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Colonization of and radiation in South America by butterflies in the subtribe Phyciodina (Lepidoptera: Nymphalidae)

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Abstract

The historical biogeography of insects in South America is largely unknown, as dated phylogenies have not been available for most groups. We have studied the phylogenetic relationships and historical biogeography of a subtribe of butterflies, Phyciodina in the family Nymphalidae, based on one mitochondrial gene (COI) and two nuclear gene regions (EF-1α and wingless). The subtribe comprises 89 species mainly found in tropical South America, with a few species in North America and the Greater Antilles. We find that the enigmatic genus *Antillea* is sister to the rest of Phyciodina, and suggest that it should be included in the subtribe. Several genera are found to be polyphyletic or nested within another genus, and are proposed to be synonymised. These are *Dagon, Castilia, Telenassa* and *Janatella*, which we propose should be synonymised with *Eresia*. Brazilian "*Ortilia*" form an independent lineage and require a new genus name. The diversification of Phyciodina has probably taken place over the past about 34 MYA. The ancestral phyciodine colonised South America from North America through a possible landspan that connected the Greater Antilles to South America about 34 MYA. A vicariance event left the ancestral *Antillea* on the Greater Antilles, while the ancestral 0e on South America colonised the Guyanan Shield and soon after the Brazilian Shield. We hypothesise that the Brazilian Shield was an important area for the diversification of Phyciodina. From there, the ancestor of *Anthanassa*, *Eresia* and *Tegosa* colonised NW South America, where especially *Eresia* diversified in concert with the rising of the Andes beginning about 20 MYA. Central America was colonised from NW South America about 15 MYA by the ancestors of *Anthanassa* and *Phyciodes*. Our study is the first to use a dated phylogeny to study the historical biogeography of a group of South American species of butterflies.

Keywords: Molecular systematics; Historical biogeography; Butterfly; Lepidoptera; Melitaeini; Nymphalidae

1. Introduction

The origin and biogeographical history of groups of organisms in the South American continent is a subject of great interest, as the continent is home to a greater part of the Earth's biodiversity (Wilson, 1992; Myers et al., 2000). This applies especially to invertebrates, including the well-known butterflies (Heppner, 1991). The Neotropical region is the richest biome for butterflies, both in species number and taxonomic representation. More than 7000 species are found in the region (Lamas, 2004), comprising about 40% of all known butterflies. The biogeographical history of butterflies in the Neotropics is not well known (Descimon, 1986; Shapiro, 1994; Miller and Miller, 1997; Viloria, 2003) and has been mainly based on speculation regarding phylogenetic relationships, as phylogenies have not been available. Some butterfly groups have been thought to have originated in the Northern Hemisphere and have subsequently colonized South

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America (Descimon, 1986; Shapiro, 1994). Other groups are thought to have sister group relations with African species, implying Gondwanan origins (Miller and Miller, 1997).

In butterflies, as in other groups of organisms, a phylogenetic framework is necessary to understand patterns of biogeographical distribution, host plant use, evolution of ecological characters and aspects of natural history in groups of related species. Comprehensive phylogenetic hypotheses have not been available for any Neotropical groups until recently (for Nymphalidae e.g. Jenkins, 1990; Brower, 1994b,a; Brower and Egan, 1997; Penz, 1999; Hill et al., 2002; Penz and DeVries, 2002; Blum et al., 2003; Viloria, 2003; Murray and Pashley Prowell, 2005; Brower et al., 2006; Willmott and Freitas, 2006). Most of these studies have not investigated the relationships of the Neotropical butterflies to possible non-Neotropical sister groups. Recently, the nymphalid tribe Melitaeini has been the focus of several phylogenetic studies (Kons, 2000; Wahlberg and Zimmermann, 2000; Zimmermann et al., 2000; Wahlberg et al., 2003, 2005). Based on the species sampled, Wahlberg and Zimmermann (2000) and Wahlberg et al. (2005) hypothesized that the Neotropical region was likely to have been colonized twice from temperate North America, once by the ancestor of Neotropical species in the subtribe Phyciodina and once by the ancestor of Neotropical members of the *Chlosyne*-group. Wahlberg (2006) found that the colonization of South America by the ancestor of Phyciodina + *Gnathotriche*-group coincided with a hypothesized land span between Venezuela and the Greater Antilles some 34 MY ago (Iturralde-Vinent and MacPhee, 1999). In this paper, we investigate the biogeographical history of Phyciodina in more detail.

Butterflies in the tribe Melitaeini are found only in the Holarctic region and in the Neotropics. They are missing completely from the tropical African, Oriental and Australian regions. The tribe comprises about 250 species, of which about 120 are found exclusively in temperate regions and about 120 are found only in the Neotropics. The recent phylogenetic studies of the tribe (Kons, 2000; Wahlberg and Zimmermann, 2000; Zimmermann et al., 2000; Wahlberg et al., 2003, 2005) have shown that there are five distinct clades that can be treated on an equal basis. Three of these have formal rank of subtribes (Euphydryina, Phyciodina and Melitaeina) and the remaining two have been referred to as the *Chlosyne*-group and the *Gnathotriche*-group (Wahlberg and Zimmermann, 2000; Wahlberg et al., 2005). Of these, Euphydryina and Melitaeina are exclusively temperate, the *Chlosyne*-group is largely temperate with some representation in tropical Central and South America. The *Gnathotriche*-group is exclusively Neotropical, but comprises only four species (Lamas, 2004). Phyciodina, the focus of this paper, is largely Neotropical (89 species) (Lamas, 2004), with a group of perhaps 12 species found in temperate North America (Scott, 1994, 1998; Wahlberg et al., 2003). The subtribe was taxonomically revised by Higgins (1981), and subsequently Lamas (2004) demoted many of Higgins's species to subspecies.

Even though almost half of the species in Melitaeini are Neotropical, there is a general lack of information on most aspects of the life histories of most species. Some species have received detailed attention in recent years (Young, 1973; Freitas, 1991), but these studies have mainly concentrated on describing larvae and their host plants. Despite this, the Neotropical Melitaeini, particularly the species in Phyciodina, appear to be an ideal group to investigate patterns of diversification in tropical regions.

2. Materials and methods

2.1. Taxonomic sampling and molecular methods

We sampled as many species as possible from almost all of the genera belonging to the subtribe Phyciodina (sensu Higgins, 1981) for a total of 87 individuals of 65 species. We were unable to acquire samples of the genus *Tisona* (a monotypic genus restricted to northern Argentina). In addition we sampled 32 outgroup species, representing all the major lineages in Melitaeini, plus one species of Kallimini. The outgroup sequences were taken from a previous study (Wahlberg et al., 2005), except for *Gnathotriche mundina*, for which new sequence was generated in this study. The sampled specimens are listed in Appendix A. Taxonomic nomenclature for genera and species follows Lamas (2004).

We extracted DNA mainly from two legs of dried butterflies using Qiagen's DNEasy extraction kit or with DNAzol® (see Junqueira et al., 2002). Extracts were eluted in a volume of 50 μl, which was found to enhance PCR success. The spread voucher specimens can be viewed at http://nymphalidae.utu.fi/Vouchers.htm. We sequenced 1450 bp of the *cytochrome oxidase subunit I* gene (COI), 1077 bp of the *Elongation Factor-1α* gene (EF-1α) and 403 bp of the *wingless* gene for all sampled individuals. Primers used are given in Table 1.

We performed all PCRs in a 20-μl reaction volume using 1 μl of DNA extract. The cycling profile for COI and *wingless* was 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 47 °C for 30 s, 72 °C for 1 min 30 s and a final extension period of 72 °C for 10 min. The cycling profile for the EF-1α primer pairs was 95 °C for 7 min, 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension period of 72 °C for 10 min. For all three genes, the PCR primers were also used for sequencing. Sequencing was done with a Beckman-Coulter CEQ8000 capillary sequencer in Stockholm, Sweden or on an ABI 377 automated sequencer in Campinas, São Paulo, Brazil. We checked the resulting chromatograms using the program BioEdit (Hall, 1999) and aligned the sequences by eye. Clear heterozygous positions in the nuclear genes (chromatogram peaks almost or exactly equal) were coded according to the IUPAC ambiguity codes. The sequences have been submitted to GenBank (Accession numbers in Appendix A).

