Cladistics 16, 347–363 (2000)
doi:10.1006/clad.2000.0136, available online at http://www.idealibrary.com on IDEAL®

Pattern of Phylogenetic Relationships among Members of the Tribe Melitaeini (Lepidoptera: Nymphalidae) Inferred from Mitochondrial DNA Sequences

Niklas Wahlberg* and Marie Zimmermann†

**Metapopulation Research Group, Department of Ecology and Systematics, Division of Population Biology, University of Helsinki, Helsinki, FIN-00014, Finland; and †UPRES Biodiversité, Laboratoire de Systématique Evolutive, Université de Provence, Centre Saint Charles, Marseille, France*

Accepted March 15, 2000; published online November 9, 2000

We report a cladistic analysis of 77 butterfly species of the tribe Melitaeini (Lepidoptera: Nymphalidae) based on mitochondrial DNA gene sequences. We sequenced ca. 536 bp from the 16S ribosomal DNA (rDNA) and a 1422-bp sequence from the cytochrome oxidase I gene. Alignments are critical to statements of homology, especially when aligning rDNA sequences. We aligned the 16S sequences using conventional methods and direct optimization. We found that direct optimization of the sequences produced the best alignments and our preferred phylogenetic hypothesis. Our results suggest that many of the previously proposed genera are paraphyletic and we conclude that there are four monophyletic groups of species in our cladogram: the *Euphydryas* group, the *Phyciodes* group, the *Chlosyne* group, and the *Melitaea* group. The following genera are found to be paraphyletic: *Castilia, Chlosyne, Didymaeformia, Eresia, Melitaea*, and *Thessalia*. In addition, recognition of the monophyletic genera *Cinclidia, Mellicta*, and *Telenassa* would render other genera paraphyletic. Our phylogenetic hypothesis indicates that the melitaeines originated in the Nearctic and have colonized the Neotropics three times and the Palaearctic twice.

INTRODUCTION

The systematics of Lepidoptera and especially butterflies has long been a controversial subject (Ehrlich, 1958; Ehrlich and Murphy, 1981; Ackery, 1984, 1988; Scoble, 1992; de Jong *et al.*, 1996). The number of families and the relationships of species groups within these families have continued to be a source of dispute among butterfly systematists, despite the wide interest in butterfly ecology and evolution (see Vane-Wright and Ackery, 1984; Dennis, 1992). The family Nymphalidae has turned out to be especially difficult, with some authors splitting it into as many as nine families. The phylogenetic structure of this speciose butterfly family is certainly not clear at the level of tribes and subfamilies at the moment.

DNA sequence data may clarify the phylogenetic relationships of groups of species that are morphologically highly variable, as DNA sequences allow for recording large numbers of characters in a relatively short period of time (Caterino and Sperling, 1999). As more sequence data become available, problems associated with different genes are becoming apparent (Simon *et al.*, 1994). It is now appreciated that different

0748-3007/00 $35.00

genes are phylogenetically informative at different hierarchical levels. In the past few years, DNA sequence data have been used successfully to elucidate the relationships of many groups of insect species at the generic level (Brower and Egan, 1997; Dietrich *et al.*, 1997; Vogler and Welsh, 1997; Foley *et al.*, 1998; Caterino and Sperling, 1999; Zimmermann *et al.*, 2000). Results of higher level phylogenies are still somewhat ambiguous in the Lepidoptera, though the increasing number of available DNA sequences may change this in the near future. In this paper we describe a cladistic analysis of the nymphalid tribe Melitaeini based on mitochondrial DNA (mtDNA) sequences.

Species belonging to the tribe Melitaeini have long been the subjects of ecological and evolutionary studies (Ehrlich *et al.*, 1975; Thomas and Singer, 1998; Hanski, 1999). While the taxonomy of the group has been intensively investigated (Higgins, 1941, 1950, 1955, 1960, 1981; Scott, 1994, 1998), a cladistic analysis has not been attempted before. In part this is due to morphological characters forming continuous transformation series through many species (Higgins, 1941; Scott, 1994, 1998), making the coding of characters difficult.

Melitaeini comprises about 250 species that have been placed into five distinct generic groups, the *Euphydryas*, *Phyciodes*, *Chlosyne*, *Gnathotriche*, and *Melitaea* groups (Higgins, 1981). Melitaeines occur throughout the Palaearctic (*Euphydryas* and *Melitaea* groups), Nearctic (*Euphydryas*, *Phyciodes* and *Chlosyne* groups), and most of the Neotropics (*Phyciodes*, *Chlosyne*, and *Gnathotriche* groups). The tribe has been revised taxonomically by L. G. Higgins over a period of 40 years (Higgins, 1941, 1950, 1955, 1960, 1978, 1981). In his last paper on melitaeines, Higgins (1981) split the tribe (Higgins treated the group as a subfamily) into 31 genera. Many authors have rejected most of these genera (e.g., Scott, 1986; Karsholt and Razowski, 1996; Tolman, 1997), while others have used Higgins' taxonomy for want of a better system (e.g., DeVries, 1987). It has been shown that some genera in Higgins' classification may be paraphyletic (Zimmermann *et al.*, 2000). Also, Zimmermann *et al.* (1999) performed a phenetic analysis of allozyme and sequence data for European melitaeines and found that species in the genus *Melitaea sensu stricto* do not cluster together. From these studies it is clear that the classification of melitaeines as a whole needs to be based on more rigorous phylogenetic hypotheses.

The taxonomic rank of the melitaeines has changed several times between tribe and subfamily (Higgins, 1941, 1981; Harvey, 1991; Karsholt and Razowski, 1996). In the most recent classification of the Nymphalidae this group represents a tribe within the subfamily Nymphalinae, with a possible sister group relation to the tribe Kallimini (which includes genera such as *Junonia* and *Hypolimnas*) (Harvey, 1991). Here we follow Harvey's (1991) classification, referring to Higgins' (1981) subfamily and tribes as tribe and subtribes, respectively (Fig. 1).

Higgins (1981) recognized three subtribes within the Melitaeini: Euphydryiti (including the *Euphydryas* group), Phycioditi (including the *Phyciodes* group), and Melitaeiti (including the *Chlosyne*, *Gnathotriche*, and *Melitaea* groups). Higgins (1941, 1950, 1955, 1960, 1978, 1981) grouped species into genera mainly on the basis of characters in the male genitalia. In an investigation

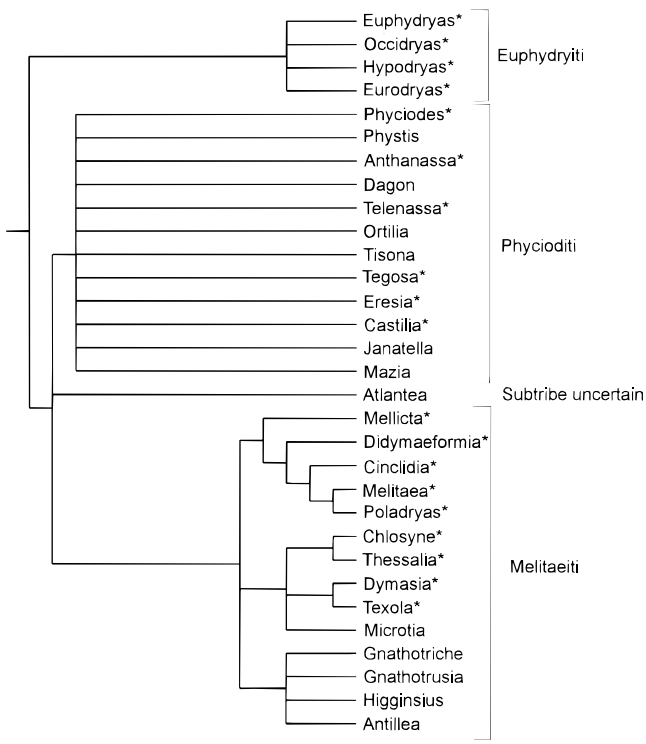

FIG. 1. Traditional classification of genera in Melitaeini after Higgins (1981) drawn according to comments such as "closely related to," "distant relationship," "closely allied," "close generic affinity," in Higgins (1941, 1950, 1955, 1960, 1978, 1981). For higher taxa we follow Harvey (1991); i.e., subfamily = tribe and tribe = subtribe. Genera marked with an asterisk are included in this study.

on the classification of the *Euphydryas* group Zimmermann *et al.* (2000) concluded that the 14 species should be placed into one genus (*Euphydryas*) instead of the four genera proposed by Higgins (1978).

