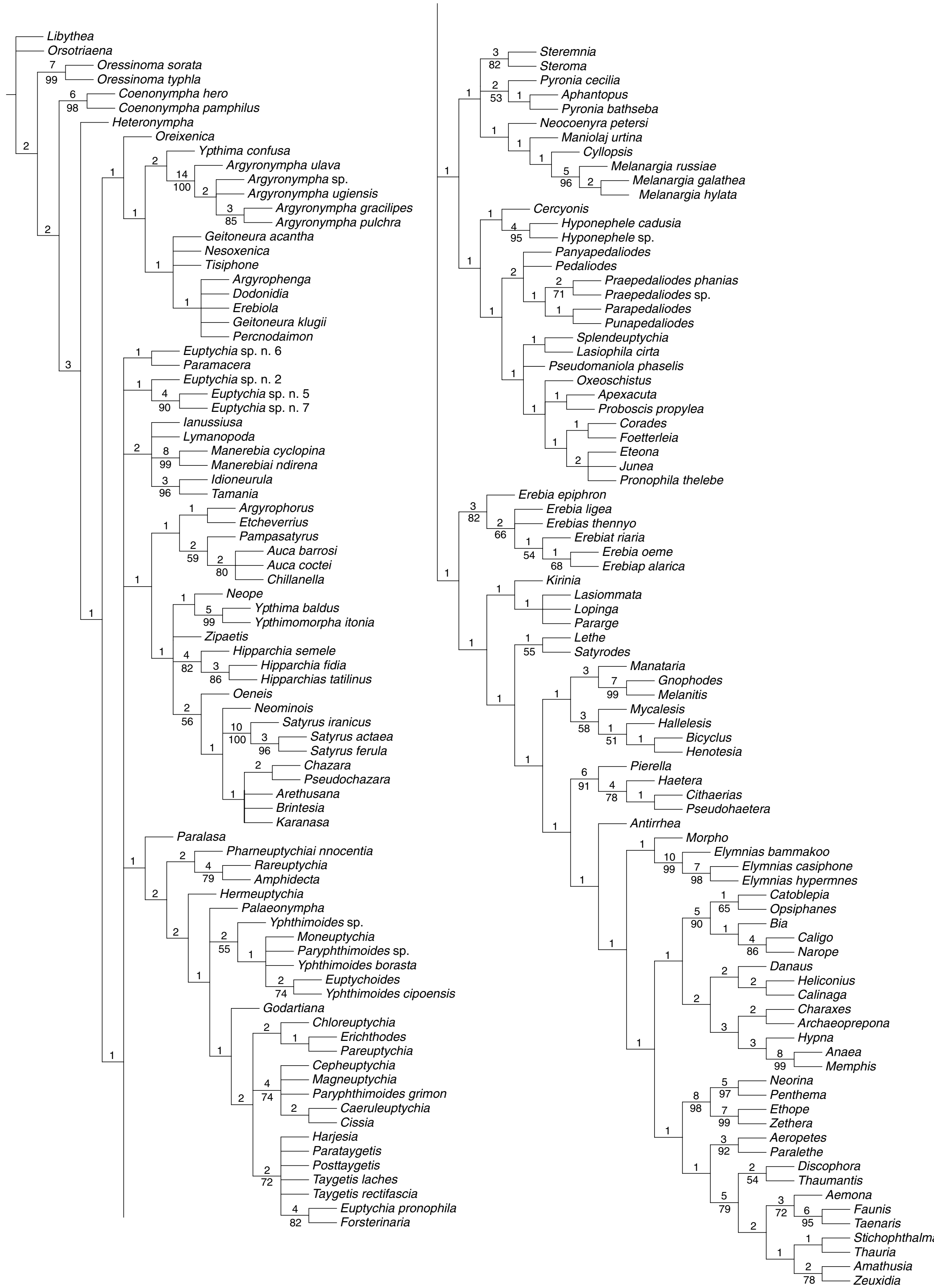

Fig. 3. Strict consensus of 1512 equally parsimonious trees from the cladistic analysis of the *wingless* gene data set (length 2982, CI 0.16, and RI 0.51). Numbers given above branches are Bremer support values and numbers below the branch are bootstrap values for the node to the right of the number.

