

PHYLOGENY AND COLOR PATTERN EVOLUTION  
IN A NEW WORLD BUMBLE BEE  
(HYMENOPTERA: APIDAE: *BOMBUS*: *PYROBOMBUS*) SPECIES COMPLEX

BY

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THESIS

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## Abstract

*Bombus ephippiatus* is one of the most common and widespread bumble bee species throughout Mexico and Central America. This robust and charismatic species possesses a highly variable color pattern throughout its wide distribution across montane and lowland regions, which raises the question of whether or not it is in fact one species. I examined the molecular phylogeny of *B. ephippiatus* populations and its sister species, *B. wilmattae* and *B. impatiens*, with the aims of resolving the species relationships, distinguishing species boundaries and examining correlations between color pattern and phylogeny. I applied DNA sequences from three genes to resolve the species-level phylogeny and to gain insight into the population structure of *B. ephippiatus*. The results reveal that *B. impatiens* is the sister group to *B. ephippiatus* + *B. wilmattae*. Results also suggest that *B. ephippiatus* is paraphyletic with respect to *B. wilmattae*, as *B. wilmattae* attaches to the Costa Rican *B. ephippiatus* clade. The *B. ephippiatus* + *B. wilmattae* complex appears to be associated with geography and color pattern, providing new insights into the evolutionary history of this complex.

*To my Mother, whom I try to emulate every day,  
and to my Father, who has done an excellent job  
fulfilling the role of both parents in my Mother's passing*

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## Table of Contents

<b>Introduction</b> .....	1
<b>Materials and Methods</b> .....	7
<i>Taxa examined</i> .....	7
<i>Gene selection</i> .....	8
<i>DNA extraction, amplification, and sequencing</i> .....	9
<i>Alignment</i> .....	10
<i>Phylogenetic analyses</i> .....	11
<i>Bayesian inference</i> .....	12
<i>Parsimony methods</i> .....	12
<i>Color pattern</i> .....	13
<b>Results</b> .....	15
<i>Tree resolution</i> .....	15
<i>Interspecific relationships</i> .....	16
<i>Intraspecific relationships</i> .....	17
<i>Color pattern</i> .....	18
<b>Discussion</b> .....	19
<i>Interspecific relationships</i> .....	19
<i>Intraspecific relationships</i> .....	21
<i>Color pattern</i> .....	23
<i>Implications for commercial rearing</i> .....	25
<i>Future directions</i> .....	26
<i>Conclusions</i> .....	28
<b>References</b> .....	47
<b>Appendix A. Alignments</b> .....	55
<i>CAD Alignment</i> .....	55
<i>PEPCK Alignment</i> .....	61
<i>COI Alignment</i> .....	67
<b>Appendix B. Sequence distance matrices</b> .....	78
<i>CAD Distance Matrix</i> .....	78
<i>PEPCK Distance Matrix</i> .....	80
<i>COI Distance Matrix</i> .....	82
<b>Appendix C. Specimen GPS coordinates</b> .....	88
<b>Appendix D. Color pattern drawings</b> .....	89

## Introduction

An important issue in evolutionary biology is the identification of forces that shape genetic structure within species and eventually contribute to their reproductive isolation. Species concepts have been widely debated in the field of systematics for decades (Dobzhansky, 1935; Mayr, 1942; Mayr, 1957; Simpson, 1951; Wiley, 1978; Mishler and Donoghue, 1982; Hull, 1997; Mayden, 1997; de Queiroz, 1999; Rossello-Mora, 2003; Reydon, 2004; Hey, 2001; Hey *et al.*, 2005; Hey, 2006a; de Queiroz, 2007; Staley, 2009; Velasco, 2009). Some systematic biologists have argued that species concepts and species delimitation need to be conceptually separate from each other (Mayden, 1999; Hey, 2006b; de Queiroz, 2007). This separation allows species delimitation to step back from the conceptual controversy so that multiple lines of evidence and data can be collected to determine operational criteria (Ross *et al.*, 2010). Regardless of debate, species delimitation has loomed in importance in recent years. Species are the functional units for a multitude of analytical methods in evolutionary, biogeographic, and ecological research (Isaac *et al.*, 2004; Balakrishnan, 2005; Weins, 2007a; Weins, 2007b; Renema *et al.*, 2008; Ross *et al.*, 2010). The determination of species has become especially important in the field of conservation biology (Agapow *et al.*, 2004; Ross and Shoemaker, 2005; Ross *et al.*, 2010;).

For centuries, bumble bees (*Bombus*: Apidae) have been a subject of fascination for naturalists (Darwin, 1859; Sladen, 1912; Plath, 1934; Heinrich, 1979; Goulson, 2003). They are important native pollinators in all of the areas they inhabit worldwide and are important in pollination services wherever they are reared commercially for greenhouse crops. Recently they have received increased attention because of their substantial global declines (e.g., United

Kingdom: Darvill *et al.* 2010; Redpath *et al.*, 2010; North America: Colla and Packer, 2008; Grixti *et al.*, 2009; Brazil: Martins and Melo, 2010). A thorough understanding of species boundaries and evolutionary history will be critically important in potential conservation efforts. Historically, species determination has been difficult in bumble bees because of morphological similarity combined with a remarkable degree of color pattern diversity across wide geographic distributions (Williams, 1998; Cameron *et al.*, 2007; Williams, 2007). The fact that species also exhibit convergent color patterns in the formation of mimicry complexes (Williams, 2007) adds to the difficulty of species delineation. As a result, bumble bees have been a taxonomic confusion of names, with over 1,100 names having been assigned to the 250 currently recognized species since Linnaeus (Williams, 1998).

The most recognizable feature of bumble bee morphology, and one which has led to much taxonomic disorder, is the pile, the long, brilliantly-colored hairs that cover the abdomen and thorax. The color of their pile is usually a striking contrast of black and yellow stripes, but many species also possess red and white pile. This coloring plays an important role in the formation of Müllerian mimicry complexes, which many species of bumble bees form throughout their worldwide distribution (Williams, 2007). Williams (2007) identified three main color pattern groups across all bumble bees: entirely black bumble bees, mostly pale (grey, yellow, brown) bumble bees, and bumble bees with contrasting bands of color. All of these color patterns show statistically significant clustering in geographic regions (Williams, 2007). The dark bees all occur in tropical areas and it is hypothesized that their color pattern may be playing a thermoregulatory or cryptic function; the pale bees occur in grasslands, so it is hypothesized that their color pattern is an example of crypsis; the intermediate banded bees occur in regions that might be unsuitable to the other color pattern groups, and it is hypothesized that they play a

thermoregulatory and/or aposematic function (Williams, 2007). Although these hypotheses have yet to be tested, these data, in combination with the fact that ancestral color patterns cannot be reliably recovered across most of the *Bombus* phylogeny (Cameron et al., 2007; Cameron *et al.*, unpublished data), imply that ecological factors are driving these color pattern complexes in bumble bees.

Prior to DNA sequencing methods, these color pattern complexes added to the difficulty of species identification in this genus. Molecular evidence (Cameron *et al.* 2007) has revealed that some sympatric species pairs and even allopatric species pairs with very different color patterns are genetically identical to each other, while other species groups that were previously thought to be possible conspecifics are in fact genetically distinct. Despite the recent comprehensive molecular phylogeny of *Bombus* (Cameron *et al.*, 2007), some species still remain to be delimited.

One *Bombus* complex that remains to be resolved involves the species *B. impatiens*, *B. ephippiatus*, and *B. wilmattae* (Hines *et al.*, 2006; Cameron *et al.*, 2007). Phylogenetic analysis of a concatenated five-gene dataset (Cameron *et al.*, 2007) suggested (with low support) that the Mesoamerican bumble bee species *B. ephippiatus* was closely related to *B. wilmattae*, a species found only in southern Chiapas, Mexico and northern Guatemala at high elevation in volcanic, semi-tropical areas (Labougle, 1990). Individual gene fragments, however, were incongruent for these three taxa: Arginine kinase (*ArgK*) sequences showed *B. ephippiatus* to be most closely related to *B. impatiens*, a North American species that is becoming increasingly important to the North American agricultural economy as a commercial pollinator (Vergara, 2008); mitochondrial *16S* showed *B. impatiens* and *B. wilmattae* to be most closely related; and the *opsin* sequences showed *B. ephippiatus* and *B. wilmattae* as sister groups (Cameron *et al.*, 2007). This lack of

concordance among datasets is typical of closely related species and it is clear that the resolution of these relationships requires further analysis.

*Bombus impatiens* is a North American bumble bee species common throughout the Eastern half of the United States, from Ontario and Maine south to Kansas and Florida (Kearns and Thomson, 2001). It is yellow and black in coloring, with the thorax and first abdominal segment completely covered in yellow pile and the rest of the abdomen covered in black pile. A narrow region in southwestern Texas separates *B. impatiens* from both *B. ephippiatus* and *B. wilmattae* (Vergara, 2008), which occur in Mexico and Central America.

*B. ephippiatus* is the most common species of bumble bee found in the Southern Nearctic; it has a geographic range extending from Northwest Mexico (Chihuahua) to Panama (Labougle, 1990), and possibly as far south as the northwestern region of South America (Ecuador and Colombia, Franklin, 1913) (Fig. 2). *B. ephippiatus* has a highly polymorphic color pattern: the thorax of workers is completely black from a dorsal view, while the abdomen and sides of the thorax are brightly colored, giving the appearance that it is wearing a “saddle” of sorts (Figs. 1a-1d). In fact, the word “*ephippiatus*” is derived from the Greek *ephippion*, which means “saddle”. In Williams’ comprehensive online guide to the color patterns of all *Bombus* species (<http://www.nhm.ac.uk/research-curation/research/projects/bombus/colour.html>), he identified two distinctive color patterns for *B. ephippiatus* queens and three patterns for workers (Figs. 1a-1d). This variation historically has caused a great deal of taxonomic confusion; it has been assigned eleven different names since Thomas Say first classified it in 1837 (Williams, 1998). Some of these names have split the species into two different subspecies based on the differences in color patterns of the workers (Friese, 1903; Friese, 1916; Frison, 1925; Milliron, 1962). The widespread geographic distribution (Franklin, 1913; Labougle, 1990) of *B. ephippiatus* over a

topologically complex region (Fig. 2) that may act as a barrier to dispersal, together with the remarkable color pattern variation, suggests the possibility that *B. ehippiatus* may have considerable population genetic structure.

*B. wilmattae* has a highly restricted range, inhabiting only volcanic, montane regions in Southern Chiapas and Northern Guatemala (Labougle, 1990). The color pattern of *B. wilmattae* is very similar to that of the darker forms of *B. ehippiatus* (Figs. 1e-1f) (Williams, 2007). When Cockerell (1912) classified this species, he considered it a subspecies of *B. ehippiatus* (then classified as *B. lateralis* (Smith, 1879)). Labougle *et al.* (1985) found consistently distinct differences in morphology between *B. ehippiatus* and *B. wilmattae*, but it is still questionable as to whether or not these species are conspecific as a result of gene topology incongruence in phylogeny (Hines *et al.*, 2006). In light of the color pattern similarities between *B. ehippiatus* and *B. wilmattae*, integrating color pattern data, geographical range, and genetics can be a powerful approach for understanding species and their boundaries (Ross *et al.* 2010).

Incorporating analyses of phenotypic characteristics with genetic analyses can be a valuable method for understanding the taxonomic status of populations and could aid in elucidating the taxonomic status of these sister species.

The study presented here addresses three main problems: 1) the phylogenetic resolution of *Bombus ehippiatus*, *B. impatiens*, and *B. wilmattae*, 2) the species status of *B. ehippiatus* and 3) the degree of color pattern concordance with the phylogeny of these three taxa. I address these questions using mitochondrial and nuclear DNA sequence data from three gene fragments to infer relationships using a variety of phylogenetic inference methods and by mapping color pattern characters onto phylogeny. The results of this study help to resolve the uncertain classification of these species and elucidate the evolution of color pattern and its function.

Knowledge about the evolutionary processes acting on these important pollinators may provide additional insight into speciation in the Neotropics.

## Materials and Methods

### *Taxa examined*

For phylogenetic estimation, I utilized sequences from representatives of three putative *Bombus* species (Table 1). Because *Bombus ephippiatus* was the focal species of this study, I used 43 individuals of this species to generate sequence data. In addition, I sequenced six individuals of *B. wilmattae* and ten individuals of *B. impatiens* for interspecific phylogeny estimation. To test the monophyly of this species complex, I included sequences from species shown to be sister taxa to this complex in previous studies, *B. huntii* and *B. vosnesenskii*.

Taxa were sampled from regions throughout their range (Table 1, Fig. 2). Specimens of *B. ephippiatus* were sampled principally from sites scattered throughout the countries of Mexico, Honduras, and Costa Rica, specimens of *B. wilmattae* were sampled from three sites within Chiapas, Mexico, and specimens of *B. impatiens* were sampled from sites in the states of Maine, Illinois, and Texas within the United States (Table 1, Fig. 2). To maintain an evenly distributed sampling across the range of the complex, approximately two specimens were sequenced from each locality. Males and females were utilized in this study and sex is indicated in Table 1.

All three taxa are represented by each of three gene fragments utilized in this study, but not every individual was sequenced successfully for each gene (see Table 1). Most of the Costa Rican *B. ephippiatus* individuals are represented only by *COI* because these specimens were all at least fifteen years old, making it difficult to amplify DNA obtained from them. Sequences available and general collection localities are presented in Table 1. Specimen vouchers for all DNA sequences are housed at the Illinois Natural History Museum in Urbana, Illinois, and I have submitted all sequences to GenBank.



### *Gene selection*

I obtained fragments of three independently evolving genes: mitochondrial cytochrome oxidase I (*COI*) and nuclear rudimentary (*CAD*) and phosphoenolpyruvate carboxykinase (*PEPCK*). Hines and Cameron (unpublished data) conducted initial sequencing of *COI* to examine this gene's phylogenetic utility in this complex. Exploratory mitochondrial *16S* sequences (Cameron lab, unpublished data) were often too unreadable to accurately align, so this gene was not used. I obtained one *PEPCK* sequence from GenBank for each of these three species, submitted by Hines *et al.* (2006). See Table 1 for complete sequence information.

Mitochondrial genes, such as *COI*, are useful for phylogenetic analyses, especially at the species level, because they usually exhibit extensive intraspecific variation, maternal transmission, and an absence of intermolecular genetic recombination (Avice, 2000). Multiple studies have used this protein-coding gene for determining between-species and within-species relationships (Avice, 2000), including other phylogenetic studies within the family Apidae (Soucy and Danforth, 2002; Bull *et al.*, 2003; Kawazoe *et al.*, 2008; Kim *et al.*, 2008) and even within the genus *Bombus* (Koulianos, 1999; Koulianos and Schmid-Hempel, 2000; Kim *et al.*, 2009). I amplified 811 base pairs (bp) from *COI* using the primers RevmtR (5'-AACCAGTAATTATTGGATATCATGA) and FormtR (5'-GGTTGAACTGTATATCCTCCA) by Hines (unpublished data).

While mitochondrial genes are useful for species-level phylogenies, they represent only a single maternally-inherited locus. Nuclear genes are necessary for determining possible interlocus variation in gene tree topologies (Maddison, 1997). Because mitochondrial DNA is passed on maternally, nuclear genes are also useful for examining histories of both sexes. The nuclear gene carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, dihydroorotase or

rudimentary (*CAD*) encodes several enzymes involved in the biosynthesis of pyrimidines. A study by Moulton and Wiegmann (2004) showed that *CAD* has considerable phylogenetic utility, especially in insect molecular systematics. It has been demonstrated that *CAD* is useful for resolving Cretaceous-age divergences in the Hymenoptera (Danforth *et al.* 2006), but a study in beetles suggests that it is also useful for more recent divergences (Wild and Maddison, 2008). I designed two forward primers, CDBF1 (5'- TGAATCCTGATTGATTCTACAGAGCAGTC) and CDBF2 (5'- TGGTCTCAGTATCGGTCAAGCG), and used the reverse primer CD688R (Wild and Maddison, 2008) to amplify 840 bases of the *CAD* gene, including two introns totaling 357 bp.

The *PEPCK* gene encodes for the enzyme phosphoenolpyruvate carboxykinase, which is involved in gluconeogenesis and glucose homeostasis (Hanson and Reshef, 1997). This gene has been successful in resolving phylogenetic relationships at multiple levels within the insects (Friedlander *et al.*, 1996; Moulton, 2000; Moulton, 2003; Sota and Vogler, 2003; Leys *et al.* 2002; Wild and Maddison, 2008), including species-level relationships within *Bombus* (Cameron *et al.*, 2007). I used *PEPCK* primers designed by Cameron *et al.* (2007) to amplify 878 bp of the gene, including two introns totaling 515 bp in length.

#### *DNA extraction, amplification, and sequencing*

I obtained tissue from specimens (preserved in 95-100% ethanol at 4°C) by removing one of the forelegs. The foreleg in *Bombus* does not possess any crucial characters for taxonomic identification, thus specimens remained useful as vouchers after I removed tissue. I re-associated the legs with specimens after the completion of DNA extractions. For the few pinned specimens used, I also removed a foreleg for DNA extraction. Tissue was digested for six or more hours in

proteinase K at 56°C on a shaking platform. I then extracted DNA using the DNeasy Blood and Tissue Kit (QIAGEN).

Standard conditions for PCR amplification included: an initial denaturation step of 95°C for 3 minutes; 35 cycles of denaturation for 60 seconds at 94°C, annealing for 60 seconds at 45-57°C, and elongation for 60 seconds at 72°C; a final extension of 3 minutes at 72°C. I modified these conditions for some of the reactions. Annealing temperatures for the genes were 44-52°C for *COI*, 48-57°C for *CAD*, and 45-52°C for *PEPCK*. For *CAD*, I carried out a nested PCR reaction, which involves using internal primers to sequence an initial, longer fragment obtained through PCR. This nested PCR of *CAD* involved using the primers CDBF2 and CD688R to amplify a longer region of the gene, then amplifying this PCR product using the primers CDBF1 and CD688R. For most PCRs I used *GoTaq* polymerase (Promega). I purified PCR products primarily using ExoSAP-IT reagent (Affymetrix). Sequencing reaction protocols used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and PCR primers. I sent sequencing products to the W.M. Keck Center for Comparative and Function Genomics at the University of Illinois, Urbana for purification and direct sequencing on an ABI 3730XL capillary sequencer.

### *Alignment*

Alignment with a computer algorithm was unnecessary due to the high similarity among sequences, therefore I did sequence editing and alignment by hand in BioEdit version 7.0.5 (Hall, 1999). I constructed concatenated datasets using Mesquite version 2.7 (Maddison and Maddison, 2009).

### *Phylogenetic analyses*

I analyzed genes both separately and as one concatenated dataset, using Bayesian methods and maximum parsimony. Because the *PEPCK* dataset contained a homologous five bp indel, I removed this region from the dataset and then coded the indel as either a single binary character (presence-absence) with equal weight to a base substitution in Bayesian analyses (Simmons and Ochoterena, 2000), or as a single “fifth” character in maximum parsimony analyses. The *CAD* sequences were heterozygous (biallelic) for some individuals. One allele possessed a nine bp indel; the other allele did not. This indel caused a frameshift in the chromatogram of heterozygous individuals, rendering the rest of the sequence trace (nearly half) unreadable after the indel. Because so many base pairs of the heterozygous sequences were lost due to unreadable chromatograms, they were excluded from these analyses. Heterozygous individuals are indicated in Table 1. For homozygous *CAD* individuals, this indel is allele-specific and because I used a mix of homozygotes for both alleles I removed it and all sites downstream associated with it. See Appendix A for sequence alignments.

