Complex MtDNA Variation and Species Delimitations in the Phyciodes tharos Species Group (Nymphalidae: Melitaeini): A Second Look in Michigan and Ohio

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COMPLEX MTDNA VARIATION AND SPECIES DELIMITATIONS IN THE *PHYCIODES THAROS* SPECIES GROUP (NYMPHALIDAE: MELITAEINI): A SECOND LOOK IN MICHIGAN AND OHIO

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ABSTRACT. The number and delimitation of species in the *Phyciodes tharos* species complex has puzzled lepidopterists for years. Previous analysis of mtDNA sequence data has suggested that *P. cocyta* is more closely related to *P. cocyta* than to *P. tharos*, in contrast to inferences from morphology and ecology. We sequenced the mitochondrial gene cytochrome oxidase I for 40 individuals of *Phyciodes tharos*, *P. cocyta*, and *P. cocyta* from Michigan and Ohio, a region at the boundaries of the geographic ranges of these species. Network and cladistic analyses reveal shared mtDNA lineages, indicating that limited hybridization occurs in this region between *P. cocyta* and the other two taxa but not between *P. tharos* and *P. cocyta*. Our evidence also supports the traditional phylogenetic assessment of *P. tharos* and *P. cocyta* as the two most closely related species in this species group. Data from nuclear genes are needed to more fully resolve this intriguing group of butterflies.

Additional key words: split network, Neighbor-Net, SplitsTree4, maximum-likelihood, introgression

Papilionoidea, the true butterflies, are no doubt the group of invertebrates most well–known to science—thousands of studies over hundreds of years having been published, especially on their ecology and evolution (Boggs et al. 2003). The taxonomy of butterflies is especially well known, with most of the worldwide taxa already described—a huge feat considering the size of the group (Ackery et al. 1999). Yet there still remain many groups of butterflies for which species boundaries remain unclear. The genus *Phyciodes* , comprised of ten currently recognized species (Pelham 2008) with mainly Nearctic distributions, is one such group.

Within *Phyciodes*, the *P. tharos* species group (*P. tharos*, *P. cocyta*, *P. cocyta*, and *P. pulchella*) has been especially interesting due to the species' phenotypic similarity, variability in diagnostic characters, and (with the exception of *P. cocyta* and *P. cocyta*) largely parapatric ranges with broad swathes of sympatry (Fig. 1) (Scott 1994). *Phyciodes tharos* and *P. cocyta* are of particular interest, having long been considered conspecifics. Oliver (1980), however, based on extensive breeding experiments that revealed a degree of hybrid breakdown between various populations of *Phyciodes tharos*, distinguished two entities which he called *tharos* Type A and *tharos* Type B. Scott (1994) assigned the name "*cocyta*" to Oliver's "*tharos* Type B" and raised it to full species status based on a few morphological characters and sympatry with *P. tharos* in some areas, despite incomplete reproductive

isolation in other areas. *Phyciodes batesii* and *P. pulchella* are more easily diagnosable, although extensive hybridization between *P. cocyta* and *P. cocyta* has also been observed in Colorado and Utah (Scott 1994, 1998). Recent authors have largely followed the lead of Scott (1994) in recognizing four distinct species in the *P. tharos* group, although agreement is by no means universal, especially as to whether *P. cocyta* should not be a subspecies of *P. tharos* (e.g. Glassberg 1999).