The *Phyciodes* group is the largest of the melitaeines, containing about 135 species that have been placed in 12 genera (Higgins, 1981). Species in *Phyciodes s.s.* are restricted to North America, while the species in the remaining genera (*Anthanassa, Eresia, Telenassa, Castilia, Tegosa, Ortilia, Dagon, Tisona, Phystis, Mazia,* and *Janatella*) are Neotropical with a few species reaching the southern United States. The bulk of the species occur in the Neotropics, primarily in northern South America. The North American species show a wing pattern similar to that of other melitaeines, but this pattern is lost in the Neotropical species. Several species are mimics of the Ithomiinae, Heliconiinae, and Acreinae species that occur in the same area.

The *Chlosyne* group comprises about 30 species that have been placed into five genera (Higgins, 1960, 1981). About half of the species are distributed throughout North America, with the other half being mainly in Central America and a few species being found in South America. Higgins (1960) placed most of the species in the genus *Chlosyne*, because he was unable to find enough differences in male genitalia to split the species into more than one genus. He found 3 species "sufficiently different" to be placed in the genus *Thessalia*. Three of Higgins' genera in the *Chlosyne* group are monotypic (*Dymasia, Microtia,* and *Texola*). Of these, *Dymasia* and *Texola* are very similar in appearance, but differ to such a degree in characters of the male genitalia that Higgins (1960) placed each of them in their own genera. Neotropical *Chlosyne* species also show wing patterns that depart from the normal melitaeine patterns exhibited by North American *Chlosyne* species.

The *Melitaea* group is almost exclusively Palaearctic with about 55 species. The group has been split into five genera (Higgins, 1941, 1955), of which the monotypic *Poladryas* occurs only in North America. The genus *Mellicta* is the only well-defined group of the Palaearctic species (Higgins, 1955). Higgins (1941) placed the rest of the species in the genus *Melitaea* and recognized three groups within the genus. Even though the groups are "connected by transitional forms" (Higgins, 1941, p. 195), Higgins (1981) elevated their status to genus (*Melitaea, Cinclidia,* and *Didymaeformia*).

There are five additional genera that are not included in the above four groups by Higgins (1981). Four genera (with eight species) belong to the *Gnathotriche* group, which occurs mainly in the Andes of South America. The remaining genus (*Atlantea*) is somewhat of a mystery as its four species occur on the large Caribbean islands, but the male genitalia are most similar to those of Palaearctic melitaeines. Higgins (1981) does not group this genus with any other species groups, suggesting that perhaps it should be placed in its own subtribe. Unfortunately we were unable to include these two groups in our study, due to difficulties in obtaining samples.

Here we present a cladistic analysis of the tribe Melitaeini (in part) based on mtDNA sequences from two genes: cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (rDNA) genes. COI sequences have been used successfully in previous generic level studies (Caterino and Sperling, 1999), while the more conserved 16S has been used mainly in higher level analyses (see Simon *et al.*, 1994). We have also sequenced a portion of the ND1 gene for some species (Zimmermann *et al.*, 2000), but this work was discontinued due to problems with amplifying many of the samples.

MATERIALS AND METHODS

Taxon Sampling

We sampled as many species in as many genera as possible (Table 1), a total of 77 species in 13 genera, plus 3 outgroup species. We had difficulties obtaining specimens from South America; thus species from this area are underrepresented in our phylogeny. Of the genera we were unable to sample, 6 belong to the *Phyciodes* group (*Ortilia, Dagon, Tisona, Phystis, Mazia,* and *Janatella*), 4 belong to the *Gnathotriche* group (*Gnathotriche, Gnathotrusia, Antillea,* and *Higginsius*), 1 belongs to the *Chlosyne* group (*Microtia*), and 1 genus (*Atlantea*) is of unknown affinity (Fig. 1). Two species belonging to the same subfamily but to different tribes, *Hypanartia lethe* (Nymphalinae: Nymphalini) and *Hypolimnas bolina* (Nymphalinae: Kallimini), were used to test the monophyly of the Melitaeini. The trees were rooted using *Asterocampa leilia* (Apaturinae). Species were identified by the collectors and some species identifications were checked by Jaakko Kullberg (Finnish

TABLE 1
Species Included in the Cladistic Analysis of the Tribe Melitaeini

Species	Sample No.	Collection locality	Collection date	COI-LCO	COI-Jerry	16S
Anthanassa						
ardys (Hewitson)	22-4	Monteverde, Costa Rica	04/09/1998	x	x	x
otanes (Hewitson)	24-4	Monteverde, Costa Rica	05/14/1998	x	x	x
ptolyca (Bates)	34-8	Manzanillo, Colima, Mexico	01/09/1994	x	x	x
texana (W. H. Edwards)	12-6	Austin, TX, USA	02/05/1998	x	x	x
tulcis (Bates)	22-1	Monteverde, Costa Rica	04/27/1998	x	x	x
Castilia						
eranites (Hewitson)	24-3	Monteverde, Costa Rica	05/01/1998	x	x	
myia (Hewitson)	24-5	Monteverde, Costa Rica	05/09/1998	x	x	x
perilla (Hewitson)	38-9	Anyañgu, Sucumbios Province, Ecuador	12/20/1994	x		x
Chlosyne						
acastus (W. H. Edwards)	35-15	Mesa Co., CO, USA	05/07/1993	x	x	
californica (Wright)	27-9	Maricopa Co., AZ, USA	04/18/1998	x	x	x
gaudealis (Bates)	37-2	La Selva, Costa Rica	02/?/1995	x	x	x
gorgone (Hübner)	34-4	Morgan Co., CO, USA	07/28/1995	x	x	x
harrisii (Scudder)	35-10	Oneida Co., NY, USA	06/14/1986	x	x	x
janais (Drury)	30-1	Butterfly farm, Costa Rica	?/?/1997	x		x
lacinia (Geyer)	32-2	Cochise Co., AZ, USA	05/20/1998	x	x	x
narva (Fabricius)	37-3	La Selva, Costa Rica	02/?/1995	x	x	x
neumoegeni (Skinner)	27-2	Pima Co., AZ, USA	03/20/1998	x	x	x
nycteis (Doubleday)	34-5	Jefferson Co., CO, USA	06/17/1992	x	x	x
palla (Boisduval)	20-4	Lake Tahoe, CA, USA	07/04/1997	x	x	x
Cinclidia						
phoebe (Denis & Schiffermüller)	15-14	Saratov, Russia	06/10/1997	x	x	x
punica Oberthür	34-11	Mohafazat Kesronan, Lebanon	05/26/1998	x	x	x
scotosia Butler	27-11	Zhangjakou, Hebei Province, China	06/26/1998	x	x	
Didymaeformia						
arduinna (Esper)	23-5	Pissoderi, Greece	07/13/1996	x	x	
deserticola Oberthür	34-12	Mohafazat Zahlé, Lebanon	04/23/1998	x	x	x
didyma (Esper)	1-7	Montpellier, Languedoc, France	04/25/1997	x	x	x
didymoides Eversmann	26-1	Dodo-Enhor, Buryatia, Russia	06/17/1998	x	x	x
latonigena Eversmann	25-3	Utitzcina, Buryatia, Russia	06/08/1998	x	x	x
persea Kollar	34-10	Les Cèdres, Mohafazat Beharré, Lebanon	05/04/1998	x	x	x
sutschana Staudinger	19-9	Kyra, Chita Region, Russia	07/20/1991	x	x	
interrupta Kolenati	17-3	Arkhyz, NW Caucasus, Russia	08/10/1997	x	x	x
trivia (Denis & Schiffermüller)	23-6	Pissoderi, Greece	07/13/1997	x	x	x
Dymasia						
dymas (W. H. Edwards)	27-7	Pima Co., AZ, USA	03/25/1998	x	x	x
Eresia						
clara (Bates)	38-1	Anyañgu, Sucumbios Province, Ecuador	11/10/1993	x		x
eunice (Hübner)	38-3	Anyañgu, Sucumbios Province, Ecuador	05/16/1994	x		x
pelonia (Hewitson)	38-7	Anyañgu, Sucumbios Province, Ecuador	02/21/1995	x		x
plaginota Röber	38-4	Anyañgu, Sucumbios Province, Ecuador	12/07/1993	x		x