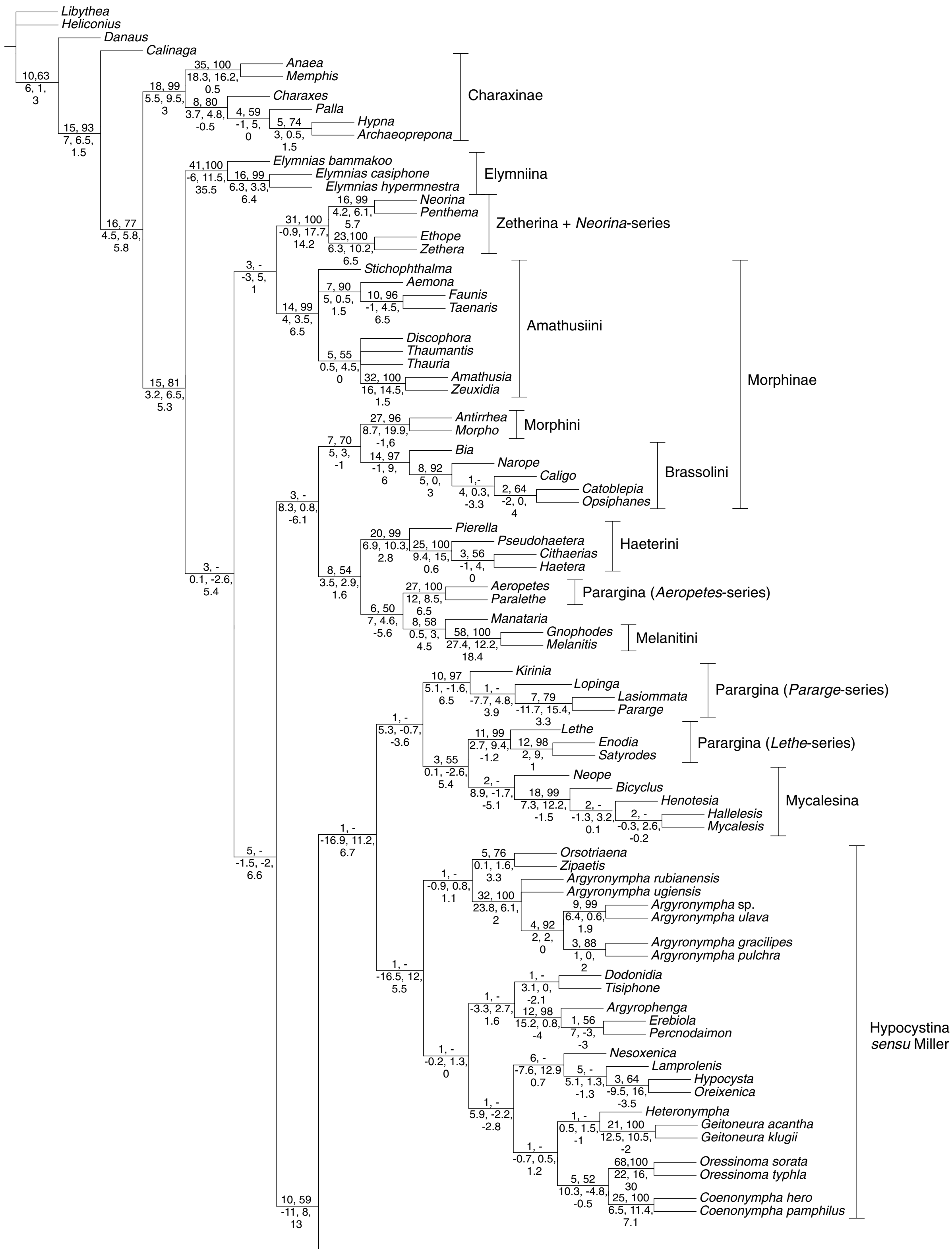

Fig. 4. Strict consensus of 16 equally parsimonious trees from the combined data set of all three genes (length 25006 CI 0.12, and RI 0.40), pruned to show basal clades. For the rest of the cladogram, see Figs. 5 and 6. The numbers given above branches are Bremer support and bootstrap values, respectively, for the node to the right of the number. The numbers below the branches are the contribution of the COI, *EF-1*α, and *wingless* data sets, respectively, to the Bremer support value of the combined analysis (results of the Partitioned Bremer Support analysis).

