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## A time-calibrated phylogeny of the butterfly tribe Melitaeini



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### ABSTRACT

The butterfly tribe Melitaeini [Nymphalidae] contains numerous species that have been the subjects of a wide range of biological studies. Despite numerous taxonomic revisions, many of the evolutionary relationships within the tribe remain unresolved. Utilizing mitochondrial and nuclear gene regions, we produced a time-calibrated phylogenetic hypothesis for 222 exemplars comprising at least 178 different species and 21 of the 22 described genera, making this the most complete phylogeny of the tribe to date. Our results suggest that four well-supported clades corresponding to the subtribes Euphydryina, Chlosynina, Melitaeina, and Phyciodina exist within the tribe. This analysis is also represents the most complete phylogenetic analysis of the Chlosynina to date, and includes several genera and species that have been previously excluded from published phylogenies of this group.

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### 1. Introduction

Lepidoptera have long been an important subject for ecological and evolutionary biology studies, but the systematics of many groups, particularly some butterflies, is often disputed. The Nymphalidae comprise the largest family of butterflies and consequently have been the subject of intense study (e.g. Wahlberg et al., 2009; Brower et al., 2010; Penz et al., 2011). Various taxonomic revisions have split (and lumped) these species, sometimes into as many as nine different families, although currently most authors treat them as a single family. At present, many phylogenetic relationships in the clade remain unresolved and the timing of diversification for the clade, in particular, is in need of additional study.

We are particularly interested in the phylogeny of a tribe of Nymphalidae, the Melitaeini. This group contains several species that serve as important models to biologists in a wide range of disciplines, from metapopulation biology to mimicry to genetics (e.g. Bates, 1862; Benson, 1972; Gilbert and Singer, 1975; Ehrlich et al., 1975, 1984; Brown and Ehrlich, 1980; Sheppard et al., 1985; Wahlberg et al., 2002). Despite this importance, evolutionary relationships of many genera (as well as species within those genera) remain unclear. Higgins (1941, 1950, 1955, 1960, 1981) and

Harvey (1991) have authored several taxonomic revisions of the group based on morphology. More recent studies have utilized molecular methods to try to clarify the evolutionary history of these species (Wahlberg and Zimmermann, 2000; Zimmermann et al., 2000; Wahlberg et al., 2005; Wahlberg and Freitas, 2007; Leneveu et al., 2009).

At present, approximately 250 species of Melitaeine are recognized (Higgins, 1981; Harvey, 1991; Wahlberg and Zimmermann, 2000). The group is distributed throughout the Palearctic, Nearctic, and Neotropical regions. During the middle and latter half of the 20th century, most of the taxonomic work on this group was done by Higgins (1941, 1950, 1955, 1960, 1981). His last taxonomic revision treated the group as a subfamily (Melitaeini) and split the group into 31 genera (Higgins, 1981) (Table 1). Many of these genera have been rejected by subsequent authors, and phylogenetic work by Zimmermann et al. (2000) and Wahlberg and Zimmermann (2000) suggested different conclusions than those of Higgins. Notably, Wahlberg and Zimmermann treated the group as a tribe, per Harvey (1991), proposed 4 species groups (subtribes), and rejected many of Higgins' generic revisions (mainly due to paraphyly or other unnatural groupings) (Higgins, 1981; Wahlberg and Zimmermann, 2000).

The phylogenetic hypothesis produced by Wahlberg and Zimmermann (2000), which utilized POY for alignment and phylogenetic inference, remains the most complete analysis of this tribe prior to this study. However, their study was only able to utilize mitochondrial gene regions, as nuclear genes were not yet widely available for these taxa. A 2005 study by Wahlberg et al. utilized

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**Table 1**  
Summary of some major revisions of the tribe Melitaeini and comparisons to the current study.

Author	Status of Melitaeini and subclades	Genera within Clades
Higgins (1981)	Subfamily containing 3 tribes: Euphydrini, Melitaeini, Phyciodini	<b>Euphydrini:</b> <i>Euphydryas</i> , <i>Eurodryas</i> , <i>Hypodryas</i> , <i>Occidryas</i> (all now considered <i>Euphydryas</i> ) <b>Melitaeini:</b> <i>Antillea</i> , <i>Cinclidia</i> , <i>Didymaeformia</i> , <i>Mellicta</i> , <i>Melitaea</i> (all now considered <i>Melitaea</i> ); <i>Chlosyne</i> , <i>Thessalia</i> (now considered <i>Chlosyne</i> ); <i>Dymasia</i> ; <i>Gnathotriche</i> , <i>Gnathotrusia</i> (now considered <i>Gnathotriche</i> ); <i>Higginsius</i> ; <i>Microtia</i> ; <i>Poladryas</i> ; <i>Texola</i> <b>Phyciodini:</b> <i>Anthanassa</i> ; <i>Castilia</i> ; <i>Dagon</i> ; <i>Eresia</i> ; <i>Janatella</i> ; <i>Mazia</i> ; <i>Ortilia</i> ; <i>Phyciodes</i> ; <i>Phystis</i> ; <i>Tegosa</i> ; <i>Telenassa</i> ; <i>Tisona</i> <b>Unplaced:</b> <i>Atlantea</i>
Harvey (1991)	Tribe containing 3 subtribes <i>sensu</i> Higgins, 1981: Euphydriti, Melitaeiti, Phycioditi	<b>Euphydriti:</b> <i>Euphydryas</i> , <i>Hypodryas</i> , <i>Occidryas</i> , <i>Eurodryas</i> <b>Melitaeiti:</b> <i>Mellicta</i> , <i>Melitaea</i> , <i>Didymaeformia</i> , <i>Cinclidia</i> ; <i>Chlosyne</i> , <i>Thessalia</i> ; <i>Poladryas</i> ; <i>Texola</i> ; <i>Dymasia</i> ; <i>Microtia</i> ; <i>Gnathotriche</i> , <i>Gnathotrusia</i> ; <i>Higginsius</i> ; <i>Antillea</i> <b>Phycioditi:</b> <i>Phyciodes</i> ; <i>Phystis</i> ; <i>Anthanassa</i> ; <i>Dagon</i> ; <i>Telenassa</i> ; <i>Ortilia</i> ; <i>Tisona</i> ; <i>Tegosa</i> ; <i>Eresia</i> ; <i>Castilia</i> ; <i>Janatella</i> ; <i>Mazia</i> <b>Unplaced:</b> <i>Atlantea</i>
Wahlberg and Zimmermann (2000)	Tribe containing 4 subtribes: Euphydriti, Melitaeiti, Phycioditi, proposed Chlosyne-group ( <i>Chlosyniti</i> )	<b>Euphydriti:</b> <i>Euphydryas</i> <b>Melitaeiti:</b> <i>Melitaea</i> ; <i>Poladryas</i> <b>Phycioditi:</b> <i>Anthanassa</i> ; <i>Eresia</i> (including <i>Telenassa</i> and <i>Castilia</i> ); <i>Phyciodes</i> ; <i>Tegosa</i> ( <b>Chlosyniti</b> ): <i>Chlosyne</i> ; <i>Dymasia</i> ; <i>Texola</i> <b>Unplaced:</b> <i>Antillea</i> ; <i>Atlantea</i> ; <i>Dagon</i> ; <i>Gnathotriche</i> ; <i>Higginsius</i> ; <i>Janatella</i> ; <i>Mazia</i> ; <i>Microtia</i> ; <i>Ortilia</i> ; <i>Phystis</i> ; <i>Tisona</i>
This study	Tribe containing 4 subtribes: Euphydryina, Melitaeina, Phyciodina, Chlosynina	<b>Euphydryina:</b> <i>Euphydryas</i> <b>Melitaeina:</b> <i>Gnathotriche</i> ; <i>Higginsius</i> ; <i>Melitaea</i> <b>Phyciodina:</b> <i>Anthanassa</i> ; <i>Antillea</i> ; <i>Atlantea</i> ; <i>Castilia</i> ; <i>Dagon</i> ; <i>Eresia</i> ; <i>Janatella</i> ; <i>Mazia</i> ; <i>Ortilia</i> ; <i>Phyciodes</i> ; <i>Phystis</i> ; <i>Tegosa</i> ; <i>Telenassa</i> <b>Chlosynina:</b> <i>Chlosyne</i> ; <i>Dymasia</i> ; <i>Microtia</i> ; <i>Poladryas</i> ; <i>Texola</i> <b>Unplaced:</b> <i>Tisona</i>

parsimony to reconstruct the phylogeny of the Nymphalinae but included fewer species of Melitaeini than did Wahlberg and Zimmerman. Previous studies were unable to resolve many of the relationships in the genus *Chlosyne*, particularly those of *C. palla*, *C. acastus*, *C. neumogeni*, *C. gabbiti*, and *C. hoffmanni*; and the relationships of several species of *Euphydryas*, notably those of *E. chalcedona* and the proposed species *E. anicia* and *E. colon*; and now previous molecular studies have placed *Atlantea*. Data are now available for considerably more species than were used in previous studies. In addition, new relaxed clock models for estimating divergence times that can simultaneously account for uncertainty in both the tree topology and divergence times have become available. Utilizing these methods, we undertook a revised analysis of the Melitaeini providing a new comprehensive estimate of phylogenetic relationships and timing of diversification for the clade.

## 2. Methods

### 2.1. Species used in study

We included sequence data from 222 exemplars belonging to the Tribe Melitaeini (Family Nymphalidae) as well as 3 outgroup species (Table 2). According to the taxonomy that we follow, we used sequence data from at least 178 different species for 21 of the 22 genera in the Melitaeini (all genera except the monotypic genus *Tisona*). The remaining exemplars represent putative subspecies or in a few cases separate populations of a given species.

Unless otherwise specified, in subsequent treatment we use the naming conventions described by the ICZN code and refer to all subtribes using the suffix ‘-ina’ (ICZN, 1999). We followed Harvey (1991) and Wahlberg and Zimmermann (2000) in treating the Melitaeini as a tribe, rather than as a subfamily as in Higgins (1981). Wahlberg and Zimmermann proposed four groups within the Melitaeini: of these, we included 34 individuals from the

Nearctic and Neotropical Chlosyniti (elsewhere referred to as the *Chlosyne*-group, e.g. Wahlberg et al., 2005, now Chlosynina), 27 individuals from the Holarctic Euphydriti (now Euphydryina), 78 individuals from the Palearctic Melitaeiti (Melitaeina (Wahlberg et al., 2005)), and 62 individuals from the Nearctic and Neotropical Phycioditi (Phyciodina (Wahlberg et al., 2005; Wahlberg and Freitas, 2007)). Twenty-one of the taxa used in our study were not categorized by Wahlberg and Zimmermann (2000). Higgins categorized six of those taxa as belonging to the clade Melitaeini (the genera *Antillea*, *Gnathotriche*, *Higginsius*, and *Microtia*), 14 individuals as Phyciodina (the genera *Dagon*, *Janatella*, *Mazia*, *Ortilia*, and *Phystis*), while he was unable to categorize the genus *Atlantea* (Higgins, 1981). Wahlberg et al. (2005) did not include *Antillea*, *Ortilia*, *Phystis* or *Dagon* in their analysis, but placed *Microtia* in the *Chlosyne*-group, *Gnathotriche* and *Higginsius* as a sister clade to Phyciodina, and included *Mazia* and *Janatella* in Phyciodina.

### 2.2. Molecular sequence data

We utilized molecular data from three separate gene regions. We used 1450 bp from the cytochrome oxidase subunit I (*COI*) mitochondrial gene region, 397 bp from the nuclear gene region wingless (*wg*), and 1240 bp from the nuclear gene region elongation factor alpha (*EF1 $\alpha$* ) (Folmer et al., 1994; Cho et al., 1995; Brower and Desalle, 1998).

For all taxa that were newly sequenced as part of this study, total genomic DNA was extracted from abdomens or legs using Qiagen DNAeasy kits per the manufacturers instructions (Qiagen Inc., Valencia, California). Each gene region for each specimen was then PCR amplified and sequenced using standard protocols (Brower, 1994; Wahlberg et al., 2005; Brower et al., 2006). The remaining sequence data were obtained from Genbank (Table 2).

Sequence data were imported into Geneious Pro 4.8.5 (Biomatters, Ltd.), and data were examined and edited manually. Contigs were assembled in Geneious for each gene region for each taxon.