Table 1
Primers used for amplifying and sequencing DNA

Gene	Direction	Primer name	Primer
COI	Forward	LCO	5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′
COI	Reverse	HCO	5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′
COI	Forward	Jerry	5′-CAA CAY TTA TTT TGA TTT TTT GG-3′
COI	Reverse	Pat	5′-ATC CAT TAC ATA TAA TCT GCC ATA-3′
EF-1α	Forward	Cho	5′-GTC ACC ATC ATY GAC GC-3′
EF-1α	Reverse	Verdi	5′-GAT ACC AGT CTC AAC TCT TCC-3′
EF-1α	Forward	EF51.9	5′-CAR GAC GTA TAC AAA ATC GG-3′
F-1α	Reverse	EFrcM4	5′-ACA GCV ACK GTY TGY CTC ATR TC-3′
Wingless	Forward	LepWG1	5′-GAR TGY AAR TGY CAY GGY ATG TCT GG-3′
Wingless	Reverse	LepWG2	5′-ACT ICG CAR CAC CAR TGG AAT GTR CA-3′

Primer pairs used shown with Forward primer first followed by Reverse primer.

2.2. Phylogenetic analyses

We searched for the most parsimonious cladograms from the combined, equally weighted and unordered data matrix consisting of 119 taxa using a heuristic search algorithm in the program TNT (Goloboff et al., 2004). The data were subjected to 100 random addition rounds of successive Sectorial, Ratchet, Drift and Tree Fusing searches (Goloboff, 1999; Moilanen, 1999; Nixon, 1999). All trees were rooted with *Doleschallia*.

It is now widely recognized that assessing incongruence among data partitions is much more complex than may be measured by a simple all-or-nothing significance test (Farris et al., 1994; Miller et al., 1997; Darlu and Lecointre, 2002). We have thus chosen to analyze the three gene regions as a single data set and have assessed the impact of each gene region on the support values of each node using Partitioned Bremer support (PBS) analyses (Baker and DeSalle, 1997; Gatesy et al., 1999).

We evaluated the character support for the clades in the resulting cladograms using Bremer support (Bremer, 1988, 1994) and bootstrap. The scripting feature of TNT was used to calculate BS values (see Peña et al., 2006). We assessed the contribution of each data partition to the BS values of the combined analyses using Partitioned Bremer support (Baker and DeSalle, 1997; Gatesy et al., 1999) using another script in TNT (scripts available from N. Wahlberg). The degree of congruence between the three separate datasets was summarised using the new Partition Congruence Index (PCI, Brower, 2006). This index is equal to the Bremer support value when there is no conflict between datasets and has negative values when there is strong conflict between datasets (Brower, 2006).

Support for clades can also be investigated through sensitivity analysis (Wheeler, 1995; Giribet, 2003). In a parsimony framework this can be done by testing the effects of differential weighting, e.g. by down-weighting third codon positions or by weighting transversions more than transitions (see e.g. Wahlberg et al., 2005). Another way of testing the stability of clades is to compare the results of unweighted parsimony analysis (which allows characters to evolve unrestricted by assumptions about their evolvability, Brower, 2000) to the results of a model-based analysis with strict assumptions about how characters can evolve through time.

We investigated the effects on the resulting phylogenetic hypothesis by restricting the evolution of the sequences to the general time reversible model with rates varying according to a gamma distribution (GTR + Γ) for each gene separately. Based on AIC values obtained using the program MrModelTest (Nylander, 2002), the best fit model for each gene was the most complex model available (GTR + Γ + I). However, it has been noted that the Γ shape parameter and the I parameter are highly correlated and are considered to be pathological when estimated together (Ren et al., 2005), thus we reduced our model to the GTR + Γ. We used Bayesian methods to estimate parameter values using the program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). The Bayesian analysis was performed on the combined data set with parameter values estimated separately for each gene region using the "unlink" command and the rate prior (ratepr) set to "variable". The analysis was run twice simultaneously for 2 million generations, with four chains (one cold and three heated) and every 100th tree sampled. The first 1000 sampled generations discarded as burn-in (based on a visual inspection of when log likelihood values reached stationarity). Results of the two simultaneous runs were compared for convergence. The purpose of this analysis was to investigate the effects on the results under restrictive assumptions of data analysis. 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.

2.3. Dating of divergences

Dating of divergences was done using the Bayesian relaxed clock method (Thorne and Kishino, 2002) on both the parsimony and Bayesian topology. The program used (see below) is limited to estimating times of divergence

for less than 100 nodes, thus the topologies were pruned down to 99 tips, with species or subspecies represented by more than one individual pruned to one individual and some outgroups removed. Polytomies in consensus trees may have unexpected effects on estimates of times of divergence (Wahlberg, 2006; Won and Renner, 2006), thus we chose one most parsimonious topology at random and took the single *a posteriori* maximum likelihood tree from the Bayesian analysis.

Since there are no fossils of melitaeines, the age constraints were taken from a recent study of times of divergences in the subfamily Nymphalinae (Wahlberg, 2006). Three nodes were constrained. The node describing Melitaeina + Phyciodina + the *Chlosyne*-group + the *Gnathotriche*-group was constrained to be no younger than 36 MYA and no older than 40 MYA, the node describing Melitaeina + Phyciodina + the *Gnathotriche*-group was constrained to be no older than 36 MYA and the node describing *Antillea* + Phyciodina was constrained to be no younger than 33 MYA. The latter two constraints were based on the assumption that the divergences can be attributed to the presence of a land span between the Greater Antilles of today and northern South America between 35 and 33 MYA (Iturralde-Vinent and MacPhee, 1999; Wahlberg, 2006).

Divergence time estimates were calculated using the program MULTIDIVTIME (available from J. Thorne, North Carolina State University), which implements a stochastic model for evolutionary rate changes over time (Kishino et al., 2001; Thorne and Kishino, 2002). Branch lengths were estimated separately for each gene on the given topology using the program ESTBRANCHES (part of the MULTIDIVTIME package) after estimating parameter values for the F84 + Γ model using PAML (Yang, 1997). In Bayesian analyses the priors are very important (Thorne and Kishino, 2002; Wiegmann et al., 2003; Sanderson et al., 2004). In this case, we had prior information about the possible age of the the ingroup and prior information about the possible rates of molecular evolution at the ingroup node (Wahlberg, 2006). The prior distribution for the time separating the ingroup node from the present (±SD) was set to 4.0 (±0.8), where one unit equals 10 MY. The prior distributions of the rate of molecular evolution at the ingroup root node (±SD) was set to 0.05 (±0.05), which is the average and standard deviation of the posterior rates of evolution of all three genes in the study of Nymphalinae (values in Table 2 of Wahlberg, 2006).

As in Wiegmann et al. (2003), initial parameter values were randomly selected to initialize the Markov chain, and then a burn-in period of 100,000 cycles of proposed changes to the current state of the Markov chain was completed before parameters were sampled from the chain. After the burn-in period, the Markov chain was run for 1,000,000 cycles with every 100 cycles sampled. Prior and posterior distributions were approximated based upon the 10,000 samples.

2.4. Biogeographical analyses

We investigated the biogeographical history of Phyciodina by subjecting our phylogenetic hypotheses to dispersal-vicariance analysis (Ronquist, 1997). The distributions of species included in our analysis were taken from Higgins (1981). The distributions were divided into 8 different regions that contained at least one endemic species (Fig. 1). The species labels on our pruned phylogenetic hypotheses were replaced by their distributions and the ancestral distributions were inferred with the program DIVA (Ronquist, 1996) using default costs (i.e. vicariance events cost nothing, dispersal and extinction events cost 1 per unit area). The maximum number of ancestral areas was constrained to be 2, 3, 4, 5, 6, 7 and 8 (the last being unconstrained).

3. Results

3.1. Phyciodina phylogeny

The combined analyses of all three genes resulted in 840 equally parsimonious trees (length 7785 steps, CI 0.24, RI 0.58), of which the strict consensus is shown in Fig. 2. Bayesian analysis resulted in a topology (average likelihood = −41955; Fig. 3) that differed at 12 ingroup nodes compared to the most parsimonious trees. All of these nodes are characterised by weak support, conflicts between data partitions and, in the Bayesian analysis, short branches. Many of the ingroup nodes are however recovered in both analyses, with many of these nodes being strongly supported and/or with no conflicting data. The subtribe Phyciodina emerged as a monophyletic group with the Caribbean genus *Antillea* as its sister genus. The clade *Antillea* + Phyciodina has good support and is stable. Phyciodina s.s. (as defined by Higgins, 1981) is also a stable clade with good support. Of the sampled genera, *Phyciodes, Tegosa, Janatella, Castilia* and *Anthanassa* are found to be monophyletic, while the genera *Ortilia, Telenassa* and *Eresia* are clearly polyphyletic, although these genera have subgroups that are strongly supported. *Dagon* has only a single species sampled and thus its monophyly is not tested here. *Mazia* and *Phystis* are monotypic genera.