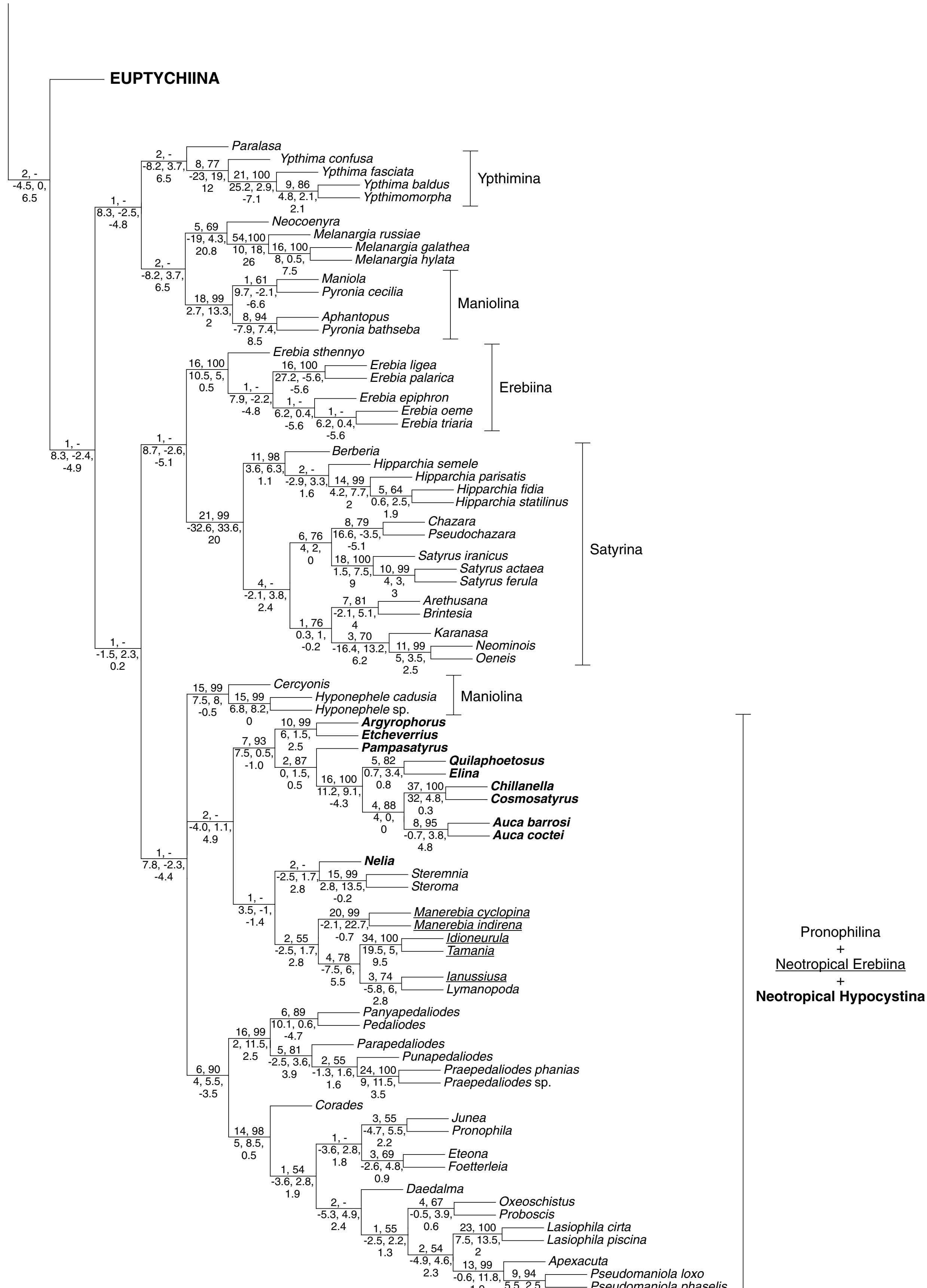

Fig. 5. Continuation of cladogram in Fig. 4. Relationships within Euptychiina appear in Fig. 6. The genera transferred by Viloria (1998, 2003; see Lamas, 2004) from Pronophilina into Erebiina and Hypocystina are underlined and in bold fonts, respectively.