I determined outgroup taxa for rooting the tree using the *Bombus* phylogeny generated by Cameron *et al.* (2007). The closest sister clade to this North American-Mesoamerican complex is a four-taxon North American clade constituting the following species: *B. bifarius* Cresson, *B. ternarius* Say, *B. huntii* Greene, and *B. vosnesenskii* Radoszkowski. Within this clade the following sister taxon relationships exist: *B. bifarius* is the closest sister to *B. ternarius*, and *B. huntii* is the closest sister to *B. vosnesenskii*. From this sister clade I chose *B. huntii* and *B. vosnesenskii* as outgroups.

### *Bayesian inference*

I implemented Bayesian phylogenetic analyses in a multiprocessor (MPI) version of MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Altekar *et al.*, 2004) using models based on Akaike information criteria (cAIC) calculated in MrModeltest 2.2 (Nylander, 2004). I partitioned nuclear genes by exon and intron regions. I also partitioned the *PEPCK* indel. The models calculated for each partition are as follows: *COI* (HKY + I), *CAD* intron (HKY), *CAD* exon (HKY + I), *PEPCK* intron (GTR + I), *PEPCK* exon (HKY), *PEPCK* indel character (standard). I conducted six independent runs (2 runs per analysis) using MrBayes to ensure that tree space had been sufficiently sampled. I simulated each run for 8,000,000 generations and 4 chains (1 hot and 3 cold chains) using flat, uninformative priors and mixed models, sampling trees every 100 generations. I compared the log likelihood plots of the two runs from a single MrBayes analysis using Tracer v1.5 (Rambaut and Drummond, 2007) to ensure proper mixing of the runs and to determine at what generation stationarity occurred so that all trees prior could be discarded (burnin). I then concatenated all genes and partitions using Mesquite version 2.7 (Maddison and Maddison, 2009). I ran this concatenated dataset as a single analysis in a multiprocessor version of MrBayes for 12,000,000 generations, 4 chains, saving trees every 100 generations using flat priors and mixed models. The analysis was repeated for a total of three analyses and 6 independent runs. Clade support was determined for each analysis by calculating posterior probabilities on all trees retained after burnin using MrBayes.

### *Parsimony methods*

Strict consensus trees for each individual gene and the concatenated dataset were constructed using parsimony algorithms implemented in PAUP\* version 4.0 (Swofford, 2003).

For each dataset, a heuristic search was performed with 1000 random additions using tree bisection and reconnection (TBR) branch swapping, retaining a maximum of 500 trees per replicate. Clade support was estimated using non-parametric bootstrapping implemented in PAUP\* v4.0 (500 pseudoreplicates, 10 random additions per pseudoreplicate, retaining 200 trees per replicate).

### *Color pattern*

To test the concordance between phylogeny and color pattern, I developed a morphological coding scheme for the metasomal color patterns of *Bombus ephippiatus* and *B. wilmattae*. I observed individual specimens and drew the color patterns by hand onto a metasomal template (Fig. 1). After I had sketched several individuals of both *B. ephippiatus* and *B. wilmattae*, I used the drawings of the individual specimens to delineate variable regions of color on the metasoma. Based on my drawings, I delimited twenty-nine variable regions or characters on the dorsum of the metasoma (Fig. 3a), and coded each character for one of ten character states, each of which represents a possible color pattern (Fig. 3b). I scored only the females for this analysis. All intact female specimens used for sequencing were coded for color pattern. I then subjected the color pattern matrix to maximum parsimony (MP) analysis implemented in PAUP\* 4.0 (Swofford, 2003). All characters were unordered and equally weighted and 1000 heuristic searches were initiated using random stepwise addition and TBR branch swapping, saving a maximum of 500 trees per replicate. Clade support was estimated using non-parametric bootstrapping with 500 pseudoreplicates, 10 random stepwise additions per replicate and TBR branch swapping. I also used neighbor-joining analysis implemented in PAUP\* to determine color pattern groupings based on distance.

The topologies of the strict consensus trees derived from parsimony analysis as well as neighbor-joining of these morphological data defined a selected number of color pattern groups to which all of the individuals belonged. Using these newly defined color pattern classifications determined by both distance and parsimony analyses, I mapped color pattern by hand onto the concatenated Bayesian phylogeny in correspondence with the major groupings identified by parsimony and neighbor-joining analyses.

## Results

### *Tree resolution*

Under parsimony and Bayesian criteria, neither nuclear gene provides much resolution at this low taxonomic level, but they do share some topological congruence (Figs. 4-7). The only ingroup resolution that *CAD* elucidates is the placement of all *B. impatiens* individuals into a monophyletic clade in Bayesian inference (Figs. 4-5). Branch lengths (Fig. 4-5) indicate that *B. impatiens* is more divergent (contains more nucleotide substitutions) than the rest of the ingroup taxa. Parsimony analysis of *CAD* (Fig. 5) provides no resolution in the ingroup. *PEPCK* shows a similar pattern (Figs. 6-7), but places *B. impatiens* basal to *B. ehippiatus* and *B. wilmattae* in both Bayesian and parsimony methods. Bootstrap support for this phylogenetic separation is low (bootstrap (bp) = 78) while posterior probability support is moderate (posterior probability (pp) = 0.92) (cut-off for strong support: pp  $\geq$  0.95; bp  $\geq$  90). These nuclear genes provide moderate support and minimal resolution for the relationships in this species complex.

*COI* is more informative for the complex than either nuclear gene (Figs. 8-9). Bayesian and parsimony analyses are congruent in topology, with the exception of a few nodes. Parsimony analysis (Fig. 9) places *B. impatiens* basal to *B. ehippiatus* and *B. wilmattae* with high support (bp = 100), but Bayesian methods (Fig. 8) place *B. impatiens* only as a more divergent lineage within a polytomy, giving high support (pp = 1.00) for the monophyly of this species. *COI* also provides resolution at the tips within species groups, which is lacking for either nuclear gene.

None of the genes utilized are informative enough to completely resolve these relationships, but combined (Fig. 10-11) they provide a more lucid picture of the evolutionary history of this species complex, with Bayesian inference presenting a slightly more defined



topology of the history of this group than maximum parsimony. From the bootstrap values present, it appears that maximum parsimony was able to reconstruct the root relationships, as well as some of the more recently diverged relationships, but was unable to recover some of the more recent tip relationships determined by Bayesian inference, specifically the grouping of the Mexican *B. ehippiatus* and a subset of the Honduran *B. ehippiatus* into a single clade (Fig. 10). In general, Bayesian analysis performed slightly better than parsimony analysis in terms of resolving relationships, but as a whole they presented very similar topologies across all genes.

### *Interspecific relationships*

There is robust support for the placement of *B. impatiens* as a monophyletic clade basal to *B. ehippiatus* and *B. wilmattae*. While *PEPCK* and *COI* are able to successfully place *B. impatiens* as basal, *CAD* and Bayesian analysis of *COI* place the *B. impatiens* clade on a longer, more divergent branch from the rest of the taxa within a polytomy. This higher amount of nucleotide sequence divergence in *B. impatiens* across all of the genes results in its basal placement in the combined gene dataset phylogeny with high support across both Bayesian and parsimony inference methods (pp = 1.00; bp = 99).

There is also evidence that *B. ehippiatus* is paraphyletic with respect to *B. wilmattae*, or at the very least that they are each other's closest sister. Both *PEPCK* and *CAD* are unable provide any resolution in the relationship between these species. While *COI* resolves some of the tip relationships within *B. ehippiatus*, the amount of variation within this gene does not distinguish *B. ehippiatus* and *B. wilmattae* as separate, reciprocally monophyletic lineages. When these genes are combined, *B. wilmattae* is placed as the closest sister to the Costa Rican *B.*

*ephippiatus*. This relationship appears only in Bayesian analyses and consistently has low posterior probability support (0.78).

#### *Intraspecific relationships*

As discussed above, *B. wilmattae* is consistently placed within or near *B. ephippiatus* across all genes and phylogenetic inference methods, but only *COI* is able to provide intraspecific resolution within *B. ephippiatus* (Fig. 8). At the root of the *B. ephippiatus* + *B. wilmattae* clade, there is an unresolved polytomy, but near the tips there is some resolution of the relationships within *B. ephippiatus*. All individuals of *B. ephippiatus* from Mexico group into a single clade, which is placed as closest sister to a subset of the individuals from Honduras. While the support for the branch connecting all of the Mexican individuals (concatenated pp = 0.86, *COI* pp = 0.76) is not substantial, there is strong support (concatenated pp = 0.98, *COI* pp = 0.95) for the placement of this subset of Honduran individuals as sister to the Mexican clade.

There is strong evidence that the Costa Rican *B. ephippiatus* (concatenated pp = 1.00, concatenated bp = 97) and *B. wilmattae* (concatenated pp = 1.00, concatenated bp = 93) are separate monophyletic clades, but, as discussed above, there is low support for their placement as sister clades. There is also robust support for the monophyly of a subset of the Honduran *B. ephippiatus* (concatenated pp = 1.00, concatenated bp = 99), but Bayesian and parsimony methods provide no resolution as to where this clade fits within the *B. ephippiatus* + *B. wilmattae* complex.

### *Color pattern*

Neighbor-joining and maximum parsimony analysis of the color pattern matrix revealed two major color pattern groupings in *B. ehippiatus* and *B. wilmattae* (Figs. 13-14). For nominal purposes, these groups will be called the “red phenotype,” which possesses yellow, red, and black hair on its abdomen, and the “black phenotype,” which possesses only yellow and black hair on its abdomen. When these phenotypes are mapped by hand onto the molecular phylogeny (Fig. 15), one can see that the red phenotype predominates in the Mexican *B. ehippiatus* clades, and the black phenotype predominates in the Costa Rican *B. ehippiatus*, Honduran *B. ehippiatus*, and *B. wilmattae* clades, but there is also some mixing. All of the individuals within the Mexican *B. ehippiatus* clade possessing the black phenotype are from localities in close proximity (see overlapping coordinate points on Fig. 16) within the Mexican state of Querétaro, a more southern state within the country. These are the only female samples from Querétaro, therefore all samples from this state possess the black phenotype. The Honduran *B. ehippiatus* possessing the red phenotype, VEP51, is from the department of Francisco Morazán, but individual VEP50 was collected from the same locality in Honduras and it possesses a black phenotype.

## Discussion

### *Interspecific relationships*

The molecular data provide robust support for the placement of *Bombus impatiens* basal to *B. ephippiatus* and *B. wilmattae*. *PEPCK* and parsimony analysis of *COI* show strong support for this basal placement. While *CAD* and Bayesian analysis of *COI* are unable to resolve *B. impatiens* as basal, the relatively higher amount of divergence in *B. impatiens* present in both of these gene topologies lends to the high support for its basal placement in the concatenated phylogeny. These data provide a different picture of these relationships than the *Bombus* phylogeny of Cameron *et al.* (2007). This previous study presents incongruent gene topologies regarding this species complex. The concatenated phylogeny of Cameron *et al.* (2007) places *B. impatiens* and *B. wilmattae* as sister taxa, yet this relationship is supported only by *16S* gene fragment data; the phylogeny for the *opsin* gene shows *B. ephippiatus* and *B. wilmattae* as sister groups, and the phylogeny for arginine kinase (*ArgK*) shows *B. impatiens* and *B. ephippiatus* as sister taxa. The placement of *B. ephippiatus* and *B. wilmattae* as sisters in the present analysis is logical due to the high morphological similarity between these two species. Because their color patterns are so similar, they have been considered variants of the same species in the past (Cockerell, 1912). The very slight differences in genitalia features (Williams, 1998) have also served as evidence towards the argument that they are conspecific. This relationship is logical also in terms of the species' geographic distribution. *B. wilmattae* exists in a highly restricted distribution within the range of *B. ephippiatus* and neither of these species comes into contact with the range of *B. impatiens* (Labougle, 1990; Vergara, 2008). The hypothesis that *B. ephippiatus* and *B. wilmattae* share a more recent common ancestor with each other than *B.*

*impatiens* seems much more probable than either of these species being sister taxa to *B. impatiens* in light of geographic distribution.

These results also raise questions regarding the ancestral distribution of this species complex. Hines (2008) estimated the ancestral distribution of this clade to have been in the Southern Nearctic, but in light of this new phylogeny, biogeographic analyses might reveal an Eastern Nearctic origin. In the phylogeny used by Hines to reconstruct ancestral distributions (2008), *B. impatiens* and *B. wilmattae* were sister taxa and *B. ephippiatus* was basal. Therefore the predicted distribution was Southern Neartic, with the clade originating in Central America. Given this biogeographic hypothesis, the speciation of *B. impatiens* was the result of a dispersal event into North America. In light of the data presented here, as well as the fact that all of the closest sister species to this complex have North American distributions (Cameron *et al.*, 2007), it is now plausible that the *B. impatiens*-*B. ephippiatus*-*B. wilmattae* clade originated in North America and then dispersed into Central America. It is presumed many other species of *Bombus* have dispersed from North America into Central and South America (Hines, 2008), but biogeographic analyses are needed to test this hypothesis.

These data also provide evidence for the paraphyly of *B. ephippiatus* with respect to *B. wilmattae*, or that they are at least each other's closest relatives. As discussed above, *B. ephippiatus* and *B. wilmattae* possess very similar color patterns, both having yellow or white pile on the first and middle of the second abdominal tergites and black pile throughout the rest of the abdomen (Fig. 1) This has led to some debate over the taxonomic status of these species (Labougle *et al.*, 1985; Labougle, 1990). There are consistent morphological differences between *B. ephippiatus* and *B. wilmattae* (Labougle *et al.*, 1985), but these genetic data seem to suggest that gene flow between these two putative species is not restricted, or at the very least that these

two species diverged recently and complete lineage sorting of alleles has not yet occurred. Without explicitly testing divergence hypotheses under an evolutionary model, inferences about gene flow and incomplete lineage sorting remain conjecture.

### *Intraspecific relationships*

There is considerable geographic structure within *B. ehippiatus* + *B. wilmattae* (Fig. 12). All *B. ehippiatus* individuals from Costa Rica form a well-supported, reciprocally monophyletic clade, as do *B. wilmattae* from Mexico. In addition there is evidence for geographic structure within *B. ehippiatus* in Mexico. All of the individuals from Guerrero (VEP05, VEP29, VEP43) form a well-supported clade with the individuals from an adjacent state, Oaxaca (VEP07, VEP25). Individuals from Chiapas (VEP17, VEP19, SC198) also form their own well-supported clade. Even though the Honduran individuals do not group into single clade, their separation is still geographically structured. Although not resolved in the Bayesian phylogeny, maximum likelihood analyses consistently group the second Honduran *B. ehippiatus* clade with the Costa Rican *B. ehippiatus* + *B. wilmattae* clade (data not shown). This makes a northern clade including the *B. ehippiatus* from Mexico and Honduras and a southern clade including *B. wilmattae* from Chiapas, Mexico and *B. ehippiatus* from Honduras and Costa Rica (Fig. 12).

The separation of the *B. ehippiatus* from Honduras between the two principle clades appears to coincide with geography from a country-level view, but when localities of each individual are examined some discrepancies are apparent. For example, individuals VEP44 and VEP45 were collected from the same small flower garden in Reserva Biológica El Uyuca in Francisco Morazán, Honduras, yet they are more genetically distinct than most of the individuals collected from across the entirety of Mexico. The same is true for individuals VEP50 + VEP51

from Parque Nacional La Tigra in Francisco Morazán and VEP52 + VEP53 from Montaña de Comayagua in Comayagua (Fig. 10). There is also geographic incongruence concerning individuals sampled from Chiapas, Mexico. The *B. ephippiatus* and *B. wilmattae* individuals from Chiapas were collected from the same localities, yet they are clearly genetically distinct.

These examples of incongruence between geography and phylogeny generate several possible hypotheses for the evolution of this *Bombus* species complex. One possible explanation for the observed patterns of gene diversity and paraphyly is ancestral polymorphism. When isolating mechanisms take effect in a population, sorting of genetic variation between lineages will occur via genetic drift, which facilitates speciation, but in recently diverged lineages it is often the case that some populations still retain the amount of genetic variation present in the ancestral population (Avice, 2000). The high genetic diversity within the individuals from Honduras could be a case of ancestral polymorphism. Honduras might represent the ancestral distribution of the *B. ephippiatus* + *B. wilmattae* clade.

Another possible explanation of these patterns is that the region between Chiapas, Mexico and Honduras is a contact zone between northern (Mexican and Honduran *B. ephippiatus*) and southern populations (Chiapan *B. wilmattae* and Honduran and Costa Rican *B. ephippiatus*) of the *B. ephippiatus*-*B. wilmattae* clade. Other studies of Mesoamerican species have found differentiation between northern taxa and southern taxa (Cavers *et al.* 2003; Novick *et al.* 2003; Devitt, 2006). For example, a study of a Mesoamerican wren species (*Campylorhynchus rufinucha*) with a distribution similar to that of *B. ephippiatus* (Vázquez-Miranda *et al.*, 2009) found patterns similar to those found here in *Bombus*, with a contact zone in Chiapas, Mexico, and determined that either of these hypotheses was plausible, but that population expansion and hybridization was more likely. Additional population genetic tests,

including model-based coalescent methods (e.g., Knowles and Maddison, 2002; Hey and Nielsen, 2004; Hickerson *et al.*, 2006), as well as larger taxa sampling and additional genetic data will be needed to distinguish between hypotheses for the evolution of the *B. ephippiatus-B. wilmattae* species complex.

### *Color pattern*

Mapping color pattern onto the *B. ephippiatus-B. wilmattae* clade suggests that color pattern variation has phylogenetic signal. The same color pattern groupings were determined from neighbor-joining methods (Fig. 13) and maximum parsimony analysis (Fig. 14). One clade represents the “red phenotype,” with individuals possessing red pile on the second and third abdominal tergites; the other clade represents the “black phenotype” with individuals possessing black pile instead of red pile. When these color pattern groupings are mapped onto the phylogeny (Fig. 15), only two cases of incongruence are apparent. First, one Honduran individual possesses the red phenotype predominant in the Mexican clade, a result that can likely be explained by limited sampling. While only twelve individuals from Honduras were sequenced for this phylogeny to keep sampling across localities consistent, 160 individual specimens from six localities were collected in total from this region, and each was scored for color pattern (data not shown). While the red phenotype does not appear common in Honduras based on sequenced individuals, these specimens are a subset of the total sample. The red phenotype is much more common in the total sample than shown from this small subset represented in the phylogeny (data not shown). There is a more evenly mixed phenotypic distribution within Honduras (Fig. 16), which is congruent with the “mixed” phylogenetic separation of the Honduran individuals (Fig. 12). This high amount of variation in color pattern in Honduras along with the high amount



of genetic diversity provides more evidence that Honduras is either a contact zone between expanding populations or represents the ancestral distribution.

The other individuals that show incongruence between color pattern and phylogeny are in the Mexican clade of *B. ephippiatus*. The individuals within the Mexican clade that possess a black phenotype all originate from localities within the state of Querétaro (Fig. 16). A pattern similar to this morphological differentiation is also present in the rufous-naped wren that shares a similar distribution (Vázquez-Miranda *et al.*, 2009); one of the subspecies along the Eastern coast of Mexico possesses a different color pattern than those along the Western coast. It is possible that increased sampling of *B. ephippiatus* closer to the Eastern coast of Mexico might reveal a black phenotype throughout the East, and a red phenotype throughout the West.