The advent of molecular analysis of DNA has been a great boon to butterfly systematics, augmenting knowledge of morphology and life histories (Sperling 2003). Wahlberg et al. (2003) conducted the first phylogenetic analysis of *Phyciodes* using DNA, with a parsimony analysis of 1450 base pairs of the mitochondrial gene cytochrome oxidase I (COI) from 140 *Phyciodes* specimens representing all ten species from across North American and Mexico. Wahlberg et al. (2003) attempted to sample specimens widely in order to capture as much geographic variation as possible. They found that the *tharos* species group formed a well-supported monophyletic clade (with the exception of two specimens). Within the clade they found that *P. tharos* was the most basal of the four species, which confirmed the suggestion of Scott (1994) based on genital and pupal characters. Surprisingly, though, they found that the haplotypes of *P. cocyta* and *P. cocyta* were interdigitated, grouping together to form several clades paraphyletic with respect to *P. pulchella*.
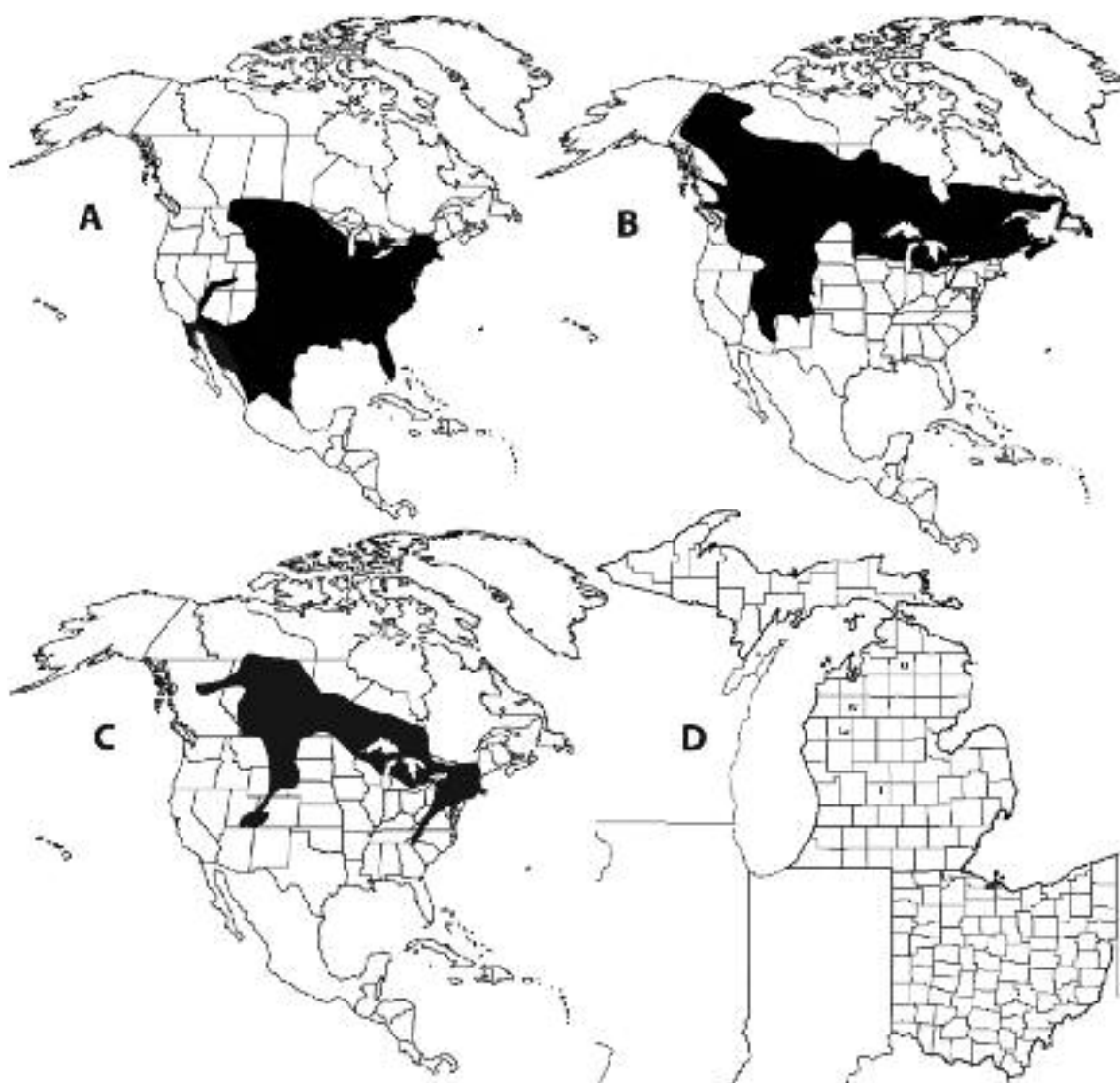
FIG. 1. Range maps of the three species in the *Phyciodes tharos* complex focused on in this study (A-C), and a map of Michigan and Ohio showing the location of the novel specimens collected for this study (D). **A:** *P. tharos.* **B:** *P. cocyta.* **C:** *P. batesii.* The range maps are modified from Opler et al. (2009) and Brock & Kaufman (2003). **D:** Letters indicate approximate locations within counties where specimens were collected for this study. O = Otsego Co.; W = Wexford Co.; La = Lake Co.; I = Ionia Co.; Lu = Lucas Co.