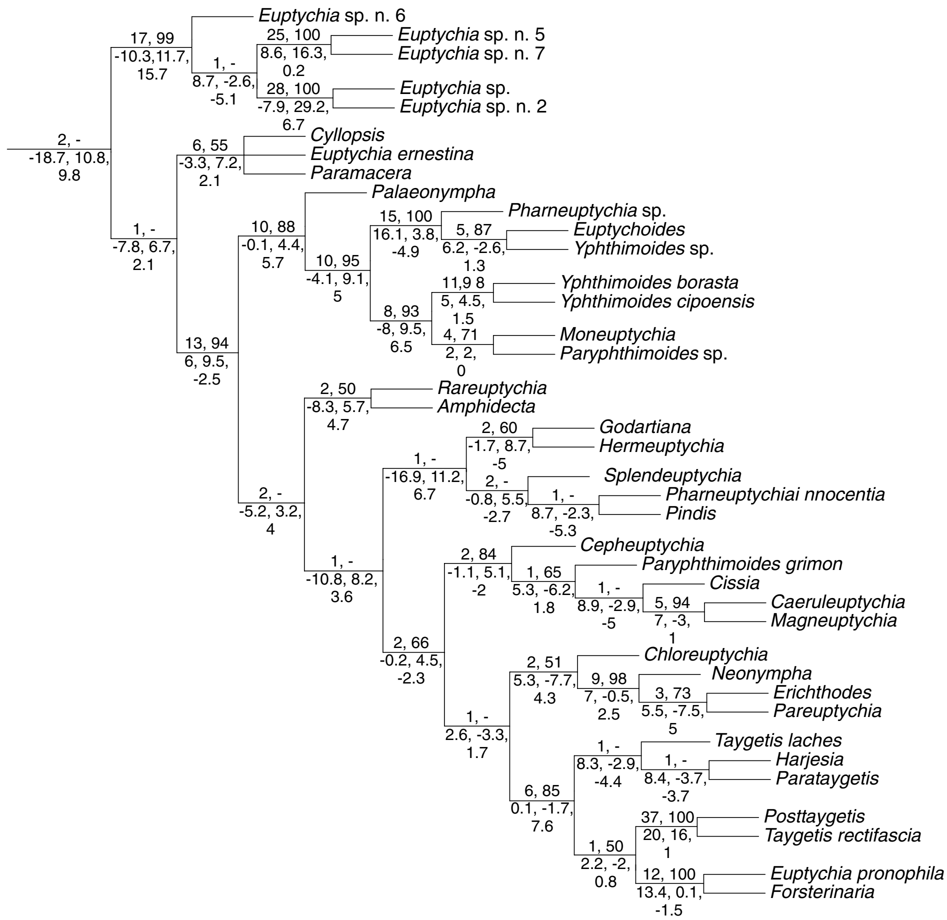

Fig. 6. Continuation of cladogram in Figs. 4 and 5. Relationships within Euptychiina.

this is not well supported and not stable to method of analysis. All of these taxa are Indo-Australian. The next clade to branch off is the Neotropical "morphine" clade, formed by Morphinae tribes Morphini and Brassolini. *Bia* appears basal in the Brassolini clade with strong Bremer and bootstrap support. The "morphine" clade (Amathusiini excluded) is recovered as sister to the "satyrine" Haeterini + Melanitini + *Manataria* + Parargina in part (*Aeropetes* and *Paralethe*).

Together, these three basal clades strongly support the hypothesis that the Satyrinae and Morphinae of current classifications are both polyphyletic, since Zetherina groups with the Amathusiini, and Haeterini + Melanitini + *Manataria* with the Neotropical Morphinae. The polyphyly of Satyrinae and Morphinae is also recovered in the Bayesian analysis. In order to circumscribe monophyletic tribes and subfamilies, it will be necessary to adjust the current status of these major lineages.

3.4.2. Relationships of the "primitive" Satyrinae

Elymniina is found to branch off first, appearing as sister to the remaining Satyrinae + Morphinae that appear forming a clade. The position of Elymniina is not stable, and in the Bayesian analysis, it is placed as sister to the Haeterini with low posterior probability. Interestingly, Elymniina members feed on palms (Arecaceae) as larvae, as do some species of Amathusiini and *Neorina* (Ackery, 1988). Few satyrine butterflies feed on Arecaceae, e.g., some Haeterini (*Dulcedo*; DeVries, 1987). The "palmflies" range from West Africa to the Indo-Australian region, and many of the species are markedly sexually dimorphic being involved in mimicry complexes with various danaine or amathusiine models, features that are quite uncommon among other satyrines. It is interesting to note that the eyespots of Elymniina, when present, are rather simple, not composed of the multiple concentric rings of differently colored scales typical of Morphinae and the rest of Satyrinae.

