Biogeographic history of the butterfly subtribe Euptychiina (Lepidoptera, Nymphalidae, Satyrinae)

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The diverse butterfly subtribe Euptychiina was thought to be restricted to the Americas. However, there is mounting evidence for the Oriental *Palaeonympha opalina* being part of Euptychiina and thus a disjunct distribution between it (in eastern Asia) and its sister taxon (in eastern North America). Such a disjunct distribution in both eastern Asia and eastern North America has never been reported for any butterfly taxon. We used 4447 bp of DNA sequences from one mitochondrial gene and four nuclear genes for 102 Euptychiina taxa to obtain a phylogenetic hypothesis of the subtribe, estimate dates of origin and diversification for major clades and perform a biogeographic analysis. Euptychiina originated 31 Ma in South America. Early Euptychiina dispersed from North to South America via the temporary connection known as GAARlandia during Eocene–Oligocene times. The current disjunct distribution of the Oriental *Palaeonympha opalina* is the result of a northbound dispersal of a lineage from South America into eastern Asia via North America. The common ancestor of *Palaeonympha* and its sister taxon *Megisto* inhabited the continuous forest belt across North Asia and North America, which was connected by Beringia. The closure of this connection caused the split between *Palaeonympha* and *Megisto* around 13 Ma and the severe extinctions in western North America because of the climatic changes of the Late Miocene (from 13.5 Ma onwards) resulted in the classic 'eastern Asia and eastern North America' disjunct distribution.

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Introduction

A general pattern of disjunct distributions between eastern Asia and eastern North America has been reported for a variety of animal and plant taxa (Carreno & Lankester 1994; Nordlander *et al.* 1996; Wang *et al.* 2003; Nie *et al.* 2006). This pattern was known from plants before Linnaean times (Xiang *et al.* 1998; Wen 1999) and has also been reported in wasps (Nordlander *et al.* 1996) and fishes (Hardman 2005). It has been proposed that this pattern is the result of the severance of a continuous belt of tropical and subtropical forests that extended throughout North

America, Europe and Asia during the Tertiary (Guo 1999; Sanmartín *et al.* 2001).

Surprisingly, even though butterflies are relatively well known biologically and are considered model organisms for numerous fields of evolutionary study (Boggs *et al.* 2003), this pattern of disjunct distribution has not yet been reported for any butterfly taxon. Although butterflies are not as diverse as some other insects (e.g. beetles and leaf-hoppers), studies on some groups of the approximately 14 000 species in the world (Ackery *et al.* 1999) have permitted the discovery of interesting biogeographic

patterns (Kodandaramaiah & Wahlberg 2007, 2009; Wahlberg & Freitas 2007; Silva-Brandão *et al.* 2008; Leneveu *et al.* 2009; Peña *et al.* 2010). As the Neotropical region harbours about 40% of all known butterfly species (Lamas 2004), one might expect that a great number of biogeographic studies would be focused on the region. However, most of our current ideas on the biogeographic past of Neotropical butterflies are based on speculative hypotheses with limited use of both phylogenetic information and dated phylogenies (Miller & Miller 1997; Viloria 2003, 2007). Dating of phylogenies is crucial to place the evolutionary history of the study groups in a temporal framework, to allow the identification of those geological events responsible for current biogeographic distributions (Sanmartín *et al.* 2001).

It is only recently that phylogenetic analyses coupled with estimation of origin and diversification times for major lineages (employing relaxed molecular clock techniques) have begun to be used with strong emphasis in order to elucidate the biogeographic history of Central and South American butterfly groups (Willmott *et al.* 2001; Mallarino *et al.* 2005; Wahlberg & Freitas 2007; Elias *et al.* 2009). In a previous study, we found that the highly diverse butterfly subfamily Satyrinae includes taxa with interesting disjunct distributions (Peña *et al.* 2006). Our molecular dataset of Satyrinae taxa and related groups provided evidence that the Oriental butterfly *Palaeonympha opalina* might be sister to some members of the subtribe Euptychiina, which was thus far entirely restricted to the Americas. This had been suggested earlier by Miller (1968), who found morphological similarities between *Palaeonympha* and members of the Euptychiina. These findings tempted him to classify the eastern Asian genus formally as a member of the Euptychiina, a subtribe that includes only a few species in North America. However, because of its disjunct distribution, Miller (1968) decided to give *Palaeonympha opalina incertae sedis* status.

The Euptychiina is one of the biggest groups in the highly diverse subfamily Satyrinae. It includes around 400 known species (Lamas 2004) distributed in the Nearctic and Neotropical regions, although the bulk of species occurs in Central and, especially, South America. As larvae, Euptychiina feed mainly on monocot plants such as grasses and bamboo (DeVries 1987; Ackery 1988), with the exception of some species in the genus *Euptychia* that feed on mosses and lycopsids (Singer *et al.* 1971; Singer & Mallet 1986). Adults of most euptychiine species are brown butterflies without the striking colours of other members of the Nymphalidae, such as *Morpho* or *Heliconius*. This may account for the lack of basic evolutionary studies on the group. Their taxonomy is in urgent need of revision, plagued by unnatural genera and many undescribed species (Lamas 2004; Murray & Prowell 2005; Peña & Lamas 2005; Freitas & Peña 2006; Freitas 2007; Pulido & Andrade 2008; Huertas *et al.* 2009).

Forster (1964) created many of the genera that currently form Euptychiina based on morphological characters of the male genitalia of species from Bolivia, but failed to provide clear diagnoses for his new genera. Miller (1968) formally established the subtribe (as Euptychiini) and included many of Forster's genera. The first phylogenetic study of Euptychiina (Murray & Prowell 2005) found it to be polyphyletic because *Oressinoma* was associated with the Ypthimina and Lethina, and *Euptychia* tended to be related to outgroup taxa. This was later explained by Peña *et al.* (2010) who found that *Euptychia* suffers from long-branch attraction artefacts and tends to be attracted towards either the root or another unrelated Satyrinae taxon. In addition, they confirmed that *Oressinoma* is not a euptychiine, but actually part of the Coenonymphina, a subtribe distributed in the Palaearctic and Indo-Australian regions (Peña *et al.* 2006; Kodandaramaiah *et al.* 2010).

The phylogenetic study by Peña *et al.* (2006) showed for the first time, the big picture of relationships within Satyrinae. They found that the Oriental *Palaeonympha* belonged to Euptychiina, as suggested by Miller (1968), and appears to be more closely related to some euptychiines endemic to the Atlantic forests of south-eastern Brazil. However, taxon sampling by Peña *et al.* (2006) was incomplete. They did not include any of the many euptychiines from North and Central America that could be closely related to *Palaeonympha opalina* and fit the classic eastern Asia–North America biogeographic pattern. Peña *et al.* (2006) did not elaborate further on the relationships of *Palaeonympha* and were unable to propose a satisfactory explanation for the disjunct eastern Asia and south-eastern Brazil pattern.

In this study, we perform a phylogenetic analysis of an extensive sampling of Euptychiina taxa to obtain a robust phylogenetic hypothesis to use in a biogeographic analysis of the group. We reconstruct the biogeographic history of Euptychiina and propose an explanation for the disjunct distribution of the Oriental *Palaeonympha*.

Materials and methods
Taxon sampling and molecular methods
We aimed to sample several species of as many genera as possible in the Euptychiina for a total of 102 Euptychiina species, including *Palaeonympha*. We could not obtain samples of the genera *Caenoptychia*, *Praefaunula*, *Pseudeuptychia* and *Taygetina*. We also included in the analyses 24 outgroups from our previous studies (Peña & Wahlberg 2008; Peña *et al.* 2010). Sequences for *Satyrotaygetis taygetina* and *Pareuptychia occirhoe* were taken from Murray

& Prowell (2005). Taxonomic nomenclature for genera and species follows Lamas (2004), with additions by Freitas (2004a, 2007) and Freitas & Peña (2006). All sequences have been deposited in the GenBank. Table 1 shows the current classification of sampled species and GenBank accession numbers.

We extracted DNA from two legs, dried or freshly conserved in 96% alcohol, using QIAGEN's (California, USA) DNeasy extraction kit. For all species, we sequenced 1487 bp of the cytochrome oxidase subunit I gene (COI) from the mitochondrial genome, and 1240 bp of the *Elongation Factor-1α* gene (*EF-1α*), 412 bp of the *wingless* gene, 691 bp of the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) and 614 bp of the ribosomal protein S5 gene (RpS5) from the nuclear genome. We used the hybrid primers for PCR amplification and sequencing from Wahlberg & Wheat (2008). Sequencing and sequence alignment were performed following protocols in Peña & Wahlberg (2008).

Phylogenetic analyses

The complete dataset consisted of 126 taxa and 4447 characters. We performed a maximum parsimony analysis (MP) treating all characters as unordered and equally weighted. We performed heuristic searches using the software TNT 1.1 (Goloboff *et al.* 2003) using a level of search 10, followed by branch-swapping of resulting trees with up to 10 000 trees held during each step. The searches were performed using the New Technology Search algorithms of TNT – successive Sectorial searches, Ratchet, Tree Drift and Tree Fusing. All cladograms were rooted with *Aeropetes*.