**Table 2**

Melitaeni and outgroup taxa used in phylogeny, with GenBank identifiers, for 3 gene regions. The study included 222 exemplars representing at least 178 species comprising 21 of the 22 Melitaeni genera, as well as outgroups *Hypolimnas*, *Vanessula*, and *Doleschallia*. Identifiers beginning with the prefix 'ECL' were sequenced as part of this study; all remaining identifiers represent GenBank accession numbers, with references given in superscripts. Any taxa and gene region for which sequence data could not be obtained were left blank.

Genus	Species	COI	Ef1-a	Wgl	
<i>Chlosyne</i>	<i>C. californica</i> (Wright 1905)	AF187750 <sup>a</sup>			
	<i>C. acastus</i> (Edwards 1874)	AF187735 <sup>a</sup>	AY788725 <sup>b</sup>	AY788486 <sup>b</sup>	
	<i>C. cyneas</i> (Godman and Salvin 1878)	AF187757 <sup>a</sup>	AY788726 <sup>b</sup>	AY788487 <sup>b</sup>	
	<i>C. erodyle</i> (Bates 1864)	GU157053 <sup>c</sup>			
	<i>C. fulvia</i> (Edwards 1879)	AF187769 <sup>a</sup>			
	<i>C. gabbii</i> (Behr 1863)	KM042286 <sup>d</sup>	KM042271 <sup>d</sup>	KM042230 <sup>d</sup>	
	<i>C. gaudialis</i> (Bates 1864)	AF187770 <sup>a</sup>	AY788727 <sup>b</sup>	AY788488 <sup>b</sup>	
	<i>C. gorgone</i> (Hübner [1810])	AF187772 <sup>a</sup>	AY788728 <sup>b</sup>	AY788489 <sup>b</sup>	
	<i>C. harrisii</i> (Scudder 1864)	AF187773 <sup>a</sup>	AY788729 <sup>b</sup>	AY788490 <sup>b</sup>	
	<i>C. hippodrome</i> (Geyer 1837)	JQ535606 <sup>e</sup>			
	<i>C. hoffmanni</i> (Behr 1863)	KM042298 <sup>d</sup>	KM042267 <sup>d</sup>	KM042225 <sup>d</sup>	
	<i>C. janais</i> (Drury 1783)	AY788620 <sup>b</sup>	AY788730 <sup>b</sup>	AY788491 <sup>b</sup>	
	<i>C. lacinia</i> (Geyer 1837)	AY090227 <sup>f</sup>	AY090195 <sup>f</sup>	AY090161 <sup>f</sup>	
	<i>C. leanira</i> (Felder and Felder 1860)	AF187781 <sup>a</sup>	KM042257 <sup>d</sup>	KM042217 <sup>d</sup>	
	<i>C. melanarge</i> (Bates 1864)	JQ548300 <sup>e</sup>			
	<i>C. narva</i> (Fabricius 1793)	AF187786 <sup>a</sup>	AY788731 <sup>b</sup>	AY788492 <sup>b</sup>	
	<i>C. neumoegei</i> ( <i>C. acastus</i> <i>neumoegei</i> Skinner 1895)	AF187787 <sup>a</sup>	KM042270 <sup>d</sup>		
	<i>C. nycteis</i> (Doubleday [1847])	AF187788 <sup>a</sup>	AY788732 <sup>b</sup>	AY788493 <sup>b</sup>	
	<i>C. palla</i> (Boisduval 1852)	AF187791 <sup>a</sup>	AY788733 <sup>b</sup>	AY788494 <sup>b</sup>	
	<i>C. palla</i> ButtsCyn	KM042295 <sup>d</sup>	KM042260 <sup>d</sup>	KM042227 <sup>d</sup>	
	<i>C. palla</i> GoatMtn	KM042296 <sup>d</sup>	KM042264 <sup>d</sup>	KM042228 <sup>d</sup>	
	<i>C. palla</i> IowaHill	KM042292 <sup>d</sup>	KM042263 <sup>d</sup>	KM042221 <sup>d</sup>	
	<i>C. palla</i> Jackson	KM042285 <sup>d</sup>	KM042261 <sup>d</sup>	KM042219 <sup>d</sup>	
	<i>C. palla</i> Leap	KM042287 <sup>d</sup>	KM042269 <sup>d</sup>		
	<i>C. palla</i> Lumgrey	KM042293 <sup>d</sup>	KM042258 <sup>d</sup>	KM042223 <sup>d</sup>	
	<i>C. palla</i> Ramshorn	KM042289 <sup>d</sup>	KM042268 <sup>d</sup>	KM042224 <sup>d</sup>	
	<i>C. palla</i> Sierraville	KM042297 <sup>d</sup>	KM042265 <sup>d</sup>	KM042226 <sup>d</sup>	
	<i>C. palla</i> Warner	KM042290 <sup>d</sup>	KM042259 <sup>d</sup>	KM042229 <sup>d</sup>	
	<i>C. palla</i> Weed	KM042294 <sup>d</sup>	KM042262 <sup>d</sup>	KM042220 <sup>d</sup>	
	<i>C. palla</i> Yuba49	KM042291 <sup>d</sup>	KM042244 <sup>d</sup>	KM042222 <sup>d</sup>	
	<i>C. theona</i> (Ménétriés 1855)	AF187808 <sup>a</sup>	AY788734 <sup>b</sup>	AY788495 <sup>b</sup>	
	<i>C. whitneyi</i> (Behr 1863)	KM042288 <sup>d</sup>	KM042266 <sup>d</sup>	KM042218 <sup>d</sup>	
	<i>Dymasia</i>	<i>D. dymas</i> (Edwards 1877)	AF187764 <sup>a</sup>	AY788785 <sup>b</sup>	AY788545 <sup>b</sup>
		<i>T. elada</i> (Hewitson 1868)	AY788659 <sup>b</sup>	AY788786 <sup>b</sup>	AY788546 <sup>b</sup>
	<i>Texola</i>	<i>E. anicia</i> (Doubleday [1847])	AF187738 <sup>a</sup>		
	<i>Euphydryas</i>	<i>E. asiatica</i> (Staudinger 1881)	FJ663556 <sup>g</sup>		
		<i>E. aurinia</i> (Rottentburg 1775)	AF187746 <sup>a</sup>	AY788743 <sup>b</sup>	AY788504 <sup>b</sup>
		<i>E. chalcedona</i> (Doubleday [1847])	AF187752 <sup>a</sup>	AY788744 <sup>b</sup>	AY788505 <sup>b</sup>
		<i>E. chalcedona</i> BullCrk	KM042272 <sup>d</sup>	KM042256 <sup>d</sup>	KM042232 <sup>d</sup>
		<i>E. chalcedona</i> Caribou	KM042277 <sup>d</sup>	KM042246 <sup>d</sup>	KM042235 <sup>d</sup>
		<i>E. chalcedona</i> CaveLk	KM042273 <sup>d</sup>	KM042254 <sup>d</sup>	KM042238 <sup>d</sup>
		<i>E. chalcedona</i> DryCrk	KM042276 <sup>d</sup>	KM042247 <sup>d</sup>	KM042237 <sup>d</sup>
		<i>E. chalcedona</i> Elphnt	KM042281 <sup>d</sup>	KM042253 <sup>d</sup>	KM042243 <sup>d</sup>
		<i>E. chalcedona</i> HuntLk	KM042284 <sup>d</sup>	KM042248 <sup>d</sup>	KM042233 <sup>d</sup>
		<i>E. chalcedona</i> KenMdw	KM042280 <sup>d</sup>	KM042250 <sup>d</sup>	KM042241 <sup>d</sup>
		<i>E. chalcedona</i> Leap	KM042274 <sup>d</sup>	KM042249 <sup>d</sup>	KM042242 <sup>d</sup>
		<i>E. chalcedona</i> Mendo	KM042279 <sup>d</sup>	KM042252 <sup>d</sup>	KM042239 <sup>d</sup>
<i>E. chalcedona</i> Mont		KM042282 <sup>d</sup>	KM042251 <sup>d</sup>	KM042236 <sup>d</sup>	
<i>E. chalcedona</i> SLO		KM042278 <sup>d</sup>		KM042240 <sup>d</sup>	
<i>E. chalcedona</i> Sn.Pass		KM042275 <sup>d</sup>	KM042255 <sup>d</sup>	KM042234 <sup>d</sup>	
<i>E. chalcedona</i> Trinity		KM042283 <sup>d</sup>	KM042245 <sup>d</sup>	KM042231 <sup>d</sup>	
<i>E. colon</i> (Edwards 1881)		AF187756 <sup>a</sup>			
<i>E. Cynthia</i> (Schiffmüller [1775])		AF153925 <sup>h</sup>			
<i>E. desfontainii</i> (Godart 1819)		AY090226 <sup>f</sup>	AY090193 <sup>f</sup>	AY090159 <sup>f</sup>	
<i>E. editha</i> (Boisduval 1852)		AF187765 <sup>a</sup>	AY788745 <sup>b</sup>	AY788506 <sup>b</sup>	
<i>E. gilletti</i> (Barnes 1897)		AF187771 <sup>a</sup>	AY788746 <sup>b</sup>	AY788507 <sup>b</sup>	
<i>E. iduna</i> (Dalman 1816)		AF187776 <sup>a</sup>			
<i>E. intermedia</i> (as <i>E. ichnea intermedia</i> Ménétriés 1859)		AF187777 <sup>a</sup>			
<i>E. maturna</i> (Linnaeus 1758)		HQ004484 <sup>i</sup>			
<i>E. merope altivolans</i> (Tuzov, 2000)		FJ663562 <sup>g</sup>			
<i>E. phaeton</i> (Drury [1773])		AF187797 <sup>a</sup>	AY788747 <sup>b</sup>	AY788508 <sup>b</sup>	
<i>Melitaea</i>		<i>M. acraeina</i> (Staudinger 1886)	FJ462229 <sup>j</sup>	FJ462289 <sup>j</sup>	FJ462164 <sup>j</sup>
		<i>M. aetherie</i> (Hübner [1826])	FJ462230 <sup>j</sup>	FJ462290 <sup>j</sup>	FJ462165 <sup>j</sup>
		<i>M. ala</i> (Staudinger 1881)	FJ462231 <sup>j</sup>	FJ462291 <sup>j</sup>	FJ462166 <sup>j</sup>
		<i>M. alatuica</i> (Staudinger 1881)	FJ663811 <sup>g</sup>		
		<i>M. ambigua</i> (Ménétriés in Schrenck 1859)	AF187736 <sup>a</sup>	FJ462292 <sup>j</sup>	FJ462167 <sup>j</sup>
		<i>M. ambrisia</i> (Higgins 1935)	FJ462232 <sup>j</sup>	FJ462293 <sup>j</sup>	FJ462168 <sup>j</sup>
	<i>M. amoenula</i> (Felder and Felder 1867)	AF187737 <sup>a</sup>	FJ462294 <sup>j</sup>	FJ462169 <sup>j</sup>	
	<i>M. arcesia leechi</i> (Alphéraky 1895)	FJ462262 <sup>j</sup>	FJ462330 <sup>j</sup>	FJ462202 <sup>j</sup>	
	<i>M. arcesia chuana</i> (Grum-Grshimailo 1893)	FJ462243 <sup>j</sup>	FJ462309 <sup>j</sup>	FJ462182 <sup>j</sup>	
	<i>M. arduinna</i> (Esper 1783)	AF187742 <sup>a</sup>	AY788774 <sup>b</sup>	AY788534 <sup>b</sup>	

(continued on next page)

Table 2 (continued)