The Neotropical *Manataria* has been reported using *Guadua angustifolia*, *Bambusa vulgaris* and *Lasiacis* sp. (Poaceae) as host plants (DeVries, 1987; Figueroa, 1953; Murillo and Nishida, 2004). *Manataria* is sister to Melanitini with good Bremer and weak bootstrap support. Sampled Melanitini are monophyletic which is not surprising since some species of *Gnophodes* have been included within the genus *Melanitis* (Larsen, 1991). The hypothesis of a close relationship between *Manataria* and Melanitini is new and well supported by our data. *Gnophodes* and *Melanitis* have crepuscular habits flying at dusk and at dawn (Braby, 2000; Larsen, 1991), while similar crepuscular activity has been recorded for *Manataria* (DeVries, 1987; Stevenson and Haber, 1996; Rydell et al., 2003). Moreover,

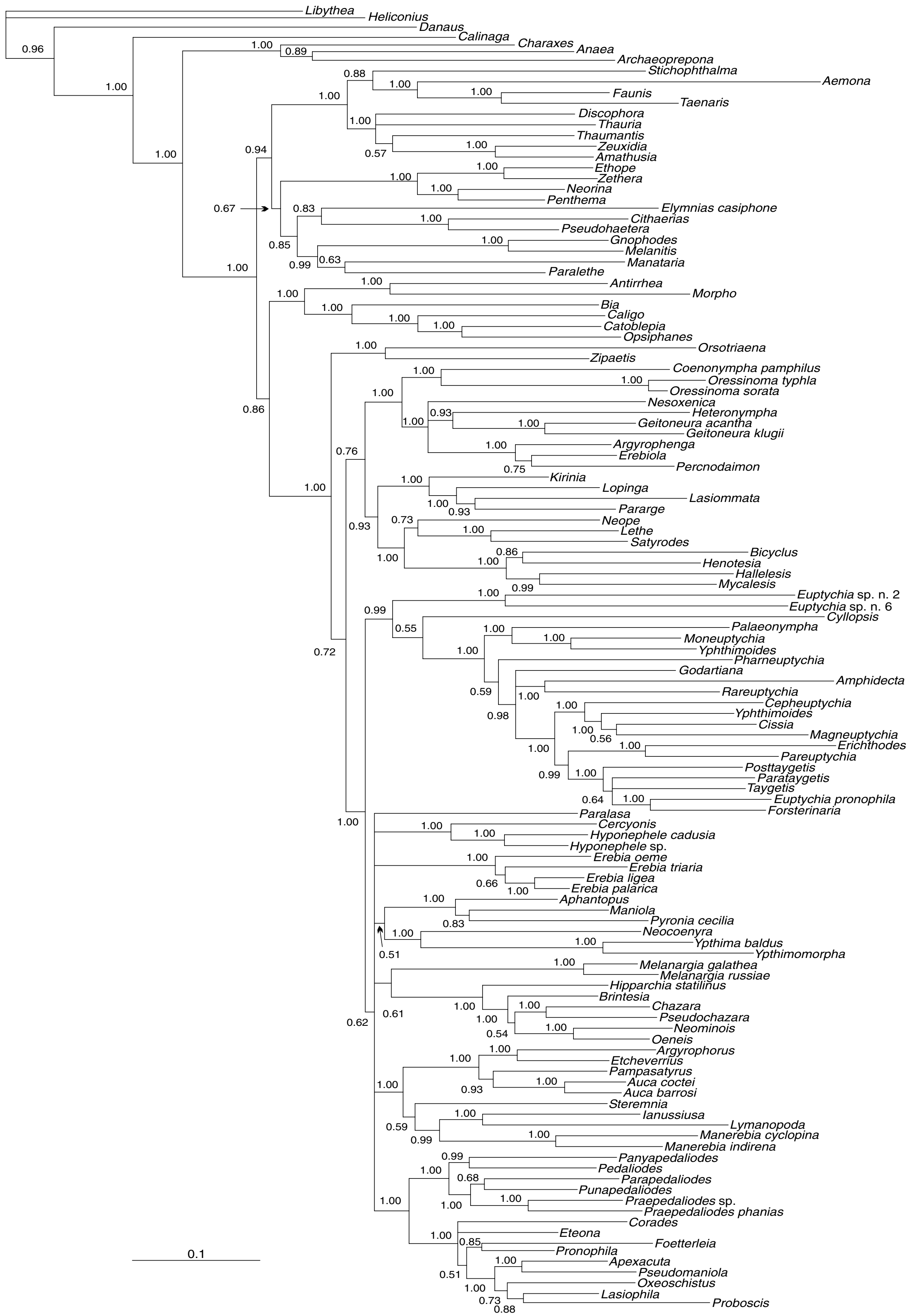

Fig. 7. Phylogenetic hypothesis based on Bayesian analysis of three genes, each modeled with a GTR + G + I model. Average log likelihood of tree −81294.34 based on two independent runs. Parameter values for models given in Table 3.

Manataria has been observed roosting in tree holes or shaded areas along forest trails in Mexico and Costa Rica, in groups up to 80 individuals (Barrera and Díaz-Batres, 1977; Murillo and Nishida, 2004; Stevenson and Haber, 1996). Interestingly, Larsen (1991) reports that *Gnophodes* species also form small congregations in forest trails. The close relationship between *Manataria* and Melanitini (Fig. 4) suggests that these striking behaviors may be due to a common origin. Whether these similarities are synapomorphies or convergence needs further investigation, since some other "satyroid" taxa are also crepuscular (e.g. most Brassolini and some *Taygetis* species in Satyrinae). Miller and Miller (1997) suggested a close affinity among *Manataria*, *Aeropetes* and *Paralethe* of the Parargina. While this relationship was supported weakly by morphological evidence, it is corroborated here by molecular data. The African genera *Aeropetes* and *Paralethe* are sister taxa appearing as sister to the clade *Manataria* + Melanitini. The clade containing Melanitini + *Manataria* + *Paralethe* is stable to method of analysis.

3.4.3. Relationships of the "advanced" Satyrinae