The inference that *P. cocyta* is most closely related to *P. cocyta* and sister group to *P. pulchella* contradicts the morphological and ecological evidence that *P. cocyta* is most closely related to *P. tharos*.

In this paper we re-investigate the relationships between *P. tharos*, *P. cocyta*, and *P. cocyta*. In contrast to Wahlberg et al. (2003), our approach was to sample *P. tharos*, *P. cocyta* and *P. cocyta* from a relatively small geographic area: the lower peninsula of Michigan and northwest Ohio, where the ranges of all three species coincide (Fig 1). This is a region not sampled by Wahlberg et al. (2003); their closest specimens to our study area were collected from Carleton Co., Ontario.

Since there is known to be incomplete reproductive isolation between these three species (Scott 1994), this area is a particularly interesting region to investigate the question of which species is most closely related to *P. cocyta*, and to see whether evidence of gene flow between species can be observed.

MATERIALS AND METHODS

Sampling and molecular techniques. Specimens of *Phyciodes* were collected between 06 June and 19 June 2006 throughout the northern part of the lower peninsula of Michigan. Since only *P. cocyta* and *P. cocyta* were found in Michigan, *P. tharos* was collected

in Ohio on 29 and 30 June 2006. All specimens were spread while fresh and are now stored at the Hillsdale College Insect Collection, Hillsdale, Michigan. Approximate collection localities are displayed in Fig 1; complete collection data are presented in Appendix 1. Identification to species level was made after examination of the color of the antennal clubs, the extent and pattern of reticulation and dark pigmentation on the dorsal wing surfaces, extent of dark coloration on the hindwings below, and overall size (Nielsen 1999; Scott 1986). In our estimation, none of the specimens from which reliable sequence data was extracted had a doubtful identification based on these characters.

Two legs were removed from each fresh specimen for DNA extraction, either immediately after capture or after several months storage in ethanol in refrigeration. Genomic DNA was extracted from the two legs of each specimen using the Qiagen DNeasy extraction kit. Two primer pairs were used to amplify 1450 base pairs of the COI gene: LCO1490-J-1514 (5'GGTCAACAAATCATAAAGATATTGG) and HCO2198-N-2175 (5'TAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994), and C1-J-2183 (5'CAACAYTTATTTTGATTTTTTGG) and TL2-N-3014 (5'ATCCATTACATATAATCTGCCATA) (Simon et al. 1994). These primers were chosen based on their previous successful amplification in *Phyciodes* (Wahlberg et al. 2003). All COI fragments were amplified with standard polymerase chain reaction (PCR) techniques. PCR products were monitored for yield, specificity, and contamination using agarose gel electro¬phoresis and cleaned with ExoSAP-IT. PCR fragments were sequenced with a dye terminator cycle sequencing kit and an Applied Biosystems 3130 Genetic Analyzer. Only one strand of each fragment was sequenced, using the forward primer. Sequence quality was assessed by the Applied Biosystems software Sequencing Analysis 5.2 and by visual inspection of the chromatograms. Areas of poor sequence quality, such as the center of the COI gene where the fragments of the two sequencing runs overlapped, were trimmed. Sequences of entirely poor quality were discarded. Forty sequences of 1319 nucleotide characters were ultimately retained.

Data analyses. Seventy-eight COI sequences of *Phyciodes tharos*, *P. cocyta*, *P. cocyta*, *P. pulchella*, and *P. phaon* from Wahlberg et al. (2003 (accession nos. AF187747, AF187785, AF187789, AF187798, AF187800, AF187783, AF187807, AY156595-AY156686), were downloaded from GenBank. Those, together with our 40 novel sequences, were aligned in Mesquite v. 2.6 (Maddison & Maddison 2009) using Clustal W v. 2.0.9 (Larkin et al. 2007) with default settings. Alignments were manually adjusted using the Align package in Mesquite (Maddison et al. 2007).

Our 40 novel sequences were opened with SplitsTree4 v. 4.10 (Huson & Bryant 2006) and a split network generated with Kimura-2 parameter (K2P) distance according to the Neighbor-Net method (Bryant & Moulton 2004); all other settings were set to default. Robustness of the splits was assessed with 1000 bootstrap repetitions.