We evaluated clade robustness 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 using the scripting feature of TNT (script pbsup.run taken from http://www.zmuc.dk/public/phylogeny/TNT/scripts/).

We also implemented a model-based phylogenetic method to analyse our dataset to test whether the resulting tree is congruent with the MP method using the software MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). We modelled the evolution of sequences according to the GTR+Γ model as in a previous study (Wahlberg *et al.* 2009), and to avoid artefacts resulting from a 'pathological' correlation between the parameter Γ and the parameter I (invariable sites) (Ren *et al.* 2005; Wahlberg & Wheat 2008). Parameter values were estimated separately for each gene region (Table 2). The analysis was run twice for 20 million generations, with every 1000th tree sampled and the first 10 000 sampled generations discarded as burn-in (based on visual inspection of the log-likelihood reaching

stationarity). We ran the analyses on an AMD 64 dual core twin processor workstation using the LAM/MPI technology for parallel computing (http://www.lam-mpi.org/). We will refer to clades that are recovered under parsimony and Bayesian analyses as robust to the addition of additional data (characters and/or taxa).

Dating of divergences

We used the Bayesian analysis software BEAST ver. 1.4.7 (Drummond & Rambaut 2007) under a log-normal relaxed molecular clock. The DNA sequences were divided in several datasets (one dataset per gene), with parameter values estimated separately for each gene region. The combined dataset was analysed under the GTR+Γ model with a relaxed clock allowing branch lengths to vary following an uncorrelated log-normal distribution (Drummond *et al.* 2006). The analysis was run twice for 15 million generations (with pre-run burn-in of 200 000 generations) with sampled trees every 2000 generations and the results compiled using both runs. The tree priors were set to a Yule speciation process and all other priors were left to the default values in BEAST.

To obtain absolute times of divergence, we used one calibration point. We fixed the crown age of Satyrini at 36.6 Ma with a standard deviation of 5.1 Ma following our previous results (Peña & Wahlberg 2008). Convergence was analysed using Tracer v1.3 and trees were summarized using TreeAnnotator v1.4.7 software, which are distributed with the BEAST package.

Biogeographic analysis

We investigated the biogeographic history of Euptychiina butterflies by evaluating our preferred phylogenetic hypothesis under a dispersal–vicariance analysis (DIVA; Ronquist 1997). The distributions were divided into five biogeographic regions (Fig. 1). Because of a limited sample of species from each outgroup lineage, some of the recovered phylogenetic relationships were spurious. To avoid the estimated areas of distribution of outgroups being affected by an incorrect topology, we used the outgroup relationships as inferred in our previous phylogenetic study of the whole tribe Satyrini Peña *et al.* (2010), which included a more extensive sampling of species. Reconstruction of ancestral distributions was inferred using default costs in the software DIVA (Ronquist 1996) – vicariance events cost zero, dispersal and extinction events cost 1 per unit area.

Results

Euptychiina phylogeny

The combined dataset produced nine equally most parsimonious cladograms of length 20 678 steps (CI 0.17; RI 0.42), the strict consensus of which is shown in Fig. 2.

Table 1 Information of specimens used for molecular studies.

Subfamily	Tribe	Subtribe	Species	Specimen code	Source of specimen	COI	EF-1α	GAPDH	RpS5	Wingless
Satyrinae	Dirini		Aeropetes tulbaghia	CP13-01	S. AFRICA: Mpumalanga Verloren Valei	DQ338579	DQ338907	EU528381	EU528419	DQ338634
Satyrinae	Haeterini		Haetera piera	CP01-84	PERU: Madre de Dios	DQ018959	DQ018926	EU141475	EU141371	DQ018897
Satyrinae	Melanitini		Melanitis leda	NW66-6	AUSTRALIA: Cairns, Queensland	AY090207	AY090173	EU141508	EU141408	AY090140
Satyrinae	Satyrini	Coenonymphina	Hypocysta pseudirius	NW123-5	AUSTRALIA: Newcastle	DQ338826	DQ338974	GQ357413	EU528440	–
Satyrinae	Satyrini	Coenonymphina	Coenonympha pamphilus	EW7-3	SWEDEN: Öland	DQ338777	DQ338920	EU528385	EU528428	DQ338637
Satyrinae	Satyrini	Coenonymphina	Oressinoma sorata	CP06-89	PERU: Oxapampa	GQ357209	GQ357278	GQ357440	GQ357570	GQ357342
Satyrinae	Satyrini	Coenonymphina	Oressinoma typhla	CP07-71	PERU: La Solitaria	DQ338802	DQ338949	GQ357441	EU528452	DQ338666
Satyrinae	Satyrini	Erebiina	Erebia oeme	EW24-7	FRANCE: Languedoc	DQ338780	DQ338923	EU141479	EU141375	DQ338640
Satyrinae	Satyrini	Eritina	Coelites euptychioides	CP16-14	INDONESIA: Kalimantan	GQ357247	GQ357313	GQ357509	GQ357635	GQ357381
Satyrinae	Satyrini	Eritina	Erites argentina	CP16-13	INDONESIA: Kalimantan	EU528321	EU528298	EU528390	EU528435	EU528277
Satyrinae	Satyrini	Eritina	Zipaetis saitis	D30	INDIA	DQ338831	DQ338981	EU528418	EU528472	DQ338696
Satyrinae	Satyrini	Lethina	Lethe minerva	NW121-17	INDONESIA: Bali	DQ338768	DQ338909	EU141492	EU141387	DQ338616
Satyrinae	Satyrini	Maniolina	Maniola jurtina	EW4-5	SPAIN: Sant Climent	AY090214	AY090180	EU141481	EU141376	AY090147
Satyrinae	Satyrini	Melanargina	Melanargia galathea	EW24-17	FRANCE: Languedoc	DQ338843	DQ338993	EU528398	EU528444	DQ338706
Satyrinae	Satyrini	Mycalesina	Mycalesis terminus	EW18-8	AUSTRALIA: Cairns	DQ338765	DQ338905	EU528400	EU528446	DQ338632
Satyrinae	Satyrini	Mycalesina	Orsotriaena medus	EW25-17	BANGLADESH: Sylhet Div.	DQ338766	DQ338906	EU528405	EU528453	DQ338633
Satyrinae	Satyrini	Parargina	Pararge aegeria	EW1-1	FRANCE: Carcassonne	DQ176379	DQ338913	EU141476	EU141372	DQ338620
Satyrinae	Satyrini	Satyrina	Satyrus actaea	NW162-21	FRANCE: Aude, Villegly	GQ864807	GQ864901	GQ865030	GQ865494	GQ864495
Satyrinae	Satyrini	Ypthimina	Paralasa jordana	CP-AC23-35	RUSSIA: Karasu	DQ338597	DQ339027	EU532176	EU528455	DQ338736
Satyrinae	Satyrini	Incertae sedis	Hyponephele cadusia	CP10-07	IRAN: Hamadan	DQ338839	DQ338989	EU528395	EU528441	DQ338702
Satyrinae	Satyrini	Pronophilina	Lasiophila cirta	CP04-36	PERU: JU, Quebrada Malambo	DQ338851	DQ339002	GQ357477	GQ357606	DQ338714
Satyrinae	Satyrini	Pronophilina	Manerebia cyclopina	CP03-63	PERU: Quebrada Siete Jeringas	DQ338785	DQ338928	EU528397	EU528443	GQ864477
Satyrinae	Satyrini	Pronophilina	Pampasatyrus gyrtone	NW126-12	BRAZIL: Campos do Jordão	DQ338837	DQ338988	EU528406	EU528454	DQ338701
Satyrinae	Satyrini	Pronophilina	Pedaliodes spn117	CP09-66	PERU: S.N. de Ampay	DQ338856	DQ339008	EU528407	EU528456	DQ338719
Satyrinae	Satyrini	Euptychiina	Amphidecta calliomma	NW126-21	BRAZIL: Mato Grosso	DQ338879	DQ339037	GQ357423	GQ357552	DQ338745
Satyrinae	Satyrini	Euptychiina	Archeuptychia cluena	NW149-9	BRAZIL: São Paulo	GQ864736	GQ864830	GQ864926	GQ865392	GQ864424
Satyrinae	Satyrini	Euptychiina	Caeruleuptychia helios	CP01-11	PERU: Madre de Dios	GU205822	GU205878	GU205934	GU205994	GU206055
Satyrinae	Satyrini	Euptychiina	Caeruleuptychia lobelia	CP01-67	PERU: Madre de Dios	DQ338788	DQ338930	GQ357424	GQ357553	DQ338648
Satyrinae	Satyrini	Euptychiina	Caeruleuptychia scopulata	CP01-95	PERU: Madre de Dios	GU205823	GU205879	GU205935	GU205995	GU206056
Satyrinae	Satyrini	Euptychiina	Caeruleuptychia umbrosa	CP01-09	PERU: Madre de Dios	GU205824	GU205880	GU205936	GU205996	GU206057
Satyrinae	Satyrini	Euptychiina	Caeruleuptychia ziza	CP02-43	PERU: Madre de Dios	GU205825	GU205881	GU205937	GU205997	GU206058
Satyrinae	Satyrini	Euptychiina	Capronnieria galesus	NW167-5	BRAZIL: Santa Catarina	GU205826	GU205882	GU205938	GU205998	GU206059
Satyrinae	Satyrini	Euptychiina	Cepheuptychia cephus	CP-C100	PERU: CICRA	GU205827	GU205883	GU205939	GU205999	GU206060
Satyrinae	Satyrini	Euptychiina	Cepheuptychia spn	CP01-31	PERU: Madre de Dios	DQ338789	DQ338931	GQ357425	GQ357554	DQ338696
Satyrinae	Satyrini	Euptychiina	Cercyeuptychia luederwaldti	CP16-02	BRAZIL: Brasilia, DF	GU205828	GU205884	GU205940	GU206000	GU206061
Satyrinae	Satyrini	Euptychiina	Chloreuptychia arnaca	CP06-76	PERU: Cordillera del Cóndor	GU205829	GU205885	GU205941	GU206001	GU206062
Satyrinae	Satyrini	Euptychiina	Chloreuptychia catharina	CP01-68	PERU: Madre de Dios	GQ864749	GQ864843	GQ864942	GQ865413	GQ864437
Satyrinae	Satyrini	Euptychiina	Chloreuptychia chlorimene	CP06-72	PERU: Cordillera del Cóndor	GU205830	GU205886	GU205942	GU206002	GU206063
Satyrinae	Satyrini	Euptychiina	Chloreuptychia herseis	CP01-72	PERU: Madre de Dios	DQ338790	DQ338932	GQ357426	GQ357555	DQ338650
Satyrinae	Satyrini	Euptychiina	Chloreuptychia marica	CP02-50	PERU: Madre de Dios	GU205831	GU205887	GU205943	GU206003	GU206064
Satyrinae	Satyrini	Euptychiina	Cissia myncea	CP01-58	PERU: Madre de Dios	GU205832	GU205888	GU205944	GU206004	GU206065
Satyrinae	Satyrini	Euptychiina	Cissia penelope	CP07-58	PERU: La Solitaria	GU205833	GU205889	GU205945	GU206005	GU206066
Satyrinae	Satyrini	Euptychiina	Cissia proba	CP01-30	PERU: Madre de Dios	GQ864751	GQ864845	GQ864945	GQ865416	GQ864439