The remainder of Figs. 4–7 represent the "satyrine" clade, which is recovered with strong Bremer support and posterior probability, but weak bootstrap support, and includes groups traditionally considered as "advanced" Satyrinae, i.e. all the satyrine representatives in this study but Elymniina, Zetherina, Melanitini, Haeterini, *Manataria* and part of the Parargina (*Neorina*, *Ethope*, *Aeropetes* and *Paralethe* as stated above). The "satyrine" clade is partially resolved, recovering as monophyletic entities only a few of its tribes and subtribes (sensu Harvey, 1991) with poor support for relationships among subtribes.

Basal within the "advanced" satyrine butterflies is a robust monophyletic group formed by some of the Parargina—*Pararge*, *Lopinga*, *Lasiommata* and *Kirinia*—which correspond to one of Miller's (1968) subdivisions of his "Lethini", his *Pararge*-series. Part of Miller's *Lethe*-series (represented by *Lethe*, *Neope*, *Enodia* and *Satyrodes* in this study) appears as sister to a clade containing the Mycalesina and *Neope*, although in the Bayesian analysis *Neope* comes out as sister to the *Lethe*-series.

Members of Miller's *Pararge*-series (*Pararge*, *Kirinia*, *Lasiommata* and *Lopinga*) form a cohesive clade. Miller's other subdivisions of "Lethini" form independent clades congruent with each of Miller's series. Obviously each of Miller's sections represents very different lineages, *Neorina*-series group with Zetherina, *Lethe*-series group with Mycalesina, *Aeropetes*-series group with *Manataria* and Melanitini, and *Pararge*-series is on its own. These results suggest that Parargina (sensu Miller) should no longer be used.

Traditional Mycalesina is not monophyletic, and the monophyly of Mycalesina as a cohesive clade (18 Bremer and 99% bootstrap support, 100% posterior probability) without *Orsotriaena* is quite surprising. *Orsotriaena* is generally recognized as being closely related to *Mycalesis* (Braby, 2000; Parsons, 1999), mainly due to adult morphology. However, larval and pupal morphology of *Mycalesis* and *Orsotriaena* are strikingly different. If *Orsotriaena* is not related to the other Mycalesina as implied by our results and morphological differences of immature stages, the adult morphological similarities are not homologous. Moreover, only the forewing vein Sc of *Orsotriaena* is basally inflated, while all veins, except the forewing radial vein, are basally inflated in *Mycalesis* (Parsons, 1999). *Orsotriaena* appears in Hypocystina as sister to the genus *Zipaetis*, a sister relationship which is strongly supported and stable.