Comparison of the results from the two methods (Figs. 2 and 3) shows that the positions of the genera *Tegosa, Dagon* and *Janatella*, and the species *Eresia levina, Eresia lansdorfi* and *Ortilia ithra* differ substantially, while all other major clades remain stable. The exact placement of these taxa will remain enigmatic until more data can be collected. There are some differences in relationships within the major clades, e.g. the *Phyciodes tharos*-group (sensu Wahlberg et al., 2003), which is monophyletic in the parsimony tree, but paraphyletic in the Bayesian tree. Also the relationships of the species *Tegosa orobia* and *Tegosa infrequens* are not congruent in the two trees.

According to our analyses, the early branching events are relatively clear, with a clade consisting of *Phystis* and

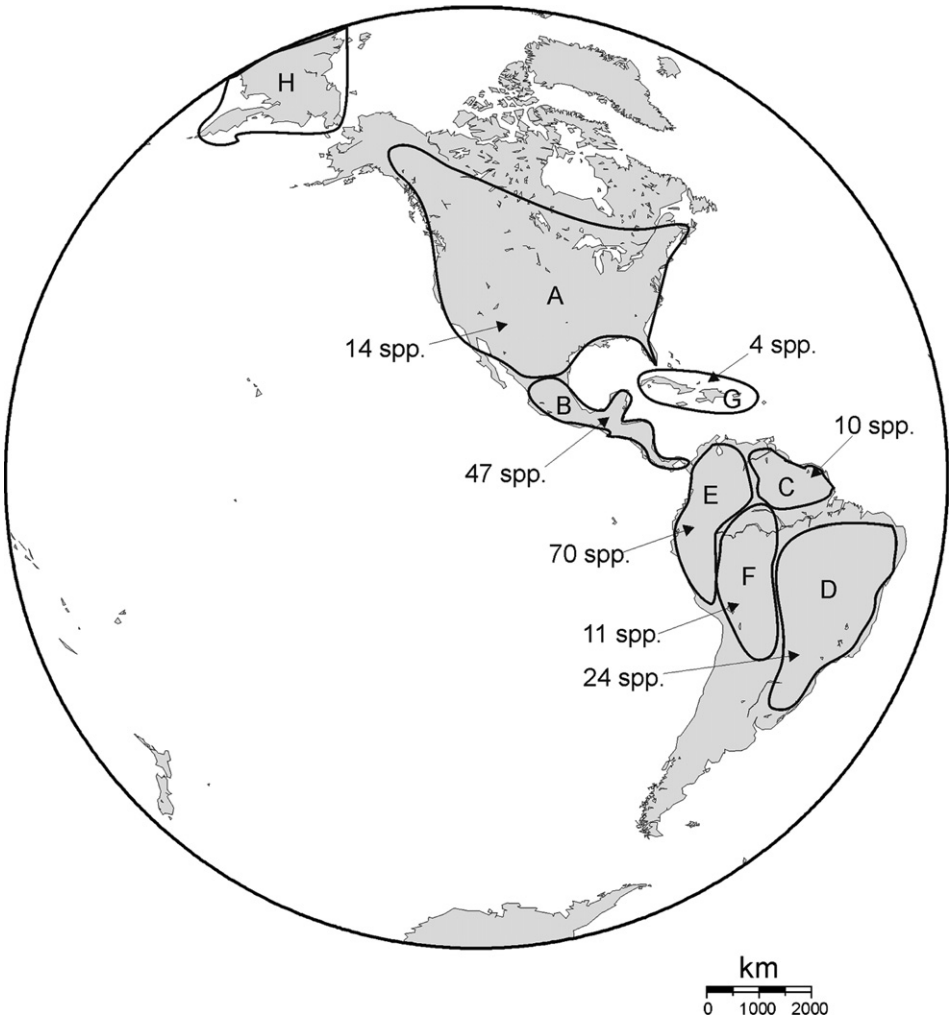

Fig. 1. The 8 different regions containing at least one endemic meliteaine species. A, North America; B, Central America; C, Guyanan Shield; D, Brazilian Shield; E, North western South America; F, Western Amazonia, G, Greater Antilles and H, Palaearctic region. Numbers of Phyciodina species found in each region shown, except for the Palaearctic region where Phyciodina does not occur.

"*T.*" *fontus* branching off after the *Antillea* lineage had diverged, and subsequently another species poor clade branching off consisting of *Mazia* + *Ortilia* s.s. (Figs. 2 and 3). The rest of the species fall into four well-supported clades, which form a monophyletic group, but whose relationships are not clear. These are the *Tegosa*, *Phyciodes*, Brazilian "*Ortilia*" and *Anthanassa* + *Eresia* s.l. clades (shown in Figs. 2 and 3). In addition to species of *Eresia*, the clade defined as *Eresia* s.l. contains the genera *Dagon*, *Castilia*, *Telenassa* (excluding "*T.*" *fontus*) and *Janatella*, as well as the species "*O.*" *ithra*.

Relationships of the potential sister groups differ somewhat between analyses and to previous studies (Wahlberg and Zimmermann, 2000; Wahlberg et al., 2005; Wahlberg, 2006). Based on the sequences of three gene regions, the sister group of Phyciodina is either the *Gnathotriche*-group (Fig. 2 and Wahlberg et al., 2005; Wahlberg, 2006) or the *Gnathotriche*-group + Melitaeina (Fig. 3). It appears evident that these three groups form a monophyletic clade to the exclusion of the *Chlosyne*-group (in contrast to Kons, 2000).

3.2. Times of divergence

Our analysis using a Bayesian relaxed clock showed fairly narrow credibility intervals for all estimated times of divergence (Fig. 4). The two topologies (Figs. 2 and 3) were both analysed and were found not to affect the estimated times of divergences for common clades at all, thus results for only the parsimony topology are shown (Fig. 4). Our estimates of absolute times of divergence within Phyciodina are contingent on the assumption that the initial split in Phyciodina (between the Caribbean *Antillea* and the rest of Phyciodina) was due to a vicariance event that happened when the brief landspan between South America and the Greater Antilles disappeared about 33 million years ago (MYA) (Iturralde-Vinent and MacPhee, 1999; Wahlberg, 2006). If this is the case, then it would appear that the major phyciodine lineages diverged in the Oligocene, leading to the origin of the *Mazia, Phyciodes, Tegosa* and *Eresia* lineages (Fig. 4). Most diversifications at the species level have happened in the Miocene between 23 and 5 MYA.