Table 1 (Continued).

Subfamily	Tribe	Subtribe	Species	Specimen code	Source of specimen	COI	EF-1α	GAPDH	RpS5	Wingless
Satyrinae	Satyrini	Euptychiina	Cissia myncea	NW108-6	BRAZIL: São Paulo	DQ338581	DQ338933	GQ357427	GQ357556	DQ338651
Satyrinae	Satyrini	Euptychiina	Cyllopsis pertepida	NW165-3	MEXICO: Guanajuato	GQ357204	GQ357274	GQ357428	GQ357557	GQ357338
Satyrinae	Satyrini	Euptychiina	Erichthodes antonina	CP02-24	PERU: Madre de Dios	DQ338792	DQ338935	GQ357429	GQ357558	DQ338653
Satyrinae	Satyrini	Euptychiina	Erichthodes julia	CP04-65	PERU: Quebrada Siete Jeringas	GU205834	GU205890	GU205946	GU206006	GU206067
Satyrinae	Satyrini	Euptychiina	Euptychia enyo	CP06-73	PERU: Cordillera del Cóndor	GQ357205	GQ357275	GQ357430	GQ357559	GQ357339
Satyrinae	Satyrini	Euptychiina	Euptychia ernestina	NW136-14	BRAZIL: São Paulo	DQ338793	DQ338936	GU205947	GU206007	GU206068
Satyrinae	Satyrini	Euptychiina	Euptychia ordinata	CP01-14	PERU: Madre de Dios	GU205835	GU205891	GU205948	GU206008	GU206069
Satyrinae	Satyrini	Euptychiina	Euptychia spn2	CP01-33	PERU: Madre de Dios	DQ338794	DQ338937	EU528392	EU528437	DQ338654
Satyrinae	Satyrini	Euptychiina	Euptychia spn5	CP01-53	PERU: Madre de Dios	DQ338795	DQ338938	GQ357431	GQ357560	DQ338655
Satyrinae	Satyrini	Euptychiina	Euptychia spn6	CP04-55	PERU: JU. 1 km S Mina Pichita	DQ338796	DQ338939	GQ357432	GQ357561	DQ338656
Satyrinae	Satyrini	Euptychiina	Euptychia spn7	CP02-58	PERU: Quebrada Siete Jeringas	GQ357206	DQ338940	GQ357433	GQ357562	DQ338657
Satyrinae	Satyrini	Euptychiina	Euptychoides castrensis	NW126-9	BRAZIL: Ribeirão das Pedras	DQ338798	DQ338942	GQ357434	GQ357563	DQ338659
Satyrinae	Satyrini	Euptychiina	Euptychoides hotchkissi	CP04-51	PERU: JU. 1 km S Mina Pichita	GU205836	GU205892	GU205949	GU206009	GU206070
Satyrinae	Satyrini	Euptychiina	Forsterinaria boliviana	CP04-88	PERU: Quebrada Siete Jeringas	DQ338799	DQ338943	GQ357435	GQ357564	DQ338660
Satyrinae	Satyrini	Euptychiina	Forsterinaria necys	NW126-10	BRAZIL: Ribeirão das Pedras	GU205837	GU205893	GU205950	–	–
Satyrinae	Satyrini	Euptychiina	Forsterinaria proxima	CP08-09	PERU: La Solitaria	GU205838	GU205894	GU205951	GU206010	GU206071
Satyrinae	Satyrini	Euptychiina	Forsterinaria quantius	CP14-07	BRAZIL: Sao Luiz do Paraitingo, SP	GQ864772	GQ864866	GQ864972	GQ865442	GQ864460
Satyrinae	Satyrini	Euptychiina	Godartiana muscosa	NW127-8	BRAZIL: Serra do Japi, SP	DQ338582	DQ338944	GQ864974	GQ865443	DQ338661
Satyrinae	Satyrini	Euptychiina	Guaianaza pronophila	NW127-20	BRAZIL: Extrema, MG	DQ338797	DQ338941	GQ864975	GQ865444	DQ338658
Satyrinae	Satyrini	Euptychiina	Harjesia blanda	CP01-13	PERU: Madre de Dios	DQ338800	DQ338945	GQ357436	GQ357565	DQ338662
Satyrinae	Satyrini	Euptychiina	Harjesia oreba	CP-CI107	PERU: CICRA	GU205839	GU205895	GU205952	GU206011	GU206072
Satyrinae	Satyrini	Euptychiina	Hermeuptychia cucullina	CP04-11	PERU: Quebrada Siete Jeringas	GU205840	GU205896	GU205953	GU206012	GU206073
Satyrinae	Satyrini	Euptychiina	Hermeuptychia fallax	CP04-37	PERU: Río Colorado, Quebrada Perla	GU205841	GU205897	GU205954	GU206013	GU206074
Satyrinae	Satyrini	Euptychiina	Hermeuptychia harmonia	CP06-93	PERU: Oxapampa	GU205842	GU205898	GU205955	GU206014	GU206075
Satyrinae	Satyrini	Euptychiina	Hermeuptychia hermes	NW127-16	BRAZIL: Extrema, MG	DQ338583	DQ338946	GQ357437	GQ357566	DQ338663
Satyrinae	Satyrini	Euptychiina	Hermeuptychia pimpla	CP04-10	PERU: Quebrada Siete Jeringas	GU205843	GU205899	GU205956	GU206015	GU206076
Satyrinae	Satyrini	Euptychiina	Hermeuptychia spn5	CP02-17	PERU: Madre de Dios	GU205844	GU205900	GU205957	GU206016	GU206077
Satyrinae	Satyrini	Euptychiina	Magneuptychia fugitiva	CP01-18	PERU: Madre de Dios	GU205845	GU205901	GU205958	GU206017	GU206078
Satyrinae	Satyrini	Euptychiina	Magneuptychia harpyia	CP02-27	PERU: Madre de Dios	GU205846	GU205902	GU205959	GU206018	GU206079
Satyrinae	Satyrini	Euptychiina	Magneuptychia moderata	CP01-36	PERU: Madre de Dios	GU205847	GU205903	GU205960	GU206019	GU206080
Satyrinae	Satyrini	Euptychiina	Magneuptychia ocypete	CP01-32	PERU: Madre de Dios	GU205848	GU205904	GU205961	GU206020	GU206081
Satyrinae	Satyrini	Euptychiina	Magneuptychia pallema	CP02-41	PERU: Madre de Dios	GU205849	GU205905	GU205962	GU206021	GU206082
Satyrinae	Satyrini	Euptychiina	Magneuptychia spn4	CP01-91	PERU: Madre de Dios	DQ338584	DQ338947	–	GQ357568	DQ338664
Satyrinae	Satyrini	Euptychiina	Magneuptychia spn2	CP02-12	PERU: Madre de Dios	GU205850	GU205906	GU205963	GU206022	GU206083
Satyrinae	Satyrini	Euptychiina	Megeuptychia antonoe	CP05-01	PERU: Cordillera del Cóndor	GU205851	GU205907	–	GU206023	GU206084
Satyrinae	Satyrini	Euptychiina	Megeuptychia monopunctata	CP06-70	PERU: Cordillera del Cóndor	GU205852	GU205908	GU205964	GU206024	GU206085
Satyrinae	Satyrini	Euptychiina	Megisto cymela	CP21-04	USA: Valley Falls, R.I	GQ357208	GQ357277	GQ357439	GQ357569	GQ357341
Satyrinae	Satyrini	Euptychiina	Moneuptychia griseldis	NW127-17	BRAZIL: Extrema, MG	GU205853	GU205909	GU205965	GU206025	GU206086
Satyrinae	Satyrini	Euptychiina	Moneuptychia soter	CP12-07	BRAZIL: São Paulo, Serra do Japi	GU205854	GU205910	–	GU206026	GU206087
Satyrinae	Satyrini	Euptychiina	Moneuptychia paeon	NW126-11	BRAZIL: Ribeirão das Pedras	GQ864792	GQ864886	GQ865004	GQ865473	GQ864481
Satyrinae	Satyrini	Euptychiina	Moneuptychia soter	CP18-01	BRAZIL: São Paulo	GU205855	GU205911	GU205966	GU206027	GU206088