The genera forming the Hypocystina clade correspond with Miller's (1968) taxa, but not with Viloria's (2003) temperate South American taxa. The Hypocystina, including the "non-hypocystine" genera *Orsotriaena*, *Coenonympha* and *Oressinoma*, appear in a clade with weak Bremer and no bootstrap support. The Neotropical euptychiine genus *Oressinoma* appears as sister to *Coenonympha*. The position of *Oressinoma*, far from Euptychiina is perhaps not so surprising: *Oressinoma* has a much differentiated adult morphology, some authors being inclined to consider *Oressinoma* as an aberrant genus (Miller, 1968). This may explain why *Oressinoma* did not group with Euptychiina in Murray and Prowell's (2005) study. The disjunct distribution of *Oressinoma* and its hypocystine relatives implies either an ancient Gondwanan common origin or more recent dispersal across wide oceanic barriers.

The Euptychiina appears as sister to a partially resolved clade, formed by representatives of Ypthimina, Maniolina, Pronophilina, Melanargiina, Erebiina and Satyrina. The odd Neotropical *Amphidecta* is clearly within Euptychiina, though it has traditionally been associated with Pronophilina and is currently classified *incertae sedis* (Lamas, 2004). Characters from immature stages of *Amphidecta reynoldsi* were not conclusive in resolving its affinities (Freitas, 2004b). Euptychiina without *Oressinoma*, but including *Amphidecta*, is monophyletic. Another surprising result is the inclusion of the Oriental genus *Palaeonympha* (thus far of uncertain position) within Euptychiina, which is a group thought to be entirely restricted to the Americas. This association is robust and is likely to remain stable to the addition of more data. If this hypothesis is corroborated through a comparative morphological study of *Palaeonympha* and the Euptychiina, the former would be the only euptychiine taxon distributed outside the Americas. More interesting is the fact that, in our data set, *Palaeonympha* appears related to species from the Southeastern Atlantic forests of Brazil. Miller (1968) commented on the similarity of the genus to euptychiines, but was not willing to place *Palaeonympha* in Euptychiina because of the disjunct distribution of this taxon. This relationship presents great potential for biogeographic studies.

Ypthimina without *Neocoenyra* is recovered as monophyletic, grouping with *Paralasa* and being sister to Melanargiina + Maniolina. The Bayesian analysis recovers Ypthimina with *Neocoenyra* as monophyletic, and places Melanargiina as sister to Satyrina.

Maniolina appears to be polyphyletic. The core Maniolina, including *Aphantopus* but excluding *Cercyonis* and *Hyponephele*, is stable with strong Bremer and bootstrap support (18 steps; 99%). *Cercyonis* and *Hyponephele* come out as sister groups with strong support, but their position with regard to the other clades is unresolved in both analyses. This study corroborates the transfer of *Aphantopus* from the Coenonymphina into Maniolina (Martin et al., 2000). Because *Cercyonis* and *Hyponephele* appear in an unresolved position, more sampling of Maniolina taxa is needed to resolve its affinities.

Satyrina without *Paralasa* is monophyletic with strong Bremer and bootstrap support (21 steps; 99%) and sister to Erebiina (only *Erebia* species), a result which is not stable to method of analysis.

The speciose Pronophilina sensu Miller (1968) appears in two clades within a polytomy, with Maniolina (*Cercyonis* and *Hyponephele*) with weak support. One clade includes Viloria's (1998, 2003; see Lamas, 2004) Neotropical "Hypocystina" and "Erebiina", in addition to *Steremnia*, *Steroma* and *Lymanopoda*, while the other clade includes the remaining Pronophilina. This analysis shows that the genera that Viloria (1998, 2003) transferred from Pronophilina into Erebiina and Hypocystina are actually more closely related to the genera currently retained in Pronophilina (Lamas, 2004), and are, distantly related to the Australian Hypocystina and Palaeartic Erebiina. Our results thus refute Viloria's hypothesis (in Lamas, 2004) that a great part of the Pronophilina belong to Hypocystina and Erebiina. Viloria's Neotropical Hypocystina and Erebiina are not supported, and his hypothesis of a Gondwanan origin for his Hypocystina should be discarded.