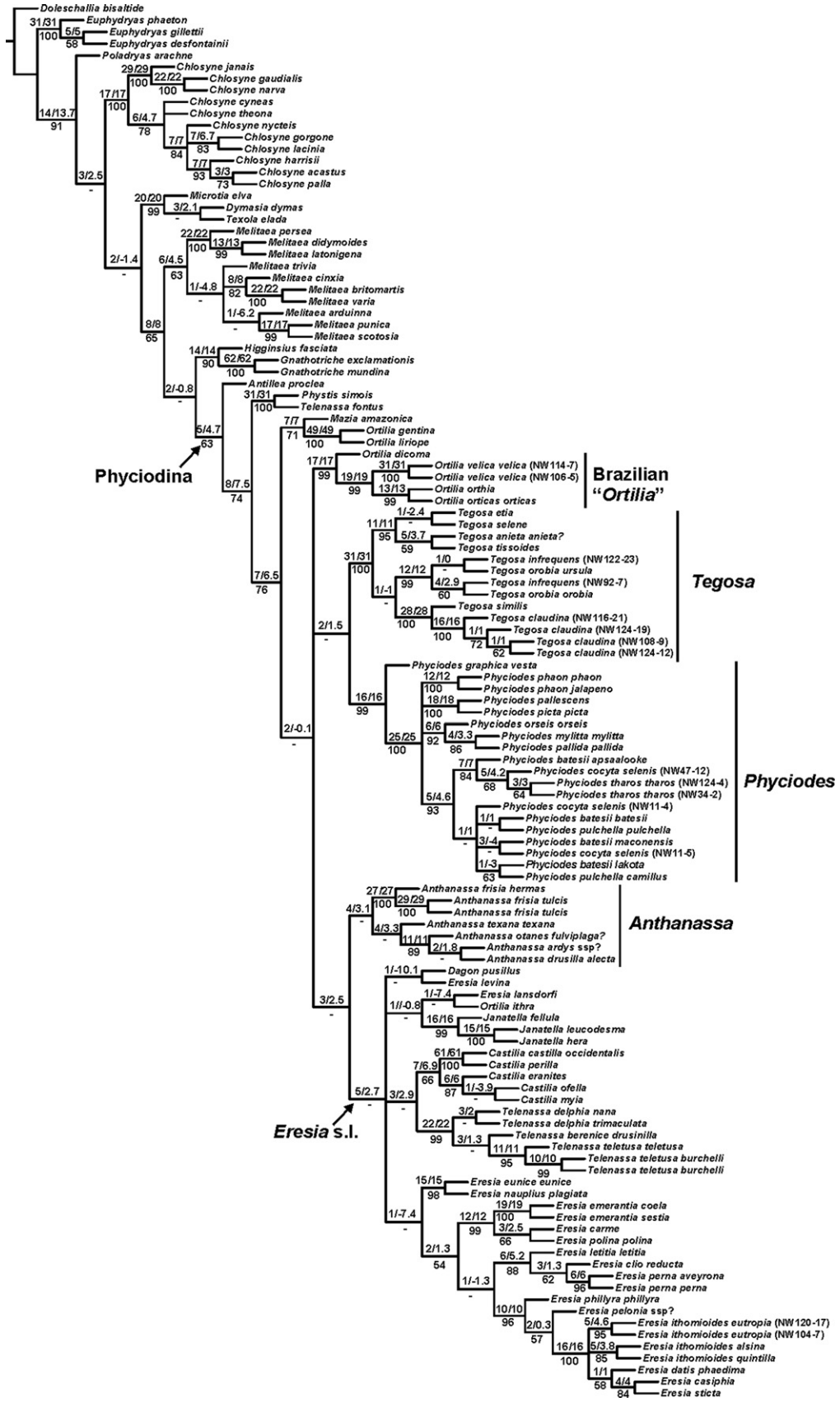

Fig. 2. A strict consensus tree of 840 equally parsimonious trees (length 7785 steps, CI 0.24, RI 0.58) found for the comined COI, EF-1α and wingless dataset. Numbers above branches are Bremer support values and Partition Congruence Indices (see text for description) for the node to the right. Numbers below are bootstrap values >50% for the node to the right.

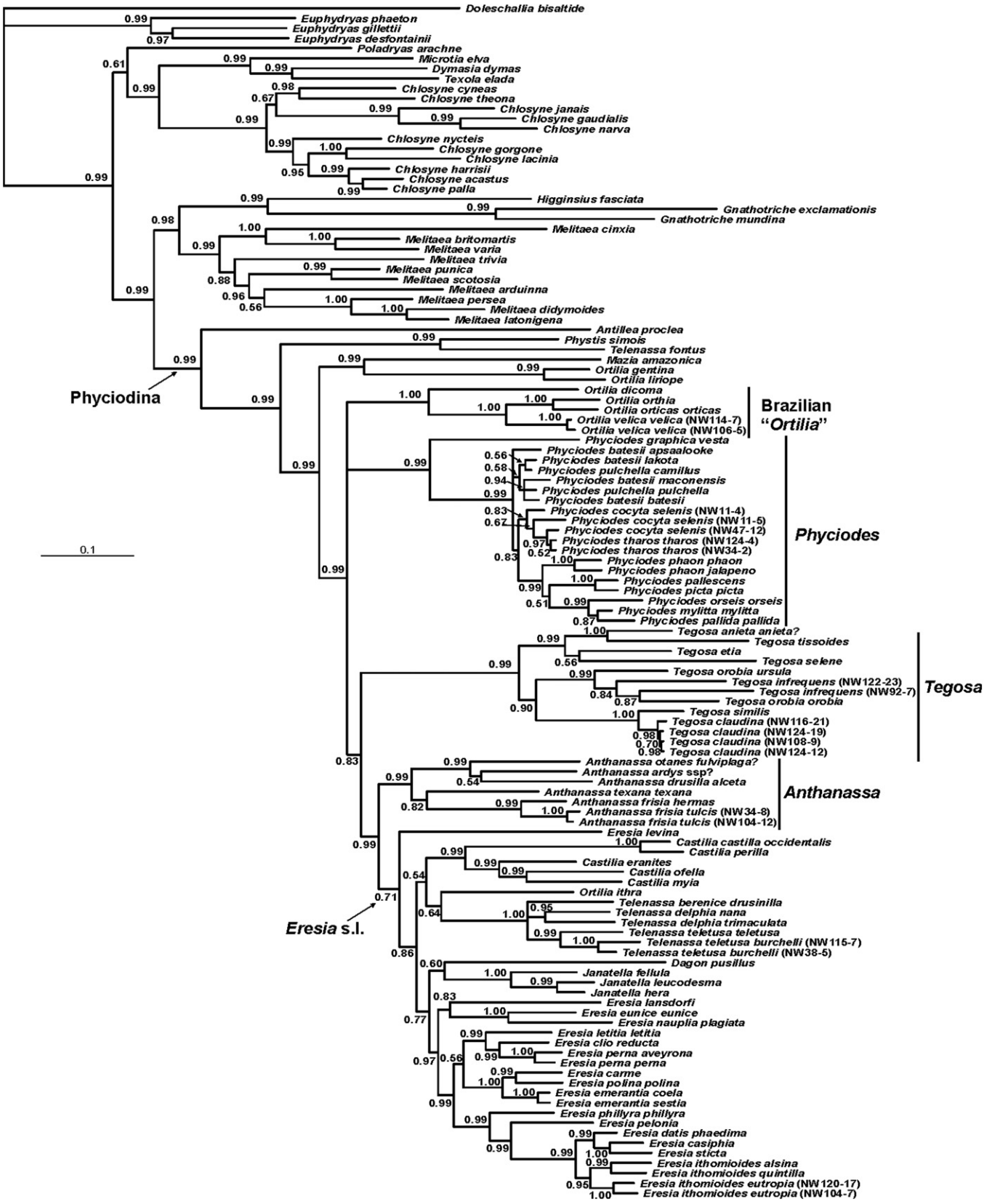

Fig. 3. The 50% majority rule phylogram of 38,000 sampled trees from the Bayesian analysis of the combined COI, EF-1α and *wingless* dataset. Numbers to the left of nodes are the posterior probabilities of those nodes.