Genus	Species	COI	Ef1-a	Wgl
	<i>M. asteria</i> (Freyer 1828)	FJ462233 <sup>j</sup>	FJ462296 <sup>i</sup>	
	<i>M. athalia</i> (Rottemburg 1775)	FJ462234 <sup>j</sup>	FJ462297 <sup>i</sup>	FJ462171 <sup>j</sup>
	<i>M. athene</i> (Staudinger 1881)	FJ663783 <sup>g</sup>	FJ462298 <sup>i</sup>	FJ462172 <sup>j</sup>
	<i>M. aurelia</i> (Nickerl 1850)	AF187745 <sup>i</sup>	FJ462299 <sup>i</sup>	FJ462173 <sup>j</sup>
	<i>M. avinovi</i> (Aheljuzhko 1914)	FJ462235 <sup>j</sup>	FJ462300 <sup>i</sup>	
	<i>M. bellona</i> (Leech 1892)	FJ462236 <sup>j</sup>	FJ462301 <sup>i</sup>	FJ462174 <sup>j</sup>
	<i>M. britomartis</i> 15-13 (Assmann 1 1847)	AF187748 <sup>i</sup>	FJ462302 <sup>i</sup>	FJ462175 <sup>j</sup>
	<i>M. britomartis</i> NW69-8	AY788655 <sup>b</sup>	AY788775 <sup>b</sup>	AY788535 <sup>b</sup>
	<i>M. cassandra</i> (Kolesnichenko and Churkin, 2001)	FJ462237 <sup>j</sup>	FJ462303 <sup>i</sup>	FJ462176 <sup>j</sup>
	<i>M. casta</i> (Kollar 1848)	FJ462238 <sup>j</sup>	FJ462304 <sup>i</sup>	FJ462177 <sup>j</sup>
	<i>M. caucasogenita</i> (Verity 1930)	FJ462239 <sup>j</sup>	FJ462305 <sup>i</sup>	FJ462178 <sup>j</sup>
	<i>M. celadussa</i> (as <i>M. athalia celadussa</i> Fruhstorfer 1910)	FJ462240 <sup>j</sup>	FJ462306 <sup>i</sup>	FJ462179 <sup>j</sup>
	<i>M. centralasiae</i> (Wnukowsky 1929)	FJ462241 <sup>j</sup>	FJ462307 <sup>i</sup>	FJ462180 <sup>j</sup>
	<i>M. chitralensis</i> (Moore 1901)	FJ462242 <sup>j</sup>	FJ462308 <sup>i</sup>	FJ462181 <sup>j</sup>
	<i>M. cinxia</i> (Linnaeus 1758)	AY788656 <sup>b</sup>	AY788776 <sup>b</sup>	AY788536 <sup>b</sup>
	<i>M. collina</i> (Lederer 1861)	FJ462244 <sup>j</sup>	FJ462311 <sup>i</sup>	FJ462183 <sup>j</sup>
	<i>M. consulis</i> (Wiltshire 1941)	FJ462245 <sup>j</sup>	FJ462312 <sup>i</sup>	FJ462184 <sup>j</sup>
	<i>M. deione</i> JL126 (Geyer 1832)	FJ462246 <sup>j</sup>	FJ462313 <sup>i</sup>	FJ462185 <sup>j</sup>
	<i>M. deione</i> NW95-5	AY788657 <sup>b</sup>	AY788777 <sup>b</sup>	AY788537 <sup>b</sup>
	<i>M. deserticola</i> 34-12 (Oberthür 1909)	AF187759 <sup>a</sup>	FJ462315 <sup>i</sup>	FJ462187 <sup>j</sup>
	<i>M. deserticola</i> JL3-10	FJ462248 <sup>j</sup>	FJ462316 <sup>i</sup>	FJ462188 <sup>j</sup>
	<i>M. diamina</i> (Lang 1789)	AF187761 <sup>a</sup>	FJ462317 <sup>i</sup>	FJ462189 <sup>j</sup>
	<i>M. didyma</i> AC7-8 (Esper 1778)	FJ462252 <sup>j</sup>	FJ462321 <sup>i</sup>	FJ462193 <sup>j</sup>
	<i>M. didyma</i> NW107-5	FJ462253 <sup>j</sup>	FJ462322 <sup>i</sup>	FJ462194 <sup>j</sup>
	<i>M. didyma</i> AC3-3	FJ462250 <sup>j</sup>	FJ462319 <sup>i</sup>	FJ462191 <sup>j</sup>
	<i>M. didyma</i> AC6-7	FJ462251 <sup>j</sup>	FJ462320 <sup>i</sup>	FJ462192 <sup>j</sup>
	<i>M. didyma</i> NW99-12	FJ462249 <sup>j</sup>	FJ462318 <sup>i</sup>	FJ462190 <sup>j</sup>
	<i>M. didymoides</i> NW26-1 (Eversmann 1847)	AF187762 <sup>a</sup>	AY090194 <sup>f</sup>	AY090160 <sup>f</sup>
	<i>M. didymoides</i> 2814	FJ462254 <sup>j</sup>	FJ462323 <sup>i</sup>	FJ462195 <sup>j</sup>
	<i>M. elisabethae</i> (Avinoff 1910)	FJ462255 <sup>j</sup>	FJ462324 <sup>i</sup>	FJ462196 <sup>j</sup>
	<i>M. enarea</i> (Fruhstorfer 1917)	FJ462256 <sup>j</sup>	FJ462325 <sup>i</sup>	FJ462197 <sup>j</sup>
	<i>M. enarea permuta</i> (Higgins, 1941)	FJ462272 <sup>j</sup>	FJ462340 <sup>i</sup>	FJ462212 <sup>j</sup>
	<i>M. fergana</i> (Staudinger 1882)	FJ462257 <sup>j</sup>	FJ462326 <sup>i</sup>	FJ462198 <sup>j</sup>
	<i>M. fermaracandica</i> (Staudinger 1882)	FJ462266 <sup>j</sup>	FJ462334 <sup>i</sup>	FJ462206 <sup>j</sup>
	<i>M. gina</i> (Higgins 1941)	FJ462258 <sup>j</sup>		
	<i>M. infernalis</i> (Grum-Grshimailo 1891)	FJ462259 <sup>j</sup>	FJ462327 <sup>i</sup>	FJ462199 <sup>j</sup>
	<i>M. interrupta</i> (Kolenati 1846)	FJ462260 <sup>j</sup>	FJ462328 <sup>i</sup>	FJ462200 <sup>j</sup>
	<i>M. jezabel</i> (Oberthür 1888)	EF683670 <sup>k</sup>	EF683664 <sup>k</sup>	
	<i>M. latonigena</i> (Eversmann 1847)	FJ462261 <sup>j</sup>	FJ462329 <sup>i</sup>	FJ462201 <sup>j</sup>
	<i>M. ludmilla</i> (Churkin, Kolesnichenko & Tuzov, 2000)	FJ462263 <sup>j</sup>	FJ462331 <sup>i</sup>	FJ462203 <sup>j</sup>
	<i>M. lunulata</i> (Staudinger 1901)	FJ462265 <sup>j</sup>	FJ462333 <sup>i</sup>	FJ462205 <sup>j</sup>
	<i>M. lutko</i> (Evans 1932)	FJ462264 <sup>j</sup>	FJ462332 <sup>i</sup>	FJ462204 <sup>j</sup>
	<i>M. menetriesi</i> (Caradja 1895)	FJ462267 <sup>j</sup>	FJ462335 <sup>i</sup>	FJ462207 <sup>j</sup>
	<i>M. minerva</i> (Staudinger 1881)	FJ462268 <sup>j</sup>	FJ462336 <sup>i</sup>	FJ462208 <sup>j</sup>
	<i>M. ninae</i> (Sheljuzhko 1935)	FJ462269 <sup>j</sup>	FJ462337 <sup>i</sup>	FJ462209 <sup>j</sup>
	<i>M. pallas</i> (Staudinger 1886)	FJ462270 <sup>j</sup>	FJ462338 <sup>i</sup>	FJ462210 <sup>j</sup>
	<i>M. parthenoides</i> (Keferstein 1851)	FJ462271 <sup>j</sup>	FJ462339 <sup>i</sup>	FJ462211 <sup>j</sup>
	<i>M. persean</i> NW120-11 (Kollar 1850)	FJ462273 <sup>j</sup>	FJ462341 <sup>i</sup>	FJ462213 <sup>j</sup>
	<i>M. persean</i> NW34-1	AF187796 <sup>a</sup>	AY788779 <sup>b</sup>	AY788539 <sup>b</sup>
	<i>M. phoebe</i> AC6-6 (Goeze 1779)	FJ462275 <sup>j</sup>	FJ462343 <sup>i</sup>	FJ462215 <sup>j</sup>
	<i>M. phoebe</i> NW15-14	FJ462274 <sup>j</sup>	FJ462342 <sup>i</sup>	FJ462214 <sup>j</sup>
	<i>M. plotina</i> (Bremer 1861)	FJ462277 <sup>j</sup>	FJ462345 <sup>i</sup>	FJ462217 <sup>j</sup>
	<i>M. protomeia</i> (Ménétriés 1859)	FJ462278 <sup>j</sup>	FJ462346 <sup>i</sup>	FJ462218 <sup>j</sup>
	<i>M. punica telonia</i> (Fruhstorfer 1908)	FJ462279 <sup>j</sup>	FJ462347 <sup>i</sup>	FJ462219 <sup>j</sup>
	<i>M. punica</i> JL3-7 (Oberthür 1876)	FJ462276 <sup>j</sup>	FJ462344 <sup>i</sup>	FJ462216 <sup>j</sup>
	<i>M. punica</i> NW34-11	AF187803 <sup>a</sup>	AY788781 <sup>b</sup>	AY788541 <sup>b</sup>
	<i>M. romanovi</i> (Grum-Grshimailo 1891)	FJ462280 <sup>j</sup>	FJ462348 <sup>i</sup>	FJ462220 <sup>j</sup>
	<i>M. saxatilis</i> (Christoff 1876)	FJ462281 <sup>j</sup>	FJ462349 <sup>i</sup>	FJ462221 <sup>j</sup>
	<i>M. scotosia</i> (Butler 1878)	AF187804 <sup>a</sup>	AY788780 <sup>b</sup>	AY788540 <sup>b</sup>
	<i>M. shandura</i> (Evans, 1924)	FJ462282 <sup>j</sup>	FJ462350 <sup>i</sup>	FJ462222 <sup>j</sup>
	<i>M. sibina</i> (Alphéraky 1881)	FJ462283 <sup>j</sup>	FJ462351 <sup>i</sup>	FJ462223 <sup>j</sup>
	<i>M. solona</i> (Alphéraky 1881)	FJ462284 <sup>j</sup>	FJ462352 <sup>i</sup>	FJ462224 <sup>j</sup>
	<i>M. sultanensis</i> (Staudinger 1886)	FJ462285 <sup>j</sup>	FJ462353 <sup>i</sup>	FJ462225 <sup>j</sup>
	<i>M. sutschana</i> (Staudinger 1892)	AF187805 <sup>a</sup>	FJ462354 <sup>i</sup>	FJ462226 <sup>j</sup>
	<i>M. trivina</i> (Denis and Schiffermüller 1775)	FJ462286 <sup>j</sup>	AY788782 <sup>b</sup>	FJ462227 <sup>j</sup>
	<i>M. varia</i> (Meyer-Dür 1851)	FJ462287 <sup>j</sup>	FJ462356 <sup>i</sup>	AY788543 <sup>b</sup>
	<i>M. wiltshirei</i> (Higgins 1941)	FJ462288 <sup>j</sup>	FJ462357 <sup>i</sup>	FJ462228 <sup>j</sup>
Poladryas	<i>P. arachne</i> (Edwards 1869)	AF186928 <sup>a</sup>	AY788799 <sup>b</sup>	AY788559 <sup>b</sup>
Anthanassa	<i>A. frisia tulcis</i> (as <i>A. tulcis</i> Bates 1864)	AY788612 <sup>b</sup>	AY788717 <sup>b</sup>	AY788478 <sup>b</sup>
	<i>A. ardys</i> (Hewitson 1864)	AF187743 <sup>a</sup>	AY788713 <sup>b</sup>	AY788474 <sup>b</sup>
	<i>A. argentea</i> (Godman and Salvin 1882)	HM890823 <sup>e</sup>		
	<i>A. drusilla</i> (Felder and Felder 1861)	AY788611 <sup>b</sup>	AY788714 <sup>b</sup>	AY788475 <sup>b</sup>
	<i>A. frisia hermas</i> (as <i>A. hermas</i> Hewitson 1864)	EF493929 <sup>i</sup>	EF493977 <sup>i</sup>	EF493870 <sup>i</sup>
	<i>A. otones</i> (Hewitson 1864)	AF187790 <sup>a</sup>	AY788715 <sup>b</sup>	AY788476 <sup>b</sup>
	<i>A. ptolyca</i> (Bates 1864)	AF187802 <sup>a</sup>		