Table 1 (Continued).

Subfamily	Tribe	Subtribe	Species	Specimen code	Source of specimen	COI	EF-1α	GAPDH	RpS5	Wingless
Satyrinae	Satyrini	Euptychiina	Neonympha areolatus	CP22-03	USA:	GU205856	GU205912	GU205967	GU206028	GU206089
Satyrinae	Satyrini	Euptychiina	Palaeonympha opalina	EW25-21	TAIWAN: Hsiaokuehu	DQ338880	DQ339038	GQ865010	GQ865479	DQ338746
Satyrinae	Satyrini	Euptychiina	Paramacera allyni	CP15-10	USA: Arizona	GU205857	GU205913	GU205968	GU206029	GU206090
Satyrinae	Satyrini	Euptychiina	Paramacera xicaque	CP15-08	MEXICO: Distrito Federal	GQ357210	GQ357279	GQ357442	GQ357571	GQ357343
Satyrinae	Satyrini	Euptychiina	Parataygetis albinotata	CP04-53	PERU: JU, 1 km S Mina Pichita	DQ338804	DQ338950	GQ357443	GQ357572	DQ338668
Satyrinae	Satyrini	Euptychiina	Pareuptychia binocula	CP02-42	PERU: Madre de Dios	GU205858	GU205914	GU205969	GU206030	GU206091
Satyrinae	Satyrini	Euptychiina	Pareuptychia hesionides	CP01-66	PERU: Madre de Dios	DQ338805	DQ338951	GQ357444	GQ357573	DQ338669
Satyrinae	Satyrini	Euptychiina	Pareuptychia ocirrhoe	NW126-6	BRAZIL: Atibaia, SP	GU205859	GU205915	GU205970	GU206031	–
Satyrinae	Satyrini	Euptychiina	Pareuptychia metaleuca	CP06-67	PERU: Cordillera del Cóndor	GU205860	GU205916	GU205971	GU206032	GU206092
Satyrinae	Satyrini	Euptychiina	Pareuptychia ocirrhoe	DNA99-064	ECUADOR: Napo Province	AY508568	AY509094	–	–	–
Satyrinae	Satyrini	Euptychiina	Paryphthimoides grimon	CP10-01	BRAZIL: Saibadela	DQ338806	DQ338952	GQ865015	GQ865483	DQ338670
Satyrinae	Satyrini	Euptychiina	Paryphthimoides poltys	CP02-19	PERU: Madre de Dios	GU205861	GU205917	GU205972	GU206033	GU206093
Satyrinae	Satyrini	Euptychiina	Paryphthimoides phronius	NW126-7	BRAZIL: Atibaia, SP	DQ338807	DQ338953	GU205973	GU206034	DQ338671
Satyrinae	Satyrini	Euptychiina	Phameuptychia innocentia	CP12-06	BRAZIL: Serra do Cipó	DQ338808	DQ338954	GU205974	GU206035	DQ338672
Satyrinae	Satyrini	Euptychiina	Phameuptychia sp.	NW127-18	BRAZIL: Extrema, MG	DQ338809	DQ338955	GU205975	GU206036	–
Satyrinae	Satyrini	Euptychiina	Pindis squamistriga	NW165-5	MEXICO: Guanajuato	GQ357211	GQ357280	GQ357445	GQ357574	GQ357344
Satyrinae	Satyrini	Euptychiina	Taygetis rectifascia	NW127-28	BRAZIL: Intervales, C. Bonito, SP	GU205862	GU205918	GU205976	GU206037	DQ338673
Satyrinae	Satyrini	Euptychiina	Posttaygetis penelea	NW126-13		DQ338813	DQ338959	GQ357446	GQ357575	DQ338682
Satyrinae	Satyrini	Euptychiina	Pseudodebis marpesa	CP01-42	PERU: Madre de Dios	GU205863	GU205919	GU205977	GU206038	GU206094
Satyrinae	Satyrini	Euptychiina	Pseudodebis valentina	CP-CI64	PERU: CICRA	GU205864	GU205920	GU205978	GU206039	GU206095
Satyrinae	Satyrini	Euptychiina	Rareuptychia clio	CP01-23	PERU: Madre de Dios	DQ338810	DQ338956	GQ865028	GQ865492	GQ864494
Satyrinae	Satyrini	Euptychiina	Satyrotaygetis satyrina	DNA97-006	COSTA RICA: Puntarenas Province	AY508575	AY509101			
Satyrinae	Satyrini	Euptychiina	Splendeuptychia ashna	CP01-19	PERU: Madre de Dios	GU205865	GU205921	GU205979	GU206040	GU206096
Satyrinae	Satyrini	Euptychiina	Splendeuptychia boliviensis	CP02-48	PERU: Madre de Dios	GU205866	GU205922	GU205980	GU206041	GU206097
Satyrinae	Satyrini	Euptychiina	Splendeuptychia doxes	NW126-8	BRAZIL: Atibaia, SP	GU205867	GU205923	GU205981	GU206042	GU206098
Satyrinae	Satyrini	Euptychiina	Splendeuptychia furina	CP02-39	PERU: Madre de Dios	GU205868	GU205924	GU205982	GU206043	GU206099
Satyrinae	Satyrini	Euptychiina	Splendeuptychia itonis	CP02-44	PERU: Madre de Dios	DQ338811	DQ338957	GQ357447	GQ357576	DQ338684
Satyrinae	Satyrini	Euptychiina	Splendeuptychia purusana	CP-CI39	PERU: CICRA	GU205869	GU205925	GU205983	GU206044	GU206100
Satyrinae	Satyrini	Euptychiina	Taydebis peculiaris	NW149-11	BRAZIL: São Paulo	GQ864811	GQ864905	GQ865036	GQ865499	–
Satyrinae	Satyrini	Euptychiina	Taygetis virgilia	NW108-3	BRAZIL: São Paulo	DQ338812	DQ338958	EU141487	EU141383	DQ338683
Satyrinae	Satyrini	Euptychiina	Taygetis mermeria	CP-CI95	PERU: CICRA	GU205870	GU205926	GU205984	GU206045	GU206101
Satyrinae	Satyrini	Euptychiina	Taygetis rufomarginata	NW129-27	BRAZIL: Saibadela	GU205871	GU205927	GU205985	GU206046	–
Satyrinae	Satyrini	Euptychiina	Taygetis rufomarginata	CP-CI125	PERU: CICRA	GU205872	GU205928	GU205986	GU206047	GU206102
Satyrinae	Satyrini	Euptychiina	Taygetis yphthima	NW149-8	BRAZIL: São Paulo	GU205873	GU205929	GU205987	GU206048	GU206103
Satyrinae	Satyrini	Euptychiina	Taygetomorpha celia	CP22-02	COLOMBIA: Antioquía	GU205874	GU205930	GU205988	GU206049	–
Satyrinae	Satyrini	Euptychiina	Taygetomorpha puritana	CP22-04	ECUADOR: Morona-Santiago	GU205875	GU205931	GU205989	GU206050	GU206104
Satyrinae	Satyrini	Euptychiina	Yphthimoides angularis	CP12-08		GU205876	GU205932	GU205990	GU206051	GU206105
Satyrinae	Satyrini	Euptychiina	Yphthimoides borasta	CP10-03	BRAZIL: São Paulo	DQ338585	DQ338960	GU205991	GU206052	DQ338680
Satyrinae	Satyrini	Euptychiina	Yphthimoides cipoensis	CP10-02	BRAZIL: Serra do Cipó	DQ338814	DQ338961	GQ357448	GQ357577	DQ338681
Satyrinae	Satyrini	Euptychiina	Yphthimoides leguialimai	CP08-88	PERU: Ampay	GU205877	GU205933	GU205992	GU206053	GU206106
Satyrinae	Satyrini	Euptychiina	Moneuptychia itapeva	CP12-04	BRAZIL: Serra do Cipó	DQ338815	DQ338962	GU205993	GU206054	DQ338675
Satyrinae	Satyrini	Euptychiina	Zischkaia pacarus	CP14-02	BRAZIL: Serra do Cipó	GQ864819	GQ864914	GQ865049	GQ865512	GQ864506

Table 2 Parameter values estimated using Bayesian phylogenetic methods. Values estimated separately for each gene region.