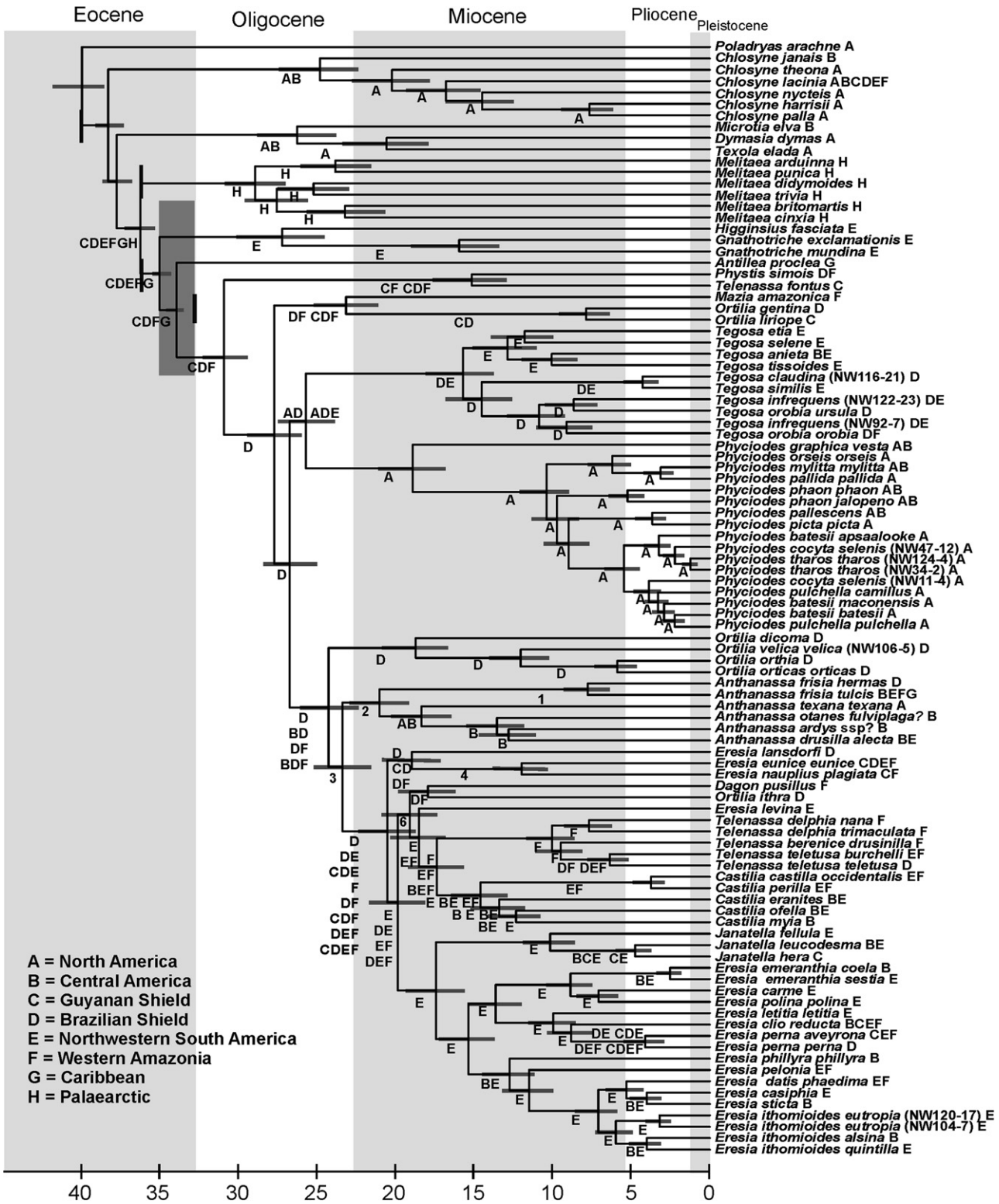

Fig. 4. Chronogram based on the parsimony topology with associated posterior credibility limits. Vertical bars show the time constraints imposed (maximum to the left of a node, minimum to the right of a node) when estimating times of divergence. Dark grey box shows the time period of the hypothesised land span connecting the Greater Antilles with South America. Results of a dispersal-vicariance analysis, with unrestricted ancestral areas, are shown for each node. For some nodes there were too many possible ancestral distributions to fit on the figure. They are given here: 1, BD DE BDE DF BDF DEF BDEF DG BDG DEG BDEG DFG BDFG DEFG BDEFG; 2, B AD BD ABD ADE BDE ABDE AF BF ABF ADF BDF ABDF AEF BEF ABEF ADEF BDEF ABDEF ADG BDG ABDG ADEG BDEG ABDEG AFG BFG ABFG ADFG BDFG ABDFG AEFG BEFG ABEFG ADEFG BDEFG ABDEFG; 3, D BD BCD BDE BCDE F BF DF ADF BDF ABDF BCDF DEF ADEF BDEF ABDEF BCDEF DFG ADFG BDFG ABDFG DEFG ADEFG BDEFG ABDEFG; 4, C CD CDE F DF CDF DEF CDEF.

3.3. Biogeographical history

Our dispersal-vicariance analysis of the melitaeine clade suggests that dispersals have played a large role in the formation of the current distributions of the butterflies in South America. Regardless of the phylogenetic hypothesis used or the maximum number of ancestral areas, the DIVA analysis suggests that 61–65 dispersal events are necessary to explain current distributions (Fig. 4). Restricting the maximum number of ancestral areas does not change the major patterns as most reconstructions of ancestral distributions comprise of 2 or 3 areas (Fig. 4).

Despite the high number of dispersal events, there appear to be several clear patterns regarding the historical biogeography of the group (Fig. 4). Based on the unconstrained maximum ancestral areas analysis, the ancestor of the subtribe (including the genus *Antillea*) is inferred to have been widespread in the Caribbean, the Guyanan shield, western Amazon and the Brazilian shield (Fig. 4). This reduces to an ancestral disjunct distribution in the Caribbean and the Brazilian shield when the maximum number of ancestral areas is restricted to 5 or less.

The split between *Antillea* and the rest of Phyciodina appears to have been a vicariance event, leaving the ancestor of *Antillea* on the Greater Antillean islands and the ancestor of the rest of Phyciodina on the South American continent, possibly in the Brazilian Shield area. From the Brazilian Shield, ancestral Phyciodina spread to other parts of tropical South America, especially to NW South America, which is inferred to be an important ancestral area for several lineages such as *Eresia, Castilia, Telenassa* and *Tegosa*. The ancestral area of the genus *Anthanassa* is unclear, but this genus is undersampled and a larger sample of the mainly Central American species will help to resolve the historical biogeography of the genus. The genus *Phyciodes* has colonized North America from South America and diversified there, according to our phylogenetic hypotheses.

4. Discussion

4.1. Phyciodina phylogeny

Our molecular phylogeny of phyciodine butterflies shows that the subtribe Phyciodina, as circumscribed by Higgins (1981), forms a monophyletic group which is sister to the Caribbean endemic *Antillea*. The position of *Antillea* was not anticipated by Higgins (1981), who suggested that it is related to the *Gnathotriche*-group, and was not found in a morphological study of the tribe Melitaeini, where it was found to be sister to the *Chlosyne*-group (Kons, 2000). In fact, most of the unique characters of *Antillea* described in the above works are clear apomorphies, as expected to occur in such a long branch. Our result is robust to method of analysis and has good support, and is likely to withstand the addition of new data. We thus propose to include *Antillea* in the subtribe Phyciodina.

The revision of Phyciodina by Higgins (1981) introduced 9 new genera, whose monophyly has never been tested. We find that several genera do form well-supported, robust monophyletic groups, but that other genera are paraphyletic and even polyphyletic. Clearly Phyciodina is in need of a careful generic level revision that takes into account our results. We have found several robust lineages, which are candidates for stable genera and can be termed the *Antillea, Phystis, Mazia, Ortilia* s.s., *Tegosa, Phyciodes*, Brazilian *''Ortilia'', Anthanassa* and *Eresia* lineages. The *Eresia* lineage (henceforth *Eresia* s.l.) includes the genera *Dagon, Janatella, Castilia* and *Telenassa* s.s. Species in these genera had been included in *Eresia* prior to Higgins (1981) revision, and this is perhaps where they should be returned.

The genus *Phyciodes* has been studied before based on mitochondrial DNA (Wahlberg et al., 2003) and our results here with more sequence data are similar. We are able to corroborate the monophyly of the genus, and it is clear that *Phyciodes graphica* is sister to the rest of the species. Adding nuclear sequence data does not clear up the patterns of non-monophyly of species belonging to the *P. tharos*-group (see Wahlberg et al., 2003). The Bayesian tree in fact suggests that the group is paraphyletic with regard to the *Phyciodes mylitta* and *Phyciodes phaon*-groups, a result not supported by parsimony analysis. The Bayesian analysis does however suggest that *Phyciodes cocyta* and *P. tharos* are sister species, as are *Phyciodes batesii* and *Phyciodes pulchella* (Fig. 3).

The genus *Ortilia* as circumscribed by Higgins (1981) is found to be polyphyletic. The type species of the genus is *Ortilia liriope* (Higgins, 1981), and thus the Brazilian "*Ortilia*"-lineage will require a new genus name. The position of "*O.*" *ithra* is unclear at the moment and is probably best placed in the genus *Eresia*. The genus *Telenassa* is also found to be polyphyletic, with the species "*T.*" *fontus* grouping with *Phystis simois*. "*T.*" *fontus* was tentatively placed in the genus *Dagon* by Higgins (1981) and subsequently moved to *Telenassa* by D'Abrera (1987). This taxon was not adequately studied by Higgins (1981), who had available only one male without an abdomen, and was not included in the study of Kons (2000), so it is not surprising that its systematic position has remained uncertain for so long. According to our results, "*T.*" *fontus* could be placed in or near the genus *Phystis*.

The position of *E. levina* is unclear at the moment, with parsimony placing it within the *Eresia* s.l. and Bayesian inference placing it as sister to *Eresia* s.l. This species does not have any apparent close relatives (Higgins, 1981) and is likely to represent an early divergence in the *Eresia* s.l. clade. Other enigmatic species in the *Eresia* s.l. clade are *Dagon pusillus* and "*O.*" *ithra*, whose positions are not stable to method of analysis. All three species are characterised by long branches (Fig. 3) which may explain their "rogue" behaviour. The genus *Dagon* contains two more species that were not sampled in this study, sampling them may help in breaking up the long branch leading to

D. pusillus. *E. levina* and "*O.*" *ithra* on the otherhand do not appear to have any closely related species, and thus their positions will require more data to resolve.