Table 2 (continued)

Genus	Species	COI	Ef1-a	Wgl	
"Castilia"	<i>A. texana</i> (Edwards 1863)	AF187806 <sup>a</sup>	AY788716 <sup>b</sup>	AY788477 <sup>b</sup>	
	<i>E. castilia</i> (as <i>C. castilia</i> Felder and Felder 1862)	EF493930 <sup>l</sup>	EF493872 <sup>l</sup>	EF493872 <sup>l</sup>	
	<i>C. myia</i> (Hewitson 1864)	AF187784 <sup>a</sup>	EF493980 <sup>l</sup>	EF493873 <sup>l</sup>	
	<i>C. perilla</i> (Hewitson 1852)	EF493931 <sup>l</sup>	EF493981 <sup>l</sup>	EF493874 <sup>l</sup>	
<i>Eresia</i>	<i>C. ofella</i> (Hewitson 1864)	AY788618 <sup>b</sup>	AY788723 <sup>b</sup>	AY788484 <sup>b</sup>	
	<i>E. burchellii</i> (as <i>Telenassa telatusa burchellii</i> Moulton 1909)	AF187749 <sup>a</sup>			
	<i>E. carme</i> (Doubleday [1847])	EF493935 <sup>l</sup>	EF493985 <sup>l</sup>	EF493878 <sup>l</sup>	
	<i>E. casiphia</i> (Hewitson 1869)	EF493936 <sup>l</sup>	EF493986 <sup>l</sup>	EF493879 <sup>l</sup>	
	<i>E. clara</i> (as <i>E. clio clara</i> Bates 1864)	AF187754 <sup>a</sup>			
	<i>E. clio</i> (Linnaeus 1758)	AY788622 <sup>b</sup>	AY788736 <sup>b</sup>	AY788497 <sup>b</sup>	
	<i>E. coela</i> (as <i>E. emerantia coela</i> Druce 1874)	AY788623 <sup>b</sup>	AY788737 <sup>b</sup>	AY788498 <sup>b</sup>	
	<i>E. datis</i> (Hewitson [1864])	EF493942 <sup>l</sup>	EF493992 <sup>l</sup>	EF493885 <sup>l</sup>	
	<i>E. eranites</i> (as <i>Castilia eranites</i> Hewitson 1857)	AY788617 <sup>b</sup>	AY788722 <sup>b</sup>	AY788483 <sup>b</sup>	
	<i>E. eunice</i> (Hübner 1807)	AY788624 <sup>b</sup>	AY788738 <sup>b</sup>	AY788499 <sup>b</sup>	
	<i>E. ithomioides alsina</i> (Hewitson 1869)	EF493933 <sup>l</sup>	EF493983 <sup>l</sup>	EF493876 <sup>l</sup>	
	<i>E. ithomioides eutropiaNW104-7</i> (Hewitson 1874)	EF493940 <sup>l</sup>	EF493990 <sup>l</sup>	EF493883 <sup>l</sup>	
	<i>E. ithomioides eutropiaNW120-17</i>	EF493937 <sup>l</sup>	EF493987 <sup>l</sup>	EF493880 <sup>l</sup>	
	<i>E. lansdorfii</i> (Godart 1819)	EF493938 <sup>l</sup>	EF493988 <sup>l</sup>	EF493881 <sup>l</sup>	
	<i>E. letitia</i> (Hewitson 1869)	AY788625 <sup>b</sup>	AY788739 <sup>b</sup>	AY788500 <sup>b</sup>	
	<i>E. levina</i> (Hewitson 1872)	EF493939 <sup>l</sup>	EF493989 <sup>l</sup>	EF493882 <sup>l</sup>	
	<i>E. nauplius</i> (Linnaeus 1758)	EF493944 <sup>l</sup>	EF493994 <sup>l</sup>	EF493887 <sup>l</sup>	
	<i>E. pelonia</i> (Hewitson 1852)	AY788626 <sup>b</sup>	AY788740 <sup>b</sup>	AY788501 <sup>b</sup>	
	<i>E. perna aveyrona</i> (Bates 1864)	EF493934 <sup>l</sup>	EF493984 <sup>l</sup>	EF493877 <sup>l</sup>	
	<i>E. perna perna</i> (Hewitson 1852)	EF493941 <sup>l</sup>	EF493991 <sup>l</sup>	EF493884 <sup>l</sup>	
	<i>E. philyra</i> (Hewitson 1852)	EF493943 <sup>l</sup>	EF493993 <sup>l</sup>	EF493886 <sup>l</sup>	
	<i>E. plaginota</i> (as <i>E. nauplius plagiata</i> Röber 1913)	AF187801 <sup>a</sup>			
	<i>E. polina</i> (Hewitson 1852)	EF493945 <sup>l</sup>	EF493995 <sup>l</sup>	EF493888 <sup>l</sup>	
	<i>E. quintilla</i> (as <i>E. ithomioides quintilla</i> Higgins 1981)	AY788627 <sup>b</sup>	AY788741 <sup>b</sup>	AY788502 <sup>b</sup>	
	<i>E. sestia</i> (as <i>E. emerantia sestia</i> Hewitson 1869)	AY788628 <sup>b</sup>	AY788742 <sup>b</sup>	AY788503 <sup>b</sup>	
	<i>E. sticta</i> (Schaus 1913)	EF493946 <sup>l</sup>	EF493996 <sup>l</sup>	EF493889 <sup>l</sup>	
	<i>Phyciodes</i>	<i>P. batesii</i> (Reakirt 1866)	AF187747 <sup>a</sup>	EF494005 <sup>l</sup>	EF493898 <sup>l</sup>
		<i>P. cocyta</i> (Cramer 1777)	AF187755 <sup>a</sup>	AY090192 <sup>f</sup>	AY090158 <sup>f</sup>
		<i>P. graphica</i> (Felder 1869)		AY788790 <sup>b</sup>	AY788550 <sup>b</sup>
<i>P. mylitta</i> (Edwards 1861)		AF187785 <sup>a</sup>	AY788791 <sup>b</sup>	AY788551 <sup>b</sup>	
<i>P. orseis</i> (Edwards 1871)		AY156631 <sup>m</sup>	AY788792 <sup>b</sup>	AY788552 <sup>b</sup>	
<i>P. pallascens</i> (Felder 1869)		AY156640 <sup>m</sup>	AY788793 <sup>b</sup>	AY788553 <sup>b</sup>	
<i>P. pallida</i> (Edwards 1864)		AF187792 <sup>a</sup>	AY788794 <sup>b</sup>	AY788554 <sup>b</sup>	
<i>P. phaon</i> (Edwards 1864)		AF187798 <sup>a</sup>	AY788795 <sup>b</sup>	AY788555 <sup>b</sup>	
<i>P. picta</i> (Edwards 1865)		AF187800 <sup>a</sup>	AY788796 <sup>b</sup>	AY788556 <sup>b</sup>	
<i>P. pulchella</i> (Boisduval 1852)		AY156662 <sup>m</sup>	AY788797 <sup>b</sup>	AY788557 <sup>b</sup>	
<i>P. tharos</i> (Drury 1773)		AF187807 <sup>a</sup>	AY788798 <sup>b</sup>	AY788558 <sup>b</sup>	
<i>Tegosa</i>		<i>T. anieta</i> (Hewitson 1864)	AY788681 <sup>b</sup>	AY788819 <sup>b</sup>	AY788579 <sup>b</sup>
		<i>T. claudina</i> (Eschscholtz 1821)	EF493957 <sup>l</sup>	EF494015 <sup>l</sup>	EF493908 <sup>l</sup>
		<i>T. etia</i> (Hewitson 1868)	EF493961 <sup>l</sup>	EF494019 <sup>l</sup>	EF493912 <sup>l</sup>
	<i>T. guatemalena</i> (Bates 1864)	HM431605 <sup>e</sup>			
	<i>T. infrequens</i> (Higgins 1981)	EF493962 <sup>l</sup>	EF494020 <sup>l</sup>	EF493913 <sup>l</sup>	
	<i>T. orobia</i> (Hewitson 1864)	EF493967 <sup>l</sup>	EF494025 <sup>l</sup>	EF493918 <sup>l</sup>	
	<i>T. selene</i> (Röber 1913)	EF493965 <sup>l</sup>	EF494023 <sup>l</sup>	EF493916 <sup>l</sup>	
	<i>T. similis</i> (Higgins 1981)	EF493966 <sup>l</sup>	EF494024 <sup>l</sup>	EF493917 <sup>l</sup>	
	<i>T. tissoides</i> (Hall 1928)	AY788682 <sup>b</sup>	AY788820 <sup>b</sup>	AY788580 <sup>b</sup>	
	<i>T. berenice</i> (Felder and Felder 1862)	EF493968 <sup>l</sup>	EF494026 <sup>l</sup>	EF493919 <sup>l</sup>	
<i>Telenassa</i>	<i>T. delphia</i> (Felder and Felder 1861)	EF493970 <sup>l</sup>	EF494029 <sup>l</sup>	EF493922 <sup>l</sup>	
	<i>T. fontus</i> (Hall 1928)	EF493974 <sup>l</sup>	EF494033 <sup>l</sup>	EF493926 <sup>l</sup>	
	<i>T. teletusa</i> (Godart 1824)	EF493971 <sup>l</sup>	EF494030 <sup>l</sup>	EF493923 <sup>l</sup>	
	<i>T. trimaculata</i> ( <i>T. delphia trimaculata</i> Hewitson 1869)	AY788683 <sup>b</sup>	AY788821 <sup>b</sup>	AY788581 <sup>b</sup>	
<i>Antillea</i>	<i>A. pelops</i> (Drury 1773)	GQ864733 <sup>n</sup>	GQ864827 <sup>n</sup>	GQ864421 <sup>n</sup>	
	<i>A. proclea</i> (Doubleday 1874)	EF493928 <sup>l</sup>	EF493976 <sup>l</sup>	EF493869 <sup>l</sup>	
<i>Atlantea</i>	<i>A. pantone</i> (Kaye 1906)	GQ864741 <sup>n</sup>	GQ864835 <sup>n</sup>	GQ864429 <sup>n</sup>	
<i>Dagon</i>	<i>D. pusilla</i> (Salvin 1869)	EF493932 <sup>l</sup>	EF493982 <sup>l</sup>	EF493875 <sup>l</sup>	
<i>Gnathotriche</i>	<i>G. exclamationis</i> (Kollar 1850)	AY788629 <sup>b</sup>	AY788748 <sup>b</sup>	AY788509 <sup>b</sup>	
	<i>G. mundina</i> (Druce 1876)	EF493927 <sup>l</sup>	EF493975 <sup>l</sup>	EF493868 <sup>l</sup>	
<i>Higginsius</i>	<i>H. fasciata</i> (Hopffer 1874)	AY788630 <sup>b</sup>	AY788749 <sup>b</sup>	AY788510 <sup>b</sup>	
<i>Janatella</i>	<i>J. fellula</i> (Schaus 1902)	EF493947 <sup>l</sup>	EF493997 <sup>l</sup>	EF493890 <sup>l</sup>	
	<i>J. hera</i> (Cramer 1779)	EF493973 <sup>l</sup>	EF494032 <sup>l</sup>	EF493925 <sup>l</sup>	
	<i>J. leucodesma</i> (Felder and Felder 1861)	AY788641 <sup>b</sup>	AY788761 <sup>b</sup>	AY788521 <sup>b</sup>	
	<i>Mazia</i>	<i>M. amazonica</i> (Bates 1864)	AY788654 <sup>b</sup>	AY788773 <sup>b</sup>	AY788533 <sup>b</sup>
<i>Microtia</i>	<i>M. elva</i> (Bates 1864)	AY788660 <sup>b</sup>	AY788787 <sup>b</sup>	AY788547 <sup>b</sup>	
<i>Ortilia</i>	<i>O. orthia</i> (Hewitson 1864)	EF493951 <sup>l</sup>	EF494001 <sup>l</sup>	EF493894 <sup>l</sup>	
	<i>O. dicoma</i> (Hewitson 1864)	EF493948 <sup>l</sup>	EF493998 <sup>l</sup>	EF493891 <sup>l</sup>	
	<i>O. gentina</i> (Higgins 1981)	EF493950 <sup>l</sup>	EF494000 <sup>l</sup>	EF493893 <sup>l</sup>	
	<i>O. ithra</i> (Kirby 1900)	EF493949 <sup>l</sup>	EF493999 <sup>l</sup>	EF493892 <sup>l</sup>	
	<i>O. liriopie</i> (Cramer 1775)	EF493972 <sup>l</sup>	EF494031 <sup>l</sup>	EF493924 <sup>l</sup>	
	<i>O. orticas</i> (Schaus 1902)	EF493952 <sup>l</sup>	EF494002 <sup>l</sup>	EF493895 <sup>l</sup>	
	<i>O. velicaNW106-5</i> (Hewitson 1864)	EF493954 <sup>l</sup>	EF494004 <sup>l</sup>	EF493897 <sup>l</sup>	
	<i>O. velicaNW114-7</i>	EF493953 <sup>l</sup>	EF494003 <sup>l</sup>	EF493896 <sup>l</sup>	

(continued on next page)

Table 2 (continued)

Genus	Species	COI	Ef1-a	Wgl
<i>Phystis</i>	<i>P. simois</i> (Hewitson 1864)	EF493956 <sup>l</sup>	EF494014 <sup>l</sup>	EF493907 <sup>l</sup>
<i>Vanessula</i>	<i>V. milca</i> (Hewitson 1873)	AY788691 <sup>b</sup>	AY788829 <sup>b</sup>	AY788589 <sup>o</sup>
<i>Hypolimnas</i>	<i>H. bolina</i> (Linnaeus 1758)	EF683668 <sup>k</sup>	AY090190 <sup>f</sup>	AF412775 <sup>b</sup>
<i>Doleschallia</i>	<i>D. bisaltidae</i> (Cramer 1777)	AY788621 <sup>b</sup>	AY788735 <sup>b</sup>	AY788496 <sup>b</sup>

<sup>a</sup> Wahlberg and Zimmermann (2000).