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
COI	25.340	0.074	0.035	0.032	0.010	0.845	0.004	0.396	0.069	0.131	0.404	0.263
EF-1a		0.065	0.256	0.084	0.047	0.501	0.048	0.279	0.214	0.225	0.282	0.238
Wingless		0.074	0.284	0.117	0.029	0.426	0.070	0.167	0.323	0.357	0.154	0.380
GAPDH		0.075	0.281	0.100	0.050	0.442	0.052	0.266	0.205	0.228	0.301	0.314
RpS5		0.107	0.235	0.144	0.039	0.444	0.031	0.260	0.197	0.216	0.327	0.274
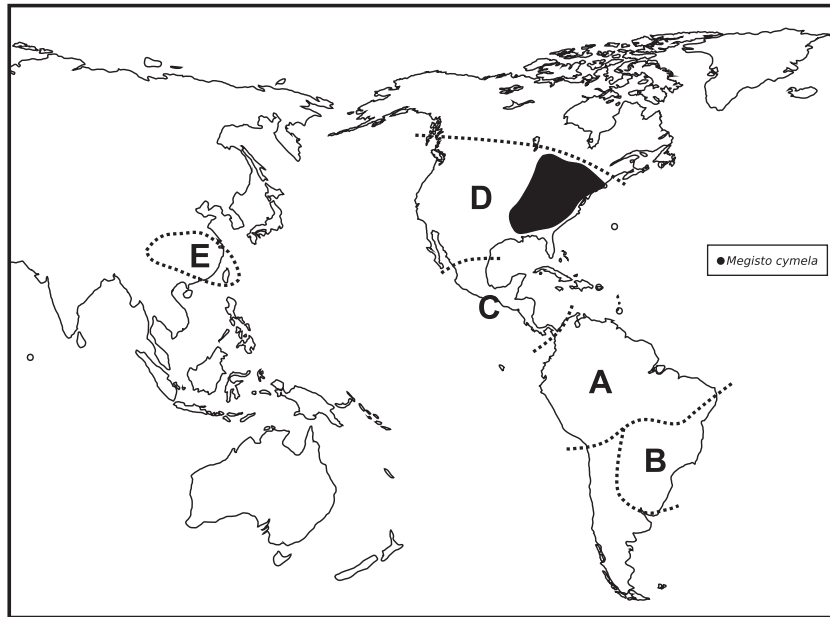
Fig. 1 A–E. The five different biogeographical areas used in this study for the DIVA analysis. —A. Northern South America. —B. South-eastern South America. —C. Central America. —D. North America. —E. Distribution of *Palaeonympha opalina* taken from Shirôzu (1960). The distribution of *Megisto cymela* (sister of *Palaeonympha opalina*) is also shown.

The Bayesian analysis produced a tree (Fig. 3) that is broadly congruent with the most parsimonious cladograms. Parameter values for the models used in the analysis are given in Table 2. The major difference is in the positions of *Chloreuptychia arnaca*, *Taydebis peculiaris* and *Satyrotaygetis satyrina*. In the parsimony analysis, *C. arnaca* appears sister to a clade containing *Cepheuptychia cephus*, *Chloreuptychia chlorimene*, *Chloreuptychia herseis*, *Chloreuptychia marica* and *Archeuptychia cluena*, whereas in the Bayesian tree, it appears sister to a clade that mainly includes species of the genera *Caeruleuptychia* and *Magneuptychia*. In the parsimony analysis, *Taydebis peculiaris* appears as sister to a clade containing *Splendeuptychia doxes* and *Splendeuptychia furina*, *Satyrotaygetis*, *Erichthodes antonina* and *Erichthodes julia*, *Neonympha*, *Megeuptychia* and *Pareuptychia*, whereas in the Bayesian analysis, *Taydebis peculiaris* appears sister to *Splendeuptychia doxes* and *S. furina*. The monotypic *Satyrotaygetis* appears sister to *Erichthodes antonina* and *E. julia* in the most parsimonious cladograms, whereas in the Bayesian tree, it is sister to a clade including *Erichthodes*, *Neonympha*, *Megeuptychia* and *Pareuptychia*.