4. Concluding remarks

This study represents the most extensive cladistic analysis to date of the long-neglected satyrine butterflies. Although we were not able to sample some taxa that might represent major lineages in the subfamily, our results are both robust and challenging, suggesting new relationships, refuting recent hypotheses and classifications, and strongly implying the need of major revision for some of the traditionally recognized subfamilies in the Nymphalidae. More importantly, our results highlight the satyrines' strong potential as a model for research in biogeography and evolutionary biology.

The results of the combined analysis of the three genes show that, of the named suprageneric taxa in the current classification of Satyrinae, only Haeterini is a natural group, while the other tribes and subtribes are either para- or polyphyletic assemblages. This study also suggests new interesting relationships of taxa long considered of uncertain affinities (e.g. *Manataria*, *Amphidecta* and *Palaeonympha*). We offer a tentative new higher classification of Satyrinae in Table 1 based on our current results, but anticipate further changes, especially regarding the status and circumscription of the subfamilies Satyrinae and Morphinae.

The traditionally recognized tribe Haeterini and subtribe Satyrina without *Paralasa* are recovered as cohesive entities, and additional data, we believe, are not likely to modify their monophyletic status. Similarly, "non-primitive" satyrine butterflies as a clade (which includes the Mycalesina, Satyrini and some members of Parargina, *Pararge* and *Lethe* series) has good support and will probably remain robust with addition of data. Euptychiina and Mycalesina also appear to be well supported monophyletic groups. However, some genera traditionally associated with these subtribes are clearly not related to them: e.g. *Orsotriaena* does not group with Mycalesina and *Oressinoma* is not part of Euptychiina.

Comparison of the parsimony and Bayesian analyses (Figs. 4–7) suggest that several relationships will require more scrutiny in the form of increased taxon sampling and/or increased character sampling (i.e. more genes sequenced). Branch lengths of many of the uncertain relationships are very short in the Bayesian analysis (Fig. 7) suggesting rapid diversification of several clades, including the clade consisting of Morphini, Brassolini, Amathusiini, the "primitive" satyrines and the "advanced" satyrines, as well as in the clade containing Satyrina, Erebiina, Pronophilina, Maniolina and Ypthimina. Taxa which require close scrutiny are *Elymnias* and the clade *Zipaetis* + *Orsotriaena*. Relationships of these taxa in the two analyses imply very different evolutionary scenarios and resolving these conflicts will require increased character sampling.

Our results imply some intriguing biogeographical patterns. We identify taxa with disjunct distributions that may have dispersed over oceanic barriers or their disjunct distributions resulted from vicariance due to the break up of ancient land masses. This is the case of the Neotropical genera *Manataria* and *Oressinoma* that are related to the Melanitini (African and Indo-Australian) and Hypocystina (Indo-Australian), respectively. *Palaeonympha* also shows an intriguing wide disjunction with its closest relatives in the Americas. All of these patterns will require closer scrutiny with more taxa sampled from the respective clades, in order to find the most likely sister groups of the disjunct taxa in question.

The "primitive" Satyrinae feed mostly on palms (fam. Arecaceae), while the species-rich "advanced" Satyrinae clade feed mostly on grasses from the family Poaceae. Strong support for the two main groups in Satyrinae, the "advanced" and "primitive" satyrines is corroborated by their different host plant preferences, and suggests that the shift from feeding on palms to grasses was a dramatic step in the evolution of the subfamily, driving the diversification of the bulk of Satyrinae, the speciose, mainly Neotropical subtribes Pronophilina and Euptychiina. Ehrlich and Raven (1964) stated that phytophagous insects diversify together with their hosts by mutual interaction along history. However, there is evidence that the crown Poaceae diversified in the Late Cretaceous (80 Mya ago; Prasad et al., 2005) while the origin of butterflies may have taken place around 70 Mya ago (Vane-Wright, 2004), and certainly, the Satyrinae is a younger lineage of butterflies. Further studies will investigate the age of the satyrines and the evolution of host plant use in the most diverse subfamily of butterflies (Peña et al., in prep.).

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