<sup>b</sup> Wahlberg et al. (unpublished).

<sup>c</sup> Janzen and Hajibabaei (unpublished).

<sup>d</sup> This study

<sup>e</sup> International Barcode of Life (iBOL)

<sup>f</sup> Wahlberg et al. (2003a,b).

<sup>g</sup> Lukhtanov et al. (2009).

<sup>h</sup> Zimmermann et al. (2000).

<sup>i</sup> Dinca et al. (2011).

<sup>j</sup> Leneuve et al. (2009).

<sup>k</sup> Min et al. (unpublished).

<sup>l</sup> Wahlberg and Freitas (2007).

<sup>m</sup> Wahlberg et al. (2003a,b).

<sup>n</sup> Wahlberg et al. (2009).

<sup>o</sup> Nylin et al. (2001).

We generated alignments of all taxa for each gene region using MUSCLE as implemented in Geneious Pro 4.8.5. Alignments were then edited manually, including translating each gene region to check for premature stop codons and to otherwise check the accuracy of the alignment. We generated additional alignments using ClustalW as implemented in Geneious Pro 4.8.5 and compared these to the MUSCLE alignments but detected no significant variation. We used jModelTest to select the most appropriate model of nucleotide substitution for each gene region (Posada, 2008).

### 2.3. Time calibration

We constrained the ages of the divergences between the outgroups (*Doleschallia bisaltide* (Kallimini), *Hypolimnas bolina* (Junoiiini) and *Vanessula milca* (Nymphalidae)) and the Melitaeini, and between outgroup species *H. bolina* and *V. milca* based on Wahlberg et al. (2009), who utilized fossil calibration data for seven species to estimate minimum divergence times (Emmel et al., 1992; Nel et al., 1993; Scott and Wright, 1990; Peñalver and Grimaldi, 2006; Kawahara, 2009). Because we used a secondary estimate of node calibration, we specified a normal distribution prior to estimate root divergence times (Ho and Phillips, 2009). We constrained the timing of the divergence between the outgroup species (*Doleschallia bisaltide* (Kallimini), *Hypolimnas bolina* (Junoiiini) and *Vanessula milca* (Nymphalidae)), and the Melitaeini as a mean of 42.53 MYA with a standard deviation of 2.4 MY. We constrained the divergence of the *H. bolina* and *V. milca* branches as 39.2 MYA with a stdev of 3 MY (Wahlberg et al., 2009).

### 2.4. Phylogenetic analysis

We estimated phylogeny for the combined dataset and each gene independently using MrBayes v. 3.2.2 under models of substitution chosen using jModelTest (Huelsenbeck et al., 2001; Huelsenbeck and Ronquist, 2001; Posada, 2008). We ran 4 replicated analyses each with one cold and three incrementally heated chains (temperature = 0.1) for 50 million iterations, logging the current state every 5000 iterations. We assessed convergence and mixing of the chains using Tracer and AWTY (Wilgenbusch et al., 2004; Rambaut et al., 2013), ensuring that all 4 analyses were sampling from the same distribution and that no clear trends were visible in the MCMC samples. We removed the first 25% of each analysis as burnin and summarized the results using MrBayes internal functions.

We also estimated tree topology and timing of divergence simultaneously under a relaxed clock. Using the CIPRES portal (Miller et al., 2010), we performed BEAST (v 1.7.1) analyses on the aligned gene regions (Drummond et al., 2012). Each of the three genes was partitioned separately, as was each codon position. For each gene, the substitution model was set to GTR with estimated base frequencies. Among-site rate heterogeneity was modeled using a discrete gamma distribution,  $\Gamma$ . We assumed an uncorrelated lognormal distribution to describe the prior probability density on branch-specific substitution rates (Drummond et al., 2006). We specified a birth–death stochastic branching process model to generate a prior on node ages. The MCMC chain was set to run for 50 million iterations and logged every 5000 generations. We repeated the BEAST analysis six times.

Log files from the six replicate analyses were combined using LogCombiner (v 1.7.1) from the BEAST package. We also used TreeAnnotator (v 1.7.1) from the BEAST package to summarize the posterior sample of trees to produce a maximum clade credibility tree and to summarize the posterior estimates of the input parameters. We assessed convergence of the MCMC using Tracer (v 1.5), and upon visual inspection we set the burn-in for the combined runs to 2.5 million iterations.

### 2.5. Data availability

We deposited all newly generated sequence data to Genbank (Accession numbers KM042217–KM042298). Our alignment and the resulting phylogenies are available in TreeBase (<http://purl.org/phylo/treebase/phyloids/study/TB2:S16045>).

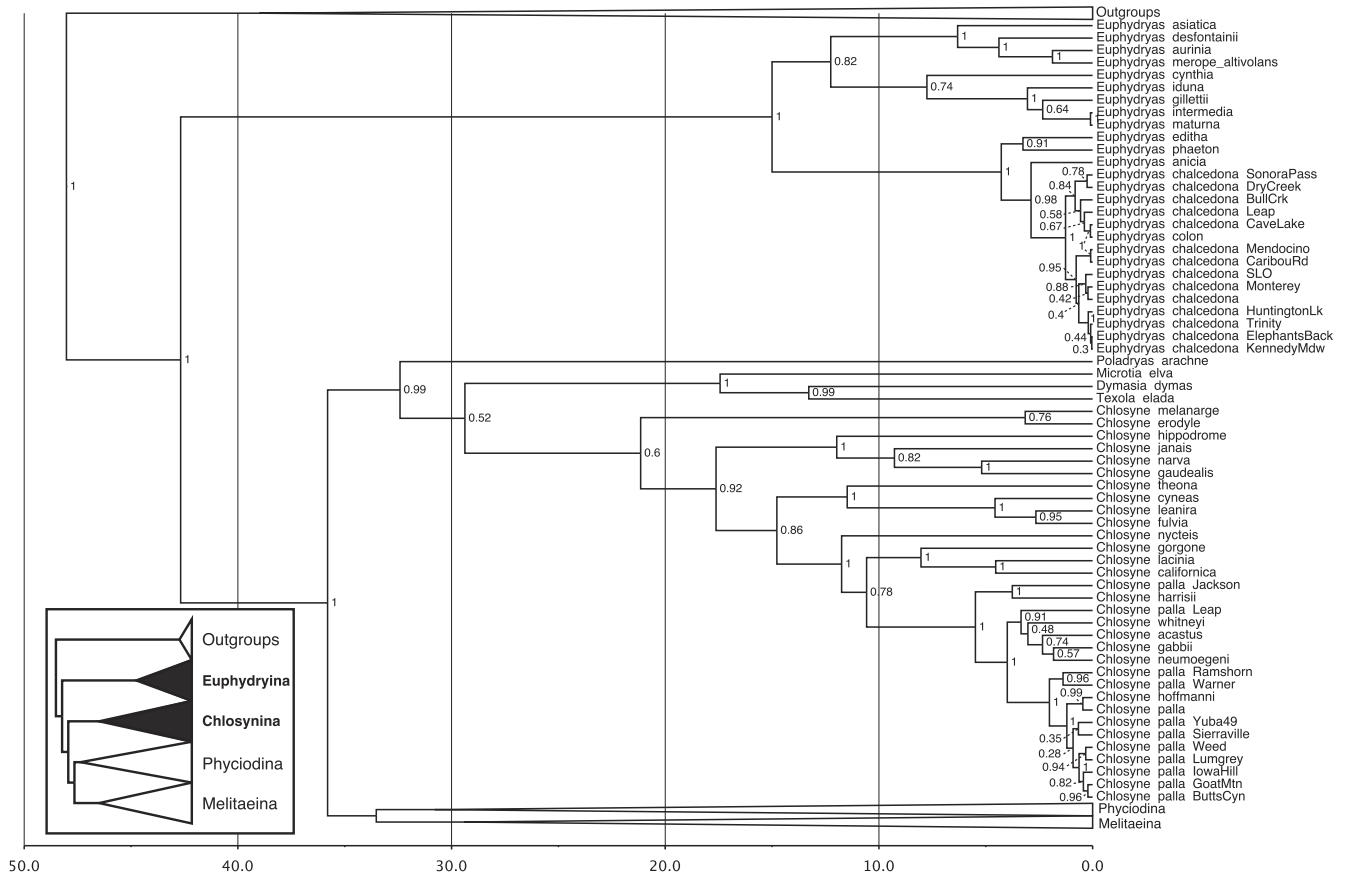
## 3. Results

### 3.1. Comparison of MrBayes gene trees vs. consensus tree

At the subtribe level the trees recovered by the nuclear markers *EF1 $\alpha$*  and *wg* via MrBayes analyses were largely uninformative, while the *COI* tree and consensus tree were largely in agreement with each other. We therefore restrict our remaining discussion to the MrBayes and BEAST consensus trees.

### 3.2. Comparison of MrBayes consensus tree and BEAST tree

Both the BEAST tree (Figs. 1–3, Supplementary File 1) and MrBayes consensus tree (Supplementary File 2) recovered four well-supported clades within the Melitaeini; both trees were also



**Fig. 1.** Phylogeny of Melitaeini depicting relationships for subtribes Euphydryina and Chlosynina (remaining clades have been collapsed for clarity of presentation) as recovered by BEAST analysis, utilizing mitochondrial gene region *COI* and nuclear gene regions *EF1 $\alpha$*  and *wg*. Node labels refer to clade posterior probabilities, while the x-axis represents time in millions of years. *Inset*: Backbone phylogeny of the Melitaeini.

in agreement on the branching order of these clades as well as on the genus composition of each. The species composition of these four clades roughly correspond to the subtribes described by Wahlberg and Zimmermann (2000), while the branching order more closely resembled that of Wahlberg et al. (2005).

### 3.3. Four clades

#### 3.3.1. Euphydryina

The clade containing all members of the genus *Euphydryas* diverged from the rest of the Melitaeini ~42.68 MYA (divergence time clade posterior probability, DTCPP = 1) (Fig. 1). The most recent ancestor (MRCA) to this monogeneric clade corresponding to Wahlberg and Zimmermann's Euphydryina (Euphydryina in later usage) underwent a speciation event ~15 MYA (DTCPP = 1).

The trees recovered by the BEAST analysis and the MrBayes analysis differ in their placement of *E. anicia*, *E. chalcedona*, and *E. colon*. Scott (1987) combines these three taxa into one species, *E. chalcedona*, while Pelham (2008) splits them. Here we retain the GenBank labels for *E. anicia* and *E. colon*, while labeling all new specimens that we collected for this project as *E. chalcedona*. Treating *E. anicia* as a separate species renders *E. chalcedona* paraphyletic in both analyses.