In order to compare directly our data with Wahlberg et al. (2003), a phylogenetic tree was generated using the maximum-likelihood method implemented in Garli 1.0 (Zwickl 2006). We used the HKY+I+G model of evolution, which was selected by ModelTest 3.7 (Posada & Crandall 1998) as the most likely to fit our data according to the AIC criterion. During the analysis, Garli was allowed to estimate rate parameters, base frequencies, and proportion of invariable sites. Fifteen search replicates were performed to find the best tree of score -4897.3347. Two hundred bootstrap replicates were performed in order to estimate branch support. The tree was rooted with the three *P. phaon* sequences and two aberrant *P. pulchella* sequences (47-6 CA3 and 49-13 CA3).

RESULTS

Network analysis. Our 40 *Phyciodes* sequences are represented by a split network in Fig. 2. A split network can be thought of as a generalized combination of many phylogenetic trees; in fact, phylogenetic trees are a sub-category within split networks. With any data set, such as DNA sequences, any given character can be used to split a set of taxa into separate groups. For example, in this matrix

<div align="center">

w AACGTG
x ACCTGG
y TACGTG
z TACTTG

</div>

the first column splits [w,x] from [y,z], the second column splits [x] from [w,y,z], the third column does not split any taxa, because it is constant, and the fourth column splits [w,y] from [x,z]. Any set of splits can be compatible or incompatible. A phylogenetic tree is a visual representation of a set of compatible splits; a split network can represent a set of splits whether compatible or incompatible. When all splits are compatible, they can each be represented graphically by a single branch: this is a phylogenetic tree. With most real data sets, however, this is not the case. Incompatible splits in a split network are represented
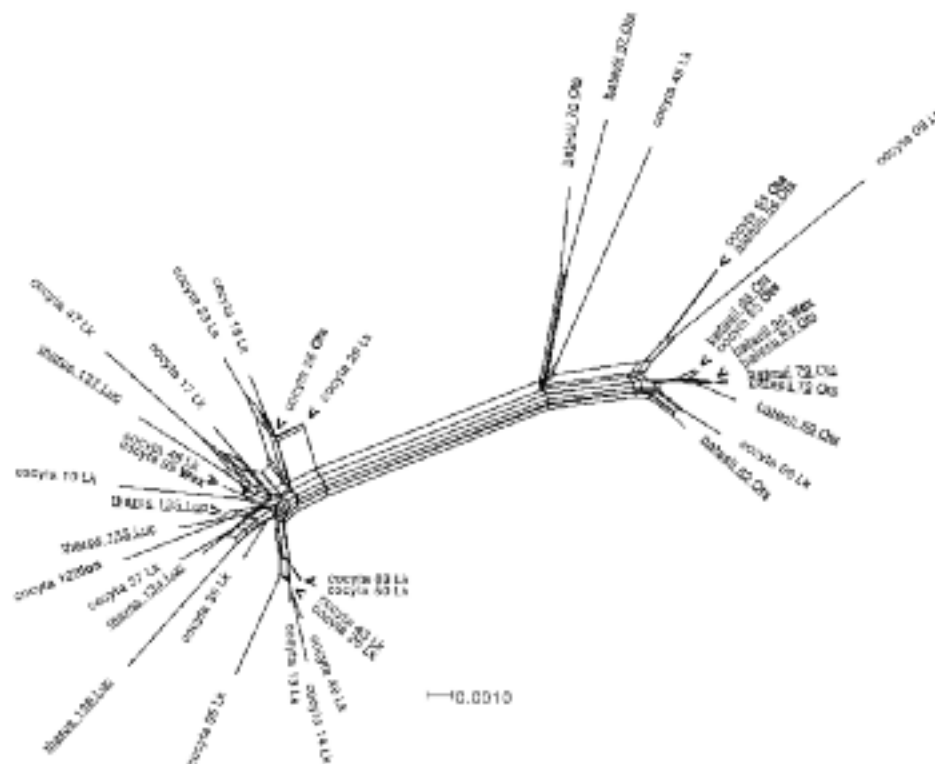
FIG. 2. A split network of 40 specimens of three species of Phyciodes, constructed by the Neighbor-Net method from 1319 base pairs of the cytochrome oxidase I gene. Each edge represents a split separating one or more taxa from the rest; multiple parallel edge indicate multiple incompatible splits. Length of edges corresponds to genetic distance according to the K2P model. Individuals of *P. tharos* and *P. batesii* are underlined. The specific epithet of the individual, a unique identifier, and the county of collection given at each terminal node. Lk = Lake Co.; Ots = Otsego Co.; Wex = Wexford Co.; Ion = Ionia Co.; Luc = Lucas Co. Bolded county abbreviations indicate either a specimen of *P. cocyta* not from Lake Co. or a specimen of *P. batesii* not from Otsego Co. Chevrons indicate either multiple specimens terminating at nodes too close to differentiate or a single specimen terminating with a branch too short to show.