We have sampled 65 of the 104 species, and of the missing 39 species (11 species of *Anthanassa*, 4 species of *Eresia* s.s., 8 species of *Castilia*, 2 species of *Dagon*, 3 species of *Telenassa*, 2 species of Brazilian "*Ortilia*", 8 species of *Tegosa* and one species of *Tisona*), only *Tisona saladillensis* is morphologically enigmatic, with Higgins (1981) commenting that it most closely resembles *Tegosa*. We thus believe we have found the major lineages of Phyciodina, with the possible exception of *Tisona*. As such, it is clear that the first three lineages to branch off (ie. *Antillea, Phystis* and *Ortilia* + *Mazia*) either have not speciated very frequently or have suffered more extinction events than the other lineages of Phyciodina.

4.2. The biogeographic history of Phyciodina

To understand the evolutionary history of Phyciodina, it is useful to briefly review the geological history of northern South America during the Oligocene and Miocene, given our hypothesis of colonization of South America by phyciodine ancestors via the landspan that may have existed 35–33 MYA. South America of the early Oligocene was very different to what it is currently. The central and northern Andes were still fairly low hills, with elevations below 1000 m (Gregory-Wodzicki, 2000). The Amazon River flowed northwards into the Caribbean Sea, while the geologically stable Guyanan and Brazilian Shields (see Fig. 1) were much like they are today (Lundberg et al., 1998). About 20 MYA, the central Andes began to rise at an accelerated rate, rising to about half their current elevation by 10 MYA (Gregory-Wodzicki, 2000). Western Amazonia is thought to have been covered by a large lake, known as Lake Pebas, between 23 and 8 MYA (Wesselingh et al., 2002), although this is controversial. In any case, it is clear that Western Amazonia was a very wet region during this period (Lundberg et al., 1998). At about 8 MYA, the northern Andes began rising and cut off access to the Caribbean by the Amazon River, which then changed course to the present eastward flow (Lundberg et al., 1998). The northern Andes rose at a relatively fast rate between 5 and 3 MYA, reaching their current elevations some 2.7 MYA (Gregory-Wodzicki, 2000). Events leading to the formation of the Panamanian Isthmus began some 15 MYA with the final formation occurring some 3 MYA, allowing dispersal over land between North and South America (Coates and Obando, 1996).

We have based our timing of divergences on the results of a previous study on the whole subfamily Nymphalinae (Wahlberg, 2006), which found that the split between the South American melitaeines and Holarctic melitaeines happened approximately 30 MYA. This date coincides with a hypothesized landspan between the Greater Antilles and South America 35–33 MYA, the so-called GAARlandia hypothesis (Iturralde-Vinent and MacPhee, 1999). We have taken this geological hypothesis as our calibration point for the split between the Caribbean endemic *Antillea* and the rest of Phyciodina. We stress that the age of Phyciodina determined by Wahlberg (2006) was based on fossil evidence, not biogeographic scenarios. Our DIVA analysis indicates that this split was indeed a vicariance event, with the ancestral species being widespread in the Caribbean region and in northeastern South America. An alternative scenario would be a simultaneous colonization of the Caribbean region and South America through a dispersal event at an unknown point in time. However, melitaeines are well-known to be relatively sedentary and form local populations that are prone to extinction (Ehrlich and Hanski, 2004). Melitaeines are unkown from oceanic islands and are missing from large parts of the world. The only endemic island melitaeines that are known are on the islands of the Greater Antilles in the Caribbean, where there are two species of the endemic genus *Antillea*, 4 species of the endemic genus *Atlantea* (possibly related to the Chlosyne-group, Kons, 2000), one species of *Phyciodes* (*P. phaon*, which may be introduced by humans to Cuba) and one species of *Anthanassa* (*Anthanassa frisia* which is widespread from subtropical North America to Brazil) (Smith et al., 1989). The evidence thus suggests that melitaeines do not disperse large distances over water.

An alternative hypothesis is for melitaeines to have colonized South America from North America about 3 MYA, when the Panamanian Isthmus formed. Such a hypothesis does not explain why the sister group to the South American clade is found in the Greater Antilles and would assume that speciation has been extremely rapid in these butterflies once they colonized South America. We find such an hypothesis untenable and contrary to the evidence presented (e.g. Wahlberg, 2006). There is some evidence of emergent land in the Panamanian region during the early Middle Miocene (Iturralde-Vinent and MacPhee, 1999), which may have played a role in phyciodine dispersal, but in the opposite direction (see below).

The ancestral Phyciodina colonized the landspan and spread south to the Guyanan Shield and then quickly to the Brazilian shield. Two species that branch off relatively early in the phyciodine clade, *O. liriope* and "*T.*" *fontus*, are endemic to the Guyanan Shield area. The ancestral populations split vicariantly to form *Antillea* and the ancestral species of the rest of Phyciodina at around 33 MYA. This ancestral species then spread over South America from the Guyanan Shield via the Brazilian Shield to Western Amazonia, forming the current lineages of *Phystis, Mazia, Ortilia* s.s. and the ancestor of the rest of Phyciodina over the period 30–25 MYA. The Brazilian Shield appears to have been an important area for the diversification of phyciodines. The ancestral lineage continued to diversify in this region to form the current lineages of *Tegosa* and the Brazilian "*Ortilia*" clade about 25 MYA. The Brazilian Shield set the stage for further colonisations of South America.

The clade including *Mazia* and *Ortilia* s.s. is not likely to contain any other species and its biogeographical history is well correlated with geological events. The common ancestor of the three species is inferred to have been widespread in the Guyanan Shield, Brazilian Shield and Western Amazonia (Fig. 4). Roughly, 23 MYA the ancestor diverged into the *Mazia* and *Ortilia* s.s. lineages. This coincides with the formation of extensive wetlands in Western Amazonia. Interestingly, *Mazia amazonica* is found in very wet habitats, such as near oxbow lakes, although it is also found in other open disturbed habitats as well (K. Willmott, pers. comm.), while *Ortilia* s.s. are found in drier forest habitats. The common ancestor of the two *Ortilia* s.s. species is inferred to have been widespread in the Guyanan and Brazilian Shields, with a vicariance event leading to the divergence of the *O. liriope* and *Ortilia gentina* lineages about 8 MYA. Once again, this coincides with the change in direction of the Amazon River, which about 8 MYA began to flow between the Guyanan and Brazilian Shields. Whether the change in direction of the flow of the river isolated the ancestral populations is a matter of speculation at this point. Indeed the change in the direction of the Amazon River is not inferred to play a central role in any other divergences, perhaps because there are currently very few species in Eastern Amazonia, and phyciodines are almost absent from Central Amazonia.

NW South America was colonised by phyciodines 25–20 MYA either twice (by the ancestor of *Tegosa* and the ancestor of *Eresia* s.l.) based on the parsimony topology (Fig. 4), or once by the ancestor of *Tegosa–Anthanassa–Eresia* s.l. based on the Bayesian topology. Most likely, the colonisation occurred from the Brazilian Shield area. *Eresia* s.l. appears to have speciated heavily in this region subsequent to colonisation. The time period coincides with the rise of the Andes (Gregory-Wodzicki, 2000), which may have affected the diversification rates in phyciodines, as has been hypothesised for *Hypanartia* (Willmott et al., 2001), ithomiines (Whinnett et al., 2005; Jiggins et al., 2006), *Heliconius* (Brower, 1996) and riodinids (Hall, 2005).

Central America appears to have been colonised several times, once by the ancestor of *Anthanassa* 20–16 MYA, once by the ancestor of *Castilia* 17–15 MYA, and several times in other *Eresia* s.l. lineages around the time of the formation of the Panamanian Isthmus. Given the confidence intervals of the possible divergence dates, it is possible that both earlier colonisations of Central America coincide with a period of time during which there possibly was emergent land between South and Central America in the early Middle Miocene (16–14 MYA), but no land connection between North and South America (Iturralde-Vinent and MacPhee, 1999).

The North American genus *Phyciodes* presents a biogeographical puzzle. According to our DIVA analyses, the ancestor was found in North America, and the common ancestor of *Phyciodes* and its sister group was widespread on the Brazilian Shield and North America and possibly NW South America. However, the DIVA analyses do not account for the fact that several of the early diverging lineages in *Phyciodes* are currently also found in Central America (Fig. 4). Taking into account this and the long branch leading to the first divergence in *Phyciodes* suggests that the ancestor to *Phyciodes* may have colonised Central America at about the same time as the ancestors of *Anthanassa* and *Castilia*, and subsequently spread north to North America, where it diversified about 10 MYA.