This amount of congruence between color pattern and phylogeny is atypical in *Bombus*. Recent efforts to reconstruct ancestral color patterns using the *Bombus* phylogeny (Cameron *et al.* 2007) have been unsuccessful due to strong convergence in color patterns associated with the abundance of Müllerian mimicry complexes within the genus (Cameron *et al.*, unpubl.). The recent divergence of the *B. ephippiatus-wilmattae-impatiens* clade in concert with a lack of mimicking species might explain this unusual amount of morphological and phylogenetic concordance.

With the exception of the individuals from Querétaro, these color pattern groupings are also concordant with geography (Fig. 16). The distribution of morphology across geography is similar to the distribution of phylogenetic clades (Fig. 12), and both exhibit intermediate geographic regions where color pattern groupings or phylogenetic clades overlap. The distribution of the red, bright forms in the North and the black, darker forms in the South follows patterns typical of bumble bee species in general. Williams (2007) found that the all-black and

mostly black species of bumble bees are most frequent near the equator and the palest forms are most frequent at mid latitudes (e.g. Neotropics). The biological phenomena behind this pattern have yet to be determined, but *B. ephippiatus* might prove to be an exemplar system in which to study the effect of bioclimatic variables on color pattern.

#### *Implications for commercial rearing*

Bees have been imported and introduced for the commercial pollination of crops around the world, and the importation of bumble bees for greenhouse pollination has increased in recent years. The two main species used for greenhouse pollination are the European *Bombus terrestris* and *B. impatiens*. *B. terrestris* has been introduced to Australia, Asia, and South America and has established in many areas of its introduction (Macfarlane and Gurr, 1995; Goulson, 2003; Thorp, 2003; Frietas *et al.*, 2003). Where the importation of this species is causing the most problems are Japan, where interspecific mating of *B. terrestris* with *B. hypocrita* is causing significant reproductive disturbance (Kondo *et al.*, 2009), and Korea, where it is actively hybridizing with the native species, *B. ignitus* (Yoon *et al.* 2009).

Currently, *B. impatiens* is being imported throughout the United States, Canada, and Mexico for greenhouse pollination (Velthuis and van Doorn, 2006). The Dutch agricultural company Koppert Biological Systems has had a *B. impatiens* rearing facility in Querétaro, Mexico since 2001 and imports bees for greenhouse pollination to Sinaloa, Sonora, Baja California, Jalisco Michoacán, and Querétaro (Vergara, 2008). The data presented here show that *B. impatiens* is closely related to *B. ephippiatus* and *B. wilmattae*, as closely related as *B. terrestris* is to *B. ignitus* (Cameron *et al.* 2007). This suggests that *B. impatiens* might be fully capable of hybridizing with *B. ephippiatus* and *B. wilmattae* if it were to escape from its

greenhouse enclosures and naturalize in the wild. The sighting of a *B. impatiens* queen in Ciudad Guzmán, Jalisco implies that naturalization might already be occurring and the possibility of hybridization has been confirmed by experimental breeding in the Bee Lab at the University of Guadalajara in Autlán (Vergara, 2008). If *B. impatiens* were to establish in the wild in Mexico, not only does it pose a hybridization threat, but it could also transmit diseases to native species as well as cause competition for floral resources and habitat (Thorp, 2003). *B. ephippiatus* is a widespread, common species throughout Mesoamerica, and the fact that it is already being reared in laboratories (Vergara, 2008) shows that it could serve as an excellent commercial pollinator for Mexico and Central America.

#### *Future directions*

While these genetic results are insightful at the species-level, they are only able to provide a general picture in regard to the relationships within *B. ephippiatus*-*B. wilmattae*. Even though mitochondrial genes represent a single maternally inherited locus, adding another mitochondrial gene might be able to provide more resolution at the species-level and aid in constructing mitochondrial haplotype networks to reveal population structure. Microsatellites would be better suited to elucidating population structure even further, so that more detailed biogeographic hypotheses can be formulated and tested. In order to accurately determine the population structure of *B. ephippiatus*-*B. wilmattae*, additional sampling is needed. This study represents only scattered populations of *B. ephippiatus* and *B. wilmattae* throughout their ranges. *B. wilmattae* specimens from Guatemala and other sites in Chiapas, Mexico are needed. Sampling of populations of *B. ephippiatus* in Guatemala, Nicaragua, and Panama, as well as further sampling in Mexico are also needed. Extensively sampling the species throughout its

entire range will make population genetic analyses more complete and will also make it easier to identify possible geographic barriers to gene flow.

The morphological results do provide some insight into the evolution of color pattern within this species complex, but mapping traits onto phylogeny is by no means a definitive way to reconstruct evolutionary patterns. Because this group does not seem to have convergence in color pattern like most *Bombus* species (Williams, 2007), it may be possible to reconstruct ancestral color patterns using Bayesian inference methods, which can be implemented in programs like BayesTraits (Pagel *et al.*, 2004), but only once all polytomies within the phylogeny have been resolved. It is also possible that environmental factors are influencing color pattern as well. Conducting independent contrasts between color pattern data and bioclimatic variables, as well as altitude, latitude, and longitude can identify if there are any correlations between habitat and morphology.

Conducting colony-level experiments could also help to elucidate how much of an influence both variables, genetics and environment, are having on color pattern development. Hybrid experiments have been used in bumble bees in the past to conduct Mendelian genetic experiments (Owen and Plowright, 1980) involving color pattern; this study found that a single pair of alleles at one locus was controlling the expression of a red color pattern or a black color pattern in another *Pyrobombus* species, *B. melanopygus*. Crosses between black Costa Rican colonies and red Mexican colonies could provide insight into the genetic mechanisms controlling color pattern in this species. If bioclimatic variables relating to temperature are correlated with color pattern, raising *B. ephippiatus* and *B. wilmattae* colonies at different temperatures could aid in determining the role environment plays in color pattern development.

## *Conclusions*

This study provides strong support that *B. impatiens* is the more distant sister within this New World species complex, diverging earlier than *B. ehippiatus* and *B. wilmattae*. This research also supports the paraphyly of *B. ehippiatus* with respect to *B. wilmattae*, bringing in to question the taxonomic status of these two species. The *B. ehippiatus*-*B. wilmattae* clade appears to possess geographic structure, but further population genetic analyses are needed to confirm this conclusion. The morphology of this recently diverged clade appears to be concordant with phylogeny as well as geography, but continued investigation is needed to provide insight into the mechanisms behind color pattern evolution.

In a world suffering extreme losses in biodiversity, the identification of species and the forces shaping their evolutionary history is of undeniable importance in the fields of biology and conservation. This study provides insight into the evolutionary relationships among important native pollinators in the New World, while generating biogeographic hypotheses about speciation processes in the Neotropics.

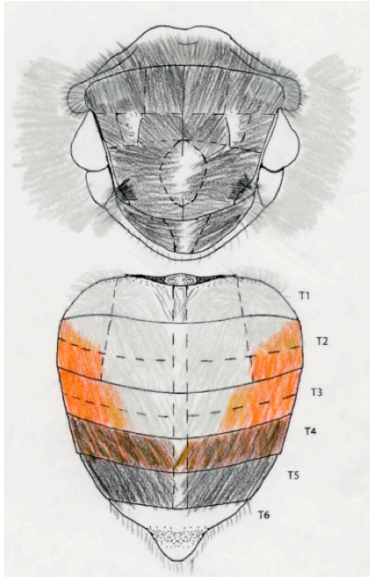
**Table 1.** Collection localities and sequence sources of taxa examined. Unless otherwise indicated with a ♂, all specimens are female. A + indicates that sequence was obtained by the author. A (+) indicates that sequence was obtained by Hines and Cameron (unpublished data). A [+] indicates that sequence was obtained by Cameron *et al.* (2007). A “Ht” indicates that individual was heterozygous for *CAD* and therefore was not used in analyses. All spaces left blank under the gene categories indicates that sequencing of that gene was unsuccessful for that individual. Accession numbers are given for those PEPCK sequences that were obtained from GenBank.

Species	Collection locality	Collector	V.#	COI	CAD	PEPCK	Acc. #
<i>ephippiatus</i>	Michoacan, Mexico	H. Hines, R. Ayala	VEP01	(+)	Ht	+	
<i>ephippiatus</i>	San Jose, Costa Rica	L. Masner	VEP02	(+)			
<i>ephippiatus</i>	Chihuahua, Mexico	K. Shaw	VEP03	(+)	+	+	
<i>ephippiatus</i>	Guerrero, Mexico	H. Hines, R. Ayala	VEP05	(+)	+	+	
<i>ephippiatus</i>	Oaxaca, Mexico	H. Hines, R. Ayala	VEP07	(+)	+	+	
<i>ephippiatus</i>	Puntarenas, Costa Rica	K. Martines	VEP09	(+)	Ht	+	
<i>ephippiatus</i>	Jalisco, Mexico	R. Ayala	VEP12	(+)	+	+	
<i>ephippiatus</i>	Queretaro, Mexico	R. Ayala	VEP13	(+)	+		
<i>ephippiatus</i>	Chihuahua, Mexico	K. Shaw	VEP14	+			
<i>ephippiatus</i>	Chiapas, Mexico	D. Sanchez	VEP17	+	+	+	
<i>ephippiatus</i>	San Jose, Costa Rica	P. Hanson	VEP18	+			
<i>ephippiatus</i>	Chiapas, Mexico	D. Sanchez	VEP19	+	Ht	+	
<i>ephippiatus</i>	Cartago, Costa Rica	P. Hanson	VEP20	+	+		
<i>ephippiatus</i>	Queretaro, Mexico	R. Ayala	VEP21	+	+	+	
<i>ephippiatus</i>	Jalisco, Mexico	R. Ayala	VEP22	+	+		
<i>ephippiatus</i>	Villa Mills, Costa Rica	A. Picado, B. Gamboa	VEP23	+	Ht	+	
<i>ephippiatus</i>	Cartago, Costa Rica	P. Hanson	VEP24	+			
<i>ephippiatus</i>	Oaxaca, Mexico	H. Hines, R. Ayala	VEP25	+	+	+	
<i>ephippiatus</i>	Chihuahua, Mexico	K. Shaw	VEP26	+	+		
<i>ephippiatus</i>	Cartago, Costa Rica	P. Hanson	VEP27	+			
<i>ephippiatus</i>	Puntarenas, Costa Rica	K. Martines	VEP28	+			
<i>ephippiatus</i>	Guerrero, Mexico	H. Hines, R. Ayala	VEP29	+	+		
<i>ephippiatus</i>	San Jose, Costa Rica	P. Hanson	VEP30	+			
<i>ephippiatus</i>	Jalisco, Mexico	R. Ayala	VEP33	+			
<i>ephippiatus</i>	Queretaro, Mexico	R. Ayala	VEP34	+	+	+	
<i>ephippiatus</i>	Queretaro, Mexico ♂	R. Ayala	VEP35	+	+	+	
<i>ephippiatus</i>	Jalisco, Mexico	R. Ayala	VEP36	+	+		
<i>ephippiatus</i>	Jalisco, Mexico ♂	R. Ayala	VEP37	+	+	+	
<i>ephippiatus</i>	Jalisco, Mexico	R. Ayala	VEP38	+	+		
<i>ephippiatus</i>	Guerrero, Mexico	H. Hines, R. Ayala	VEP43	+	+		
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP44	+	Ht	+	
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP45	+	+	+	
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP46	+	+	+	
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP47	+	+	+	
<i>ephippiatus</i>	El Paraiso, Honduras ♂	M. Duennes, J. Torres	VEP48	+		+	
<i>ephippiatus</i>	El Paraiso, Honduras ♂	M. Duennes, J. Torres	VEP49	+	+	+	
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP50	+			
<i>ephippiatus</i>	Francisco Morazan, Honduras	M. Duennes, J. Torres	VEP51	+			

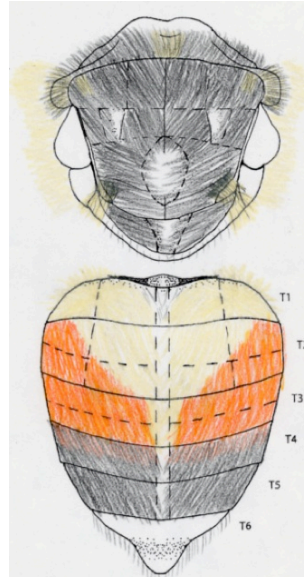
**Table 1. (cont.)**

<i>ephippiatus</i>	Comayagua, Honduras	M. Duennes, J. Torres	VEP52	+		+	
<i>ephippiatus</i>	Comayagua, Honduras	M. Duennes, J. Torres	VEP53	+		+	
<i>ephippiatus</i>	Santa Barbara, Honduras	M. Duennes, J. Torres	VEP54	+		+	
<i>ephippiatus</i>	Santa Barbara, Honduras	M. Duennes, J. Torres	VEP55	+			
<i>ephippiatus</i>	Chiapas, Mexico	M. Guzman	SC198	(+)		[+]	EF050844
<i>wilmattae</i>	Chiapas, Mexico ♂	S. Cameron	Vwilm03	(+)	+		
<i>wilmattae</i>	Chiapas, Mexico	D. Sanchez	Vwilm04	+	+		
<i>wilmattae</i>	Chiapas, Mexico	J. Nieh, R. Vandame	Vwilm06	+	Ht	+	
<i>wilmattae</i>	Chiapas, Mexico	J. Nieh, R. Vandame	Vwilm07	+	Ht		
<i>wilmattae</i>	Chiapas, Mexico	J. Nieh, R. Vandame	Vwilm10	+			
<i>wilmattae</i>	Chiapas, Mexico	R. Vandame	SC199	(+)		[+]	EF050843
<i>impatiens</i>	Maine, USA	S. Cameron, J. Whitfield	Vimpa02	+	+		
<i>impatiens</i>	Illinois, USA	H. Hines	Vimpa03	+	Ht	+	
<i>impatiens</i>	Illinois, USA ♂	H. Hines	Vimpa04	+	+		
<i>impatiens</i>	Maine, USA	S. Cameron, J. Whitfield	Vimpa06	+	+		
<i>impatiens</i>	Texas, USA	I. Stewart	IM412	+			
<i>impatiens</i>	Texas, USA	I. Stewart	IM420	+			
<i>impatiens</i>	Texas, USA	I. Stewart	IM423	+			
<i>impatiens</i>	Texas, USA	I. Stewart	IM424	+			
<i>impatiens</i>	Texas, USA	I. Stewart	IM426	+			
<i>impatiens</i>	Illinois, USA	H. Hines	SC060			[+]	EF050842

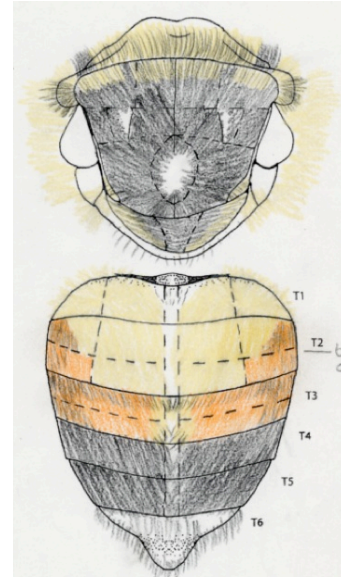
**Figure 1.** Worker color patterns of *B. ephippiatus* and *B. wilmattae*. All cartoon drawings were illustrated by the author.