Neither of the methods of analysis found the subtribe Euptychiina as a monophyletic entity. The Bayesian analysis recovered *Euptychia sensu stricto* (represented here by *Euptychia enyo* and *Euptychia* spp. n.) as sister to a clade formed by some outgroups and another clade that includes all other Euptychiina (Fig. 3). MP's strict consensus recovered *Euptychia sensu stricto*, all other euptychiines and some outgroups collapsed in a polytomy (Fig. 2). All other patterns of relationships are recovered consistently in both analyses, implying a strong phylogenetic signal that is recovered independent of method of analysis used. Of the sampled genera and species, we found that *Paramacera*, *Yphthimoides*, *Zischkaia*, *Hermeuptychia*, *Taygetomorpha*, *Erichthodes*, *Megeuptychia*, *Pareuptychia* and *Caeruleuptychia* are monophyletic. However, it should be borne in mind that we did not sample all species for some of these genera and it is possible that including the remaining species of large genera, such as *Yphthimoides* and *Caeruleuptychia*, will render them non-monophyletic. We could not test the monophyly of *Cyllopsis*, *Megisto*, *Amphidecta*, *Pharneuptychia*, *Godartiana*,
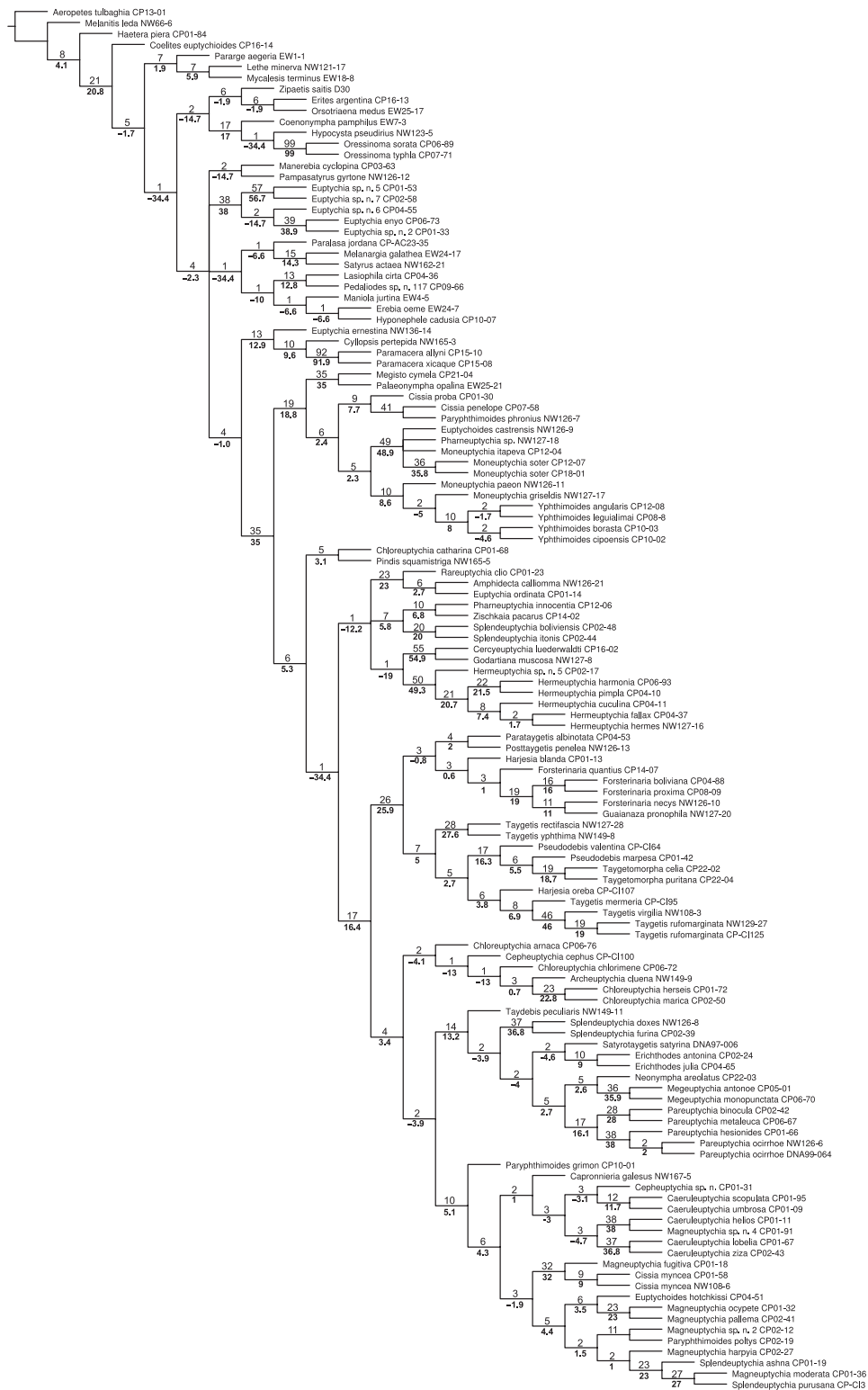
Fig. 2 Strict consensus of three equally parsimonious trees (20 799 steps; CI = 0.17; RI = 0.42) from the maximum parsimony analysis. Numbers given above branches are Bremer support values and numbers below the branch are PCI values for the node to the right of the number.
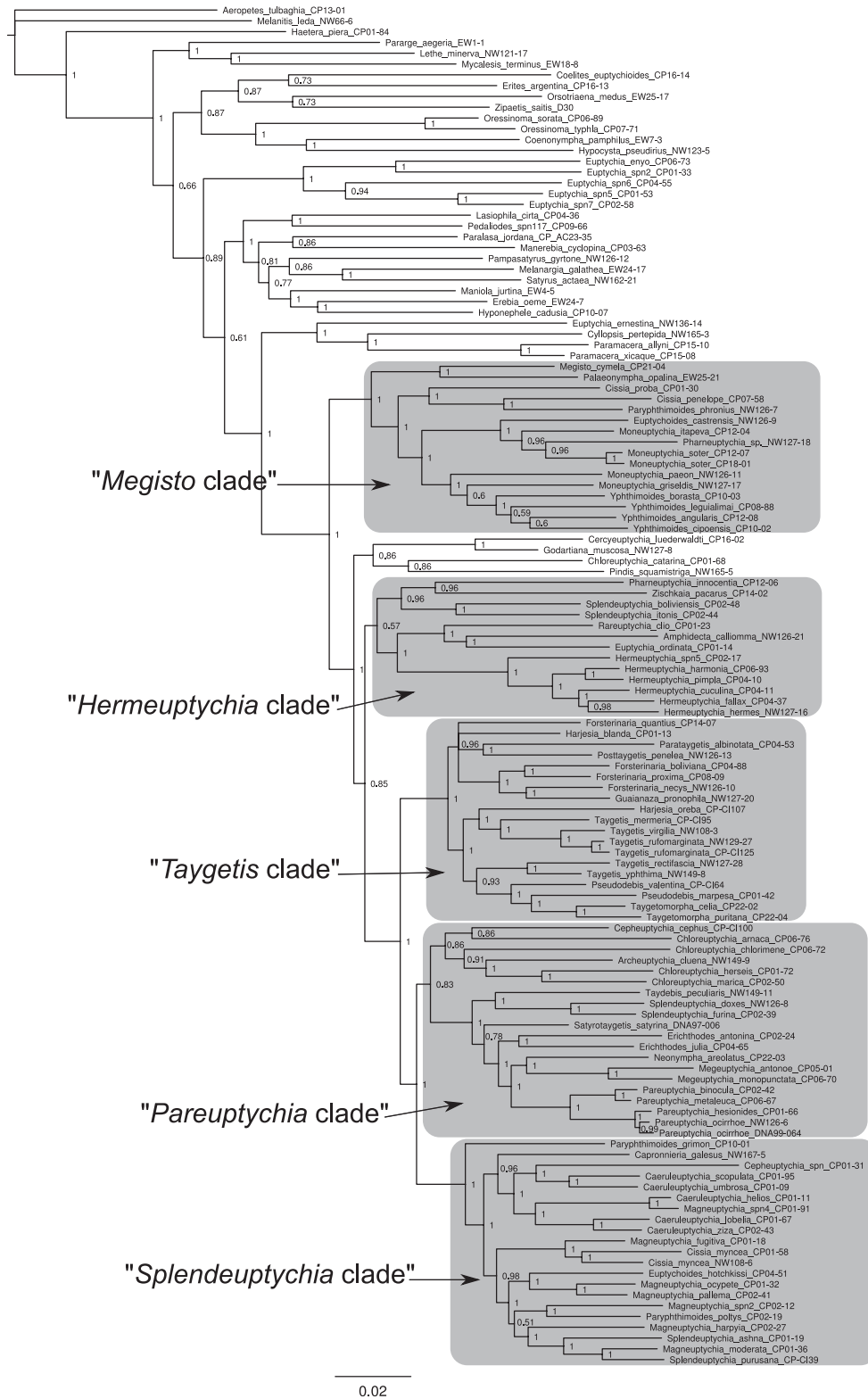
Fig. 3 Majority rule cladogram based on Bayesian inference, modelled with a GTR+Γ model. Numbers at the branches are posterior probability values for the node to the right of the number.

Cepheuptychia and *Neonympha* as our dataset included only a single species. We could not test whether the subspecies in *Palaeonympha opalina* form a monophyletic entity.

The North and Central American euptychiines do not group together and are related to different ingroup taxa: *Paramacera* and *Cyllopsis* are sister genera and closely related to *Euptychia ernestina*; *Megisto cymela* is sister to the Oriental *Palaeonympha opalina*; *Pindis squamistriga* appears as sister to *Chloreuptychia arnaca*; and *Neonympha* is sister to the genus *Megeuptychia* or *Erichthodes*.

It is possible to identify five major clades in the Euptychiina (Fig. 3). Of these, probably the '*Megisto* clade' is the most interesting because it includes the North American *Megisto* and East Asian *Palaeonympha* as sister to a clade of species that are mainly endemic to south-eastern Brazil (cf. Fig. 1). We found this pattern in a previous study (Peña *et al.* 2006), although *Megisto* was not included in that dataset. The '*Hermeuptychia* clade' includes the monophyletic *Hermeuptychia*, *Amphidecta calliomma* and the monotypic genera *Rareuptychia* and *Cercyeuptychia*. Our third clade corresponds to the '*Taygetis* clade' found by Murray & Prowell (2005), while our fifth clade is the '*Cissia* clade' in Murray & Prowell (2005) that we call '*Splendeuptychia* clade' because the type species of *Cissia* (*Cissia penelope*) does not appear in this group. Our '*Splendeuptychia* clade' also includes the Brazilian endemic *Capronnieria galesus*. Our fourth clade consists in a disparate collection of taxa, which, among others, includes the '*Pareuptychia* clade' of Murray & Prowell (2005), *Megeuptychia*, and the monotypic *Archeuptychia* and *Satyrotaygetis*.

Times of divergence

Our time estimates from the relaxed molecular clock technique produced wide confidence intervals for most nodes (Fig. 4). This results from taking account of the standard error for the estimated age of Satyrini as 36.6 ± 5.1 Ma (from Peña & Wahlberg 2008). Wider intervals are expected when employing secondary calibration points (Graur & Martin 2004). Our estimated times indicate that the Euptychiina appeared during the Oligocene at around 31 Ma. The genus *Euptychia* is an old lineage that diversified at around 23 Ma. The five major clades in Euptychiina diverged in the early Miocene, and most of the diversification at the genus and species level occurred during the mid to late Miocene (16–7 Ma) (Fig. 4). In the '*Megisto* clade', the split between *Palaeonympha* and *Megisto* is estimated to be as early as 13 Ma, while the split between these two taxa and the Brazilian endemics occurred at ~21 Ma. It is interesting to note that some North and Central American euptychiines are relatively old lineages: *Cyllopsis* split from *Paramacera* almost 20 Ma;

and *Pindis* branched off around 21 Ma. This contrasts with the relatively young ages of *Neonympha* and *Satyrotaygetis*, dated at around 11 Ma.

Biogeographic history

Our biogeographic analysis in DIVA suggests that dispersal events have been important in the biogeographic history of the Euptychiina. DIVA suggests that 67 dispersal events are needed to explain the current distributions of our sampled euptychiines (Fig. 4). Restricting the number of maximum ancestral areas only affects the ancestral distributions of five nodes, and the major biogeographic patterns and implications are not affected.