by multiple parallel branches (Huson & Bryant, 2006). Hence a split network can incorporate a measure of uncertainty within a data set. The network in Fig. 2 was constructed by the Neighbor-Net method, in which the data matrix used to generate the split network is a matrix of the genetic distances between taxa.

The sequences in Fig. 2 separate into two clusters with a large relative distance between them, supported by 100% bootstrap support (not shown). All of the five *P. tharos* are in one cluster, all of the 10 *P. cocyta* are in the other, and the 25 *P. cocyta* are distributed among both (20 with the *P. tharos* grouping and five with the *P. cocyta* grouping). There is no one set of splits that encompasses one species exclusively. Of the five *P. cocyta* that group with the *P. cocyta*, two are two of the three *P. cocyta* from Otsego Co.—the location where

most of the *P. cocyta* in our study were collected, and the only place we sampled where *P. cocyta* was the most commonly encountered *Phyciodes* species. Four of the five *P. tharos* sequences are included in a group of six haplotypes separated from the rest by a single set of splits (Fig. 2). Of the two *P. cocyta* sequences that complete that group, one is the lone *P. cocyta* specimen from Ionia Co., by far the closest geographically to the location where the *P. tharos* specimens were collected (Fig. 1D).

Phylogenetic tree analysis. When we constructed a maximum-likelihood tree from our 40 novel sequences in addition to the 78 *Phyciodes* sequences from Wahlberg et al. (2003), the branching pattern and branch support closely resemble the parsimony tree found in Wahlberg et al. (2003) (Fig. 3). All the clades
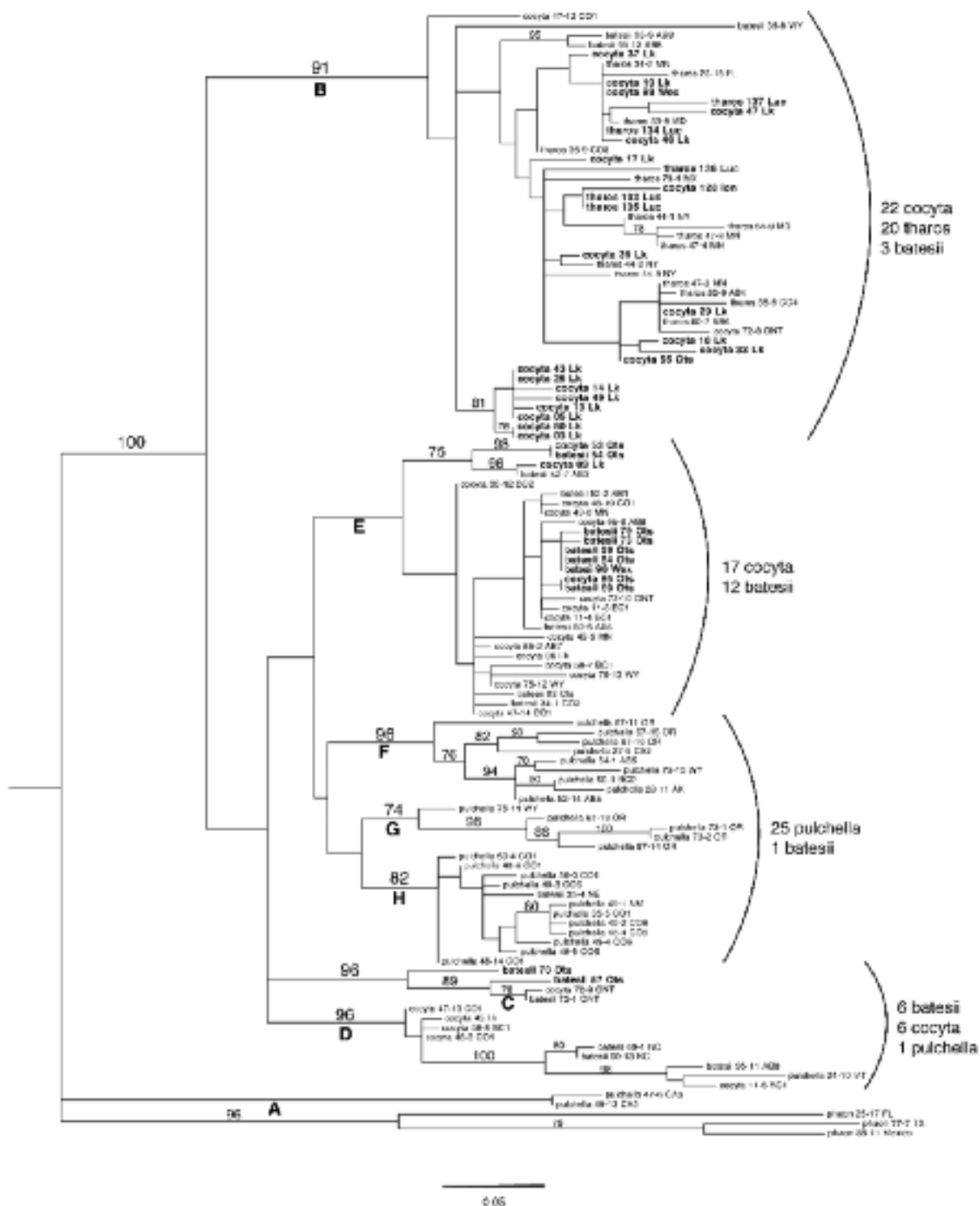