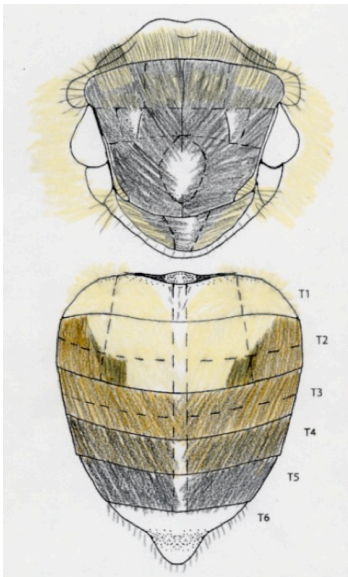
**1a.** *B. ephippiatus*



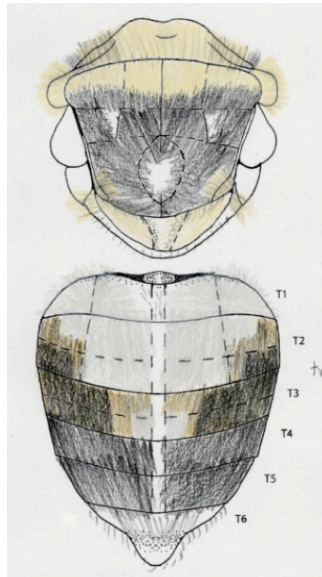
**1b.** *B. ephippiatus*



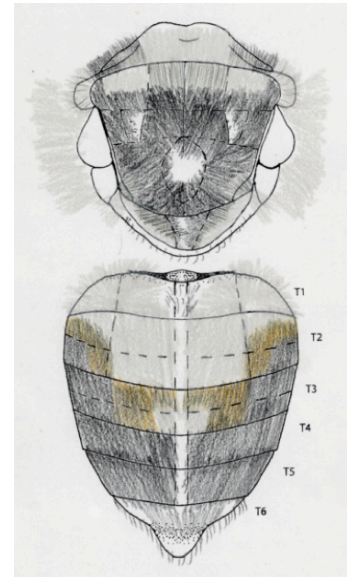
**1c.** *B. ephippiatus*



**1d.** *B. ephippiatus*

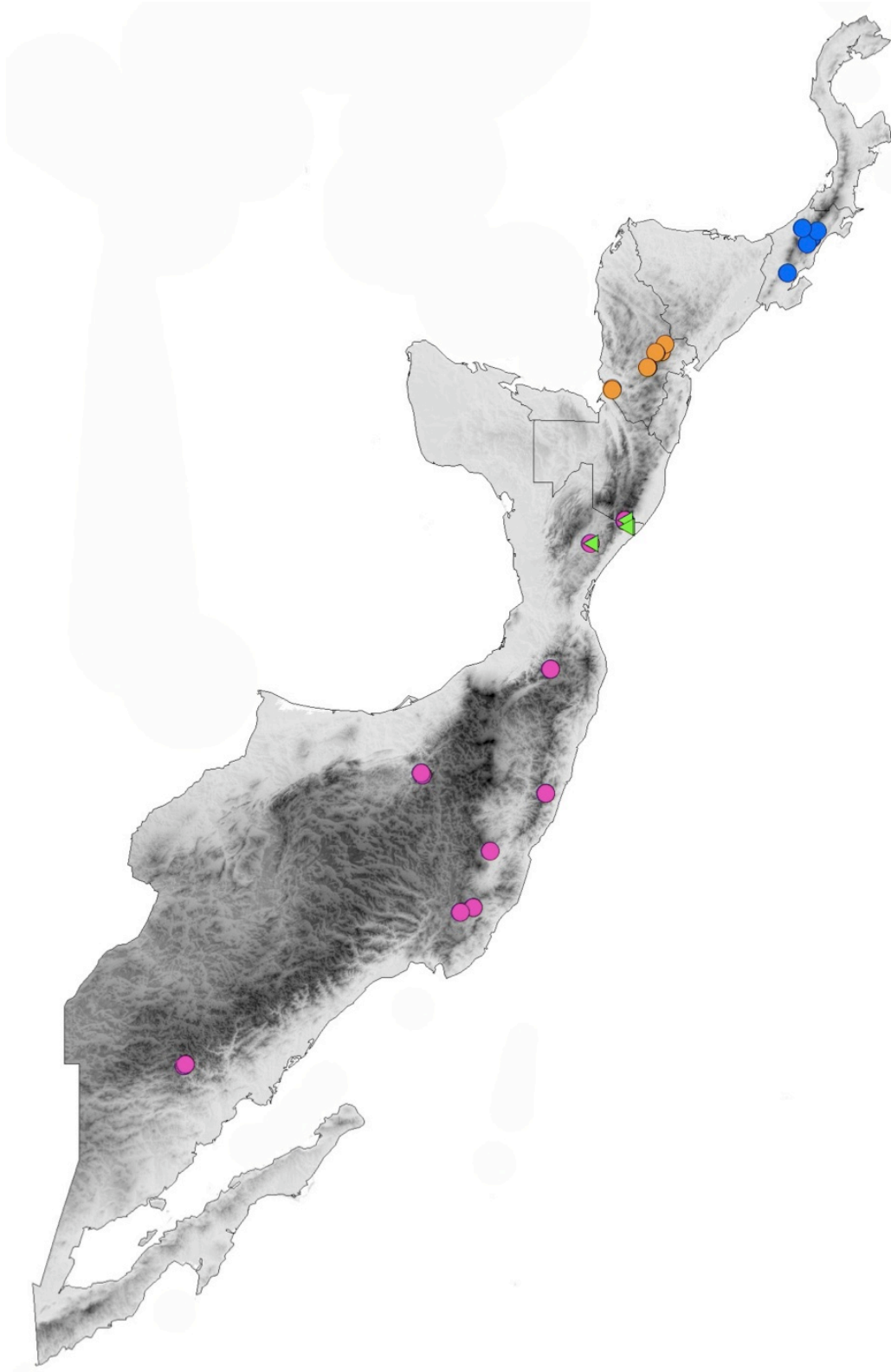


**1e.** *B. wilmattae*



**1f.** *B. wilmattae*

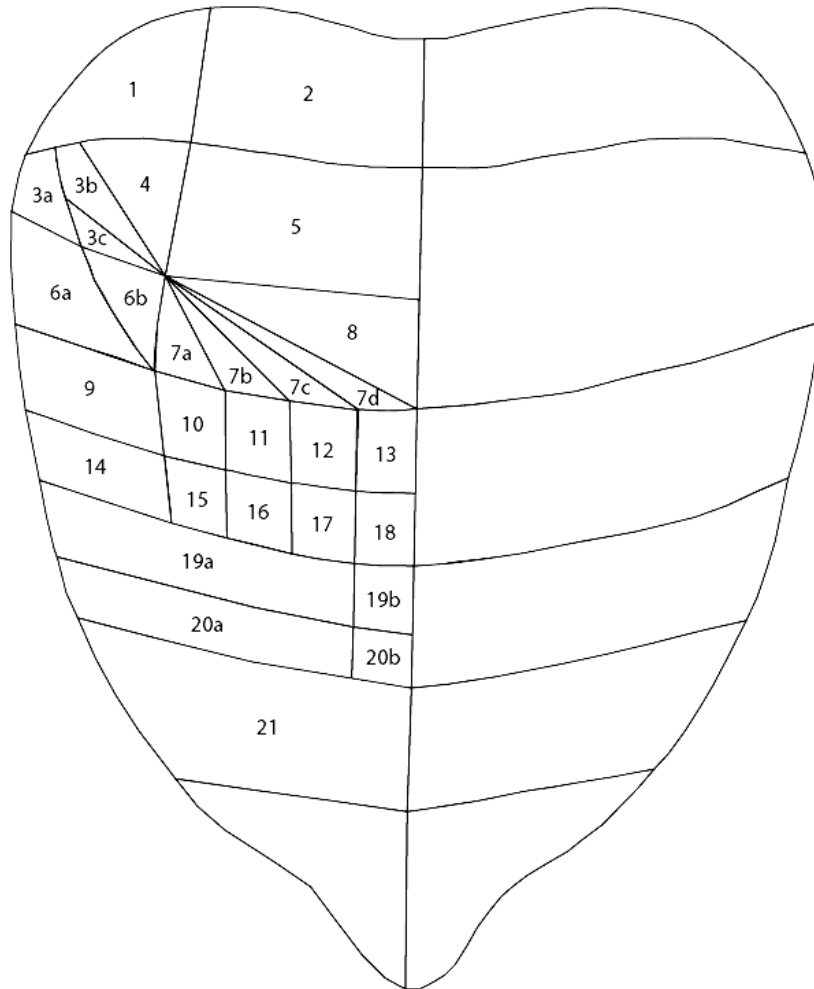




**Figure 2.** Map of collection localities of all specimens examined, excluding *B. impatiens*. Circles are *B. ephippiatus* specimens and triangles are *B. wilmattae* specimens. The colors of the points represent the country of origin. Pink = *B. ephippiatus* from Mexico; green = *B. wilmattae* from Chiapas, Mexico; orange = *B. ephippiatus* from Honduras; blue = *B. ephippiatus* from Costa Rica. Individual points on the map represent multiple samples of specimens.

**Figure 3.** Regions (characters) of the metasoma used for morphological coding and the different colors (character states) that each region can possess.

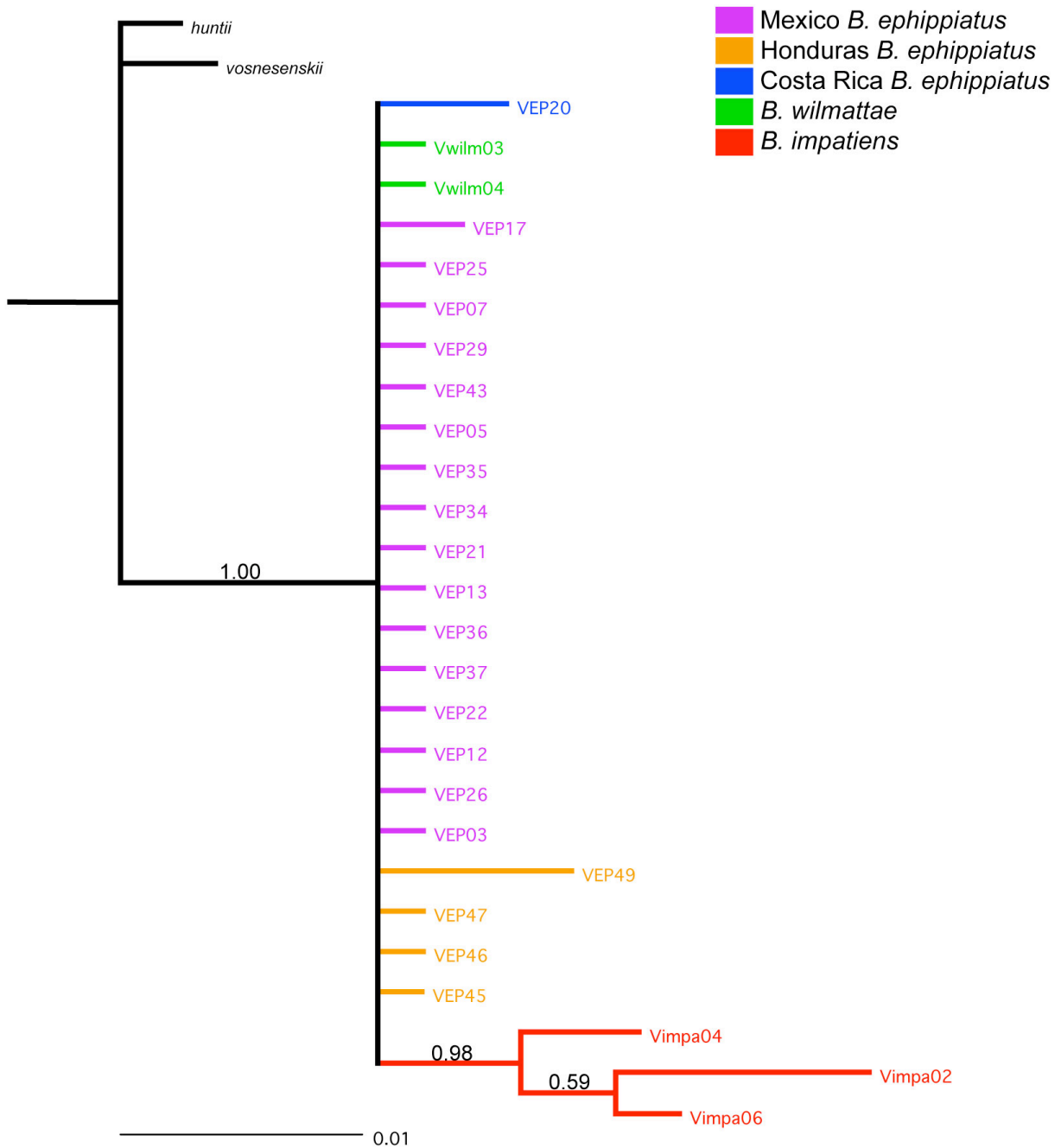
**Figure 3a.**



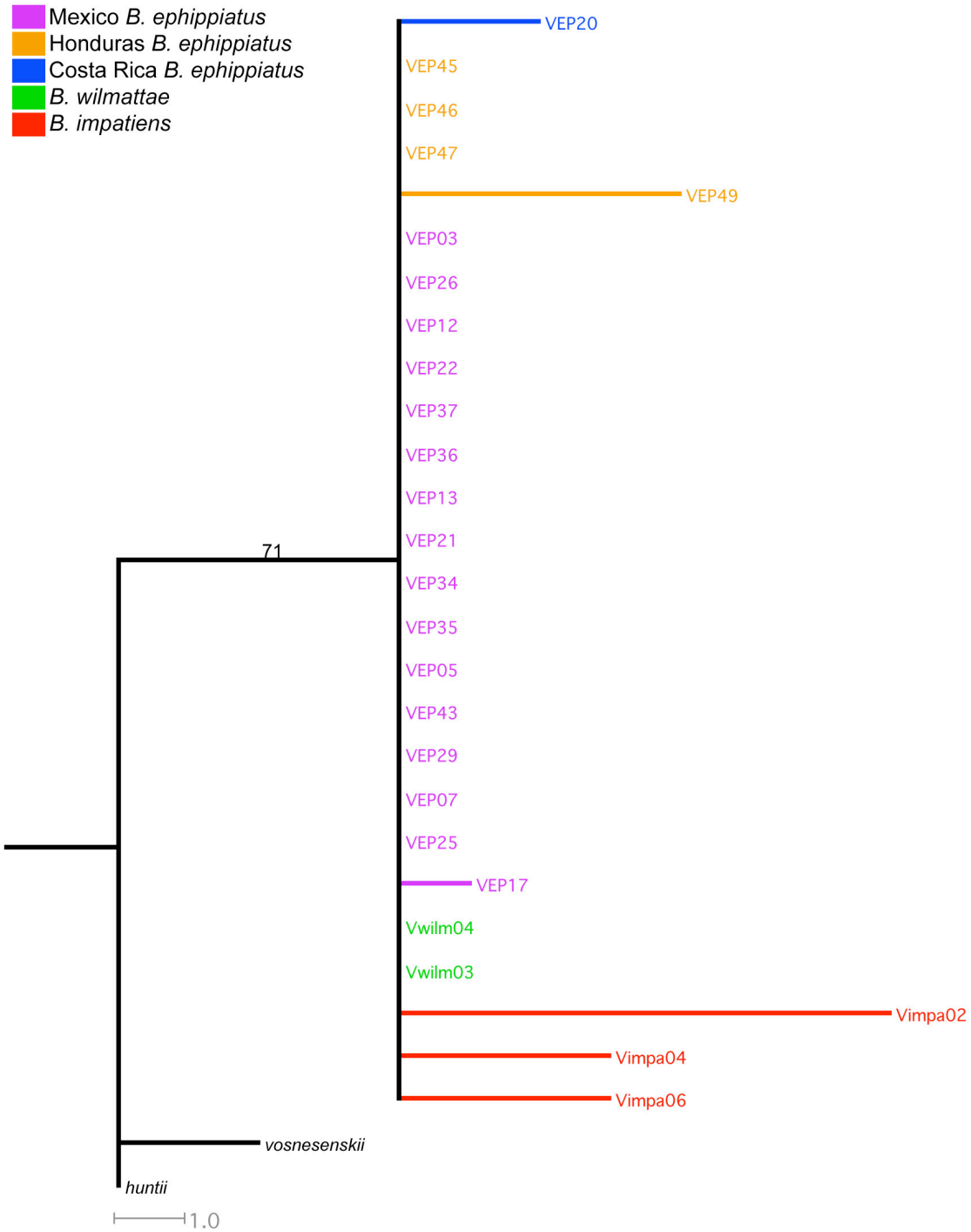
**Figure 3b.**

**Character states**

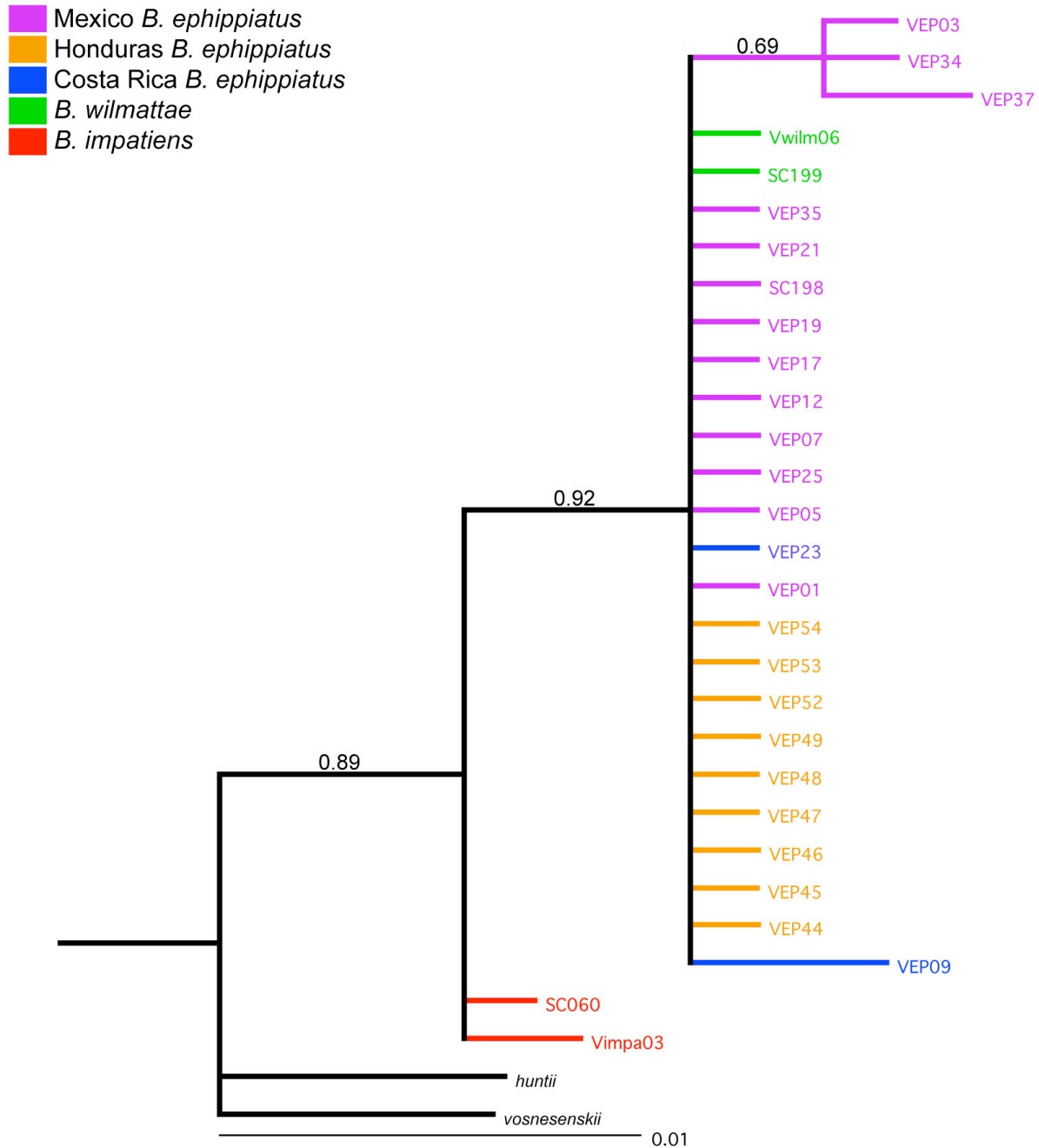
- 1 white
- 2 ivory
- 3 yellow
- 4 orange
- 5 black/orange mix
- 6 two-toned
- 7 black
- 8 black/ivory or two-tone/ivory mix
- 9 black/yellow mix
- 10 orange/yellow mix



**Figure 4.** Bayesian phylogeny based on the *CAD* dataset. Phylogeny estimated from 159,600 trees (2 runs: 8,000,000 generations, 4 chains, sampling every 100 trees, burnin=200 trees per run) using flat priors and a HKY model for the intron region (nst=2) and a HKY + I model for the exon region (nst=2). Colors on the branches correspond to geographic location and species, as color-coded on the legend in the right hand corner. Scale bar indicates % substitution that the branch lengths represent.