TABLE 1—*Continued*

Species	Sample No.	Collection locality	Collection date	COI-LCO	COI-Jerry	16S
Euphydryas						
anicia (Doubleday & Hewitson)	23-16	Spring Mountain, ID, USA	?/?/1997	x	x	x
aurinia (Rottemburg)	6-4	Cervières, Alpes, France	07/02/1995	x	x	x
chalcedona (Doubleday & Hewitson)	14-4	Santa Barbara, CA, USA	03/24/1998	x	x	x
colon (Edwards)	11-7	Trail, BC, Canada	07/05/1997	x	x	x
cynthia (Denis & Schiffermüller)	6-2	Albulapass, Switzerland	07/27/1995	x	x	x
desfontainii (Godart)	6-5	Montes Universales, Spain	05/?/1995	x	x	x
editha (Boisduval)	5-8	Fresno Co., CA, USA	?/?/1994	x	x	x
gillettii (Barnes)	24-6	Pondera Co., MT, USA	07/15/1996	x	x	x
iduna (Dalman)	28-1	Muotka, Inari, Finland	07/12/1998	x	x	x
intermedia (Ménétriés)	6-3	Névache, Alpes, France	07/01/1995	x	x	x
maturna (L.)	1-8	Joutseno, Finland	07/01/1997	x	x	x
phaeton (Drury)	13-3	Anne Arundle Co., MD, USA	06/10/1997	x	x	x
Melitaea						
amoenula Felder	23-15	Taglong Ladak, India	07/03/1997	x	x	x
arcesia Bremer	10-9	Tov Aimak, Mongolia	06/18/1997	x	x	x
cinxia (L.)	23-13	Pissoderi, Greece	07/13/1997	x	x	x
diamina (Lang)	10-24	Tov Aimak, Mongolia	06/18/1997	x	x	x
Mellicta						
ambigua (Ménétriés)	10-1	Tov Aimak, Mongolia	06/18/1997	x	x	x
athalia (Rottemburg)	5-5	Joutseno, Finland	07/01/1997	x	x	x
aurelia (Nickerl)	23-2	Rolle, France	06/24/1995	x	x	x
britomartis (Assmann)	15-13	Saratov, Russia		x	x	x
centralasiae (Wnukowsky)	19-15	Djirga, Buryatia, Russia	07/13/1995	x	x	x
deione (Geyer)	1-4	Massingmeu, Savoie, France	04/25/1997	x	x	x
parthenoides (Keferstein)	3-4	Massingmeu, Savoie, France	09/29/1997	x	x	x
varia (Meyer-Dür)	24-13	Laus de Cervières, Alpes, France	08/13/1995	x	x	x
Phyciodes						
batesii (Reakirt)	35-4	Sioux Co., NE, USA	08/11/1994	x	x	x
cocyta (Cramer)	11-4	Trail, BC, Canada	07/12/1997	x	x	x
mylitta (Edwards)	11-10	Trail, BC, Canada	08/17/1997	x	x	x
orseis (Edwards)	37-1	Siskiyou Co., CA, USA	08/08/1994	x	x	x
pallida (Edwards)	34-6	Boulder Co., CO, USA	06/08/1994	x	x	x
phaon (Edwards)	35-11	Mazatlan, Mexico	05/26/1991	x	x	x
picta (Edwards)	34-7	Morgan Co., CO, USA	07/28/1995	x	x	x
pulchella (Boisduval)	27-5	Mono Co., CA, USA	07/09/1994	x	x	x
tharos (Drury)	34-2	Freeborn Co., MN, USA	06/25/1998	x	x	x
Poladryas						
arachne (Edwards)	27-4	Inyo Co., CA, USA	05/11/1996	x	x	x
Tegosa						
anieta (Hewitson)	22-2	Monteverde, Costa Rica	04/21/1998	x	x	x
Telenassa						
burchelli Moulton	38-5	Anyañgu, Sucumbios Province, Ecuador	07/18/1994	x		x
Texola						
elada (Hewitson)	27-16	Pima Co., AZ, USA	03/20/1998	x	x	x
Thessalia						
cyneas Godman & Salvin)	38-17	Cochise Co., AZ, USA	09/20/1998	x	x	x
fulvia (Edwards)	27-10	Pima Co., AZ, USA	03/25/1998	x	x	x
leanira (C. & R. Felder)	27-1	Inyo Co., CA, USA	05/11/1996	x	x	x
theona (Ménétriés)	27-6	Cochise Co., AZ, USA	04/20/1997	x	x	x
Outgroup species						
Asterocampa leilia (Edwards)	27-12	Pima Co., AZ, USA	03/20/1998	x	x	x
Hypolimnas bolina (L.)	29-5	Ulu Gombak, Selangor, Malaysia	09/01/1995	x	x	x
Hypanartia lethe (Fabricius)	36-6	Bello Horizonte, Brazil	12/?/1998	x	x	x

Note. Genera are as in Higgins (1981). An (x) in the last three columns means that the respective sequence was succesfully sequenced. Species taxonomy follows Higgins (1981), Scott (1986), DeVries (1987), Ferris (1989), and Karsholt and Razowski (1996). Sample number refers to the tube in which the DNA sample is stored at the University of Helsinki.

Museum of Natural History) and James Scott (Colorado).

Molecular Techniques

The details of the molecular techniques used are described in Zimmermann *et al.* (2000). Total genomic DNA was extracted mainly from two legs of dried specimens using a standard phenol–chloroform extraction protocol. The remainder of these individuals are deposited at the Finnish Museum of Natural History in the University of Helsinki as voucher specimens. PCR was performed using three primer pairs. Two primer pairs were used to amplify the COI gene, LCO1490-J-1514 / HCO2198-N-2175 (Folmer *et al.*, 1994) and C1-J-2183 / TL2-N-3014 (Simon *et al.*, 1994), and the primer pair LR-J-12887 / LR-N-13398 (Simon *et al.*, 1994) was used to amplify the 16S ribosomal gene. The PCR conditions are described in Zimmermann *et al.* (2000). The two COI sequences did not overlap and were therefore analyzed as two separate sequences (COI-LCO and COI-Jerry). The 633-bp sequence of COI-LCO corresponds to positions 1539–2172 in the *Drosophila yakuba* Burla mtDNA sequence (Clary and Wolstenholme, 1985). The 789-bp sequence of COI-Jerry corresponds to positions 2201–2990 in the *D. yakuba* sequence. The ca. 536-bp sequence of the large (16S) ribosomal subunit corresponds to positions 12,873–13,407 in the *D. yakuba* sequence.

PCR fragments were directly sequenced using an ABI 377 Automated Sequencer and a dye terminator cycle sequencing kit. Each fragment was sequenced in both directions to maximize the accuracy of the sequence. The 16S sequences were not compared to sequences of other species prior to phylogenetic analysis, as this may have affected their alignments (see below). The COI sequences were aligned by eye on checking ABI output using an entire *Melitaea didymoides* COI sequence (position 1539–2990 in *D. yakuba*) as a standard melitaeine COI sequence. The sequences are available from GenBank (Accession Nos. AF186849–AF186921 for 16S, AF186922–AF186993 for COI-Jerry, and AF153925 and AF187734–AF187812 for COI-LCO sequences).

Phylogenetic Analyses

Due to alignment problems with the 16S sequences (see Results), the data sets were analyzed using two methods. We analyzed the data sets first using conventional methods; i.e., the 16S sequences were aligned using a computer program and the resulting alignment was then subjected to parsimony analysis. The second method creates alignments through direct optimization of the unaligned sequences (Wheeler, 1996, 1998).

Alignment for 16S sequences was performed using MALIGN (Wheeler and Gladstein, 1994). To maximize the efficiency of the search, only sequences with flanking primer areas successfully sequenced were aligned using this method ($n = 70$). Five replications were done for the following gap cost settings: 1:1, 3:5, 1:2, 1:3, 1:4, 1:6, and 1:8 (transformation of a base pair:change to a gap). Note that Whiting *et al.* (1997) suggest that a gap cost of 3:5 is generally good for arthropods. Leading and trailing gaps had a cost of 16, in order to let the flanking primer areas align. Each resulting alignment was subjected to parsimony analysis using the program NONA 1.8 (Goloboff, 1993), with five random sequence additions and the mult* option, which searches for trees using a TBR (tree bisection–reconnection) branch-swapping algorithm. Transitions and transversions were weighted equally. Gaps were coded as missing data. The sequence alignment giving the shortest tree was chosen for further analysis, as we felt that this was the only objective criterion available to us.

We analyzed the COI and 16S data sets separately and simultaneously using maximum parsimony. Transitions and transversions were weighted equally and gaps in the 16S sequences were coded as missing data. The most parsimonious trees were searched for with the program NONA 1.8 (Goloboff, 1993), using the command sequence hold*; hold / 20; mult*100. If necessary, more trees were searched for using the sswap* option, which also uses TBR branch-swapping but only at sister nodes. Bremer support indices (Bremer, 1994) and jackknife values (Farris *et al.*, 1996) were calculated for all the data sets. The large number of taxa in this study precluded the calculation of exact Bremer support indices above two steps. We calculated approximate Bremer support indices by setting the maximum number of trees held in the computer's memory to the maximum number it could process using the command bsupport;, which was 32,760 trees. Jackknife values were calculated from 10,000 replicates at a cut-off point of 50% using the program Parsimony Jackknifer (Farris, 1995).