FIG. 3. A maximum-likelihood tree constructed under the HKY+I+G model. Haplotypes in regular type are from Wahlberg et al. (2003); numbers and letters after the specific epithet refer to the specimen and region of collection identifiers, respectively. . Lk = Lake Co.; Ots = Otsego Co.; Wex = Wexford Co.; Ion = Ionia Co.; Luc = Lucas Co. Haplotypes in bold and larger type are our novel sequences; numbers and letters after the specific epithet refer to the specimen and county of collection identifiers, respectively. Numbers above branches refer to the bootstrap support for that branch out of 200 bootstrap repetitions. Bootstrap support is shown for all branches with support of 70 or higher. Bold letters beneath branches identify clades which correspond to analogous clades in Fig. 4, Wahlberg et al. (2003). Scale bar is proportional to number of changes per site.

TABLE 1. Number of haplotypes of three species of *Phyciodes* found in certain clades of the phylogenetic tree generated from the data of Wahlberg et al. (2003), the number of haplotypes of each species from our data added to those clades when our data was analyzed with that of Wahlberg et al. (2003), and the total number of haplotypes of each species in those clades (see Fig. 3).

	B "tharos" clade	C, D, E "cocyta/batesii" clades	F–H "pulchella" clades
Wahlberg et al. (2003)	15 tharos 2 cocyta 3 batesii	18 cocyta 8 batesii 1 pulchella	25 pulchella 1 batesii
Added	5 tharos 20 cocyta	5 cocyta 10 batesii	- -	-
Totals	22 cocyta 20 tharos 3 batesii	23 cocyta 18 batesii 1 pulchella	25 pulchella 1 batesii

marked with capital letters by Wahlberg et al. (2003) as being important for discussion in their tree are also found in our tree, and with comparable branch supports. The phylogeny appears robust to the addition of new sequences and to the method of phylogenetic inference, parsimony or maximum likelihood. The topology of the tree can be summarized as three principal clades: a *"tharos"* clade (clade B), a *"cocyta/batesii"* clade (clades C, D, E), and a *"pulchella"* clade (clades F – H). All five of our novel *P. tharos* sequences and 20 of our 25 novel *P. cocyta* sequences clustered within the *"tharos"* clade; the remaining five *P. cocyta* sequences and all ten of our novel *P. cocyta* sequences clustered within the *"cocyta/batesii"* clade (Table 1).