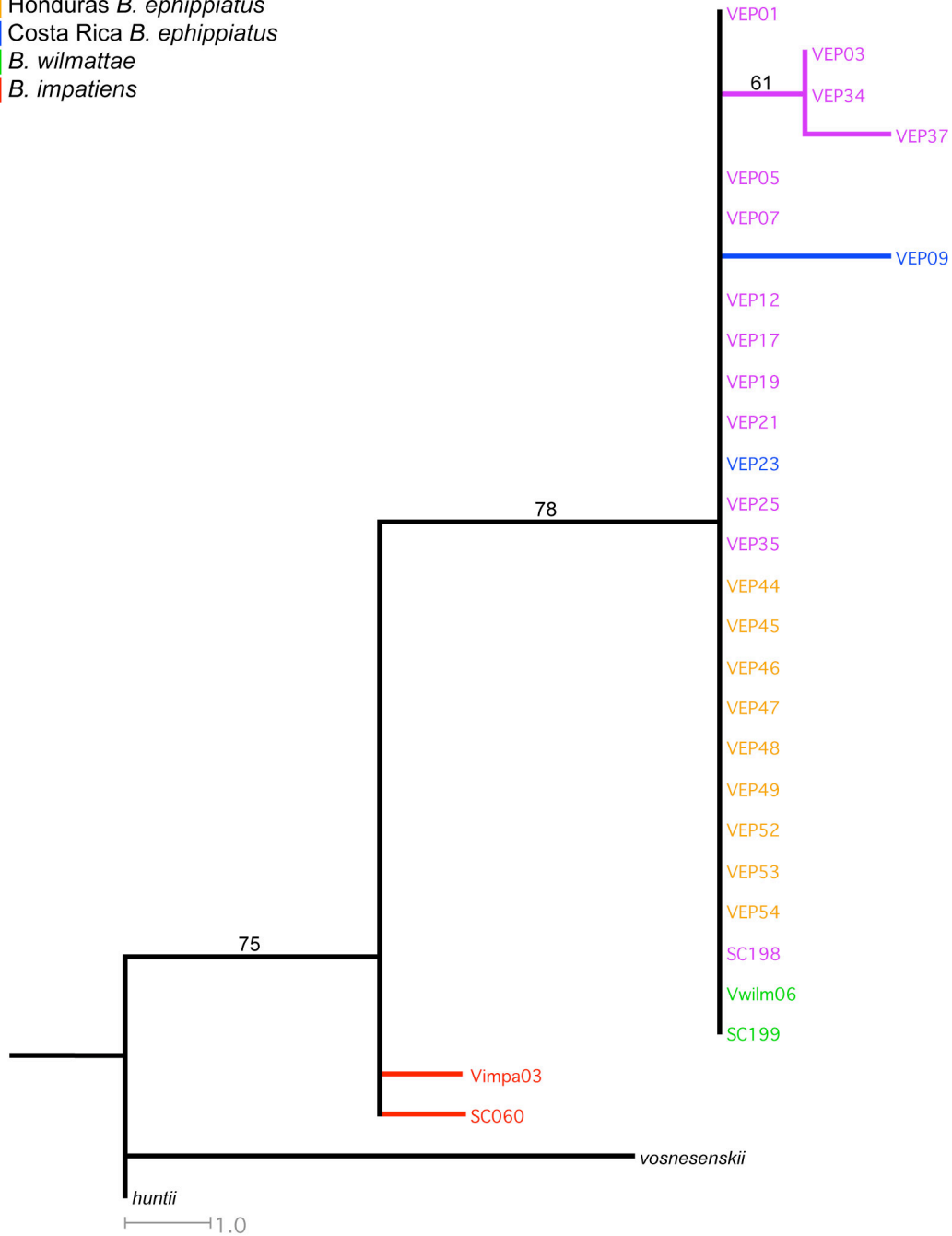


**Figure 5.** Parsimony-based phylogeny of the *CAD* dataset. Strict consensus of 9 most parsimonious trees obtained using a heuristic search (1000 random additions, TBR branch swapping, a maximum of 500 trees saved per random addition). Numbers above branches indicate all bootstrap support values  $\geq 50$ . Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates the number of substitutions that the branch lengths represent.

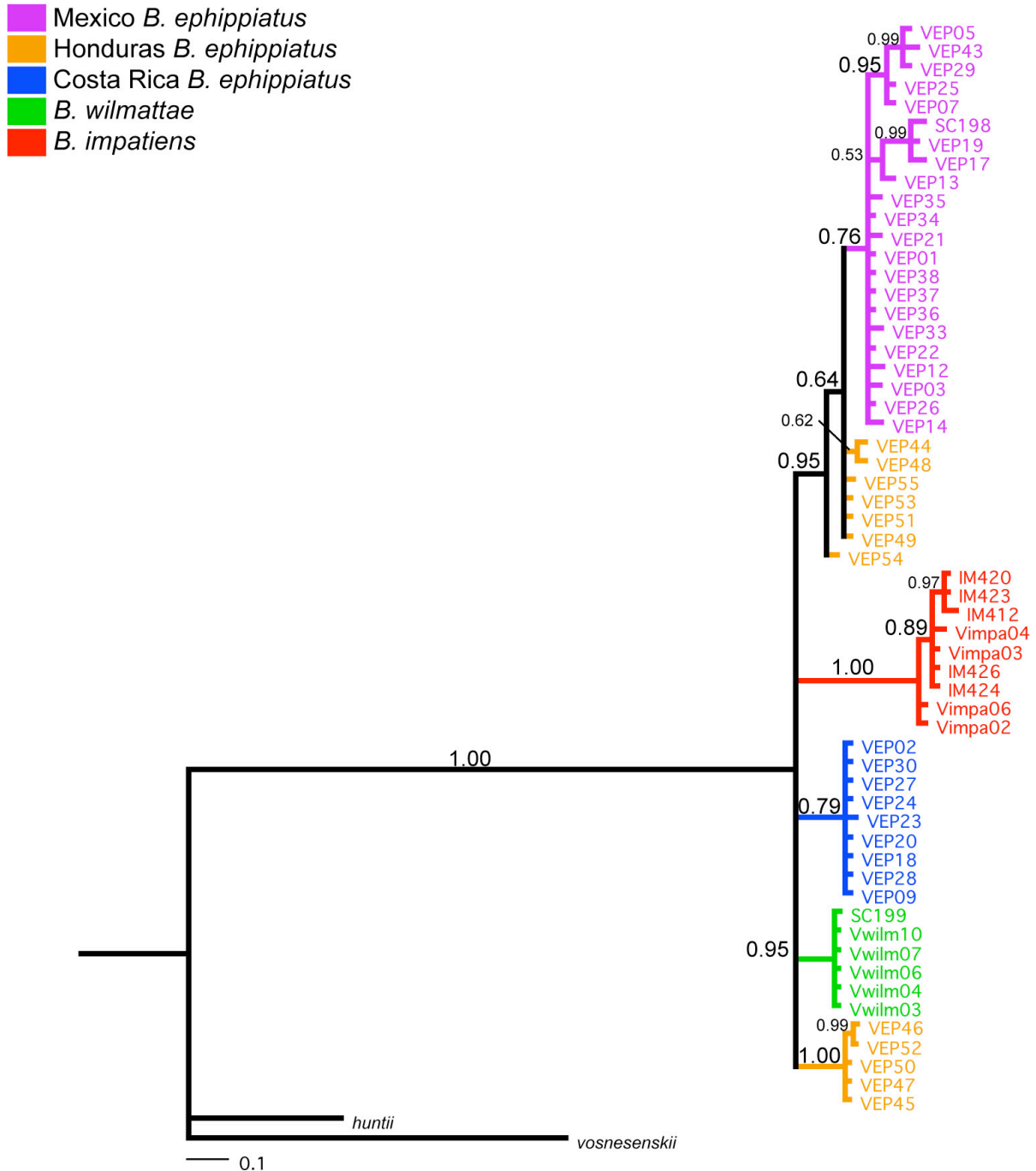


**Figure 6.** Bayesian phylogeny based on the *PEPCK* dataset. Phylogeny estimated from 159,600 trees (2 runs: 8,000,000 generations, 4 chains, sampling every 100 trees, burnin=200 trees per run) using flat priors and a GTR + I model for the intron region (nst=6), a HKY model for the exon region (nst=2) and a standard model for gap characters. Clade support values are Bayesian posterior probabilities. Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates % substitution that the branch lengths represent.

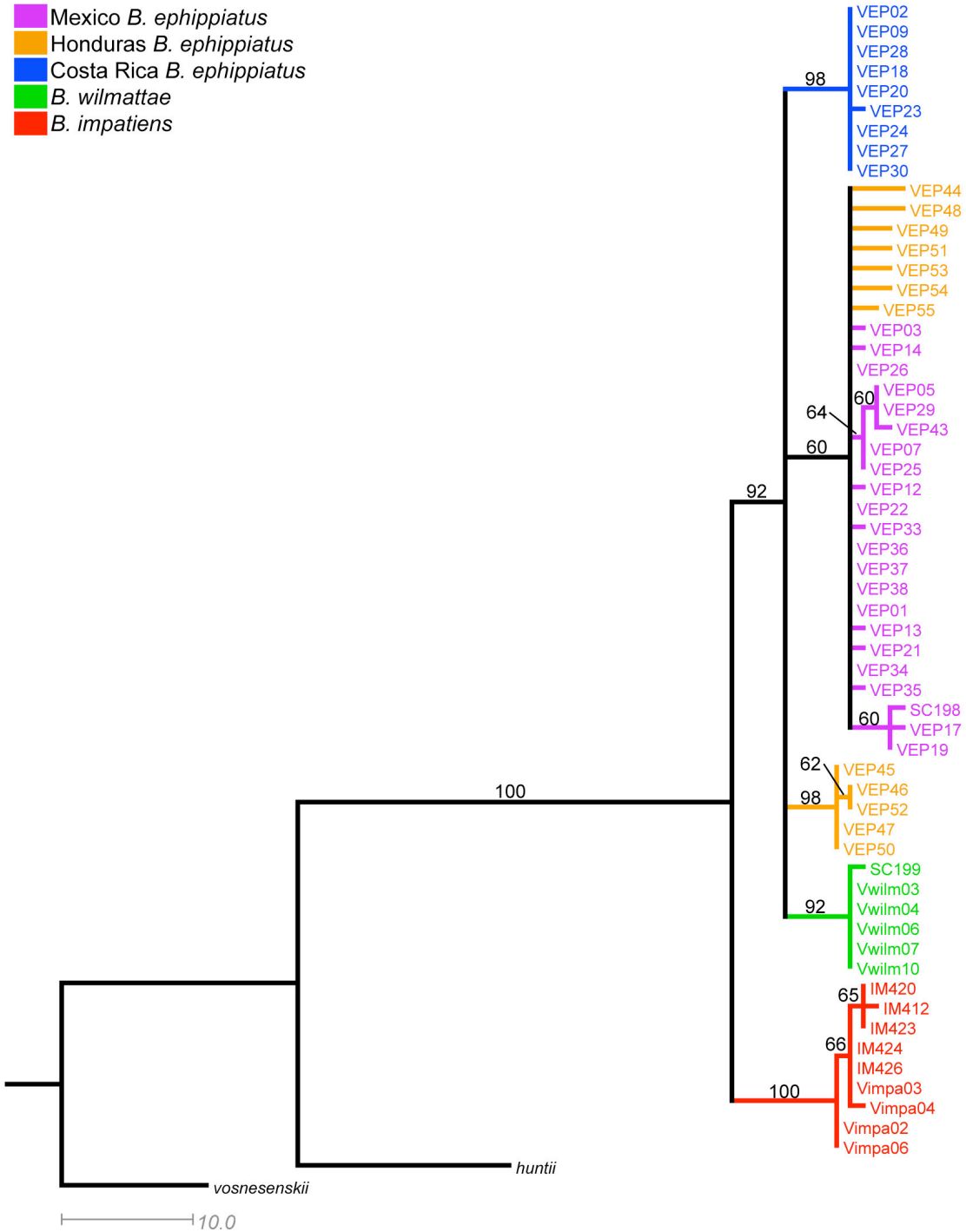
- Mexico *B. ehippiatus*
- Honduras *B. ehippiatus*
- Costa Rica *B. ehippiatus*
- *B. wilmattae*
- *B. impatiens*



**Figure 7.** Parsimony-based phylogeny of the *PEPCCK* dataset. Strict consensus of 3 most parsimonious trees obtained using a heuristic search (1000 random additions, TBR branch swapping, a maximum of 500 trees saved per random addition). Gaps were treated as a unique character state. Numbers above branches indicate all bootstrap support values  $\geq 50$ . Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates the number of substitutions that the branch lengths represent.

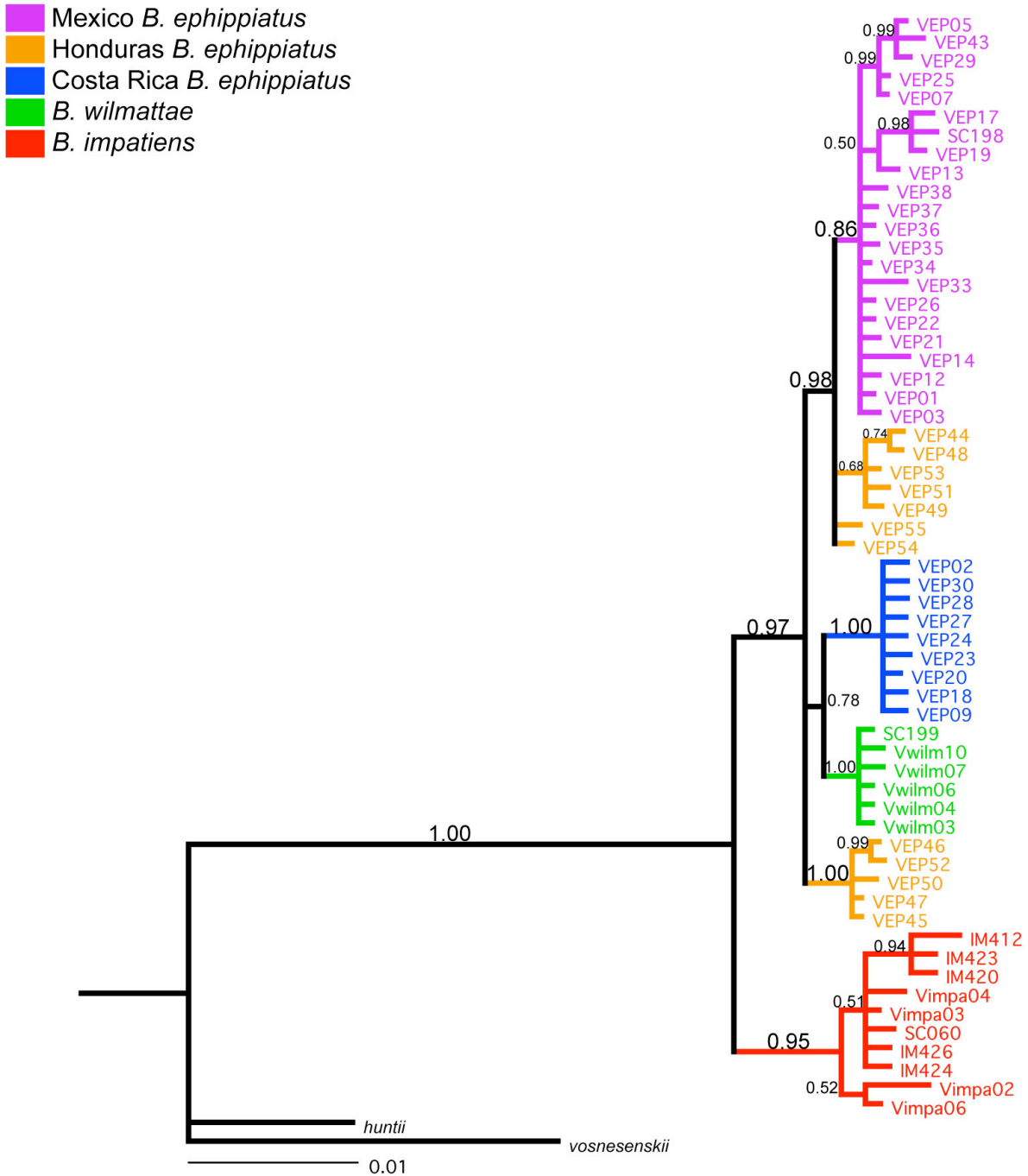


**Figure 8.** Bayesian phylogeny based on the *COI* dataset. Phylogeny estimated from 159,000 trees (2 runs: 8,000,000 generations, 4 chains, sampling every 100 trees, burnin=500 trees per run) using flat priors and a GTR + I model (nst=6). Clade support values are Bayesian posterior probabilities. Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates % substitution that the branch lengths represent.

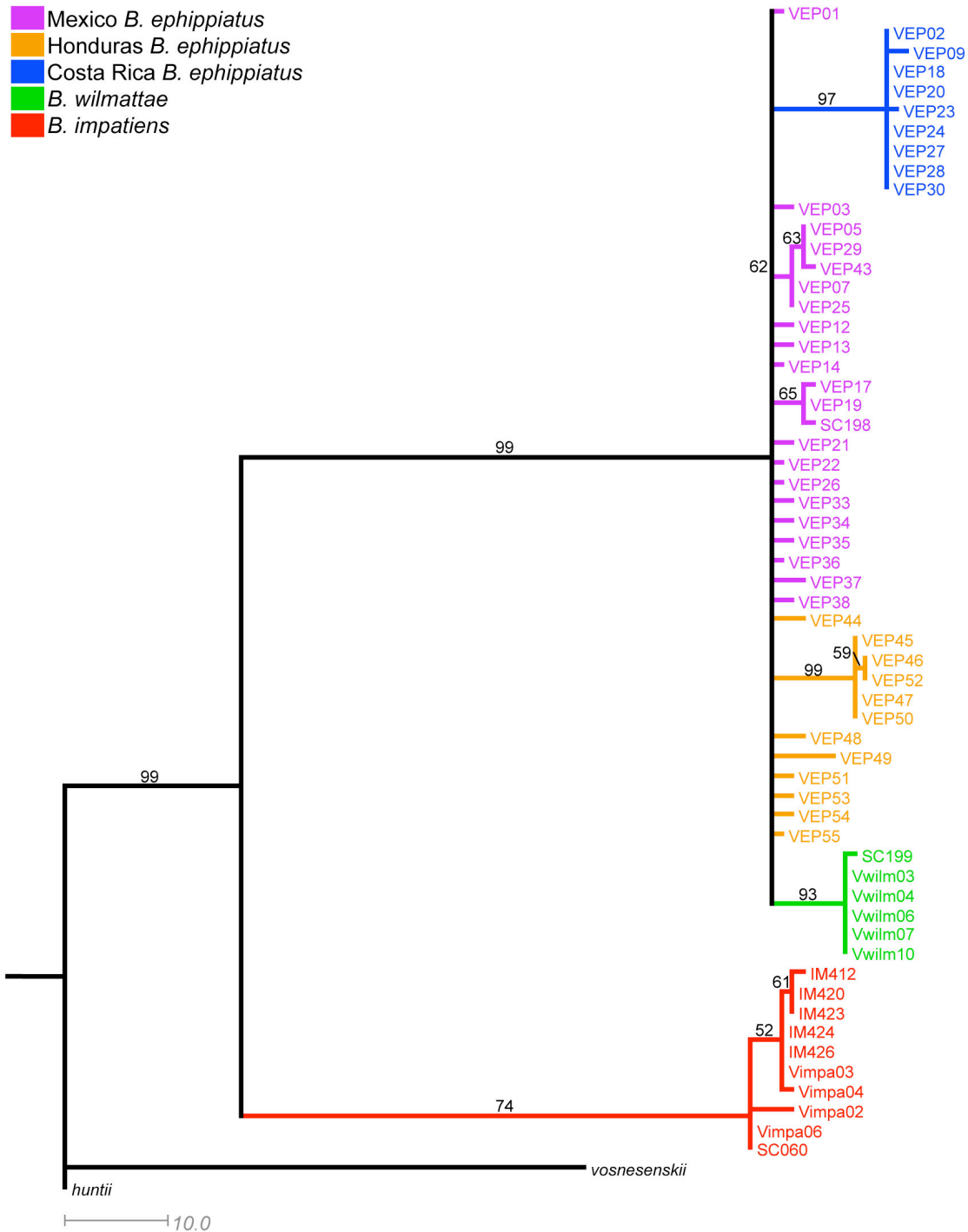


**Figure 9.** Parsimony-based phylogeny of the *COI* dataset. Strict consensus of 11,482 most parsimonious trees obtained using a heuristic search (1000 random additions, TBR branch swapping, a maximum of 500 trees saved per random addition). Numbers above branches indicate all bootstrap support values  $\geq 50$ . Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates the number of substitutions that the branch lengths represent.