The area of origin of Euptychiina is not clear. Our DIVA reconstructions indicate that the ancestor of Euptychiina originated somewhere in South America (A + B). Alternatively, DIVA estimates an implausible disjunct area of origin in South and North America (A + D) (Fig. 4).

Early in the evolution of Euptychiina, at least two dispersal events into south-eastern Brazil and Central or North America gave rise to (*Paramacera* + *Cyllopsis*) and *Euptychia ernestina* (Fig. 4). The lineage that remained in central South America underwent similar dispersal events producing at least two lineages. First, a dispersal into south-eastern Brazil and subsequent diversification gave rise to most of the euptychiines endemic to the Brazilian Atlantic forests (taxa in the '*Megisto* clade'). The other lineage corresponds to the Nearctic *Megisto* + Oriental *Palaeonympha*, which is inferred to be the result of dispersal into North America and the Oriental region (Fig. 4). Our DIVA analysis demonstrates that the diversification of all other euptychiines occurred in South America, and that incursions to south-eastern Brazil, Central America and North America were not rare (Fig. 4).

Discussion

Euptychiina phylogeny

The robust phylogenetic hypotheses in this study confirm that Euptychiina, as delimited by Lamas (2004), is a polyphyletic group. We present a revised checklist of a monophyletic Euptychiina, which includes two genera long considered *incertae sedis*: the Neotropical *Amphidecta* and the Oriental *Palaeonympha opalina* (Table 3). Previous studies using morphological characters from adult and immature stages of *Amphidecta* (Miller 1968; Viloria 2003; Freitas 2004b) were inconclusive and failed to define the position of this genus.

The study of Murray & Prowell (2005) concluded that the genus *Euptychia* does not share a common ancestor with other euptychiines. It is possible that the results of Murray & Prowell (2005) were affected by long-branch attraction artefacts (Bergsten 2005). Our previous study of the Satyrini
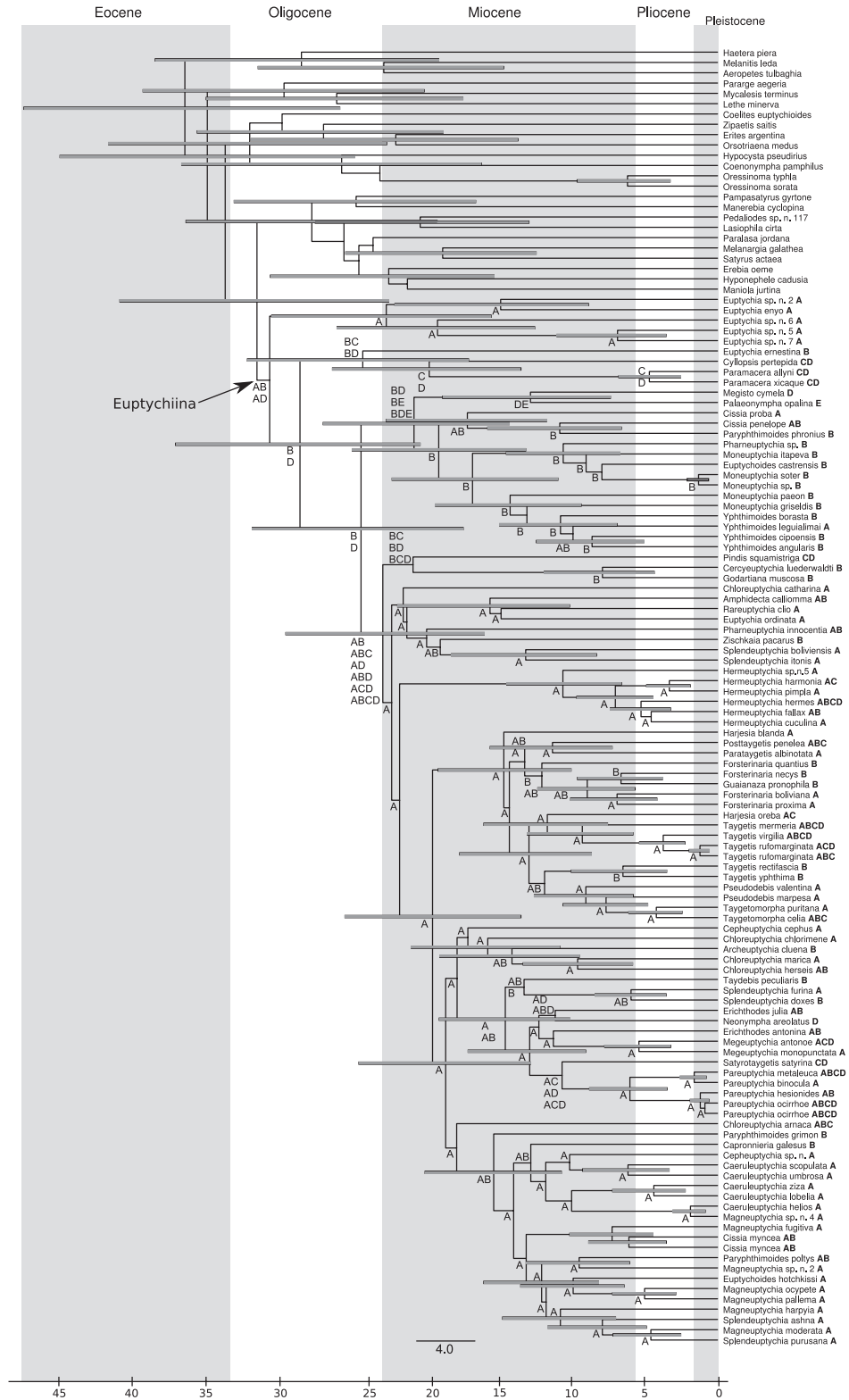
Fig. 4 Estimated times of divergence derived from the BEAST analysis. Results of a dispersal–vicariance analysis, with unrestricted ancestral areas shown for each node. Error margins for estimated times are shown as horizontal bars at the nodes.

Table 3 Revised checklist of genera in the subtribe Euptychiina.

Amphidecta Butler, 1867
Archeuptychia Forster 1964
Caenoptychia Le, Cerf 1919
Caeruleuptychia Forster, 1964
Capronnieria Forster, 1964
Cepheuptychia Forster, 1964
Cercyeuptychia Miller & Emmel, 1971
Chloreuptychia Forster, 1964
Cissia Doubleday, 1848
Coeruleotaygetis Forster, 1964
Cyllopsis Felder, 1869
Erichthodes Forster, 1964
Euptychia Hübner, 1818
Euptychoides Forster, 1964
Forsterinaria Gray, 1973
Godartiana Forster, 1964
Guaianaza Freitas & Peña, 2006
Harjesia Forster, 1964
Hermeuptychia Forster, 1964
Magneuptychia Forster, 1964
Megeuptychia Forster, 1964
Megisto Hübner, [1819]
Moneuptychia Forster, 1964
Neonympha Hübner, 1818
Palaeonympha Butler, 1871
Paramacera Butler, 1868
Parataygetis Forster, 1964
Pareuptychia Forster, 1964
Paryphthimoides Forster, 1964
Pharneuptychia Forster, 1964
Pindis Felder, 1869
Posttaygetis Forster, 1964
Praefaunula Forster, 1964
Pseudeuptychia Forster, 1964
Pseudodebis Forster, 1964
Rareuptychia Forster, 1964
Satyrotaygetis Forster, 1964
Splendeuptychia Forster, 1964
Taydebis Freitas, 2003
Taygetina Forster, 1964
Taygetis Hübner, [1819]
Taygetomorpha Miller, 2004
Yphthimoides Forster, 1964
Zischkaia Forster, 1964

(Peña *et al.* 2010) found that *Euptychia* is on a long branch that suffers from attraction to several other satyrines. When other long branches, such as *Calisto*, some taxa in Ypthimina and even some Brassolinae and Morphinae, are included in a phylogeny, *Euptychia* is prone to be attracted to them (Peña *et al.* 2010). This could be due to *Euptychia* being a relatively old lineage that branched off early in the history of Euptychiina, around 22 Ma (Fig. 4), and then underwent rapid diversification. *Euptychia ernestina* does not appear related to *Euptychiia sensu stricto*, which is expected because morphological traits of the lectotype indicate that this species should be removed from the genus *Euptychia*.

Even though our sampling of Euptychiina is incomplete, it is evident that the subtribe is plagued by polyphyletic genera and needs a great deal of taxonomic work. Although recent studies have begun to tackle this problem (Freitas 2003, 2004a, 2007; Peña & Lamas 2005; Freitas & Peña 2006; Pulido & Andrade 2008; Huertas *et al.* 2009), the current classification of genera in Euptychiina remains basically unchanged since the work of Forster (1964).