Of the missing species, *Tisona salladilensis* is perhaps the most important for biogeographical inferences. It is found northern Argentina and thus would be placed in our Brazilian Shield area. This is an important area in the basal divergences of the group and it is likley that *Tisona* will be placed in one of the more basal lineages, perhaps sister to *Tegosa* (Higgins, 1981), thus inclusion of *Tisona* would likely corroborate our hypothesis that the Brazilian Shield area has been important for the diversification of Phyciodina. The poor sampling in the genus *Anthanassa* does not allow us to make very strong inferences about the biogeographical history of the group. This genus is mainly found in Central America, and a broader sampling of the species would allow a better understanding of the colonisation of Central America by Phyciodina.

4.3. Historical biogeography of butterflies in South America

The biogeography of Neotropical butterflies has been the subject of many speculations in the last years (Descimon, 1986; Brower et al., 1992; Shapiro, 1994; Viloria, 2003), but conclusions were mostly limited by the general lack of well-supported phylogenies for most of the groups, absence of adequate fossil records and no confident timing of divergences estimates. It is worth noting that in some of the above studies, a special treatment was given to the butterfly fauna of the Antilles. In fact for many reasons, including the maintenance of some relict taxa and extinctions of widespread lineages, Antillean butterflies can provide valuable material for understanding the evolution of Neotropical butterflies as a whole (see also Fox, 1963; Miller and Miller, 1989; Hall et al., 2004; Peñalver and Grimaldi, 2006), and in this paper the Antilles played an important role in understanding the early historical biogeography of the Phyciodina.

Our study is the first to infer the historical biogeography of a group of butterflies in South America based on a detailed time scale. However, Phyciodina may be somewhat atypical of South American butterflies in that they are inferred to be relatively recent colonists of the continent, whereas most groups are thought to be endemic to the continent (Descimon, 1986; Shapiro, 1994; Miller and Miller, 1997; Viloria, 2003; Wahlberg, 2006). Thus, its history cannot be considered a model to be expanded to mostly Neotropical butterfly groups. Nevertheless, the geological events affecting these butterflies have also affected those groups endemic to the continent over the past 30 MYA, and in this sense we do have a good starting

point to better understand the biogeographical patterns of Neotropical butterflies.

In this scenario, we could expect that when new data come to light, many Neotropical butterfly groups will show similar patterns of recent diversification, linked to the main post-Eocene geological events in Central and South America, especially the geological evolution of the Antilles, the rising of the Andes and the formation of the Panamanian Isthmus. We will also expect several early diverging butterfly lineages to be endemic to the mountains of SE Brazil and Guyanan shield, since these two formations are very old and could have harbored the ancestors of many modern lineages, that later spread through the newly drained Amazon basin, and further through the rising Andes. Again, we need well-supported phylogenies with divergence times to investigate these hypotheses.

It is clear to us that South America has played a major role in the evolution of the Nymphalidae, but how and when we will not be able to investigate in detail until we have robust estimates of the times of divergence for the whole family, including the origin of all major clades. Several Nymphalidae subfamilies are exclusively Neotropical, and this region also harbors about 40% of the species in the family (Lamas, 2004). The early historical biogeography of Neotropical butterflies, that may go as far back as the mid-Cretaceous (see Braby et al., 2006; Wahlberg, 2006), is mostly unknown at this point in time.

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Appendix A. Specimen collection data and GenBank Accession numbers (Editor: sequences have been submitted to GenBank Accession numbers pending)

Species	Voucher code	Collection locality	COI	EF-1α	Wingless
Doleschallia bisaltide	NW64-5	Butterfly farm supplier	AY788621	AY788735	AY788496
Euphydryas gillettii	NW24-6	USA: Montana	AF187771	AY788746	AY788507
Euphydryas desfontainii	NW70-4	SPAIN: El Guix	AY090226	AY090193	AY090159
Euphydryas phaeton	NW13-3	USA: Maryland	AF187797	AY788747	AY788508
Chlosyne acastus	NW35-15	USA: Colorado	AF187735	AY788725	AY788486
Chlosyne cyneas	NW38-17	USA: Arizona	AF187757	AY788726	AY788487
Chlosyne gaudialis	NW37-2	COSTA RICA: La Selva	AF187770	AY788727	AY788488
Chlosyne gorgone	NW34-4	USA: Colorado	AF187772	AY788728	AY788489
Chlosyne harrisii	NW35-10	USA: New York	AF187773	AY788729	AY788490
Chlosyne janais	NW62-1	COSTA RICA: Butterfly farm supplier	AY788620	AY788730	AY788491
Chlosyne lacinia	NW62-4	COSTA RICA: Butterfly farm supplier	AY090227	AY090195	AY090161
Chlosyne narva	NW37-3	COSTA RICA: La Selva	AF187786	AY788731	AY788492
Chlosyne nycteis	NW34-5	USA: Colorado	AF187788	AY788732	AY788493
Chlosyne palla	NW20-4	USA: California	AF187791	AY788733	AY788494
Chlosyne theona	NW27-6	USA: Arizona	AF187808	AY788734	AY788495
Gnathotriche exclamationis	NW89-9	ECUADOR: Sucumbios	AY788629	AY788748	AY788509
Gnathotriche mundina	KS-ac29	PERU: Junin	EF493927	EF493975	EF493868
Higginsius fasciatus	PE-10-20	PERU: Cuzco, Quebrada Chaupimayo	AY788630	AY788749	AY788510
Melitaea arduinna	NW23-5	GREECE: Pissoderi	AF187742	AY788774	AY788534
Melitaea britomartis	NW69-8	SWEDEN	AY788655	AY788775	AY788535
Melitaea cinxia	NW73-14	SWEDEN: Stockholm	AY788656	AY788776	AY788536
Melitaea didymoides	NW26-1	RUSSIA: Buryatia	AF187762	AY090194	AY090160
Melitaea latonigena	NW25-3	RUSSIA: Buryatia	AF187780	AY788778	AY788538
Melitaea persea	NW34-10	LEBANON: Mohafazat Beharré	AF187796	AY788779	AY788539
Melitaea punica	NW34-11	LEBANON: Mohafazat Kesronan	AF187803	AY788781	AY788541
Melitaea scotosia	NW27-11	CHINA: Hebei Province	AF187804	AY788780	AY788540

Appendix A (*continued*)