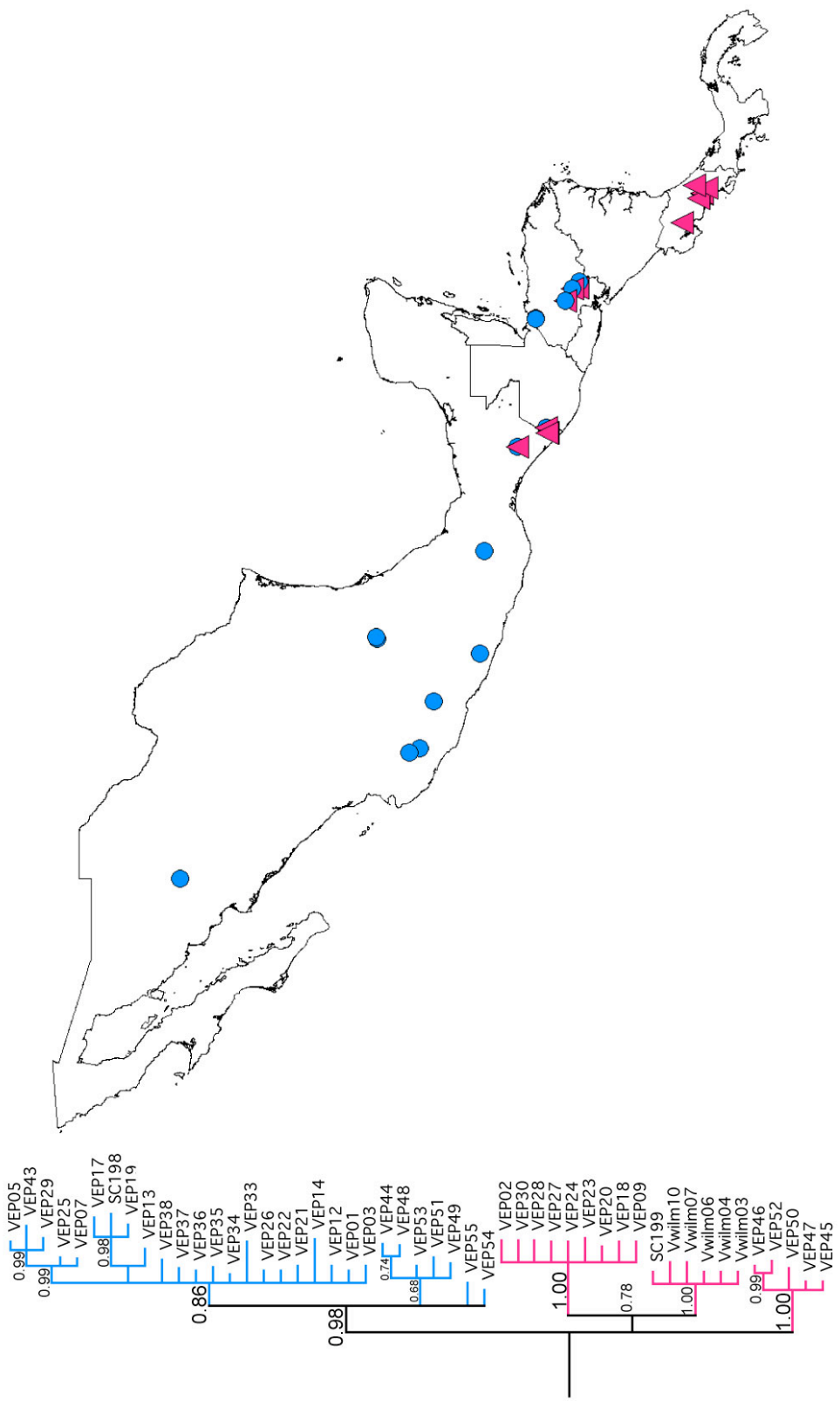




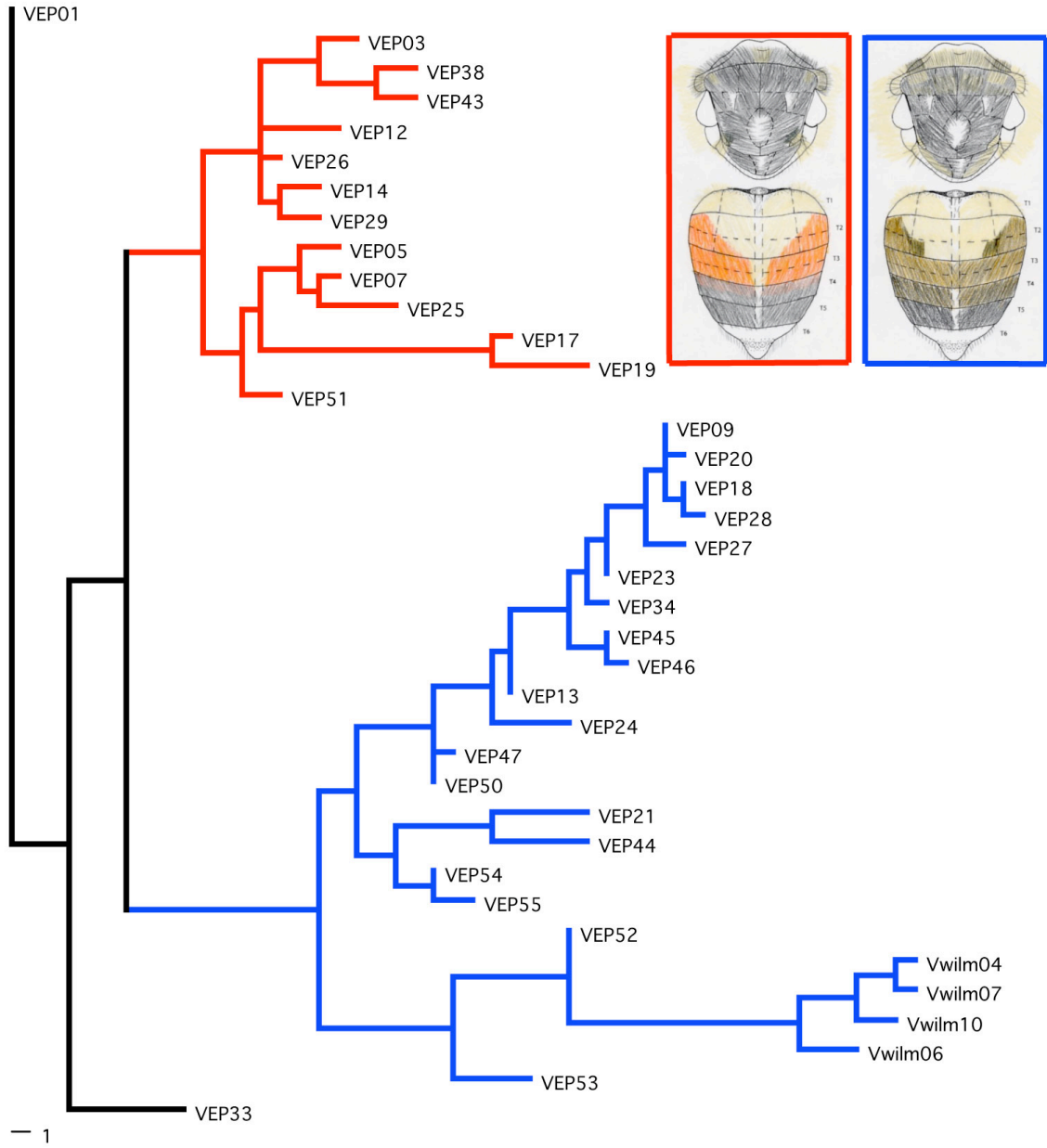
**Figure 10.** Bayesian phylogeny based on the combined gene dataset (*CAD* + *PEPCK* + *COI*). Phylogeny estimated from 237,000 trees (2 runs: 12,000,000 generations, 4 chains, sampling every 100 trees, burnin=1,500 trees per run) using flat priors, mixed models, and partitioning by gene, exon/intron, and gap characters when suitable. Clade support values are Bayesian posterior probabilities. Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates % substitution that the branch lengths represent.



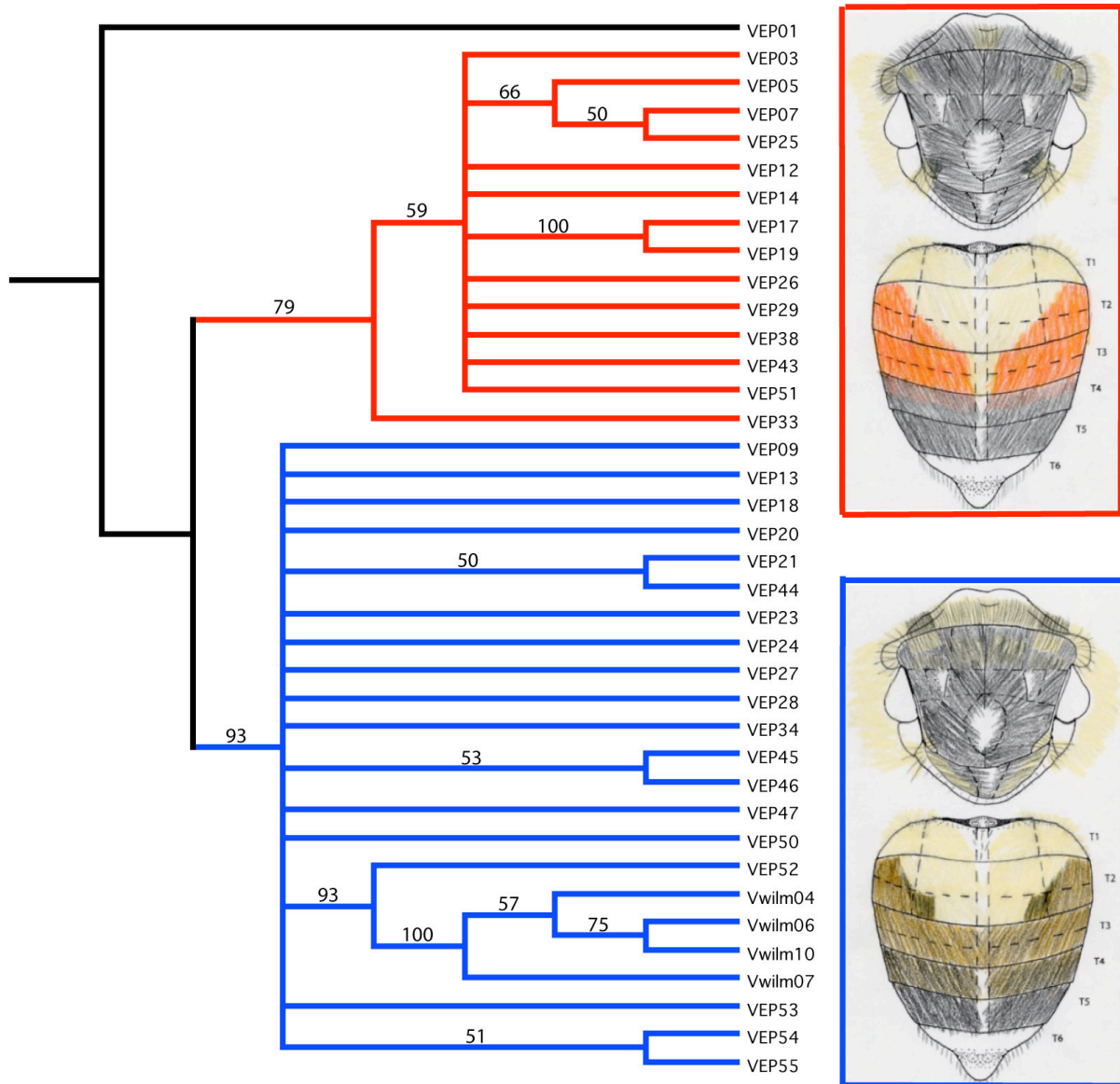
**Figure 11.** Parsimony-based phylogeny of the combined gene dataset (*CAD* + *PEPCK* + *COI*). Strict consensus of 500,000 most parsimonious trees obtained using a heuristic search (1000 random additions, TBR branch swapping, a maximum of 500 trees saved per random addition). Numbers above branches indicate all bootstrap support values  $\geq 50$ . Colors on the branches correspond to geographic location and species, as color-coded on the legend in the left hand corner. Scale bar indicates the number of substitutions that the branch lengths represent.



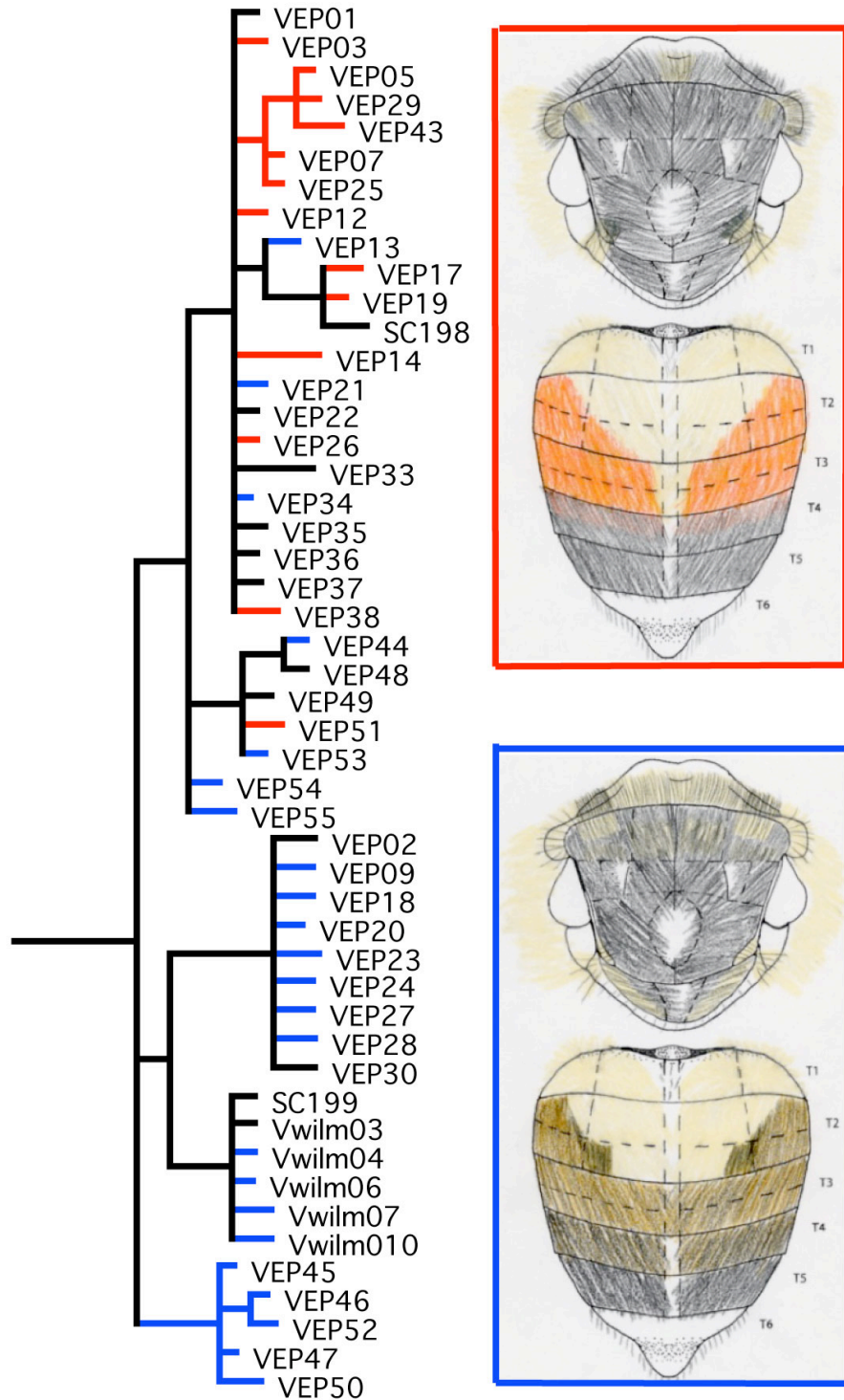
**Figure 12.** Map of collection localities of all specimens examined, excluding *B. impatiens*. This map is coded by phylogenetic clade placement. Blue circles represent specimens placed in the Mexico-Honduras clade. Pink triangles represent specimens placed in the Chiapas-Honduras-Costa Rica clade.



**Figure 13.** Neighbor-joining analysis of color pattern data for voucher specimens used in DNA phylogenies. The colors of the branches in the distance-based phylogeny correspond with color pattern phenotype, which is represented by the drawings of color pattern done by the author in the right hand corner.

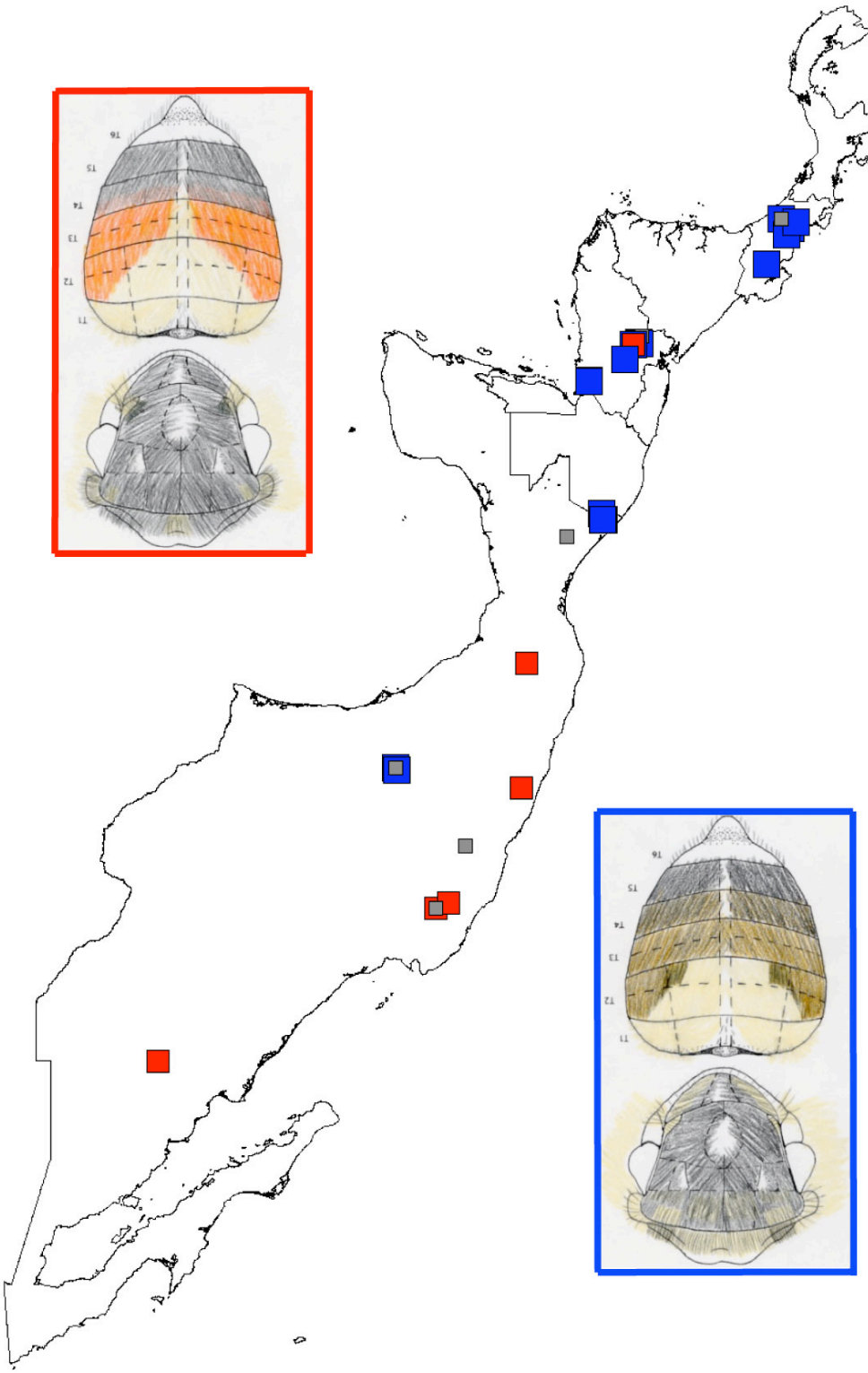


**Figure 14.** Parsimony-based phylogeny of the color pattern morphological data. Strict consensus of 45,873 most parsimonious trees obtained using a heuristic search (1000 random additions, TBR branch swapping, a maximum of 500 trees saved per random addition). Numbers above branches indicate all bootstrap support values  $\geq 50$ . Colors on the branches correspond to color pattern phenotype, as color-coded around the drawings on the right.