#### 3.3.2. Chlosynina

The divergence event that gave rise to the MRCA of Chlosynina and the MRCA of Melitaeina and Phyciodina occurred approximately 35.8 MYA (DTCPP = 1) (Fig. 1). Our divergence time

estimate analysis recovers strong posterior support (DTCPP = 0.99) for the split of *Poladryas* from the rest of the Chlosynina ~32.4 MYA; however, the Bayesian consensus tree recovers this node with only weak support (MBCPP = 0.56).

#### 3.3.3. Melitaeina

The divergence event that gave rise to the two remaining subtribes occurred approximately 33.52 MYA (MBCPP = 1) (Fig. 2). One of these groups, corresponding to Melitaeina, consists of the genera *Melitaea*, *Higginsius*, and *Gnathotriche*. The MRCA of this group diverged ~29.43 MYA (MBCPP = 1).

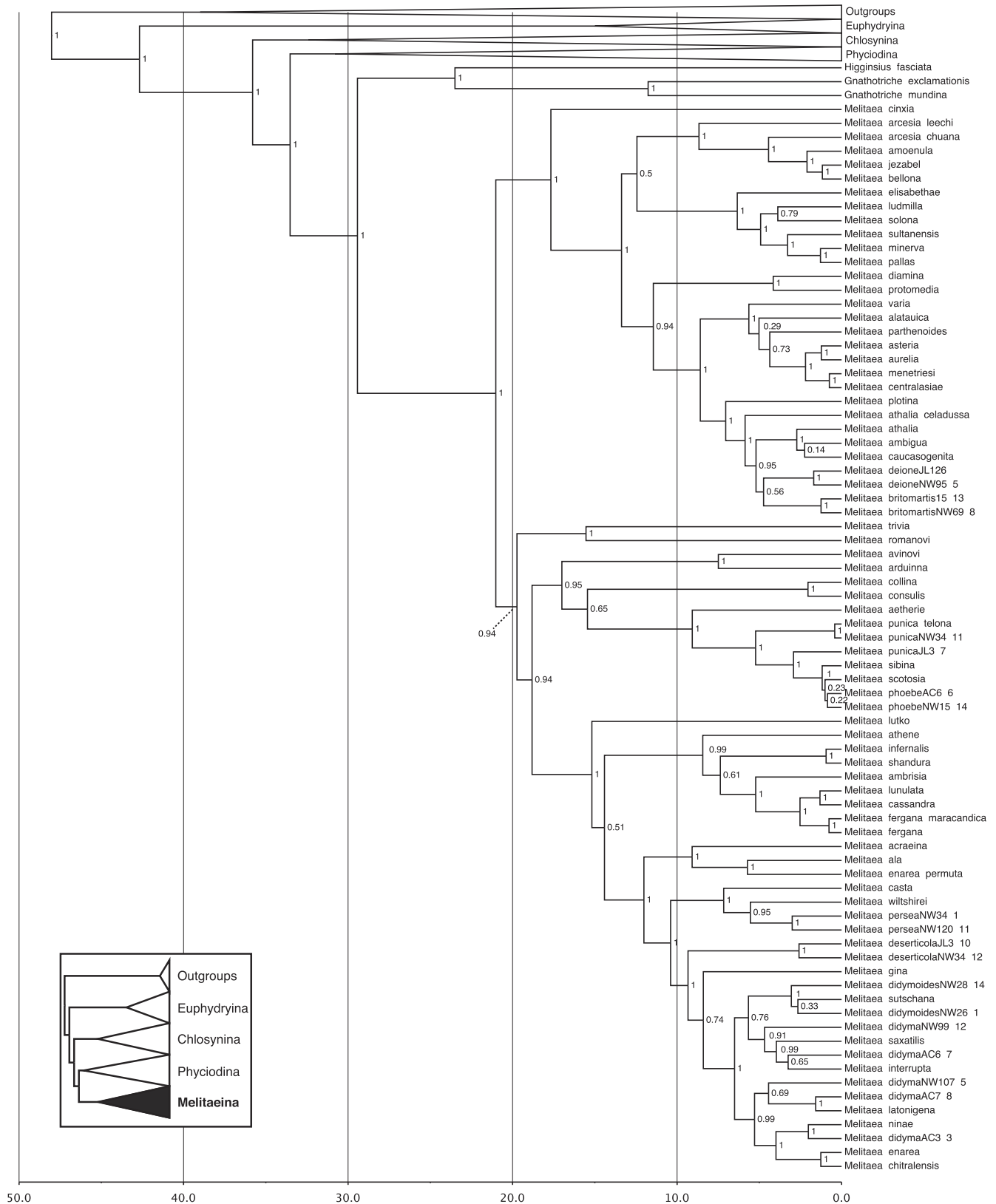
Within the subtribe Melitaeina, we noted several differences between the topology of the MrBayes tree and the BEAST tree. The two analyses recovered different topologies regarding the following species: *M. solana*; *M. ambigua* vs. *M. athalia*; and *M. consulis* + *M. collina* vs. *M. avinonvi* + *M. arduinna*. The MrBayes analysis returned polytomies at several places where the BEAST analysis was resolved: the *M. alatuica* branch; the *M. lutko* branch; the *M. gina* branch; the *M. didyma*NW99\_12 branch; and the *M. phoebe*AC\_6 vs. *M. phoebe*NW15\_14 branches.

#### 3.3.4. Phyciodina

The other group, corresponding to Phyciodina, consists of the genera *Mazia*, *Ortilia*, *Phyciodes*, *Tegosa*, *Eresia*, *Castilia*, *Telenassa*, *Dagon*, *Janatella*, *Anthanassa*, *Phystis*, *Atlantea*, and *Antillea*, who share a MRCA ~30.8 MYA (MBCPP = 1) (Fig. 3).

The topology of the trees recovered by the MrBayes and BEAST analyses differed in regard to several taxa within the Phyciodina.

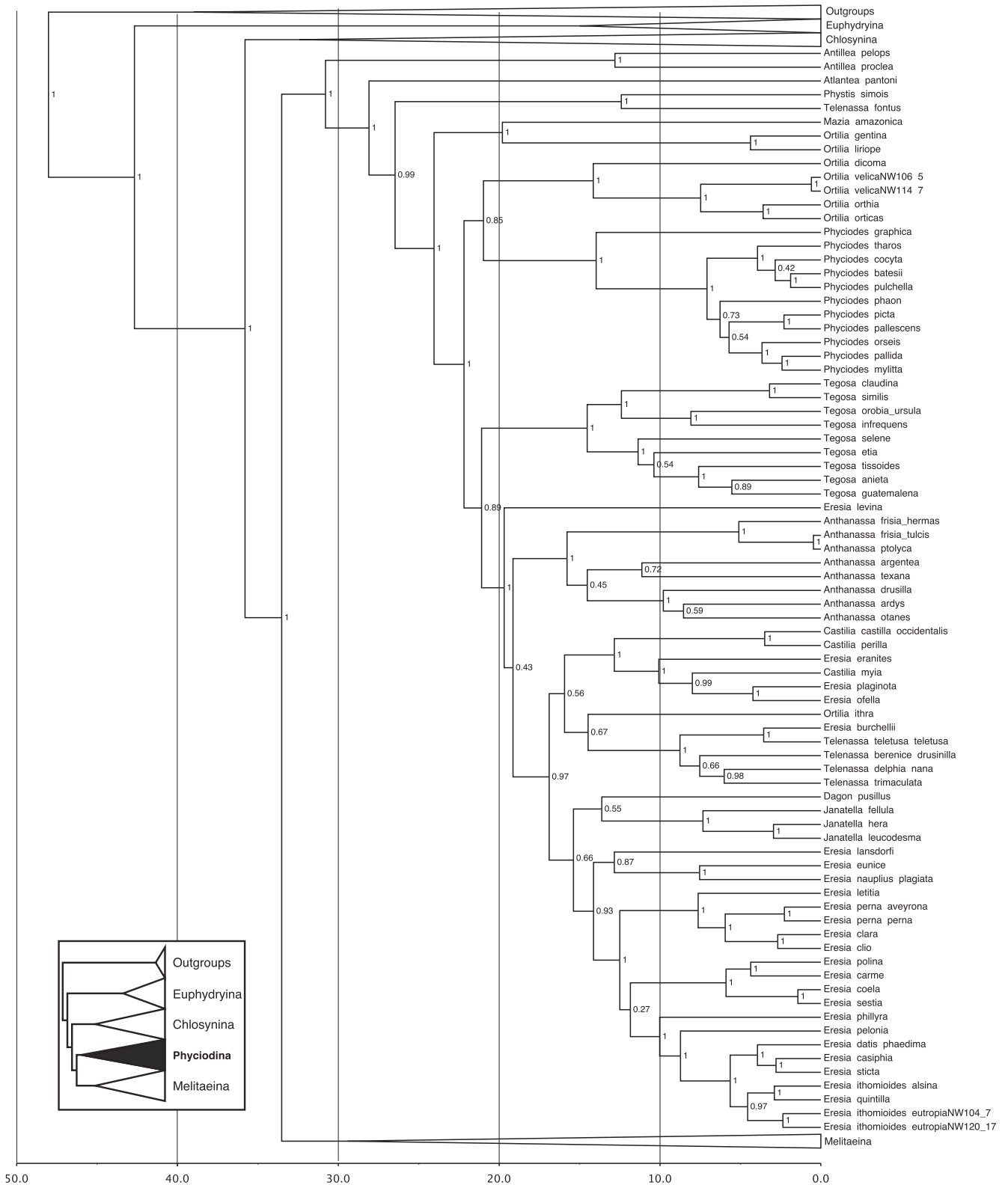




**Fig. 2.** Phylogeny of Melitaeini depicting relationships for subtribe Melitaeina (remaining clades have been collapsed for clarity of presentation) as recovered by BEAST analysis, utilizing mitochondrial gene region *COI* and nuclear gene regions *EF1 $\alpha$*  and *wg*. Node labels refer to clade posterior probabilities, while the x-axis represents time in millions of years. *Inset*: Backbone phylogeny of the Melitaeina.

The *Anthanassa* polytomy recovered by MrBayes analysis is resolved in the BEAST analysis by placing the *A. frisia* + *A. ptolyca* as subtending the rest of the genus. The two methods also differ

in the placement of *A. otanes* and *A. drusilla*, as well as in the placement of several *Eresia* species; the placement of *Tegosa selena* and *T. etia*; and the placement of *Phycodes batesii* and *P. pulchella*.



**Fig. 3.** Phylogeny of Melitaeini depicting relationships for subtribe Phyciodina (remaining clades have been collapsed for clarity of presentation) as recovered by BEAST analysis, utilizing mitochondrial gene region *COI* and nuclear gene regions *EF1 $\alpha$*  and *wg*. Node labels refer to clade posterior probabilities, while the x-axis represents time in millions of years. *Inset*: Backbone phylogeny of the Melitaeini.

**4. Discussion**

Our finding of four distinct, well-supported clades within the Melitaeini is consistent with previous findings by Wahlberg and

Zimmermann (2000) and Wahlberg et al. (2005), which is unsurprising given the fact that the studies use similar gene regions. However, we found that the subtribe branching order described by Wahlberg and Zimmermann (2000) differs from the hypothesis

proposed here, and while subtribe branching order is consistent with Wahlberg et al. (2005) there were other differences between this study and our results, as well. We discuss these differences below.

#### 4.1. Four clades: comparison to previous hypotheses

Our phylogenetic analysis recovered four distinct, well-supported clades within Melitaeini, roughly corresponding to those described by Wahlberg and Zimmermann (2000), although the branching order more closely resembled that of Wahlberg et al. (2005). Here we discuss these results in the context of previous phylogenetic hypotheses reported for each of the four clades.