DISCUSSION

Our results in Fig. 2 suggest that: 1. mitochondrial introgression may have taken place between *P. cocyta* and *P. cocyta* and between *P. cocyta* and *P. tharos*, and 2. that *P. cocyta* in our area of study is more closely related to *P. tharos* than to *P. cocyta*. The first inference is supported by the fact that none of the three species are separated into an exclusive cluster. It is also suggestive that of the two *P. cocyta* that cluster with the *P. tharos* sequences group A in Fig. 2, one is from Ionia Co., the closest location geographically to where all the *P. tharos* were collected. Similarly, two of the three *P. cocyta* collected from Otsego Co., where almost all of the *P. cocyta* were collected, cluster with the *P. cocyta* sequences despite three-quarters of the *P. cocyta* clustering at the *P. tharos* end of the figure. The second

inference is supported because 15 of the 20 *P. cocyta* sequences are far closer to the *P. tharos* sequences than to the *P. cocyta*. Moreover, when our data are added to that of Wahlberg et al. (2003) (Fig. 3), the same 15 *P. cocyta* sequences, as well as the five *P. tharos* sequences, come out in the *"tharos"* clade (clade B; Table 1). The *P. cocyta* from our study area are not only more similar to the *P. tharos* than the *P. cocyta* from our study area, but from all around the continent.

Species that are recently diverged and closely related are expected to sustain some gene flow (Coyne & Orr 2004). As long as they maintain genetic integrity across their range, this does not compromise their status as good species (Sperling 2003). Gene flow between recently diverged species is not unusual and has been documented in a wide variety of animal taxa (e.g. Nosil 2008; Friar 2007). The members of the *Phyciodes tharos* species complex are likely just such recently diverged species. They diverged on the basis of adaptation to different ecological pressures (Oliver 1980), and differences in flight periods in response to environmental conditions was likely a key factor in the speciation (Oliver 1980; Scott 1994). At the geographic boundary between species' ranges (especially of parapatric species, like *tharos* and *cocyta*) where ecological pressures are similar on both, hybridization is particularly likely.

Hybridization and mitochondrial introgression may best explain the pattern in our data (Fig. 2). Similar patterns, where mitochondrial lineages correlate better with geographic than phylogenetic distances between populations, have been observed in other Lepidoptera (Schmidt & Sperling 2008). It may not be the best explanation for the non-monophyly of especially *P. cocyta* and *P. cocyta* in Wahlberg et al. (2003), although non-monophyly in mitochondrial gene trees has been demonstrated to be an indicator of mitochondrial introgression in various other taxa (Linnen & Farrell 2007; Shaw 2002; Gompert et al. 2008). Nearly all of the *P. tharos*, *P. cocyta* and *P. cocyta* in Wahlberg et al. (2003) were sampled from or near areas where all three species are found and yet, unlike our data, different species collected from the same locality fell out in different parts of their tree.

Many species, especially recently diverged ones, are paraphyletic with respect to their gene trees due to gene introgression via hybridization or incomplete lineage sorting from variable ancestral populations (Maddison 1997; Funk & Omland 2003). Wahlberg et al. (2003) explained the patterns of non-monophyly of the species in the *P. tharos* complex as largely due to incomplete lineage sorting, but with hybridization and mitochondrial gene introgression playing a role

especially between *P. cocyta* and *P. cocyta*. Across the largely sympatric ranges of *P. cocyta* and *P. cocyta* (Fig. 1), there is only one place where morphology indicates hybridization may be currently occurring regularly between them: the Rocky Mountains of Utah and Colorado (Scott 1998). Elsewhere their phenotypes are quite distinct (Scott 1994, 1998). Confusion in species identification in the *P. tharos* species complex usually occurs when *P. tharos* and *P. cocyta* are confused, as in the case of Porter and Mueller (1998) whose conclusions in hybridization experiments between *P. tharos* and *P. cocyta* were challenged by a claim by J. Scott that they had misidentified a subspecies of *P. cocyta* as *P. tharos* (Wahlberg et al. 2003). Furthermore, hybridization and hybrid viability has been observed several times between *P. tharos* and *P. cocyta*, both in the laboratory and in the wild (Oliver 1980; Scott 1986b). Our data corroborate the conclusion of Wahlberg et al. (2003) that introgression has likely occurred between *P. cocyta* and both *P. cocyta* and *P. tharos*, but, in contrast to their conclusions, suggest that introgression between *P. cocyta* and *P. tharos* has been more widespread than between *P. cocyta* and *P. cocyta*, at least in our area of study.