The data sets were also analyzed using the program POY (Gladstein and Wheeler, 1998), which implements the direct optimization regime of Wheeler (1996). This method has consistently found shorter trees than those found by conventional methods when analyzing rDNA sequence data (Wheeler, 1996, 1998). The idea behind direct optimization is that gaps are not observable entities, but rather that indels (insertion / deletion events) are processes that create sequences of different length. The optimization procedure uses unordered optimization (Farris, 1970) to optimize sequence data directly into a phylogenetic tree with indels invoked to reconstruct hypothetical ancestral sequences (Wheeler, 1996). We analyzed the three data sets (16S, COI-LCO, and COI-Jerry) simultaneously for all species using this method. Since the outgroup node affects the alignment procedure (Wheeler, 1996), the initial trees were built 20 times with a random outgroup node and random sequence additions. After the random sequence additions, branch-swapping was performed on the lowest cost tree(s) using first SPR (subtree pruning regrafting) and then TBR in succession. This was replicated 10 times with the lowest cost tree being kept until the end of the run. We used a cost of 1 for base transformation and a cost of 2 for gaps, as suggested by our analyses by MALIGN (see Results). We also analyzed the data sets with a gap cost of 4. The POY analyses ran for 4 days on a 350 MHz Pentium PC. We analyzed the resulting alignments in NONA using 10 random additions and TBR branch-swapping and compared the lengths of the trees to those produced by the MALIGN aligned sequences. We also calculated Bremer support indices and jackknife values from 100 replicates (which took several days on the same computer mentioned above) for the resulting trees. Bremer support indices, as implemented in POY, are calculated based on a complete TBR search on a lowest cost tree by keeping track of the cost of trees where each group is absent. The index is then simply the difference between the minimum cost tree and the cost of the trees in which each group is absent. This method may overestimate the actual values.

To examine the broad historical biogeography of the group, we optimized the distribution of each species (coded as Nearctic, Palaearctic, or Neotropical, unordered) onto our phylogenetic hypothesis. The distribution of each species was taken from the literature (Higgins, 1941, 1950, 1955, 1960, 1978, 1981).

RESULTS

Patterns of Change in the Sequences

For the majority of the species, we were successfully able to sequence all three fragments. All species amplified for CO1-LCO, but seven species did not amplify for 16S and eight species did not amplify for COI-Jerry (Table 1). However, all species included in the analysis had at least two of the three sequences in the data matrix.

The lengths of the 16S sequences varied between 534 and 556 bp (mean $\pm$ SD = 536.9 $\pm$ 2.9 bp, mode = 535 bp, n = 69). The aligned sequences (by MALIGN) that gave the shortest tree had a total of 572 sites, of which 64 character sites had at least one gap inserted in one sequence. This large number of sites is due mainly to the large number of sequences that had to be aligned. Most of the indels are restricted to six areas of the sequence; i.e., the putative loop areas of the rRNA molecule and the areas in between contained at most one indel. The gap cost ratio that gave the shortest tree (659 steps long) was 1:2 and the cost of the alignment was 933. The number of variable sites (ignoring gaps) was 190, of which 126 sites were parsimony informative. However, only one of the searches performed using the gap cost of 1:2 gave the shortest tree. All other searches with the same parameter values produced trees several steps longer (666–687 steps). Gap costs of 1:1, 3:5, and 1:2 all yielded trees of similar length (662–670 steps).

The simultaneous analysis of all three data sets by POY with a gap cost of 2 produced 12 alignments of the 16S data set (no indel events were inferred for the COI data sets). Six of these alignments had a total of 618 sites and the other 6 alignments had 619 sites. We analyzed the 12 alignments in NONA, excluding three sequences that were not included in the MALIGN analyses to make the results comparable. Four of the alignments produced trees that were 650 steps long (2 alignments with 618 sites and 2 alignments with 619 sites), while the other alignments gave rise to trees that were 651 steps long. The cost of the 12 POY alignments as diagnosed by MALIGN varied from 881 to 884, which is about 50 units less than the lowest cost alignment found by MALIGN at the same gap cost ratio. The alignment chosen for further scrutiny had 618 character sites, had a MALIGN cost of 881, and produced trees

650 steps long (only one alignment out of 12). This alignment featured 190 variable sites (ignoring gaps), of which 124 were parsimony informative. Note that the numbers of variable and parsimony informative sites are about equal to those in the MALIGN aligned sequences. The data matrix is available on the Web at http://www.helsinki.fi/science/metapop/sequences. Increasing the gap cost to 4 in POY resulted in 8 alignments that had 616 character sites and produced trees that were 656 steps long.

The 1422-bp COI sequence had 587 variable sites of which 455 sites were parsimony informative (including the outgroup sequences). As is typical for protein coding genes, most of the variation was in the third-codon position: of the potentially informative characters, 75 were first-position sites, 14 were second-position sites, and 366 were third-position sites. Also typical of insect mtDNA (DeSalle *et al.*, 1987; Simon *et al.*, 1994), the sequences were AT-rich, especially at the third-codon position (overall average incidence of A = 32%, T = 39%, C = 15%, and G = 14%; average incidence at third position of A = 45%, T = 47%, C = 7%, and G = 1%). The uncorrected divergences of all pairwise comparisons for COI ranged from 0.01 to 0.15. The average uncorrected sequence divergence within the species groups is about 0.06, while the between species group average is around 0.10 (Table 2).

Phylogenetic Analyses

The two genes yielded cladograms with different resolutions after parsimony analysis. The MALIGN aligned 16S data set produced 570 equally parsimonious trees. The strict consensus of these trees recovers several groups well, but the relationships of these groups and many species are unclear (Fig. 2A). However, most of the variation was caused by the sequence of just one species, *Tegosa anieta. T. anieta* is part of the *Phyciodes* group, but comes out as the most basal taxon after the root when analyzing the chosen alignment. In other alignments this species comes out basal to the *Phyciodes–Chlosyne–Melitaea* groups or, in the case of gap cost ratio 1:3, within the *Phyciodes* group. The POY aligned 16S data set (including the three species for which flanking primer regions were not successfully sequenced) produced over 4417 equally parsimonious trees and their strict consensus recovers the *Euphydryas* group as basal to the *Phyciodes–Chlosyne–Melitaea* groups (Fig. 2B). *T. anieta* has an unresolved position within the latter clade. We do not believe that the aberrant behavior of the *T. anieta* sequence is the result of contamination, but that the large difference between this sequence and the rest of the *Phyciodes* group sequences is due to differential evolution. The extent of this differential evolution needs to be studied in more detail by sampling more extensively in *Tegosa*, a genus containing about 14 species.

The tree topology obtained from the MALIGN aligned 16S data set was highly dependent on the alignment, as has been found in other studies (Fitch and Smith, 1983; Morrison and Ellis, 1997). Some alignments (with gap costs ranging from 1:1 to 1:3) yielded trees with better resolution, but the topologies of these

TABLE 2
The Uncorrected Average Sequence Divergence of the COI Sequences for the Four Species Groups and Outgroups

Species groups	Outgroup (n^a = 3)	*Euphydryas* (n = 11)	*Phyciodes* (n = 17)	*Chlosyne* (n = 16)	*Melitaea* (n = 25)
Outgroup	0.120 (0.113–0.125)	0.105 (0.095–0.115)	0.117 (0.107–0.131)	0.115 (0.099–0.134)	0.116 (0.105–0.129)
Euphydryas		0.048 (0.003–0.068)	0.093 (0.081–0.112)	0.092 (0.071–0.118)	0.096 (0.065–0.108)
Phyciodes			0.073 (0.012–0.111)	0.100 (0.084–0.128)	0.096 (0.068–0.124)
Chlosyne				0.068 (0.018–0.105)	0.090 (0.065–0.128)
Melitaea					0.069 (0.017–0.097)

Note. Numbers in parentheses give the range of divergences for the given comparison. The species groups are as described in association with Fig. 4.
[a] Number of species.

FIG. 2. (A) The strict consensus of 570 trees found for the MALIGN aligned 16S data set (L 659, CI 0.40, RI 0.65). (B) The strict consensus of 4417 trees found for the POY aligned 16S data set including the three sequences for which the flanking primer areas were not successfully sequenced (L 677, CI 0.40, RI 0.66). Numbers above the branches refer to jackknife values (only values above 50% in 10,000 replications are reported) and numbers below the branches are the Bremer support indices. Branch lengths are arbitrary in both figures.

trees were not congruent between the different alignments, especially at the deeper nodes (not illustrated). The trees were also several steps longer (up to 15 steps) than that presented in Fig. 2A. The unaligned data set is obviously unreliable when analyzed with conventional methods and calls into question studies that accept one alignment without exploring the parameter space in more detail.