**Figure 15.** Color pattern groupings based on neighbor-joining and parsimony analyses mapped onto the molecular Bayesian phylogeny of the combined gene dataset. Colors on the branches represent the two main color pattern groupings, which are depicted to the right. The black terminal branches are associated with individuals that were not coded for color pattern, either because they were male or were too damaged.





**Figure 16.** Map of collection localities of all specimens examined, excluding *B. impatiens*. This map represents the distribution of the two main color patterns across geographic range. Red squares represent specimens that possess the red phenotype. Blue squares represent specimens that possess the black phenotype. Smaller grey squares represent other voucher specimens that were included in the molecular analyses, but were not coded for color pattern, either because they were male or were too damaged.

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**Appendix A. Alignments.** Alignments used for phylogenetic inference. Regions excluded from analyses are indicated with X's above the alignment and with red letters. For nuclear genes, brackets enclose introns and numbers above the alignment indicate codon position. Dashes enclosed in vertical lines above the alignment are gap regions and numbers associated with them indicate the number of the gap character starting with the first '0' or '1' at the end of the matrix for that gene.

*CAD Alignment*

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      |.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
      5      10     15     20     25     30     35     40     45     50     55     60     65     70
VEP03  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP05  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP07  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP12  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP13  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP17  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP20  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP21  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP22  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP25  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP26  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP29  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP34  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP35  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP36  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP37  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP43  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP45  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP46  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP47  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
VEP49  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
Vwilm03 GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
Vwilm04 GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
Vimpa02 GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
Vimpa04 GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGAACCCGAGTGGACGTAGGATTTTATGTCTT
Vimpa06 GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTACTCCGAGTGGACGTAGGATTTTATGTCTT
huntii  GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTAYTCCGAGTGGACGTAGGATTTTATGTCTT
vosnesenskii GATTCTACAGAGCAGTCGCTTTTCTTTTCTGGCAAATTTTGATGTATTCCGAGTGGACGTAGGATTTTATGTCTT

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      80     85     90     95     100    105    110    115    120    125    130    135    140    145
VEP03  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP05  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP07  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP12  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP13  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP17  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP20  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP21  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP22  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP25  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP26  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP29  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP34  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP35  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP36  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP37  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP43  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP45  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP46  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP47  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
VEP49  TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
Vwilm03 TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
Vwilm04 TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT
Vimpa02 TGAATATTTTCAGGTAATACAATCGGAGCGGCCCGATGGTGTCCCTTTGACATTTGGCGGACAGACTGCTCTCAAT

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Appendix A. (cont.)

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305 310 315 320 325 330 335 340 345 350 355 360 365 370

VEP03 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP05 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP07 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP12 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP13 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP17 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP20 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP21 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP22 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP25 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP26 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP29 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP34 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP35 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP36 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP37 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP43 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP45 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP46 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP47 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

VEP49 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

Wvilm03 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

Wvilm04 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

Vimpa02 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

Vimpa04 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC-----GATTAATTCGGATGTGATGTGGCGGATCTGAA

Vimpa06 GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

huntii GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

vosnesenskii GCGGTCTATTCTATCGAAGAGGTAAGAGAATCGC**AAGTCTCGC**GATTAATTCGGATGTGATGTGGCGGATCTGAA

X

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380 385 390 395 400 405 410 415 420 425 430 435 440 445

VEP03 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP05 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP07 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP12 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP13 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP17 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP20 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP21 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP22 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP25 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP26 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP29 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP34 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP35 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP36 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP37 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP43 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP45 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP46 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP47 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

VEP49 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

Wvilm03 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

Wvilm04 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

Vimpa02 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATAGAAAGTACGATC

Vimpa04 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATAGAAAGTACGATC

Vimpa06 TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATAGAAAGTACGATC

huntii TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

vosnesenskii TTGCGAGAGCCATCTTCGGCGATCTATACTTTCGGAAAATACAGATTCCTACACAGGCTATCGAAAGTACGATC

.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|

455 460 465 470 475 480 485 490 495 500 505 510 515 520

VEP03 TATTCACGTGAGTTTGAAGTTTCACAGTTTATTCATTATCGATATTCAAATCGATTATTCACAGGAGGATAT

VEP05 TATTCACGTGAGTTTGAAGTTTCACAGTTTATTCATTATCGATATTCAAATCGATTATTCACAGGAGGATAT

VEP07 TATTCACGTGAGTTTGAAGTTTCACAGTTTATTCATTATCGATATTCAAATCGATTATTCACAGGAGGATAT

VEP12 TATTCACGTGAGTTTGAAGTTTCACAGTTTATTCATTATCGATATTCAAATCGATTATTCACAGGAGGATAT





## Appendix A. (cont.)

VEP49 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 Vwilm03 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 Vwilm04 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 Vimpa02 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 Vimpa04 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 Vimpa06 CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 huntii CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC  
 vosnesenskii CATCGATAAATCATTGAAAGGCTGGAAAGAGGTGGAATACGAGGTCGTTCCGGATGCGTACGACAATTGTATCAC

....|....|....|  
 830 835

VEP03 AGTTTGCAACATGGA  
 VEP05 AGTTTGCAACATGGA  
 VEP07 AGTTTGCAACATGGA  
 VEP12 AGTTTGCAACATGGA  
 VEP13 AGTTTGCAACATGGA  
 VEP17 AGTTTGCAACATGGA  
 VEP20 AGTTTGCAACATGGA  
 VEP21 AGTTTGCAACATGGA  
 VEP22 AGTTTGCAACATGGA  
 VEP25 AGTTTGCAACATGGA  
 VEP26 AGTTTGCAACATGGA  
 VEP29 AGTTTGCAACATGGA  
 VEP34 AGTTTGCAACATGGA  
 VEP35 AGTTTGCAACATGGA  
 VEP36 AGTTTGCAACATGGA  
 VEP37 AGTTTGCAACATGGA  
 VEP43 AGTTTGCAACATGGA  
 VEP45 AGTTTGCAACATGGA  
 VEP46 AGTTTGCAACATGGA  
 VEP47 AGTTTGCAACATGGA  
 VEP49 AGTTTGCAACATGGA  
 Vwilm03 AGTTTGCAACATGGA  
 Vwilm04 AGTTTGCAACATGGA  
 Vimpa02 AGTTTGCAACATGGA  
 Vimpa04 AGTTTGCAACATGGA  
 Vimpa06 AGTTTGCAACATGGA  
 huntii AGTTTGCAACATGGA  
 vosnesenskii AGTTTGCAACATGGA







































**Appendix B. Sequence distance matrices. Matrices of Uncorrected ‘p’ distances for sequence datasets.**

*CAD Distance Matrix*

	VEP20	VEP45	VEP46	VEP47	VEP49	VEP03	VEP26	VEP12	VEP22	VEP37	VEP36	VEP13	VEP21	VEP34	VEP35	VEP05
VEP20																
VEP45	0.00242															
VEP46	0.00242	0														
VEP47	0.00242	0	0													
VEP49	0.00725	0.00483	0.00483													
VEP03	0.00242	0	0	0.00483												
VEP26	0.00242	0	0	0.00483	0											
VEP12	0.00242	0	0	0.00483	0	0										
VEP22	0.00242	0	0	0.00483	0	0	0									
VEP37	0.00242	0	0	0.00483	0	0	0	0								
VEP36	0.00242	0	0	0.00483	0	0	0	0	0							
VEP13	0.00242	0	0	0.00483	0	0	0	0	0	0						
VEP21	0.00242	0	0	0.00483	0	0	0	0	0	0	0					
VEP34	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0				
VEP35	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0			
VEP05	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0		
VEP43	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	
VEP29	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	
VEP07	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	
VEP25	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	
VEP17	0.00366	0.00122	0.00122	0.00122	0.00121	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122	0.00122
Vwilm04	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	0
Vwilm03	0.00242	0	0	0.00483	0	0	0	0	0	0	0	0	0	0	0	0
Vimpa02	0.00848	0.00847	0.00847	0.00847	0.00484	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847	0.00847
Vimpa04	0.00363	0.00362	0.00362	0.00362	0.00846	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362
Vimpa06	0.00364	0.00362	0.00362	0.00362	0.00363	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362	0.00362
huttii	0.00485	0.00485	0.00485	0.00485	0.00727	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485	0.00485
vosnesenskii	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725	0.00725

**Appendix B. (cont.)**

	VEP43	VEP29	VEP07	VEP25	VEP17	Vwilm04	Vwilm03	Vimpa02	Vimpa04	Vimpa06	huntii
VEP20											
VEP45											
VEP46											
VEP47											
VEP49											
VEP03											
VEP26											
VEP12											
VEP22											
VEP37											
VEP36											
VEP13											
VEP21											
VEP34											
VEP35											
VEP05											
VEP43											
VEP29	0										
VEP07	0	0									
VEP25	0	0	0								
VEP17	0.00122	0.00122	0.00122	0.00122							
Vwilm04	0	0	0	0	0.00122						
Vwilm03	0	0	0	0	0.00122	0					
Vimpa02	0.00847	0.00847	0.00847	0.00847	0.00606	0.00847	0.00847				
Vimpa04	0.00362	0.00362	0.00362	0.00362	0.00365	0.00362	0.00362	0.00727			
Vimpa06	0.00362	0.00362	0.00362	0.00362	0.00244	0.00362	0.00362	0.00363	0.00242		
huntii	0.00485	0.00485	0.00485	0.00485	0.00489	0.00485	0.00485	0.0073	0.00606	0.00364	
vosnesenskii	0.00725	0.00725	0.00725	0.00725	0.00485	0.00725	0.00725	0.00726	0.00846	0.00483	0.00121



Appendix B. (cont.)

PEPCK Distance Matrix

	VEP01	VEP03	VEP05	VEP07	VEP09	VEP12	VEP17	VEP19	VEP21	VEP23	VEP25	VEP34	VEP35	VEP37	VEP44	VEP45
VEP01																
VEP03	0.00114															
VEP05	0	0.00114														
VEP07	0	0.00114	0													
VEP09	0.00228	0.00342	0.00228													
VEP12	0	0.00114	0	0	0.00228											
VEP17	0	0.00114	0	0	0.00228	0										
VEP19	0	0.00114	0	0	0.00228	0	0									
VEP21	0	0.00114	0	0	0.00228	0	0	0								
VEP23	0	0.00114	0	0	0.00228	0	0	0	0							
VEP25	0	0.00114	0	0	0.00228	0	0	0	0	0						
VEP34	0.00114	0	0.00114	0.00114	0.00342	0.00114	0.00114	0.00114	0.00114	0.00114	0.00114					
VEP35	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114				
VEP37	0.00228	0.00114	0.00228	0.00228	0.00456	0.00228	0.00228	0.00228	0.00228	0.00228	0.00228	0.00114	0.00228			
VEP44	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228		
VEP45	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	
VEP46	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP47	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP48	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP49	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP52	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP53	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
VEP54	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
SC198	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
Vvilm06	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
SC199	0	0.00114	0	0	0.00228	0	0	0	0	0	0	0.00114	0	0.00228	0	0
Vimpa03	0.00116	0.00231	0.00116	0.00116	0.00344	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116	0.00231	0.00116	0.00346	0.00116	0.00116
SC060	0	0.00115	0	0	0.00228	0	0	0	0	0	0	0	0	0.0023	0	0
hunii	0.00802	0.00917	0.00802	0.00802	0.103	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00804	0.00802	0.01032	0.00802	0.00802
vosnesenskii	0.00686	0.00802	0.00686	0.00686	0.00914	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00801	0.00686	0.00916	0.00686	0.00686

**Appendix B. (cont.)**

	VEP46	VEP47	VEP48	VEP49	VEP52	VEP53	VEP54	SC198	Vwilm06	SC199	Vimpa03	SC060	huntii
VEP01													
VEP03													
VEP05													
VEP07													
VEP09													
VEP12													
VEP17													
VEP19													
VEP21													
VEP23													
VEP25													
VEP34													
VEP35													
VEP37													
VEP44													
VEP45													
VEP46													
VEP47	0												
VEP48	0	0											
VEP49	0	0	0										
VEP52	0	0	0	0									
VEP53	0	0	0	0	0								
VEP54	0	0	0	0	0	0							
SC198	0	0	0	0	0	0	0						
Vwilm06	0	0	0	0	0	0	0	0					
SC199	0	0	0	0	0	0	0	0	0				
Vimpa03	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116	0.00116			
SC060	0	0	0	0	0	0	0	0	0	0	0		
huntii	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00802	0.00573	0.00458	
vosnesenskii	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00686	0.00573	0.00458	0.00687

## Appendix B. (cont.)

### COI Distance Matrix

	VEP02	VEP09	VEP28	VEP18	VEP20	VEP23	VEP24	VEP27	VEP30	VEP44	VEP45	VEP46	VEP47	VEP48	VEP49	VEP50
VEP02																
VEP09	0															
VEP28	0	0														
VEP18	0	0	0													
VEP20	0	0	0	0												
VEP23	0.00123	0.00123	0.00123	0.00123	0.00123											
VEP24	0	0	0	0	0	0.00123										
VEP27	0	0	0	0	0	0.00123	0									
VEP30	0	0	0	0	0	0.00123	0	0								
VEP44	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986							
VEP45	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00863						
VEP46	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00986	0.00123					
VEP47	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00863	0	0.00123				
VEP48	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986	0	0.00863	0.00986				
VEP49	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00123	0.00986	0.00123				
VEP50	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00863	0	0.00863	0.00986			
VEP51	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00123	0.00986	0.00123	0	0.00986		
VEP52	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00986	0.00123	0	0.00123	0.00986	0.0111	0.00123
VEP53	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00123	0.00986	0.0111	0.00986	0.00123	0	0.00986
VEP54	0.00863	0.00863	0.00863	0.00863	0.00863	0.00986	0.00863	0.00863	0.00863	0.00123	0.0074	0.00863	0.0074	0.00123	0.00247	0.0074
VEP55	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986	0.00247	0.00863	0.00986	0.00863	0.00247	0.00123	0.00863
VEP03	0.00991	0.00991	0.00991	0.00991	0.00991	0.01116	0.00991	0.00991	0.00991	0.00247	0.00869	0.00993	0.00869	0.00247	0.00124	0.00869
VEP14	0.00991	0.00991	0.00991	0.00991	0.00991	0.01115	0.00991	0.00991	0.00991	0.00497	0.00868	0.00993	0.00868	0.00497	0.00373	0.00868
VEP26	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP05	0.0148	0.0148	0.0148	0.0148	0.0148	0.01603	0.0148	0.0148	0.0148	0.0074	0.01356	0.0148	0.01356	0.0074	0.00617	0.01356
VEP29	0.0148	0.0148	0.0148	0.0148	0.0148	0.01603	0.0148	0.0148	0.0148	0.0074	0.01356	0.0148	0.01356	0.0074	0.00617	0.01356
VEP43	0.01603	0.01603	0.01603	0.01603	0.01603	0.01726	0.01603	0.01603	0.01603	0.00863	0.0148	0.01603	0.0148	0.00863	0.0074	0.0148
VEP12	0.01356	0.01356	0.01356	0.01356	0.01356	0.0148	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
VEP22	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP33	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00617	0.00986	0.0111	0.00986	0.00617	0.00493	0.00986

Appendix B. (cont.)

	VEP02	VEP09	VEP28	VEP18	VEP20	VEP23	VEP24	VEP27	VEP30	VEP44	VEP45	VEP46	VEP47	VEP48	VEP49	VEP50
VEP36	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP37	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP38	0.01235	0.01235	0.01235	0.01235	0.01235	0.01358	0.01235	0.01235	0.01235	0.0037	0.0111	0.01235	0.0111	0.0037	0.00247	0.0111
VEP01	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP07	0.01356	0.01356	0.01356	0.01356	0.01356	0.0148	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
VEP25	0.01356	0.01356	0.01356	0.01356	0.01356	0.0148	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
VEP13	0.01356	0.01356	0.01356	0.01356	0.01356	0.0148	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
VEP21	0.01356	0.01356	0.01356	0.01356	0.01356	0.01233	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
VEP34	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.00493	0.0111	0.01233	0.0111	0.00493	0.0037	0.0111
VEP35	0.01356	0.01356	0.01356	0.01356	0.01356	0.0148	0.01356	0.01356	0.01356	0.00617	0.01233	0.01356	0.01233	0.00617	0.00493	0.01233
SC198	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.0074	0.0111	0.01233	0.0111	0.0074	0.00617	0.0111
VEP17	0.01233	0.01233	0.01233	0.01233	0.01233	0.01356	0.01233	0.01233	0.01233	0.0074	0.0111	0.01233	0.0111	0.0074	0.00617	0.0111
VEP19	0.0111	0.0111	0.0111	0.0111	0.0111	0.01233	0.0111	0.0111	0.0111	0.00617	0.00986	0.0111	0.00986	0.00617	0.00493	0.00986
SC199	0.00863	0.00863	0.00863	0.00863	0.00863	0.00986	0.00863	0.00863	0.00863	0.00863	0.00986	0.0111	0.00986	0.00863	0.0074	0.00986
Vvilm03	0.00864	0.00864	0.00864	0.00864	0.00864	0.00987	0.00864	0.00864	0.00864	0.00864	0.00987	0.0111	0.00987	0.00864	0.0074	0.00987
Vvilm04	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986	0.00986	0.0111	0.01233	0.0111	0.00986	0.00863	0.0111
Vvilm06	0.00864	0.00864	0.00864	0.00864	0.00864	0.00988	0.00864	0.00864	0.00864	0.00865	0.00988	0.0099	0.00988	0.00865	0.00741	0.00988
Vvilm07	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986	0.00986	0.0111	0.01233	0.0111	0.00986	0.00863	0.0111
Vvilm10	0.00986	0.00986	0.00986	0.00986	0.00986	0.0111	0.00986	0.00986	0.00986	0.00986	0.0111	0.01233	0.0111	0.00986	0.00863	0.0111
IM420	0.01977	0.01977	0.01977	0.01977	0.01977	0.021	0.01977	0.01977	0.01977	0.01853	0.01976	0.021	0.01976	0.01853	0.01856	0.01976
IM412	0.02343	0.02343	0.02343	0.02343	0.02343	0.02466	0.02343	0.02343	0.02343	0.02219	0.02343	0.02466	0.02343	0.02219	0.02343	0.02343
IM423	0.0222	0.0222	0.0222	0.0222	0.0222	0.02344	0.0222	0.0222	0.0222	0.02097	0.0222	0.02222	0.0222	0.02097	0.0222	0.0222
IM424	0.02096	0.02096	0.02096	0.02096	0.02096	0.02219	0.02096	0.02096	0.02096	0.01973	0.02096	0.02219	0.02096	0.01973	0.02096	0.02096
IM426	0.01976	0.01976	0.01976	0.01976	0.01976	0.02099	0.01976	0.01976	0.01976	0.01852	0.01976	0.02099	0.01976	0.01852	0.01855	0.01976
Vimpa03	0.02096	0.02096	0.02096	0.02096	0.02096	0.02219	0.02096	0.02096	0.02096	0.01973	0.02096	0.02219	0.02096	0.01973	0.02096	0.02096
Vimpa04	0.02219	0.02219	0.02219	0.02219	0.02219	0.02343	0.02219	0.02219	0.02219	0.02096	0.02219	0.02343	0.02219	0.02096	0.02219	0.02219
Vimpa02	0.01973	0.01973	0.01973	0.01973	0.01973	0.02096	0.01973	0.01973	0.01973	0.0185	0.01973	0.02096	0.01973	0.0185	0.01973	0.01973
Vimpa06	0.01973	0.01973	0.01973	0.01973	0.01973	0.02096	0.01973	0.01973	0.01973	0.0185	0.01973	0.02096	0.01973	0.0185	0.01973	0.01973
vosnesenskii	0.08385	0.08385	0.08385	0.08385	0.08385	0.08508	0.08385	0.08385	0.08385	0.08261	0.08261	0.08261	0.08261	0.08261	0.08385	0.08261
hunii	0.06412	0.06412	0.06412	0.06412	0.06412	0.06535	0.06412	0.06412	0.06412	0.06782	0.06782	0.06782	0.06782	0.06782	0.06905	0.06782

Appendix B. (cont.)