The final word on the relationships between these butterflies has, of course, not yet been established. The keys to shedding more light on the questions in this species complex will be more intense sampling (e.g. Funk 1999) and the use of nuclear genetic markers. Numerous studies have shown the importance of sampling both mitochondrial and nuclear genes for phylogenetic analyses, since their different patterns of inheritance often lead to discordant gene trees (e.g. Berthier et al. 2006; Gomez-Zurita & Vogler 2003). Wahlberg and Freitas (2007) did analyze a mitochondrial gene and two nuclear genes for 11 species of the *P. tharos* species complex in a phylogeny of the Phyciodina tribe, but with ambiguous results: parsimony and Bayesian analyses of the combined genes gave very different arrangements of the *P. tharos* species complex. Until an analysis of nuclear genes can be incorporated into a much more intense sampling of the *P. tharos* species complex, we can only anticipate further elucidation of the complications in this fascinating group of butterflies.

ACKNOWLEDGEMENTS

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Please see Appendix on next page.

Appendix 1. Localities and dates for the specimens collected for this study. All specimens collected by the senior author.

#	Species	Location	County	Date
03	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
05	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
06	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
09	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
10	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
13	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
14	*Phyciodes cocyta*	Frank Smith Rd & Old MI 63	Lake Co., MI	06 June 2007
17	*Phyciodes cocyta*	G. H. Gordon Biological Station, Luther	Lake Co., MI	07 June 2007
18	*Phyciodes cocyta*	G. H. Gordon Biological Station, Luther	Lake Co., MI	07 June 2007
25	*Phyciodes cocyta*	Silver Creek Pathway, Pierre Marquette State Forest	Lake Co., MI	08 June 2007
29	*Phyciodes cocyta*	Silver Creek Pathway, Pierre Marquette State Forest	Lake Co., MI	08 June 2007
33	*Phyciodes cocyta*	Silver Creek Pathway, Pierre Marquette State Forest	Lake Co., MI	08 June 2007
37	*Phyciodes cocyta*	Silver Creek Pathway, Pierre Marquette State Forest	Lake Co., MI	08 June 2007
39	*Phyciodes cocyta*	King's Hwy & Old MI 63	Lake Co., MI	10 June 2007
43	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
45	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
46	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
47	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
49	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
50	*Phyciodes cocyta*	2 ½ Mile Rd & Old MI 63	Lake Co., MI	10 June 2007
53	*Phyciodes cocyta*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
54	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
55	*Phyciodes cocyta*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
57	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
59	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
65	*Phyciodes cocyta*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
69	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
70	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
73	*Phyciodes batesii*	Crapo Lake Rd & Old State Rd	Otsego Co., MI	13 June 2007
79	*Phyciodes batesii*	Grignell Rd & Old State Rd	Otsego Co., MI	13 June 2007
82	*Phyciodes batesii*	Grignell Rd & Old State Rd	Otsego Co., MI	13 June 2007
84	*Phyciodes batesii*	Grignell Rd & Old State Rd	Otsego Co., MI	13 June 2007
96	*Phyciodes batesii*	Long Lake Campground, 8 mi NE of Cadillac	Wexford Co., MI	16 June 2007
99	*Phyciodes cocyta*	Long Lake Campground, 8 mi NE of Cadillac	Wexford Co., MI	16 June 2007
128	*Phyciodes cocyta*	Hawley Rd, 1 mi S of Ellison Rd	Ionia Co., MI	20 June 2007
133	*Phyciodes tharos*	Black Rd, Monclova	Lucas Co., OH	29 June 2007
134	*Phyciodes tharos*	Black Rd, Monclova	Lucas Co., OH	29 June 2007
135	*Phyciodes tharos*	Black Rd, Monclova	Lucas Co., OH	29 June 2007
136	*Phyciodes tharos*	Black Rd, Monclova	Lucas Co., OH	30 June 2007
137	*Phyciodes tharos*	Black Rd, Monclova	Lucas Co., OH	30 June 2007