The COI data set yielded 24 equally parsimonious trees. The strict consensus of these trees is highly resolved (Fig. 3A), even though it has been suggested that the third-codon position is too variable to be a reliable character in higher level phylogenies (Swofford *et al.*, 1996). The COI data set analyzed with the third-codon position sites removed produces over 1000 equally parsimonious trees and the strict consensus of these is highly unresolved with only a few small terminal groups retained (tree not shown). It is obvious that the third position contains the most information for phylogenetic inference in this data set, confirming the result shown by Källersjö *et al.* (1999) for a large data set of plant sequences and by Björklund (1999) for vertebrate sequences. The combined data set (with the POY aligned 16S sequences) produced 32 trees. The strict consensus of these trees (Fig. 3B) is largely congruent with the other trees (Figs. 2 and 3A), except that *Poladryas* and the *Texola*/*Dymasia* clade are placed in aberrant positions. It is worth noting that the branches leading to *Poladryas* and *Texola*/*Dymasia* are very long, yet they are not attracted to each other (see Siddall and Whiting, 1999, for a discussion on supposed problems with long-branch attractions).

Direct optimization yielded 12 equally costly trees of cost 4251 when gaps were given a cost of 2 (Fig. 4). We imported these trees into NONA and analyzed them with the combined data set (with the 16S sequences aligned by POY). Eight of the POY trees were 3985 steps long and 4 trees were 3987 steps long (all trees CI 0.28, RI 0.58). The 8 trees are thus only 3 steps longer than the most parsimonious trees found by largely ignoring information on indel events. *Anthanassa texana* has an incongruent position in the POY trees as the sister to *Anthanassa tulcis* and *Anthanassa ptolyca*, whereas it has strong support in all NONA derived trees as the sister to *Anthanassa otanes* and *Anthanssa ardys*. Placing *An. texana* as the sister to *An. otanes* and *An. ardys* in the POY trees reduces the number of steps to 3983. Diagnosing the NONA trees from the combined data set in POY gave a cost 4255, which is 4 units larger than those found by POY. When gap cost was increased to 4, POY found 8 trees equally costly of cost 4517. The strict consensus of these trees is identical to that in Fig. 4. The strict consensus of the trees found through direct optimization is our preferred phylogenetic hypothesis (Fig. 4), because with this method we were able to utilize all of the available data and were able to extract the greatest amount of information from the unaligned 16S sequences. In other words, we believe that using information on indel events in addition to base transformations results in a more reliable estimate of phylogenetic relationships.

In general, the previously recognized species groups in the wider sense were recovered. We found the Melitaeini to be a monophyletic group with respect to the outgroups used with relatively good support. The relationships of the species groups appears to be (*Euphydryas* (*Phyciodes* (*Chlosyne Melitaea*))) as implied by Higgins (see Fig. 1), but the deeper nodes are not well supported. The COI data set alone suggests an alternative order, with *Phyciodes* being the sister group to *Melitaea* (Fig. 3A), but this has low support. In the following we use brackets to denote genera that we believe should be synonymized according to Table 3.

The *Euphydryas* group was monophyletic in all analyses. The present cladogram for the *Euphydryas* group (Fig. 4) is essentially identical to the cladogram that has been previously reported based on a partially different data set (Zimmermann *et al.*, 2000). The main difference is that the unresolved trichotomy of *iduna–gillettii–maturna/intermedia* is now resolved as (*gillettii* (*iduna* (*maturna intermedia*))). Our conclusions for this group remain the same; i.e., the three extra genera *[Occidryas]*, *[Hypodryas]* and *[Eurodryas]* proposed by Higgins (1978) are synonymized to *Euphydryas* (see Zimmermann *et al.*, 2000, for details).

The *Phyciodes* group was also recovered well, with the exception of *T. anieta* due to its problematic 16S sequence. Direct optimization places *T. anieta* as the basal taxon to the South American species of the *Phyciodes* group (Fig. 4). The South American species (in the genera *Anthanassa*, *[Castilia]*, *Eresia*, and *[Telenassa]*) form a monophyletic clade as do their sister group, the North American species in the genus *Phyciodes s.s.*

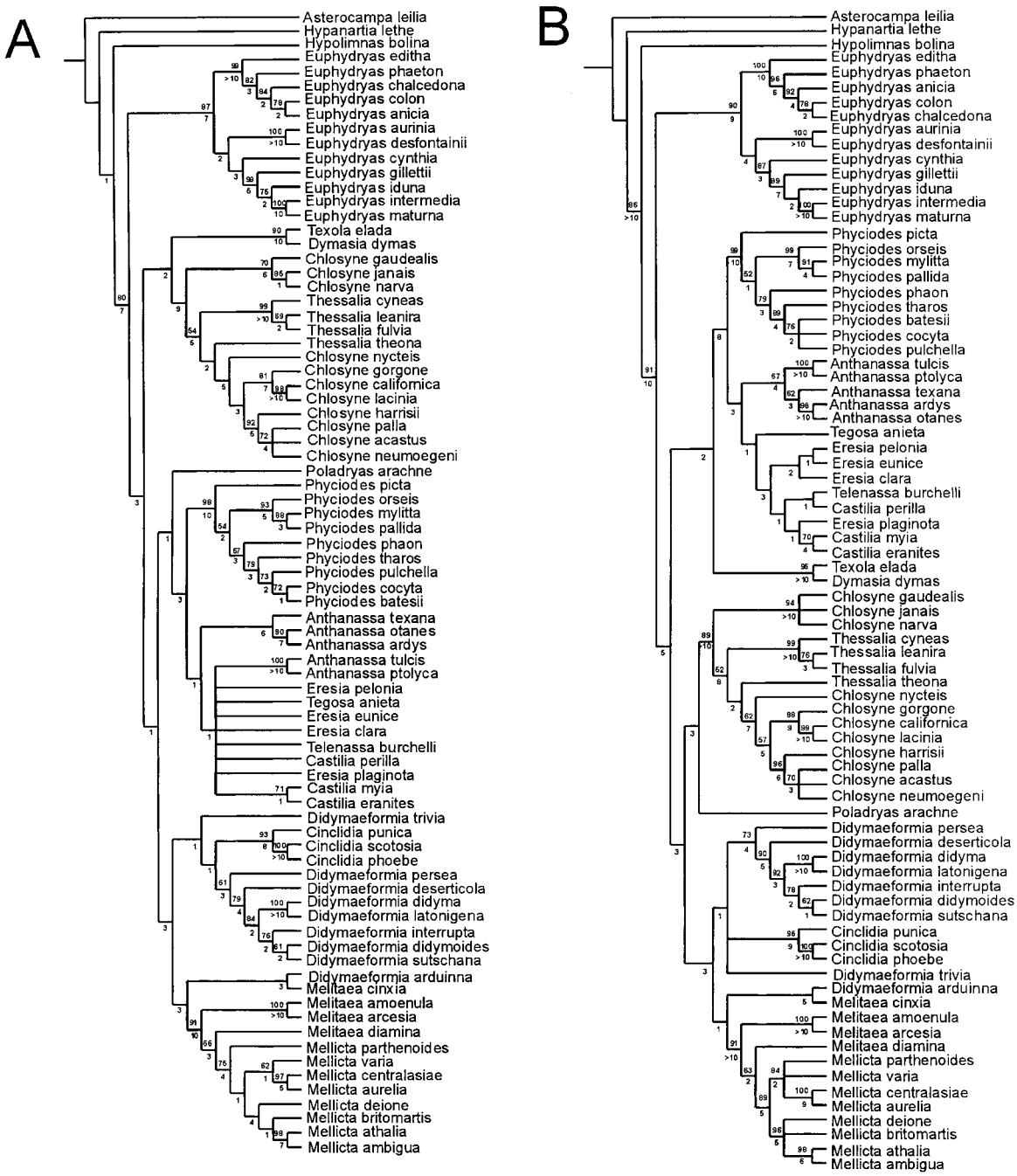

FIG. 3. (A) The strict consensus of 24 trees found for the COI data set (L 3281, CI 0.26, RI 0.57). (B) The strict consensus of 32 trees found for the combined data set (with the POY aligned 16S sequences) (L 3982, CI 0.28, RI 0.58). Numbers above the branches refer to jackknife values (only values above 50% in 10,000 replications are reported) and numbers below the branches are the Bremer support indices. Branch lengths are arbitrary in both figures.