Our results support Murray & Prowell's (2005) division of Euptychiina into several clades. We recovered basically the same *Taygetis* and *Splendeuptychia* ('*Cissia* clade' in their study) clades, but their '*Pareuptychia* clade' should be expanded to include *Megeuptychia*, *Archeuptychia* and *Satyrotaygetis*. We found two additional major clades in Euptychiina (Fig. 3): our '*Megisto* clade', which includes the Oriental *Palaeonympha opalina* as sister to a clade that includes *Megisto* and some endemic euptychiines to southeastern Brazil (Fig. 3); and our '*Hermeuptychia* clade', which includes *Cercyeuptychia*, *Zischkaia*, *Amphidecta*, *Pindis*, *Rareuptychia* and some members of *Splendeuptychia*, *Pharneuptychia* and *Godartiana* (Fig. 3).

The genus *Splendeuptychia* is polyphyletic. The type species of the genus, *Splendeuptychia ashna* (Hewitson, 1869) and *Splendeuptychia purusana* (Aurivillius, 1929) appear in a clade equivalent of Murray & Prowell's (2005) '*Cissia* clade', whereas the other sampled members are placed in two other widely separated clades. However, detailed morphological studies combined with DNA sequencing of the remaining species will be needed to reassign the species currently included in *Splendeuptychia* to other genera.

Cissia is also polyphyletic. The type species *Cissia penelope* (Fabricius, 1775) appears in our '*Megisto* clade' as sister to *Cissia proba* (Weymer, 1911) and *Paryphthimoides phronius* (Godart, [1824]). This implies that *Cissia myncea* (Cramer, 1780) needs to be transferred to another genus, probably *Magneuptychia* or *Euptychoides* because of many similarities with the type species *Magneuptychia libye* and *Euptychoides hotchkissi*.

The relationships in the '*Taygetis* clade' are not clear. Our preferred hypothesis is for a clade of *Taygetis* and relatives (including *Harjesia oreba*) sister to a polytomy formed by *Forsterinaria quantius*, *Harjesia blanda*, (*Parataygetis albinotata* + *Posttaygetis penelea*) and a clade including other *Forsterinaria* and *Guaianaza* (Fig. 3). *Guaianaza* appears to be within *Forsterinaria* and thus could be subsumed into the latter. This hypothesis could be tested with a better sampling of the genus, including most of the remaining 20 *Forsterinaria* species.

The biogeographic history of Euptychiina
We have used the age of Satyrini (36.6 ± 5.1 Ma) found by Peña & Wahlberg (2008) (which itself used 25 Ma as

the age of the fossil *Lethe corbieri*) as a secondary calibration point to estimate dates of origin of the major lineages in the Satyrinae. Peña & Wahlberg (2008) concluded that both Euptychiina and Pronophilina were the products of Palaearctic ancestors that dispersed into the Americas via the Beringian Bridge during the Eocene–Oligocene (Peña *et al.* 2010). This event corresponds to the 'Beringian Bridge I' phase of Sanmartín *et al.* (2001). According to our times of divergence and inferred ancestral distributions for Euptychiina, it appears that by approximately 31 Ma, Euptychiina had established in the New World and started diversification either in South America or alternatively in both North and South America. We speculate that early Euptychiina dispersed from North to South America via the temporary connection between the Greater Antilles and north-western South America during Eocene–Oligocene times, known as the GAARlandia landspan 35–33 Ma (Iturralde & MacPhee 1999). It has been found that this land connection may have also been important for the evolution of Phyciodina butterflies (Nymphalidae) (Wahlberg & Freitas 2007). Our hypothesis implies that early colonizers of North America went extinct and current euptychiines in North and Central America are the result of later colonizations from South America (see below).

We found that dispersal events have been very important in the evolution of the Euptychiina, resulting in a rather complex biogeographic history. We have previously shown that dispersal in this taxon, and in satyrines in general (Peña & Wahlberg 2008), is likely to have been aided by the fact that host plant occurrence does not set strong range limits, as the larvae of most species feed on a range of grasses with extensive combined distributions.

Our data indicate that during the Early Oligocene (at around 28 Ma) there was an early dispersal event into North America, which produced the genera *Cyllopsis* and *Paramacera*. The Eocene–Oligocene transition was a time of retreat of seas and land uplift because of a decrease in global temperatures that lasted until the Late Oligocene (27–25 Ma) (Iturralde & MacPhee 1999). Therefore, it is possible that the *Cyllopsis* + *Paramacera* lineage used the same GAARlandia connection between North and South America to disperse, this time, however, in a northward direction.

An almost simultaneous split at 24 Ma originated of two lineages, the ancestor of the '*Megisto* clade' and that of all remaining euptychiines. The '*Megisto* clade' split into two lineages, one of which dispersed onto the Brazilian shield and gave rise to several extant euptychiines endemic to the Atlantic forests in south-eastern Brazil. The other lineage, the ancestor of *Megisto* + *Palaeonympha*, migrated northwards. During this period, the sea level was again rising, after reaching its lowest level at 35 Ma

(Miller *et al.* 1996), and the GAARlandia land bridge underwent marine transgressions. During the Late Oligocene (27–25 Ma), it consisted in a series of terranes separated by deep marine gaps (Iturralde & MacPhee 1999). Our data suggest that the ancestor of *Megisto* + *Palaeonympha* was able to overcome these marine barriers by crossing from one terrane to the next along the axis of former GAARlandia and then disperse into North America and Asia.

Palaeonympha and *Megisto* split around 13 Ma (Fig. 4). The results of the DIVA analysis suggested that their common ancestor was distributed in both North America and Asia (areas D + E; Fig. 4). As *Megisto* inhabits open deciduous woodlands (Miller 1976) and *Palaeonympha* occurs in mountain forests (Mell 1942), it is plausible that their common ancestor could inhabit the continuous forest belt across North Asia and North America, which was connected by Beringia from the Middle–Late Miocene (14–10 Ma) to the Late Pliocene (3.5 Ma), the 'Beringian Bridge II' of Sanmartín *et al.* (2001). The global cooling of climate during the Late Miocene (from 13.5 Ma onwards) that caused extensive extinctions in western North America (Janis 1993), a continuous decrease in temperature with aridification of climate in Eurasia and replacement of forests by grasslands (Cerling *et al.* 1997) might have contributed to the extinction of *Megisto* and *Palaeonympha* in western North America and North Asia respectively. Therefore, these events and a vicariance event resulting from the closure of the Beringian Bridge caused the current disjunct distribution of these two taxa.

The major diversification of Euptychiina coincides with the last uplift of the Andes mountain chains (Late Miocene–Early Pliocene) (Gregory-Wodzicki 2000). For some butterfly groups, the Andean foothills have been reported to act as a 'species pump', pushing new species that originate in the Andes into the Amazon basin (Hall 2005; Whinnett *et al.* 2005). It appears that the 'species pump' hypothesis did not exert a major influence on the biogeographic history of the Euptychiina, as the majority of extant euptychiines are dwellers of the lowland forests in Amazonia (area A). It is remarkable that very few Euptychiina genera have species inhabiting montane habitats of the Andes (Peña & Lamas 2005; Pulido & Andrade 2008). Species in the subtribe Pronophilina are dominant in high Andean habitats while they are absent in the lowlands. It is possible that the ancestor of Pronophilina was preadapted for coping with the cold temperatures of Andean mountain forests (1500–3200 m where they are most abundant), and because of competition for resources, only few euptychiines were able to colonize those habitats.

Although there are several hypotheses to explain the megadiversity of terrestrial organisms in the Amazonia,

the reasons why this region harbours more species than other areas remain unclear. It is entirely possible that the high diversity of Euptychiina butterflies in the Amazonia has been the result of the complex history of the region – marine incursions during the Miocene (Wesselingh *et al.* 2002), dynamic riverine barriers (Hall & Harvey 2002), and climatic cooling and droughts of the controversial Pleistocene refugia (Solomon *et al.* 2008) – which disturbed communities and populations driving diversification.

Conclusions

The subtribe Euptychiina had Palaearctic ancestors that dispersed into the Americas via the Beringian Bridge during the Eocene–Oligocene and colonized the New World approximately 31 Ma. There was considerable diversification in the Amazon basin (area A) with recurrent dispersal onto the Brazilian shield and into Central and North America. It is clear from our time estimates of the diversification of Euptychiina and reconstructions of ancestral areas that the current disjunct distribution of the Oriental *Palaeonympha opalina* is the result of a northbound dispersal of a lineage from South America into North America and then west into Asia.

We conclude that the ancestor of *Megisto* and its sister taxon *Palaeonympha* probably inhabited the continuous forest belt across North Asia and North America, which was connected by Beringia. A vicariance event as a result of the closure of the Beringian Bridge combined with the global climatic changes during the Late Miocene, that caused dramatic changes in vegetation and extensive extinctions in western North America and Eurasia contributing to the extinction of *Megisto* and *Palaeonympha* in western North America and North Asia, resulted in the classic 'eastern Asia and eastern North America' disjunct distribution of *Palaeonympha* and *Megisto*. To our knowledge, this is the first time that this pattern is reported for a group of butterflies.

Acknowledgements

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