##### 4.1.1. Euphydryina

Like Zimmermann et al. (2000), we found no support for Higgins' proposed split of *Euphydryas anicia*, *E. colon*, *E. chalcedona*, and *E. editha* into a distinct genus ('*Occidryas*') (Higgins, 1981). However, together with *E. phaeton*, these species do form a monophyletic group that diverged ~15 MYA ago from the MRCA of *E. aurinia*, *E. merope altivans*, *E. desfontainii*, *E. asiatica*, *E. cynthia*, *E. iduna*, *E. intermedia*, *E. maturna*, and *E. gillettii* (DTCPP = 1). Higgins also proposed that the latter five species be designated a separate genus called '*Hypodryas*' while the former four species be designated a distinct genus called '*Eurodryas*'. We do recover these proposed taxa as monophyletic clades that diverged from one another ~12.26 MYA, although support for '*Hypodryas*' as well as the combined clade of '*Hypodryas*' and '*Eurodryas*' is modest (DTCPP = 0.74 and 0.82, respectively). However, Higgins' proposed split of *Euphydryas* has been rejected by subsequent authors (Higgins, 1981; Zimmermann et al., 2000).

##### 4.2. Chlosynina

This study presents the most complete molecular phylogeny of the *Chlosyne*-group subtribe, including 21 of the approximately 27 species in the nominate genus. The genus *Poladyras* comprises two species, one of which (*P. arachne*) is included here; the remaining genera (*Texola*, *Microtia*, and *Dymasia*) are each monotypic, and are also included in this study. Two other molecular phylogenies have been presented pertaining to this group: the first included only 15 species of *Chlosyne* and does not include *Microtia* (Wahlberg and Zimmermann, 2000), while the second included *Microtia* but only included 11 species of *Chlosyne* (Wahlberg et al., 2005).

Higgins (1981) placed *Microtia* within the *Chlosyne*-group based on morphological characters and Wahlberg et al. (2005) recovered a similar placement using molecular data. We found strong support for placement of *Microtia* within Chlosynina, sister to *Texola/Dymasia* (CPP = 1). These three genera appear to have diverged from *Chlosyne* ~29.4MYA, while *Microtia* diverged from *Texola* and *Dymasia* ~17.4 MYA. Wahlberg and Freitas (2007) estimated more ancient divergences, ~37.5 MYA and ~26MYA, respectively. Differences can most likely be attributed to difference in calibration constraints applied in the studies.

Of particular interest in our study is the placement of a group of Nearctic *Chlosyne* species occurring in the western United States. Specifically, we were interested in the taxonomic relationship of *C. palla*, *C. whitneyi*, *C. acastus* (and an included subspecies, *C. acastus neumogeni*), *C. hoffmanni*, and *C. gabbii*. We found strong posterior support for the monophyly of this group, the members of which often have parapatric or sympatric distributions. Divergence time estimates suggest that this clade diverged from the rest of *Chlosyne* ~5.5MYA (CPP = 1), near the end of the Miocene and beginning of the Pliocene. Previous studies of this group have only included *C. palla*, *C. acastus*, and *C. acastus neumogeni* (treated as a

full species by Wahlberg and Zimmermann, 2000). While the latter is not considered a full species (Pelham, 2008), we found it to be sister to *C. gabbii* rather than to *C. acastus*, having diverged ~2.3MYA (CPP = 0.75). These three taxa together are weakly supported as sister taxa to the alpine taxon *C. whitneyi*, diverging ~3MYA (CPP = 0.48). The name *C. whitneyi* was once used for high-elevation (sub-alpine) populations of a taxon now called *C. palla altasierra* (Emmel et al., 1998) (here provisionally labeled as *C. palla* "Leap"). We found this taxon to be more closely related to *C. acastus/C. gabbii/C. whitneyi* than to the nominate species *C. palla*, with which it is parapatric. This finding may explain why female forms of the California populations of *C. palla* are polymorphic and the *C. acastus/C. gabbii/C. whitneyi/C. palla altasierra* group are not. We hypothesize that this female-limited polymorphism either arose after *C. palla* diverged from the others, or the polymorphism is the ancestral state to these species and was lost in the *C. acastus/C. gabbii/C. whitneyi/C. palla altasierra* lineage. Ancestral state reconstruction of this trait was uninformative due to recent divergences and subsequent low phylogenetic signal (data not shown).

The location of *C. hoffmanni* nested within population-level specimens of *C. palla* was unexpected. These two species can be difficult to distinguish, although the specimen used here was examined by multiple experts and appears to be a good example of *C. hoffmanni*. However, we urge caution in interpreting this finding, and instead suggest that sequence data from additional specimens be analyzed to clarify this relationship.

##### 4.3. Melitaeina

A previous study by Wahlberg et al. (2009) estimated that Melitaeini diverged from Nymphalinae ~50 MYA. Our study places this divergence within a similar time frame and our 95% HPD estimate contains the Wahlberg et al. estimate. The genus *Melitaea* has sometimes been split into two genera, *Melitaea* and *Mellicta* (Asher et al., 2001; Beccaloni et al., 2013). We found that, with the exception of *M. sutschana*, the species usually included in *Mellicta* (*M. alatuica*, *ambigua*, *asteria*, *athalia*, *aurelia*, *britomartis*, *caucaso-genita*, *celadussa*, *centralasiae*, *deione*, *menetriesi*, *parthenoides*, *plotina*, and *varia*) do form a monophyletic group, but designating them as a separate genus would render *Melitaea* paraphyletic. This '*Mellicta*' group diverged from the rest of the *Melitaea* ~11.4 MYA (CPP = 0.95).

While the *Melitaea/Mellicata* split does not seem to be valid, we did find two distinct clades within *Melitaea* that have also been described by Leneveu et al. (2009) utilizing the same gene regions, the *Didymaeformia* and *Melitaea* clades. Species composition of the two clades was consistent between the two studies, however we were able to include *M. alatuica* and *M. jezebel* in the *Melitaea* clade (not included in the study by Leneveu et al.). Unlike the tree presented by Leneveu et al., however, we found strong posterior support for this node, which is estimated to have split ~21 MYA (CPP = 1).

Within-clade differences at this level were minimal in the *Didymaeformia* clade. Branching order and species relationships were similar between our study and Leneveu et al. with the exception of the *M. arduina* + *M. avinovi* branch and *M. consulis* + *M. collina* branch. Support for the deeper node (representing the divergence of *M. arduina* + *M. avinovi* in our study) is higher in our study than the Leneveu et al. study (CPP = 0.95 vs. 0.87, respectively) (Leneveu et al., 2009). In both studies the interval between this node and the next is short (>1 my).

We found much greater differences between the two studies within the *Melitaea* clade. While Leneveu et al. found the *diamina* group (*M. diamina* and *M. protomedia*) as sister to a clade comprising the *arcesia*, *minerva*, and *athalia* groups with strong posterior

node support (CPP = 1), we found the *diamina* group to be sister to the *athalia* group, again with slightly weaker support (CPP = 0.95). We also found the *arcesia* and *minerva* groups to be sister taxa, and when taken together are sister to the *athalia/diamina* clade. The previous study, however, found the *athalia* and *minerva* groups to be sister clades, subtended by the *arcesia* clade, which is itself subtended by the *diamina* clade.

#### 4.4. Phyciodina

Of the four Melitaini subtribes, the most problematic is the Phyciodina. This appears to be caused by difficulty in naming species, whether through misidentification of GenBank specimens or the need for a thorough taxonomic revision of this group (or both). Many species in this group are involved in mimicry systems, leading to phenotypic similarity and thus confusion concerning proper identification. Assuming that all species are in fact correctly identified, a major revision of this group seems called for, as many of the genera are not monophyletic. Wahlberg and Freitas (2007) encountered a similar result in a previous molecular study of this group. While that study performed both parsimony and Bayesian phylogenetic analyses, most of the discussion pertains to the parsimony result, so we will focus our comparisons primarily on that phylogeny as well.

Wahlberg and Freitas found nine stable lineages within Phyciodina (*Antillea*, *Phystis*, *Mazia*, *Ortilia* s.s., *Tegosa*, *Phyciodes*, Brazilian “*Ortilia*”, *Anthanassa*, and *Eresia* s.l.). They recommended that the genera *Dagon*, *Janatella*, *Castilia*, and *Telenassa* s.s. be returned to the *Eresia*, undoing the split proposed by Higgins (Higgins, 1981; Wahlberg and Freitas, 2007). Several of these groups contain problematic taxa, though whether this is due to misidentification or misclassification is uncertain.

Like Wahlberg and Freitas (2007) we found that *Telenassa fontus* is sister to *Phystis* (CPP = 1), despite the fact that the remaining *Telenassa* are quite distant on the Phyciodina tree. Whether this species should be classified as *Phystis* or as a separate genus should be examined more closely. Barring this, *Phystis* forms a stable monotypic genus (CPP = 0.99).

The species “*Eresia*” *burchellii* is listed as such on Genbank but is treated elsewhere as *Telenassa*. Wahlberg and Freitas (2007) treat it as a subspecies of *T. teletusa* and estimate a split between it and the nominate subspecies to be > 5MYA. While our phylogeny also supports this arrangement for *Telenassa*, we found the split between *T. teletusa teletusa* and *T. teletusa burchellii* (“*Eresia*” *burchellii*) to be more recent (~3.5MYA).

We also found the placement of several members of the genus *Ortilia* to be problematic. Our phylogeny agrees with that of Wahlberg and Freitas (2007) in placing *O. liriopae* and *O. gentina* as sister to *Mazia*, with strong posterior branch support (CPP > 0.99). Wahlberg and Freitas identified a monophyletic clade of *Ortilia* that they called the Brazilian “*Ortilia*”, consisting of *O. dicoma*, *O. orthia*, *O. orticas*, and *O. velica*. We arrived at the same conclusion, with strong posterior node support for this clade (CPP = 1), and agree that this clade requires a new name as the generic type species, *O. liriopae*, is not included in this clade (Higgins, 1981; Wahlberg and Freitas, 2007). While both studies found *O. ithra* to be paraphyletic to the other *Ortilia*, the two studies differed in the placement of this branch. The hypotheses proposed by Wahlberg and Freitas place the species as sister to *Eresia lansdorfi* (parsimony tree, bootstrap < 50), subtending *Telenassa* with weak support (MrBayes, CPP = 0.64), and as sister to *Dagon pusillus* in their chronogram. They suggest placing it within *Eresia* (see below), while our phylogeny places it as sister to *Telenassa* (CPP = 0.67), with *D. pusillus* sister to *Janatella* (CPP = 0.55).

#### 4.5. Other clades of interest

Aside from the four distinct subtribe clades discussed above, this study examines the placement of several additional lineages that have traditionally been the subject of major phylogenetic uncertainty or disagreement. We discuss each of these in turn below.

#### 4.6. Placement of *Gnathotriche* and *Higginsius*

Studies have variously placed *Gnathotriche* and *Higginsius* with *Melitaea* + *Chlosyne* based on morphology (Higgins, 1981), within Phyciodina based on molecular sequence data (Wahlberg et al., 2005; Wahlberg and Freitas, 2007), and with Melitaeina based on molecular sequence data (Wahlberg and Freitas, 2007). We found strong support for placing these species sister to *Melitaea*, within the subtribe Melitaeina. Divergence times between *Gnathotriche/Higginsius* and *Melitaea* are similar to those of deep nodes within Phyciodina, e.g. *Antillea* or *Atlantea* and the remaining Phyciodina. This evidence plus Higgins' morphological treatment suggests that these three species together constitute a subtribe. If this finding is correct, it would influence the biogeographical hypothesis proposed by Wahlberg and Freitas (2007). Revisions of this hypothesis would need to take into account the divergence of the neotropical *Higginsius* and *Gnathotriche* ~6.4 MY after the divergence of the (neotropical and nearctic) *Chlosyne*-group, and ~4.1 MY after the divergence of the (neotropical and nearctic) Phyciodina.

#### 4.7. Placement of *Atlantea*, *Antillea*, *Ortilia*, *Phystis*, *Dagon*, *Mazia*, and *Janatella*

This is the first study to place *Atlantea* in a phylogeny, whether using morphological or molecular information. Higgins (1981) was unable to place this genus within any subtribe based on morphology, and suggested that it may constitute a distinct subtribe of the Melitaeina. The phylogenetic hypothesis presented here shows strong posterior support placing *Atlantea* within Phyciodina (CPP = 1), diverging from the other genera ~2.7 MY after *Antillea* (~28.1 MYA). This subtribe is comprised of Neotropical and Nearctic species, most of which are endemic to South America. *Atlantea* is endemic to the Greater Antilles, and its phylogenetic placement near *Antillea* suggests a fairly simple biogeographic scenario. Our placement of *Antillea* as the first Phyciodina taxon to diverge is consistent with the placement presented by Wahlberg and Freitas (2007), although our findings suggest a slightly more recent divergence (~30.8MYA vs. ~34MYA) (CPP = 1).