Species	Voucher code	Collection locality	COI	EF-1α	Wingless
Melitaea trivia	NW23-6	GREECE: Pissoderi	AF187810	AY788782	AY788542
Melitaea varia	NW24-13	FRANCE: Laus de Cervières	AF187812	AY788783	AY788543
Dymasia dymas	NW27-7	USA: Arizona	AF187764	AY788785	AY788545
Microtia elva	NW61-1	MEXICO: Chiapas	AY788660	AY788787	AY788547
Texola elada	NW7-1	USA: Texas	AY788659	AY788786	AY788546
Poladryas arachne	NW27-4	USA: California	AF187740	AY788799	AY788559
Anthanassa ardys ssp?	NW22-4	COSTA RICA: Monteverde	AF187743	AY788713	AY788474
Anthanassa drusilla alecta	NW76-7	ECUADOR: Esmeraldas	AY788611	AY788714	AY788475
Anthanassa frisia hermas	NW92-6	BRAZIL: São Paulo	EF493929	EF493977	EF493870
Anthanassa frisia tulcis	NW34-8	MEXICO: Colima, Manzanillo	AF187802	EF493978	EF493871
Anthanassa frisia tulcis	NW104-12	PANAMA: Gamboa	AY788612	AY788717	AY788478
Anthanassa otanes fulviplaga?	NW24-4	COSTA RICA: Monteverde	AF187790	AY788715	AY788476
Anthanassa texana texana	NW12-6	USA: Texas	AF187806	AY788716	AY788477
Antillea proclea	NW119-18	JAMAICA	EF493928	EF493976	EF493869
Castilia castilla occidentalis	NW114-6	COLOMBIA: Cali	EF493930	EF493979	EF493872
Castilia eranites	NW76-2	ECUADOR: Pichincha	AY788617	AY788722	AY788483
Castilia myia	NW24-5	COSTA RICA: Monteverde	AF187784	EF493980	EF493873
Castilia ofella	NW105-3	PANAMA: Achiote Road	AY788618	AY788723	AY788484
Castilia perilla	NW115-11	PERU	EF493931	EF493981	EF493874
Dagon pusillus	NW134-16	PERU: Junin	EF493932	EF493982	EF493875
Eresia carme	NW110-1	COLOMBIA: Antioquia	EF493935	EF493985	EF493878
Eresia casiphia	NW104-11	ECUADOR: Alluriquin	EF493936	EF493986	EF493879
Eresia clio reducta	NW76-5	ECUADOR: Esmeraldas	AY788622	AY788736	AY788497
Eresia datis phaedima	CP07-49	PERU: Junin	EF493942	EF493992	EF493885
Eresia emerantia coela	NW104-3	PANAMA: Path to Gloria Alta	AY788623	AY788737	AY788498
Eresia emerantia sestia	NW76-8	ECUADOR: Esmeraldas	AY788628	AY788742	AY788503
Eresia eunice eunice	NW92-5	BRAZIL: São Paulo	AY788624	AY788738	AY788499
Eresia ithomioides alsina	NW104-5	PANAMA	EF493933	EF493983	EF493876
Eresia ithomioides eutropia	NW120-17	COSTA RICA: Area Conservacion Guanacaste	EF493937	EF493987	EF493880
Eresia ithomioides eutropia	NW104-7	PANAMA	EF493940	EF493990	EF493883
Eresia ithomioides quintilla	NW76-3	ECUADOR: Esmeraldas	AY788627	AY788741	AY788502
Eresia lansdorfi	NW92-14	BRAZIL: São Paulo	EF493938	EF493988	EF493881
Eresia letitia letitia	NW91-9	ECUADOR: Sucumbios	AY788625	AY788739	AY788500
Eresia levina	NW120-19	COLOMBIA: Cali	EF493939	EF493989	EF493882
Eresia nauplius plagiata	NW108-15	PERU	EF493944	EF493994	EF493887
Eresia pelonia ssp?	NW108-11	PERU	AY788626	AY788740	AY788501
Eresia perna aveyrona	NW129-8	BRAZIL: Acre	EF493934	EF493984	EF493877
Eresia perna perna	NW114-5	BRAZIL: São Paulo	EF493941	EF493991	EF493884
Eresia phillyra phillyra	NW130-5	MEXICO	EF493943	EF493993	EF493886
Eresia polina polina	NW91-16	ECUADOR: Sucumbios	EF493945	EF493995	EF493888
Eresia sticta	NW104-2	PANAMA	EF493946	EF493996	EF493889
Janatella fellula	NW110-2	COLOMBIA: Antioquia	EF493947	EF493997	EF493890
Janatella hera	NW148-3	SURINAM: Mazaroni Plateau	EF493973	EF494032	EF493925
Janatella leucodesma	NW85-16	PANAMA: Gamboa	AY788641	AY788761	AY788521
Mazia amazonica	NW76-6	ECUADOR	AY788654	AY788773	AY788533
Ortilia dicoma	NW124-13	BRAZIL: Rio Grande do Sul	EF493948	EF493998	EF493891
Ortilia gentina	NW108-1	BRAZIL: São Paulo	EF493950	EF494000	EF493893
Ortilia ithra	NW92-11	BRAZIL: São Paulo	EF493949	EF493999	EF493892

(*continued on next page*)

Appendix A (*continued*)

Species	Voucher code	Collection locality	COI	EF-1α	Wingless
Ortilia liriope	NW148-1	SURINAM: Mazaroni Plateau	EF493972	EF494031	EF493924
Ortilia orthia	NW124-14	BRAZIL: Rio Grande do Sul	EF493951	EF494001	EF493894
Ortilia orticas orticas	NW128-29	BRAZIL: Minas Gerais	EF493952	EF494002	EF493895
Ortilia velica velica	NW114-7	BRAZIL: São Paulo	EF493953	EF494003	EF493896
Ortilia velica velica	NW106-5	BRAZIL: São Paulo	EF493954	EF494004	EF493897
Phyciodes batesii apsaalooke	NW35-8	USA: Wyoming	AY156596	EF494006	EF493899
Phyciodes batesii batesii	NW72-4	CANADA: Ontario	AF187747	AY788789	AY788549
Phyciodes batesii lakota	NW35-4	USA: Nevada	AF187747	EF494005	EF493898
Phyciodes batesii maconensis	NW69-1	USA: North Carolina	AY156601	EF494007	EF493900
Phyciodes cocyta selenis	NW11-4	CANADA: British Columbia	AF187755	AY090192	AY090158
Phyciodes cocyta selenis	NW11-5	CANADA: British Columbia	AY156606	EF494008	EF493901
Phyciodes cocyta selenis	NW47-12	USA: Colorado	AY156608	EF494009	EF493902
Phyciodes graphica vesta	NW41-1	MEXICO: Mexico State	AY156684	AY788790	AY788550
Phyciodes mylitta mylitta	NW11-10	CANADA: British Columbia	AF187785	AY788791	AY788551
Phyciodes orseis orseis	NW67-3	USA: California	AY156631	AY788792	AY788552
Phyciodes pallescens	NW64-2	MEXICO: Michoacán	AY156640	AY788793	AY788553
Phyciodes pallida pallida	NW58-5	CANADA: British Columbia	AY156637	EF494011	EF493904
Phyciodes pallida pallida	NW34-6	USA: Colorado	AF187792	AY788794	AY788554
Phyciodes phaon jalapeno	NW35-11	MEXICO: Mazatlan	AF187798	AY788795	AY788555
Phyciodes phaon phaon	NW25-17	USA:Florida	AY156638	EF494012	EF493905
Phyciodes picta picta	NW34-7	USA: Colorado	AF187800	AY788796	AY788556
Phyciodes pulchella camillus	NW48-14	USA: Colorado	AY156643	EF494013	EF493906
Phyciodes pulchella pulchella	NW67-14	USA: Oregon	AY156662	AY788797	AY788557
Phyciodes tharos tharos	NW34-2	USA: Minnesota	AF187807	AY788798	AY788558
Phyciodes tharos tharos	NW124-4	USA: North Carolina	EF493955	EF494010	EF493903
Phystis simois simois	NW108-8	BRAZIL: Bahia	EF493956	EF494014	EF493907
Tegosa anieta anieta	NW91-11	ECUADOR: Sucumbios	AY788681	AY788819	AY788579
Tegosa claudina	NW108-9	BRAZIL: Rio Grande do Sul	EF493957	EF494015	EF493908
Tegosa claudina	NW116-21	BRAZIL: São Paulo	EF493958	EF494016	EF493909
Tegosa claudina	NW124-19	BRAZIL: Rio Grande do Sul	EF493959	EF494017	EF493910
Tegosa claudina	NW124-12	BRAZIL: Rio Grande do Sul	EF493960	EF494018	EF493911
Tegosa etia	CP03-42	PERU: Junin	EF493961	EF494019	EF493912
Tegosa infrequens	NW92-7	BRAZIL: São Paulo	EF493962	EF494020	EF493913
Tegosa infrequens	NW122-23	BRAZIL: São Paulo	EF493963	EF494021	EF493914
Tegosa orobia orobia	NW128-10	BRAZIL: Rio Grande do Sul	EF493964	EF494022	EF493915
Tegosa orobia ursula	NW132-3	BOLIVIA: Depto. Chuquisaca	EF493967	EF494025	EF493918
Tegosa selene	NW91-12	ECUADOR: Esmeraldas	EF493965	EF494023	EF493916
Tegosa similis	NW110-3	COLOMBIA: Antioquia	EF493966	EF494024	EF493917
Tegosa tissoides	NW76-4	ECUADOR: Esmeraldas	AY788682	AY788820	AY788580
Telenassa berenice drusinilla	NW132-5	BOLIVIA: Depto. Chuquisaca	EF493968	EF494026	EF493919
Telenassa delphia nana	CP03-96	PERU: Junin	EF493970	EF494029	EF493922
Telenassa delphia trimaculata	NW91-6	ECUADOR: Sucumbios	AY788683	AY788821	AY788581
Telenassa fontus	NW148-5	SURINAM: Mazaroni Plateau	EF493974	EF494033	EF493926
Telenassa teletusa burchelli	NW115-7	BRAZIL: Rondonia	EF493969	EF494027	EF493920
Telenassa teletusa burchelli	NW38-5	ECUADOR: Sucumbios	AF187749	EF494028	EF493921
Telenassa teletusa teletusa	NW92-12	BRAZIL: São Paulo	EF493971	EF494030	EF493923

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