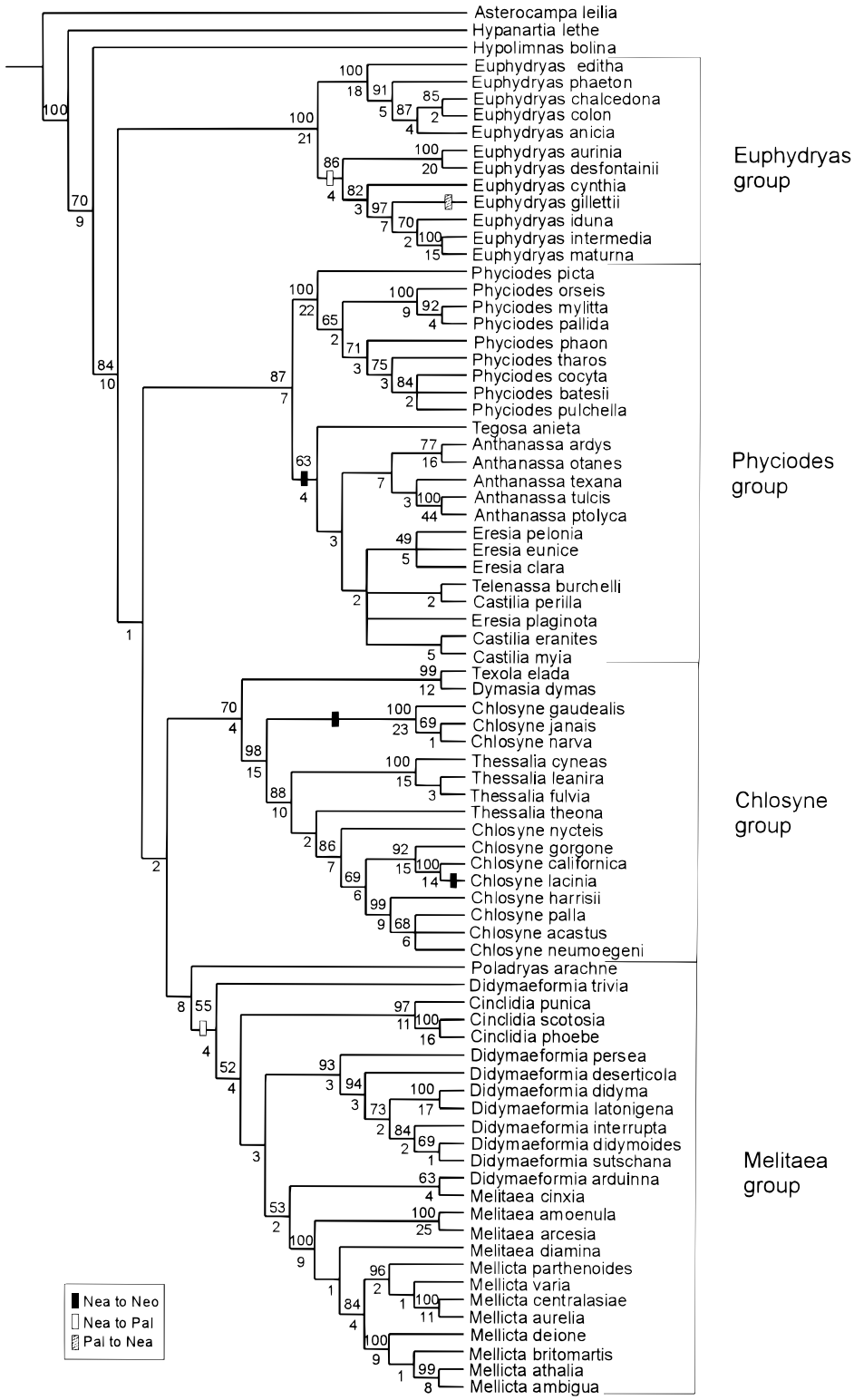

FIG. 4. Strict consensus tree of 12 trees found during a direct optimization search of the combined data set. Numbers above the branches refer to jackknife values (only values above 50% in 100 replications are reported) and numbers below the branches are the Bremer support indices. Branch lengths are arbitrary. The bars on the branches refer to inferred changes of state in the most parsimonious solution to optimizing distribution onto the cladogram (see text for discussion). Nea, Nearctic; Neo, Neotropical; Pal, Palaearctic.

TABLE 3
Proposed Classification of the Tribe Melitaeini Based on the Phylogenetic Hypothesis in Fig. 4

Tribe	Subtribe	Genus	Synonyms
Melitaeini	Euphydryiti	*Euphydryas*	*Occidryas, Eurodryas, Hypodryas*
	Phycioditi	*Phyciodes*	
		Tegosa	
		Anthanassa	
		Eresia	*Castilia, Telenassa*
	Melitaeiti	*Poladryas*	
		Melitaea	*Cinclidia, Didymaeformia, Mellicta*
		(*Chlosyne*)	*Thessalia*
		(*Texola*)	
		(*Dymasia*)	

Note. Genera in parentheses are included in the subtribe Melitaeiti here, though sequence divergences suggest that they should be in a subtribe of their own.

(Fig. 4). In the Neotropical clade the only monophyletic genus seems to be *Anthanassa.* Within this clade the position of *An. texana* should be seen as unresolved, though there is more evidence for it to be sister to *An. otanes* and *An. ardys. Eresia* appears to be paraphyletic, with the genera *[Telenassa]* and *[Castilia]* within it. Species of *[Castilia]* are also imbedded within *Eresia*, and they do not form a monophyletic group. The relationships of the North American species are mainly in accordance with morphological evidence (Scott, 1994). Scott (1994) groups *Phyciodes picta* and *Phyciodes phaon* together, but in our cladogram *P. picta* is basal to the rest of the *Phyciodes s.s.* clade and *P. phaon* is the sister species to the *Phyciodes tharos* species group (Fig. 4).

The genera *Chlosyne* and *[Thessalia]* together form a monophyletic group in the 16S (without the *T. anieta* sequences), COI, and combined data set analyses, though both genera are paraphyletic within this clade (Figs. 3 and 4). *Texola* and *Dymasia* form a clade that is basal in the *Chlosyne* group. Higgins (1960) groups *Chlosyne lacinia* with the other Neotropical *Chlosyne* species included in our cladogram, but our results give clear evidence that *C. lacinia* is a part of the North American clade and has spread to the Neotropics from the Nearctic (Fig. 4). Also, the grouping of *Chlosyne harrisii*, *Chlosyne nycteis*, and *Chlosyne gorgone* by Higgins (1960) appears artificial based on our analyses (e.g., Fig. 4).

Members of the *Melitaea* group are recovered as a monophyletic group in the 16S data set without *T. anieta* (Fig. 2B) and in the combined data set analyzed with direct optimization (Fig. 4). The COI data set places *Poladryas* as the sister to the *Phyciodes* group, though this has no jackknife support. Within *Melitaea sensu lato, [Cinclidia]* and *[Mellicta]* come out as monophyletic, while *Melitaea* and *[Didymaeformia]* are both paraphyletic. Our cladogram differs much from the phylogenetic hypothesis envisioned by Higgins (1941, 1981) for this group (see Fig. 1). In our cladogram *Poladryas* is basal to the rest of the *Melitaea* group. Of the Palaearctic *Melitaea, Melitaea [Didymaeformia] trivia* is basal and it is interesting to note that Higgins (1941) commented on the archaic nature of the male genitalia in this species. That *[Cinclidia]* form a monophyletic group in our analyses is not surprising as *Melitaea [Cinclidia] punica* and *Melitaea [Cinclidia] scotosia* are sometimes considered to be subspecies of *Melitaea [Cinclidia] phoebe*. The genus *Melitaea s.s.* forms a paraphyletic and basal group to *[Mellicta]*. Higgins (1941) commented on the similarities between *Melitaea cinxia* and *Melitaea [Didymaeformia] arduinna* in male genitalia, but concluded that *M. [D.] arduinna* female genitalia unmistakably affiliate this species with *[Didymaeformia]*. Our cladogram suggests that the morphology of female genitalia in *M. [D.] arduinna* might be plesiomorphic and that morphology of male genitalia is derived in the case of these two species. The wing patterns of *Melitaea arcesia*, *Melitaea amoenula*, and *Melitaea diamina* all resemble those of species in *[Mellicta]*, but were placed in *Melitaea* by Higgins (1941) based on genitalic similarities. Our phylogenetic hypothesis suggests that the genitalic similarities are plesiomorphic characters while the wing morphology is derived. *[Mellicta]* form a monophyletic group in our analyses, but appear to be a derived subgroup of *Melitaea*. In accordance with Higgins (1955), there are two species groups in this clade, the *Melitaea [Mellicta] athalia* group and the *Melitaea[Mellicta] aurelia* group, both of which have strong support in our analyses.

Optimizing the distribution of each species onto our preferred phylogenetic hypothesis gives clear results (Fig. 4). The most parsimonious solution suggests that the tribe Melitaeini originated in the Nearctic region. The Palaearctic region has been colonized independently twice, once by the ancestor of the *Melitaea* species and once by the ancestor of the Palaearctic *Euphydryas*. The Neotropical region has been colonized

independently three times, once by the ancestor of the *Tegosa–Anthanassa–Eresia* clade and twice from the *Chlosyne* clade.