Wahlberg and Zimmermann (2000) and Wahlberg et al. (2005) did not include *Ortilia*, *Phystis*, or *Dagon* in their analyses, while the former also were unable to include *Mazia* and *Janatella*. The phylogeny produced by Wahlberg and Freitas (2007) places all of these genera within Phyciodina, which agrees with our result. While some of the specific placements within Phyciodina proposed by these two studies differ, it appears to be clear that all of these genera belong within this subtribe. Rather than belabor the remaining differences, we will simply agree with Wahlberg and Freitas that this subtribe is in need of careful, thorough taxonomic revision. This should include a morphological analysis of the GenBank specimens included in our molecular study to ensure that some of the taxonomic and phylogenetic disagreements are not due to simple identification errors.

## 5. Conclusion

We found four, well-supported clades within the tribe Melitaeini, largely corresponding to previously proposed subtribes.

Of these four, we found Euphydryina to have diverged first, ~42.67 MYA; this was followed by the branching of the Chlosynina ~35.8 MYA, and then by the divergence of Phyciodina and Melitaeina ~33.52 MYA. Within these subtribes, our most significant findings are the placement of *Gnathotriche* and *Higginsius* within Melitaeina, the placement of *Atlantea* within Phyciodina, and the description of the Chlosynina phylogeny.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.06.010>.

## References

- Asher, J., Warren, M., Fox, R., Harding, P., Jeffcoate, G., Jeffcoate, S., 2001. The Millennium Atlas of Butterflies of Britain. Oxford University Press, Oxford.
- Bates, H.W., 1862. Contributions to an insect fauna of the Amazon Valley. Lepidoptera: Heliconiidae. Trans. Linnean Soc. London 23, 495–566.
- Beccaloni, G., Scoble, M., Kitching, I., Simonsen, T., Robinson, G., Pitkin, B., Hine, A., Lyal, C., 2013. In: Roskov, Y., Kunze, T., Paglinawan, L., Orrell, T., Nicolson, D., Culham, A., Bailly, N., Kirk, P., Bourgoin, T., Baillargeon, G., Hernandez, F., De Wever, A. (Eds.), LepIndex: The Global Lepidoptera Names Index Species 2000 and ITIS Catalogue of Life. Species 2000, Reading, UK.
- Benson, W.W., 1972. Natural selection for Müllerian mimicry in *Heliconius erato* in Costa Rica. Science 176, 936–939.
- Brower, A.V.Z., 1994. Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences (Lepidoptera: Nymphalidae). Mol. Phylogenet. Evol. 3, 159–174.
- Brower, A.V.Z., Desalle, R., 1998. Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. Insect Mol. Biol. 7, 73–82.
- Brower, A.V.Z., Freitas, A.V.L., Lee, M.-M., Silva-Brandao, K.L., Whinnett, A., Willmott, K.R., 2006. Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. Syst. Entomol. 31, 288–301. <http://dx.doi.org/10.1111/j.1365-3113.2005.00321.x>.
- Brower, A.V.Z., Wahlberg, N., Ogawa, J.R., Boppré, M., Vane-Wright, R.I., 2010. Phylogenetic relationships among genera of danaine butterflies (Lepidoptera: Nymphalidae) as implied by morphology and DNA sequences. Syst. Biodivers. 8, 75–89. <http://dx.doi.org/10.1080/14772001003626814>.
- Brown, I.L., Ehrlich, P.R., 1980. Population biology of the checkerspot butterfly, *Euphydryas chalcedona*: structure of the Jasper Ridge colony. Oecologia 47, 239–251.
- Cho, S., Mitchell, A., Regier, J.C., Mitter, C., Poole, R.W., Friedlander, T.P., Zhao, S., 1995. A highly conserved nuclear gene for low-level phylogenetics: Elongation Factor 1a recovers morphology-based tree for heliothine moths. Mol. Biol. Evol. 12, 650–656.
- Dinca, V., Zakharov, E.V., Hebert, P.D., Vila, R., 2011. Complete DNA barcode reference library for a country's butterfly fauna reveals high performance for temperate Europe. Proc. Roy. Soc. B 278 (1704), 347–355.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699–710. <http://dx.doi.org/10.1371/journal.pbio.0040088>.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. pp. 3–6.
- Ehrlich, P.R., White, R.R., Singer, M.C., McKechnie, S.W., Gilbert, L.E., 1975. Checkerspot butterflies: a historical perspective. Science 188, 221–228.
- Ehrlich, P.R., Launer, A.E., Murphy, D.D., 1984. Can sex ratio be defined or determined? The case of a population of checkerspot butterflies. Am. Nat. 124, 527–539.
- Emmel, J.F., Emmel, T.C., Mattoon, S.O., 1998. A checklist of the butterflies and skippers of California. In: Emmel, T.C. (Ed.), Systematics of Western North American Butterflies. Mariposa Press, Gainesville, FL, pp. 825–836.
- Emmel, T.C., Minno, M.C., Drummond, B.A., 1992. Florissant butterflies: a guide to the fossil and present-day species of central Colorado. Stanford University Press, Stanford, CA.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3, 294–299.
- Gilbert, L.E., Singer, M.C., 1975. Butterfly ecology. Annu. Rev. Ecol. Syst. 6, 365–395.
- Harvey, D.J., 1991. Higher classification of the Nymphalidae, Appendix B. In: Nijhout, H.F., The Development and Evolution of Butterfly Wing Patterns. Smithsonian Institution Press, Washington, DC, pp. 255–273.
- Higgins, L.G., 1941. An illustrated catalogue of the Palearctic *Melitaea* (Lep. Rhopalocera). Trans. Roy. Entomol. Soc. London 91, 175–365.
- Higgins, L.G., 1950. A descriptive catalogue of the Palearctic Euphydryas (Lepidoptera: Rhopalocera). Trans. Roy. Entomol. Soc. London, pp. 101.
- Higgins, L.G., 1955. A descriptive catalogue of the genus *Melitaea* Billberg (Lepidoptera: Nymphalidae) and its species, with supplementary notes on the genera *Melitaea* and *Euphydryas*. Trans. Roy. Entomol. Soc. London 106, 1–127.
- Higgins, L.G., 1960. A revision of the melitaeine genus *Chlosyne* and allied species (Lepidoptera: Nymphalidae). Trans. Roy. Entomol. Soc. London 112, 381–465.
- Higgins, L.G., 1981. A revision of *Phyciodes* Hubner and related genera, with a review of the classification of the Melitaeini (Lepidoptera: Nymphalidae). Bull. Br. Museum (Natural History) 43, 77–243.
- Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58, 367–380.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
- Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314. <http://dx.doi.org/10.1126/science.1065889>.
- ICZN, 1999. International code of zoological nomenclature, fourth ed. London.
- Kawahara, A.Y., 2009. Phylogeny of snout butterflies (Lepidoptera: Nymphalidae: Libytheinae): combining evidence from morphology of extant, fossil, and recently extinct taxa. Cladistics 25, 263–278. <http://dx.doi.org/10.1111/j.1096-0031.2009.00251.x>.
- Leneveu, J., Chichvarkhin, A., Wahlberg, N., 2009. Varying rates of diversification in the genus *Melitaea* (Lepidoptera: Nymphalidae) during the past 20 million years. Biol. J. Linn. Soc. 97, 346–361. <http://dx.doi.org/10.1111/j.1095-8312.2009.01208.x>.
- Lukhtanov, V.A., Sourakov, A., Zakharov, E.V., Hebert, P.D., 2009. DNA barcoding Central Asian butterflies: increasing geographical dimension does not significantly reduce the success of species identification. Mol. Ecol. Resource 9 (5), 1302–1310.
- Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, LA, pp. 1–8.
- Nel, A., Nel, J., Balme, C., 1993. Un nouveau Lépidoptère Satyrinae fossile de l'Oligocène du sud-est de la France (Insecta, Lepidoptera, Nymphalidae). Linnaea Belgica 14, 20–36.
- Nylin, S., Nyblom, K., Ronquist, F., Janz, N., Belicic, J., M. Kallersjo, M., 2001. Phylogeny of Polytonia, Nymphalis, and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis. Zoo. J. Linn. Soc-Lond 132, 441–468.
- Pelham, J.P., 2008. A catalogue of the butterflies of the United States and Canada. J. Res. Lepidoptera 40, 1–672.
- Peñalver, E., Grimaldi, D., 2006. New data on Miocene butterflies in Dominican amber (Lepidoptera: Riodinidae and Nymphalidae) with the description of a new nymphalid. Am. Museum Novitates 3519, 1–17. [http://dx.doi.org/10.1206/00030082\(2006\)3519\[1:NDOMB\]2.0.CO;2](http://dx.doi.org/10.1206/00030082(2006)3519[1:NDOMB]2.0.CO;2).
- Penz, C.M., Mohammadi, N., Wahlberg, N., 2011. Neotropical *Blepolenis* butterflies: wing pattern elements, phylogeny, and Pleistocene diversification (Lepidoptera, Nymphalidae). Zootaxa 17, 5326.
- Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256. <http://dx.doi.org/10.1093/molbev/msn083>.
- Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2013. Tracer v.1.4. <<http://beast.bio.ed.ac.uk/Tracer>>.
- Scott, J.A., 1987. The Butterflies of North America: a Natural History and Field Guide. Stanford University Press, Stanford, CA, pp. 583.
- Scott, J.A., Wright, D.M., 1990. Butterfly Phylogeny and Fossils. In: Kudrna, O. (Ed.), Butterflies of Europe, vol. II. Aula-Verlag, Weisbaden, Germany, pp. 152–208.
- Sheppard, P.M., Turner, J.R.G., Brown, K.S., Benson, W.W., Singer, M.C., 1985. Genetics and the evolution of Müllerian mimicry in *Heliconius* butterflies. Philos. Trans. Roy. Soc. London 308, 433–610.
- Wahlberg, N., Brower, A.V.Z., Nylin, S., 2005. Phylogenetic relationships and historical biogeography of tribes and genera in the subfamily Nymphalinae (Lepidoptera: Nymphalidae). Biol. J. Linn. Soc. 86, 227–251. <http://dx.doi.org/10.1111/j.1095-8312.2005.00531.x>.
- Wahlberg, N., Freitas, A.V.L., 2007. Colonization of and radiation in South America by butterflies in the subtribe Phyciodina (Lepidoptera: Nymphalidae). Mol. Phylogenet. Evol. 44, 1257–1272. <http://dx.doi.org/10.1016/j.ympev.2007.04.012>.
- Wahlberg, N., Klemetti, T., Selonen, V., Hanski, I., 2002. Metapopulation structure and movements in five species of checkerspot butterflies. Oecologia 130, 33–43. <http://dx.doi.org/10.1007/S004420100775>.
- Wahlberg, N., Leneveu, J., Kodandaramaiah, U., Peña, C., Nylin, S., LAV, Brower, A.V.Z., Freitas, V.L., 2009. Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. Proceedings of the Royal Society B: Biological Sciences, vol. 276, pp. 4295–4302. (doi:10.1098/rspb.2009.1303).

- Wahlberg, N., Oliveira, R., Scott, J.A., 2003a. Phylogenetic relationships of *Phyciodes* butterfly species (Lepidoptera: Nymphalidae): complex mtDNA variation and species delimitations. *Syst. Entomol.* 28 (2), 257–274.
- Wahlberg, N., Weingartner, E., Nylin, S., 2003b. Towards a better understanding of the higher systematics of Nymphalidae (Lepidoptera: Papilionoidea). *Mol. Phylogenet. Evol.* 28 (3), 473–484.
- Wahlberg, N., Zimmermann, M., 2000. Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera : Nymphalidae) inferred from mitochondrial DNA sequences. *Cladistics* 16, 347–363. <http://dx.doi.org/10.1006/clad.2000.0136>.
- Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. <<http://ceb.csit.fsu.edu/awty>>.
- Zimmermann, M., Wahlberg, N., Descimon, H., 2000. Phylogeny of *Euphydryas* checkerspot butterflies (Lepidoptera: Nymphalidae) based on mitochondrial DNA sequence. *Ann. Entomol. Soc. Am.* 93, 347–355.