DISCUSSION

The purpose of aligning sequences is to create a data matrix in which each character site is homologous. Character homology is especially difficult to ascertain when working with rDNA, in which insertions and deletions can happen commonly. There are no general guidelines on how to choose among different alignments, so our strategy has been to choose the alignment that is most parsimonious. In this paper we have used an unconventional method (direct optimization; Wheeler, 1996) to arrive at an alignment that produces trees that are much shorter than those found from sequences aligned in more conventional ways. The method (as implemented by POY) uses information on sequence lengths and base compositions to simultaneously align and build a cladogram. If all sequences are of the same length (as in the case of COI-LCO and COI-Jerry in our study), direct optimization works like any parsimony-based algorithm (albeit much more slowly) and the end result is a set of most parsimonious trees. If there is variation in sequence length, direct optimization reconstructs ancestral states according to rules set *a priori*. In our analyses, the rules were relatively simple: a base change added a cost of 1 to the cladogram and an indel event added a cost of 2 to the cladogram and we searched for trees that minimized these costs. The trees we found were only three steps longer (of which two steps can be attributed to the incongruent placement of one taxon) than the most parsimonious trees found when information on indel events was largely ignored (information on indel events are implicitly used when making statements of character homology in alignments). Since an inferred indel event holds as much information as an inferred base transformation, we have chosen the trees found through direct optimization as our preferred phylogenetic hypothesis.

The systematic implications of our exercise are clear. Many of the species groups and genera proposed by Higgins (1941, 1950, 1955, 1960, 1978, 1981) are not natural groups according to our analyses, and some genera are contained within larger clades. Some of our results are surprising, such as *Chlosyne lacinia* not being part of the Neotropical *Chlosyne* clade. Other results were expected, such as the paraphyly of *[Didymaeformia]* and *Melitaea*, for which, in Higgins' (1941, p. 195) own words, "it is really difficult to specify generic characters common to all." The four species groups we have considered in this paper are monophyletic, but their relationships are not altogether clear. The most parsimonious solution when analyzing all available sequence data is similar to that implied by Higgins (1981), i.e., (*Euphydryas* (*Phyciodes* (*Chlosyne Melitaea*))) (compare Figs. 1 and 4). Due to the low support, this result needs confirmation by generating more data, of both molecular and morphological origins.

Higgins (1981) proposed three subtribes for the tribe Melitaeini: Euphydryiti, Phycioditi, and Melitaeiti. Our results suggest that the *Chlosyne* group is as differentiated from the *Melitaea* group as the *Phyciodes* group (Table 2) and should probably be considered an entity of its own ("Chlosyniti"), but a formal description is beyond the scope of this paper. The four species groups are easily delimited by morphological features, and species within them are united by several ecological characters such as host plant use (N. Wahlberg, unpublished results). The status of the *Gnathotriche* group is unknown at the moment, as we were unable to obtain specimens from this group for this study.

Several genera included in our study appear to be paraphyletic. These are *[Castilia]*, *Chlosyne*, *[Didymaeformia]*, *Eresia*, *Melitaea*, and *[Thessalia]*. In addition, three monophyletic genera, *[Cinclidia]*, *[Mellicta]*, and *[Telenassa]* (one species), were members of larger clades. Our study provides the evidence needed to group many of these genera into one genus, as some authors have already done (Scott, 1986; Karsholt and Razowski, 1996). A phylogenetic classification for the species used in our study is presented in Table 3. The latest checklist for European butterflies (Karsholt and Razowski, 1996) is in agreement with our suggestion that species belonging to *[Cinclidia]*, *[Didymaeformia]*, and *[Mellicta]* should be placed in *Melitaea*. The possible subgeneric status of these groups needs to be appraised in a thorough cladistic study of *Melitaea*.

The Phycioditi can be divided into a Nearctic clade and a Neotropical clade, which can be termed the *Phyciodes s.s.* clade and the *Eresia* clade, respectively. *Tegosa* appears to be basal in the *Eresia* clade. This may be

the true position of *Tegosa* as it shares the host plant family of most *Phyciodes s.s.* species, that is, Asteraceae (Scott, 1986; DeVries, 1987). Species with known host plants in the Neotropical Phycioditi, other than those in *Tegosa*, feed on plants in the family Acanthaceae (DeVries, 1987; Brown, 1992), which may be a derived character. The nine new genera proposed by Higgins (1981) for the *Phyciodes* group most probably belong to the *Eresia* clade. Our results suggest that some of these genera may be unnatural groupings and a conservative approach would be to group all the new genera into the genus *Eresia*, pending a thorough cladistic analysis of the Neotropical Phycioditi. The basal position of *Tegosa* in the *Eresia* clade should be confirmed through wider sampling and more data.

The *Chlosyne* group is a well-defined clade in this study, though the internal relationships present some surprises. As mentioned earlier, *C. lacinia* is well within the North American clade, while the other Neotropical *Chlosyne* species are basal to this clade. Host plant use supports this hypothesis, *C. lacinia* uses plants in Asteraceae as do the other North American *Chlosyne*, except *[Thessalia]* (Scott, 1986). The Neotropical *Chlosyne* included in this study specialize on plants in the Acanthaceae (DeVries, 1987). Some Central American *Chlosyne* species also feed on Asteraceae and it would be interesting to know whether these species are more closely related to *C. lacinia* than the Neotropical clade. Another surprise is the paraphyly of *[Thessalia]*. These species have been considered a natural group for a long time (Higgins, 1960), but in our cladogram the species belonging to this genus form a basal, paraphyletic assemblage to the other North American *Chlosyne* species. A conservative approach would be to include *[Thessalia]* within *Chlosyne*, as has been done by Scott (1986).

Our biogeographical hypothesis for the tribe Melitaeini indicates that the group originated in the Nearctic and has colonized the Palaearctic and Neotropics on several separate occasions (Fig. 4). The age of the tribe is not known, but considering the possible origin of the group, the melitaeines may have diverged from their sister group with the advent of repeated glacial periods at the beginning of the Pliocene epoch (ca. 5 Mya). This presents a plausible scenario for the species radiations in melitaeines. It may be that the ancestor of the melitaeines was able to exploit the new habitat created during the glaciations and subsequently speciated. As the divergences between the different species groups are very similar (Table 2), it is possible that the ancestors of the four species groups originated in the first episode of speciation, which happened in the Nearctic (Fig. 4). The subsequent species radiations would have then happened when new regions were colonized (i.e., the Palaearctic and Neotropics).

It became apparent from our study that the tribe Melitaeini is in need of a systematic and phylogenetic revision, especially concerning the generic division. Ours is the only phylogenetic hypothesis available for the Melitaeini, which has used sequence data from two mitochondrial genes. These genes were chosen because they were likely to be informative at different hierarchical levels (Simon *et al.*, 1994). The COI data set was indeed highly informative at the species level and produced a well-resolved tree. The utility of the 16S data set was hampered by alignment difficulties and the number of informative sites was apparently too small to resolve the deeper nodes with confidence. The number of informative sites was highly dependent on the alignment as was the topology of the resulting cladograms. We did not investigate the use of nuclear genes, which have recently been used successfully to resolve higher level relationships (e.g., Brower and Egan, 1997). We are certain that direct optimization of the COI and 16S sequences has disclosed the underlying phylogenetic signal in our data set. On the whole our data set has uncovered the relationships of species in the tribe Melitaeini with good support. The phylogenetic hypothesis we present is robust enough to be used for comparative studies of species in this tribe.

ACKNOWLEDGMENTS

We are deeply indebted to the following people for providing specimens for this study: Gregory Ballmer, Alexei Belik, Deane Bowers, Willy De Prins, Henri Descimon, Phil DeVries, Claude Dutreix, Tracy Feldman, Konrad Fiedler, Krister Karttunen, Steve Kohler, Atte Komonen, Norbert Kondla, Jaakko Kullberg, Guang-Chun Lei, Onildo Marini Filho, Yuri Marusik, Klas Nyblom, Alain Olivier, Gordon Pratt, Sergei Rudykh, Jorge Salazaar, Michel Savourey, James Scott, Mike Singer, Mike Soukup, Toomas Tammaru, Bruce Walsh, Ward Watt, and Ernest Williams. Jaakko Kullberg and James Scott kindly helped with species identifications. We are grateful to Andrew Brower, Ilkka Hanski, Jaakko Hyvönen, Lauri Kaila, Jyrki Muona,

Carla Penz, and John Wenzel for comments on previous versions of the manuscript. Thanks are also extended to Ward Wheeler for help with POY.

REFERENCES

Ackery, P. R. (1984). Systematic and faunistic studies on butterflies. *In* "The Biology of Butterflies." (R. I. Vane-Wright and P. R. Ackery, Eds.), pp. 9–21. Princeton Univ. Press, Princeton.

Ackery, P. R. (1988). Host plants and classification: A review of nymphalid butterflies. *Biol. J. Linn. Soc.* **33,** 95–203.

Björklund, M. (1999). Are third positions really that bad? A test using vertebrate cytochrome b. *Cladistics* **15,** 191–197.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10,** 295–304.

Brower, A. V. Z., and Egan, M. G. (1997). Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: Heliconiiti): A revised phylogenetic position for *Eueides* based on sequences from mtDNA and a nuclear gene. *Proc. R. Soc. London B* **264,** 969–977.

Brown, K. S. Jr. (1992). Borboletas da Serra do Japi. *In* "História Natural da Serra do Japí: Ecologia e preservação de umaárea florestal no sudeste do Brasil" (P. Morelatto, Ed.), pp. 142–186. Editora da UNICAMP, Campinas-SP.

Caterino, M. S., and Sperling, F. A. H. (1999). *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. *Mol. Phylogenet. Evol.* **11,** 122–137.

Clary, D. O., and Wolstenholme, D. R. (1985). The mitochondrial DNA molecule of *Drosophila yakuba*: Nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.* **22,** 252–271.

de Jong, R. Vane-Wright, R. I., and Ackery, P. R. (1996). The higher classification of butterflies (Lepidoptera): Problems and prospects. *Ent. Scand.* **27,** 65–101.

Dennis, R. L. H. (Ed.) (1992). "The Ecology of Butterflies in Britain." Oxford Univ. Press, Oxford.

DeSalle, R., Freedman, T., Prager, E. M., and Wilson, A. C. (1987). Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila. J. Mol. Evol.* **26,** 157–164.

DeVries, P. J. (1987). "The Butterflies of Costa Rica and Their Natural History, Vol. 1, Papilionidae, Pieridae, Nymphalidae." Princeton Univ. Press, Princeton.

Dietrich, C. H., Whitcomb, R. F., and Black, W. C., IV (1997). Phylogeny of the grassland leafhopper genus *Flexamia* (Homoptera: Cicadellidae) based on mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **8,** 139–149.

Ehrlich, P. R. (1958). The comparative morphology, phylogeny and higher classification of the butterflies. *Kansas. Univ. Sci. Bull.* **39,** 305–370.

Ehrlich, P. R., and Murphy, D. D. (1981). Butterfly nomenclature: A critique. *J. Res. Lepid.* **20,** 1–11.

Ehrlich, P. R., White, R. R., Singer, M. C., McKechnie, S. W., and Gilbert, L. E. (1975). Checkerspot butterflies: A historical perspective. *Science* **188,** 221–228.

Farris, J. S. (1970). A method for computing Wagner trees. *Syst. Zool.* **34,** 21–34.

Farris, J. S. (1995). Parsimony Jackknifer, Version 4.22. Computer program, distributed by author.

Farris, J. S., Albert, V. A., Källersjö, M., Lipscomb, D., and Kluge, A. G. (1996). Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12,** 99–124.

Ferris, C. D. (1989). "Supplement to: A Catalogue/Checklist of the Butterflies of America North of Mexico, Memoir No. 3." The Lepidopterists' Society, Los Angeles, CA, USA.

Fitch, W. M., and Smith, T. F. (1983). Optimal sequence alignments. *Proc. Natl. Acad. Sci. USA* **80,** 1382–1386.

Foley, D. H., Bryan, J. H., Yeates, D., and Saul, A. (1998). Evolution and systematics of *Anopheles*: Insights from a molecular phylogeny of Australasian mosquitoes. *Mol. Phylogenet. Evol.* **9,** 262–275.

Folmer, O., Black, M. B., Hoch, W., Lutz, R. A., and Vrijehock, R. C. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Bio. Biotechnol.* **3,** 294–299.

Gladstein, D. S., and Wheeler, W. C. (1998). "Phylogeny Reconstruction via Direct Optimization POY." American Museum of Natural History, New York. [Available from authors]

Goloboff, P. A. (1993). NONA, Version 1.8. Computer program, distributed by the author.

Hanski, I. (1999). "Metapopulation Ecology." Oxford Univ. Press, Oxford.

Harvey, D. J. (1991). Higher classification of the Nymphalidae, Appendix B. *In* "The Development and Evolution of Butterfly Wing Patterns" (H. F. Nijhout), pp. 255–273. Smithsonian Institution Press, Washington, DC.

Higgins, L. G. (1941). An illustrated catalogue of the Palaearctic *Melitaea* (Lep. Rhopalocera). *Trans. R. Entomol. Soc. London* **91,** 175–365.

Higgins, L. G. (1950). A descriptive catalogue of the Palaearctic *Euphydryas* (Lepidoptera: Rhopalocera). *Trans. R. Entomol. Soc. London* **101,** 435–487

Higgins, L. G. (1955). A descriptive catalogue of the genus *Mellicta* Billberg (Lepidoptera: Nymphalidae) and its species, with supplementary notes on the genera *Melitaea* and *Euphydryas. Trans. R. Entomol. Soc. London* **106,** 1–127.

Higgins, L. G. (1960). A revision of the melitaeine genus *Chlosyne* and allied species (Lepidoptera: Nymphalinae). *Trans. R. Entomol. Soc. London* **112,** 381–465.

Higgins, L. G. (1978). A revision of the genus *Euphydryas* Scudder (Lepidoptera: Nymphalidae). *Entom. Gaz.* **29,** 109–115.

Higgins, L. G. (1981). A revision of *Phyciodes* Hübner and related genera, with a review of the classification of the Melitaeinae (Lepidoptera: Nymphalidae). *Bull. Br. Mus. Nat. Hist.* **43,** 77–243.

Källersjö, M., Albert, V. A., and Farris, J. S. (1999). Homoplasy *increases* phylogenetic structure. *Cladistics* **15,** 91–93.

Karsholt, O., and Razowski, J. (1996). "The Lepidoptera of Europe: A Distributional Checklist." Apollo Books, Stenstrup, Denmark.

Morrison, D. A., and Ellis, J. T. (1997). Effects of nucleotide sequence alignment on phylogeny estimation: A case study of 18S rDNAs of Apicomplexa. *Mol. Biol. Evol.* **14,** 428–441.

Scoble, M. J. (1992). "The Lepidoptera: Form, Function and Diversity." Oxford Univ. Press, Oxford.

Scott, J. A. (1986). "The Butterflies of North America." Stanford Univ. Press, Stanford.

Scott, J. A. (1994). Biology and systematics of *Phyciodes* (*Phyciodes*). *Papilio (New Series)* **7,** 1–120. [Privately published, available from author]

Scott, J. A. (1998). *Phyciodes* (*Phyciodes*): New discoveries, new subspecies, and convergence. *Papilio (New Series)* **10,** 1–42. [Privately published, available from author]

Siddall, M. E., and Whiting, M. F. (1999). Long-branch abstractions. *Cladistics* **15,** 9–24.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87,** 651–701.

Swofford, D. L., Olsen, G. L., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. *In* "Molecular Systematics" (D. M. Hillis, C. Morowitz, and B. K. Mable, Eds.), 2nd ed., pp. 407–514, Sinauer, Sunderland, MA.

Thomas, C. D., and Singer, M. C. (1998). Scale-dependent evolution of specialization in a checkerspot butterfly: From individuals to metapopulations and ecotypes. *In* "Genetic Structure and Local Adaptation in Natural Insect Populations" (S. Mopper and S. Y. Strauss, Eds.), pp. 343–374. Chapman and Hall, New York.

Tolman, T. (1997). "Collins Field Guide to the Butterflies of Britain and Europe." HarperCollins, London.

Vane-Wright, R. I., and Ackery, P. R. (1984). "The Biology of Butterflies." Princeton Univ. Press, Princeton.

Vogler, A. P., and Welsh, A. (1997). Phylogeny of North American *Cicindela* tiger beetles inferred from multiple mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **8,** 225–235.

Wheeler, W. C. (1996). Optimization alignment: The end of multiple sequence alignment in phylogenetics? *Cladistics* **12,** 1–9.

Wheeler, W. C. (1998). Alignment characters, dynamic programming and heuristic solutions. *In* "Molecular Approaches to Ecology and Evolution" (R. DeSalle and B. Schierwater, Eds.), pp. 243–251. Birkhäuser Verlag, Basel.

Wheeler, W. C., and Gladstein, D. S. (1994). MALIGN: A multiple sequence alignment program. *Heredity* **85,** 417–418.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D., and Wheeler, W. C. (1997). The Strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* **46,** 1–68.

Zimmermann, M., Aubert, J., and Descimon, H. (1999). Molecular systematics of the Melitaeinae (Lepidoptera: Nymphalidae). *C. R. Acad Sci.* **322,** 429–439. [In French, with English summary]

Zimmermann, M., Wahlberg, N., and Descimon, H. (2000). Phylogeny of *Euphydryas* checkerspot butterflies (Lepidoptera: Nymphalidae) based on mitochondrial DNA sequence data. *Ann. Entomol. Soc. Am.* **93,** 347–355.