	VEP02	VEP09	VEP28	VEP18	VEP20	VEP23	VEP24	VEP27	VEP30	VEP44	VEP45	VEP46	VEP47	VEP48	VEP49	VEP50	VEP51	VEP52	VEP53	VEP54	VEP55	VEP03	VEP14	VEP26	VEP05	VEP29	VEP43	VEP12	VEP22	VEP33	VEP36	VEP37						
VEP02																																						
VEP09																																						
VEP28																																						
VEP18																																						
VEP20																																						
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VEP45																																						
VEP46																																						
VEP47																																						
VEP48																																						
VEP49																																						
VEP50																																						
VEP51																																						
VEP52	0.0111																																					
VEP53	0	0.0111																																				
VEP54	0.00247	0.00863	0.00247																																			
VEP55	0.00123	0.00986	0.00123	0.00123																																		
VEP03	0.00124	0.00993	0.00124	0.00371	0.00247																																	
VEP14	0.00373	0.00993	0.00373	0.00371	0.00247	0.00248																																
VEP26	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124																															
VEP05	0.00617	0.0148	0.00617	0.00617	0.00493	0.0037	0.0037	0.00247																														
VEP29	0.00617	0.0148	0.00617	0.00617	0.00493	0.0037	0.0037	0.00247	0																													
VEP43	0.0074	0.01603	0.0074	0.0074	0.00617	0.00497	0.00498	0.0037	0.00123																													
VEP12	0.00493	0.01356	0.00493	0.00493	0.0037	0.00247	0.00248	0.00123	0.0037	0.00493																												
VEP22	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124	0	0.00247	0.0037	0.00247	0.00247	0.0037	0.00493																								
VEP33	0.00493	0.0111	0.00493	0.00493	0.0037	0.00124	0.00124	0.00123	0.0037	0.00493	0.0037	0.00123	0.0037	0.00493	0.00247	0.0037	0.0037	0.00247	0.00493	0.0037	0.0037	0.00123	0.00247	0.00123	0.0037	0.00493	0.00247	0.00247	0.00123	0.00247	0.00493	0.00247	0.00123	0.00247	0.00123	0.00247		

Appendix B. (cont.)

	VEP51	VEP52	VEP53	VEP54	VEP55	VEP03	VEP14	VEP26	VEP05	VEP29	VEP43	VEP12	VEP22	VEP33	VEP36	VEP37
VEP36	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124	0	0.00247	0.00247	0.0037	0.00123	0	0.00123		
VEP37	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124	0	0.00247	0.00247	0.0037	0.00123	0	0.00123	0	
VEP38	0.00247	0.01235	0.00247	0.00371	0.00247	0	0.00124	0	0.00247	0.00247	0.0037	0.00123	0	0.00124	0	0
VEP01	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124	0	0.00247	0.00247	0.0037	0.00123	0	0.00123	0	0
VEP07	0.00493	0.01356	0.00493	0.00493	0.0037	0.00247	0.00247	0.00123	0.00123	0.00123	0.00247	0.00247	0.00123	0.00247	0.00123	0.00123
VEP25	0.00493	0.01356	0.00493	0.00493	0.0037	0.00247	0.00247	0.00123	0.00123	0.00123	0.00247	0.00247	0.00123	0.00247	0.00123	0.00123
VEP13	0.00493	0.01356	0.00493	0.00493	0.0037	0.00251	0.00251	0.00123	0.0037	0.0037	0.00493	0.00247	0.00123	0.00247	0.00123	0.00123
VEP21	0.00493	0.01356	0.00493	0.00493	0.0037	0.00248	0.00248	0.00123	0.0037	0.0037	0.00493	0.00247	0.00123	0.00247	0.00123	0.00123
VEP34	0.0037	0.01233	0.0037	0.0037	0.00247	0.00123	0.00124	0	0.00247	0.00247	0.0037	0.00123	0	0.00123	0	0
VEP35	0.00493	0.01356	0.00493	0.00493	0.0037	0.00248	0.00248	0.00123	0.0037	0.0037	0.00493	0.00247	0.00123	0.00247	0.00123	0.00123
SC198	0.00617	0.01233	0.00617	0.00617	0.00493	0.00373	0.00373	0.00493	0.0074	0.0074	0.00863	0.00617	0.00493	0.00617	0.00493	0.00493
VEP17	0.00617	0.01233	0.00617	0.00617	0.00493	0.00373	0.00373	0.00493	0.0074	0.0074	0.00863	0.00617	0.00493	0.00617	0.00493	0.00493
VEP19	0.00493	0.0111	0.00493	0.00493	0.0037	0.00249	0.00248	0.0037	0.00617	0.00617	0.0074	0.00493	0.0037	0.00493	0.0037	0.0037
SC199	0.0074	0.0111	0.0074	0.0074	0.00617	0.00621	0.00621	0.00863	0.0111	0.0111	0.01233	0.00986	0.00863	0.0074	0.00863	0.00863
Vwilm03	0.0074	0.0111	0.0074	0.00741	0.00617	0.00621	0.00621	0.00863	0.0111	0.0111	0.01233	0.00987	0.00863	0.0074	0.00863	0.00863
Vwilm04	0.00863	0.01233	0.00863	0.00863	0.0074	0.00744	0.00744	0.00986	0.01233	0.01233	0.01356	0.0111	0.00986	0.00863	0.00986	0.00986
Vwilm06	0.00741	0.0099	0.00741	0.00741	0.00618	0.00622	0.00622	0.00864	0.0111	0.0111	0.01234	0.00988	0.00864	0.00741	0.00864	0.00864
Vwilm07	0.00863	0.01233	0.00863	0.00863	0.0074	0.00744	0.00744	0.00986	0.01233	0.01233	0.01356	0.0111	0.00986	0.00863	0.00986	0.00986
Vwilm10	0.00863	0.01233	0.00863	0.00863	0.0074	0.00744	0.00744	0.00986	0.01233	0.01233	0.01356	0.0111	0.00986	0.00863	0.00986	0.00986
IM420	0.01856	0.021	0.01856	0.0173	0.01731	0.01613	0.01611	0.01981	0.02227	0.02227	0.02352	0.02104	0.01981	0.01856	0.01981	0.01981
IM412	0.02343	0.02466	0.02343	0.02096	0.02219	0.02103	0.02102	0.02466	0.02713	0.02713	0.02836	0.02589	0.02466	0.02343	0.02466	0.02466
IM423	0.0222	0.02222	0.0222	0.01974	0.02097	0.01981	0.0198	0.02344	0.0259	0.0259	0.02714	0.02467	0.02344	0.0222	0.02344	0.02344
IM424	0.02096	0.02219	0.02096	0.0185	0.01973	0.01855	0.01854	0.02219	0.02466	0.02466	0.02589	0.02343	0.02219	0.02096	0.02219	0.02219
IM426	0.01855	0.02099	0.01855	0.01729	0.0173	0.01611	0.01609	0.0198	0.02226	0.02226	0.02351	0.02103	0.0198	0.01855	0.0198	0.0198
Vimpa03	0.02096	0.02219	0.02096	0.0185	0.01973	0.01855	0.01854	0.02219	0.02466	0.02466	0.02589	0.02343	0.02219	0.02096	0.02219	0.02219
Vimpa04	0.02219	0.02343	0.02219	0.01973	0.02096	0.01978	0.01978	0.02343	0.02589	0.02589	0.02713	0.02466	0.02343	0.02219	0.02343	0.02343
Vimpa02	0.01973	0.02096	0.01973	0.01726	0.0185	0.01731	0.0173	0.02096	0.02343	0.02343	0.02466	0.02219	0.02096	0.01973	0.02096	0.02096
Vimpa06	0.01973	0.02096	0.01973	0.01726	0.0185	0.01731	0.0173	0.02096	0.02343	0.02343	0.02466	0.02219	0.02096	0.01973	0.02096	0.02096
vosnesenskii	0.08385	0.08261	0.08385	0.08138	0.08261	0.08172	0.08172	0.08261	0.08261	0.08261	0.08261	0.08138	0.08261	0.08261	0.08261	0.08261
hunii	0.06905	0.06782	0.06905	0.06658	0.06782	0.06793	0.06793	0.07028	0.07028	0.07028	0.07028	0.06905	0.07028	0.07028	0.07028	0.07028

**Appendix B. (cont.)**

	VEP38	VEP01	VEP07	VEP25	VEP13	VEP21	VEP34	VEP35	SC198	VEP17	VEP19	SC199	Vwilm03	Vwilm04	Vwilm06	Vwilm07
VEP36																
VEP37																
VEP38																
VEP01	0															
VEP07	0.00123	0.00123														
VEP25	0.00123	0.00123	0													
VEP13	0.00123	0.00123	0.00247													
VEP21	0.00123	0.00247	0.00247	0.00247												
VEP34	0	0.00123	0.00123	0.00123	0.00123	0.00123										
VEP35	0.00123	0.00123	0.00247	0.00247	0.00247	0.00247	0.00123									
SC198	0.00494	0.00493	0.00617	0.00617	0.0037	0.00617	0.00493	0.00617								
VEP17	0.00494	0.00493	0.00617	0.00617	0.0037	0.00617	0.00493	0.00617	0.00247							
VEP19	0.00371	0.0037	0.00493	0.00493	0.00247	0.00493	0.0037	0.00493	0.00123	0.00123						
SC199	0.00865	0.00863	0.00986	0.00986	0.00986	0.00986	0.00863	0.00986	0.00863	0.00863	0.0074					
Vwilm03	0.00865	0.00863	0.00987	0.00987	0.00987	0.00987	0.00863	0.00987	0.00863	0.00863	0.0074	0				
Vwilm04	0.00988	0.00986	0.0111	0.0111	0.0111	0.0111	0.00986	0.0111	0.00986	0.00986	0.00863	0.00123	0			
Vwilm06	0.00866	0.00864	0.00988	0.00988	0.00988	0.00988	0.00864	0.00988	0.00864	0.00864	0.00741	0	0	0		
Vwilm07	0.00988	0.00986	0.0111	0.0111	0.0111	0.0111	0.00986	0.0111	0.00986	0.00986	0.00863	0.00123	0	0	0	
Vwilm10	0.00988	0.00986	0.0111	0.0111	0.0111	0.0111	0.00986	0.0111	0.00986	0.00986	0.00863	0.00123	0	0	0	0
IM420	0.01982	0.01981	0.02104	0.02104	0.02105	0.02104	0.01981	0.02103	0.01979	0.01979	0.01856	0.01853	0.01854	0.01978	0.01855	0.01978
IM412	0.02468	0.02466	0.02589	0.02589	0.02589	0.02589	0.02466	0.02466	0.02466	0.02466	0.02343	0.02343	0.02343	0.02466	0.02344	0.02466
IM423	0.02346	0.02344	0.02467	0.02467	0.02467	0.02467	0.02344	0.02344	0.02344	0.02344	0.0222	0.0222	0.02221	0.02344	0.02222	0.02344
IM424	0.02221	0.02219	0.02343	0.02343	0.02343	0.02343	0.02219	0.02219	0.02219	0.02219	0.02096	0.02096	0.02097	0.02219	0.02098	0.02219
IM426	0.01981	0.0198	0.02103	0.02103	0.02104	0.02103	0.0198	0.02101	0.01978	0.01978	0.01855	0.01852	0.01853	0.01977	0.01854	0.01977
Vimpa03	0.02221	0.02219	0.02343	0.02343	0.02343	0.02343	0.02219	0.02219	0.02219	0.02219	0.02096	0.02096	0.02097	0.02219	0.02098	0.02219
Vimpa04	0.02345	0.02343	0.02466	0.02466	0.02466	0.02466	0.02343	0.02343	0.02343	0.02343	0.02219	0.02219	0.0222	0.02343	0.02221	0.02343
Vimpa02	0.02098	0.02096	0.02219	0.02219	0.02219	0.02219	0.02096	0.02096	0.02096	0.02096	0.01973	0.01973	0.01974	0.02096	0.01975	0.02096
Vimpa06	0.02098	0.02096	0.02219	0.02219	0.02219	0.02219	0.02096	0.02096	0.02096	0.02096	0.01973	0.01973	0.01974	0.02096	0.01975	0.02096
<i>vosnesenskii</i>	0.08263	0.08261	0.08138	0.08138	0.08385	0.08385	0.08261	0.08385	0.08508	0.08508	0.08385	0.08385	0.08284	0.08261	0.08284	0.08261
<i>hunii</i>	0.0703	0.07028	0.06905	0.06905	0.07152	0.07152	0.07028	0.07152	0.07028	0.06905	0.06905	0.06658	0.06647	0.06535	0.06647	0.06535

Appendix B. (cont.)

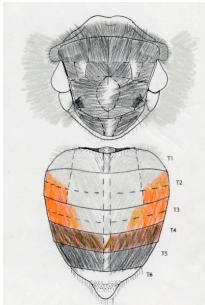
	Vvilim10	IM420	IM412	IM423	IM424	IM426	Vimpa03	Vimpa04	Vimpa02	Vimpa06	vosnesenskii
VEP36											
VEP37											
VEP38											
VEP01											
VEP07											
VEP25											
VEP13											
VEP21											
VEP34											
VEP35											
SC198											
VEP17											
VEP19											
SC199											
Vvilim03											
Vvilim04											
Vvilim06											
Vvilim07											
Vvilim10											
IM420	0.01978										
IM412	0.02466	0.00123									
IM423	0.02344	0	0.00123								
IM424	0.02219	0.00123	0.00247	0.00124							
IM426	0.01977	0.00123	0.00247	0.00124	0						
Vimpa03	0.02219	0.00123	0.00247	0.00124	0	0					
Vimpa04	0.02343	0.00248	0.0037	0.00247	0.00123	0.00123	0.00123				
Vimpa02	0.02096	0.00247	0.0037	0.00248	0.00123	0.00123	0.00123	0.00247			
Vimpa06	0.02096	0.00247	0.0037	0.00248	0.00123	0.00123	0.00123	0.00247	0		
vosnesenskii	0.08261	0.08395	0.08755	0.08631	0.08508	0.08394	0.08508	0.08385	0.08508	0.08508	
huntii	0.06535	0.06917	0.07275	0.07152	0.07028	0.06916	0.07028	0.07152	0.07028	0.07028	0.05302



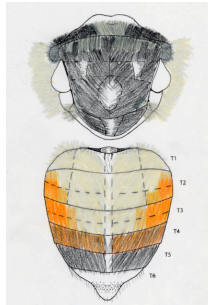
**Appendix C. Specimen GPS coordinates.** GPS coordinates for specimens used in this study, excluding *B. impatiens*.

<b>Species</b>	<b>Voucher #</b>	<b>Latitude</b>	<b>Longitude</b>
<i>ephippiatus</i>	SC198	16.140094	-92.777981
<i>ephippiatus</i>	VEP01	19.118402	-101.893329
<i>ephippiatus</i>	VEP02	9.564722	-83.742222
<i>ephippiatus</i>	VEP03	28.221098	-108.251971
<i>ephippiatus</i>	VEP05	17.474758	-100.178778
<i>ephippiatus</i>	VEP07	17.31736	-96.495108
<i>ephippiatus</i>	VEP09	10.28396	-84.75961
<i>ephippiatus</i>	VEP12	20.0085	-103.70833
<i>ephippiatus</i>	VEP13	21.17631	-99.57348
<i>ephippiatus</i>	VEP14	28.168724	-108.2131
<i>ephippiatus</i>	VEP17	15.117761	-92.107067
<i>ephippiatus</i>	VEP18	9.829227	-83.443688
<i>ephippiatus</i>	VEP19	15.120847	-92.096547
<i>ephippiatus</i>	VEP20	9.7	-83.9
<i>ephippiatus</i>	VEP21	21.13517	-99.63268
<i>ephippiatus</i>	VEP22	19.6143	-103.56765
<i>ephippiatus</i>	VEP23	9.564651	-83.707901
<i>ephippiatus</i>	VEP24	9.7	-83.9
<i>ephippiatus</i>	VEP25	17.31736	-96.495108
<i>ephippiatus</i>	VEP26	28.168724	-108.2131
<i>ephippiatus</i>	VEP27	9.4	-83.52
<i>ephippiatus</i>	VEP28	10.28396	-84.75961
<i>ephippiatus</i>	VEP29	17.474758	-100.178778
<i>ephippiatus</i>	VEP30	9.829227	-83.443688
<i>ephippiatus</i>	VEP34	21.13517	-99.63268
<i>ephippiatus</i>	VEP35	21.17631	-99.57348
<i>ephippiatus</i>	VEP36	19.6143	-103.56765
<i>ephippiatus</i>	VEP37	19.6143	-103.56765
<i>ephippiatus</i>	VEP38	19.6143	-103.56765
<i>ephippiatus</i>	VEP33	20.0085	-103.70833
<i>ephippiatus</i>	VEP43	17.474758	-100.178778
<i>ephippiatus</i>	VEP44	14.03055556	-87.08
<i>ephippiatus</i>	VEP45	14.03055556	-87.08
<i>ephippiatus</i>	VEP46	14.018834	-87.096436
<i>ephippiatus</i>	VEP47	14.018834	-87.096436
<i>ephippiatus</i>	VEP48	13.93166667	-86.87666667
<i>ephippiatus</i>	VEP49	13.93166667	-86.87666667
<i>ephippiatus</i>	VEP50	14.19444444	-87.12972222
<i>ephippiatus</i>	VEP51	14.19722222	-87.12583333
<i>ephippiatus</i>	VEP52	14.43611111	-87.56083333
<i>ephippiatus</i>	VEP53	14.4375	-87.56083333
<i>ephippiatus</i>	VEP54	15.49638889	-88.18083333
<i>ephippiatus</i>	VEP55	15.49222222	-88.22666667
<i>wilmattae</i>	Vwilm03	16.140094	-92.777981
<i>wilmattae</i>	Vwilm04	15.120847	-92.096547
<i>wilmattae</i>	Vwilm06	15.05	-92.283333
<i>wilmattae</i>	Vwilm07	15.05	-92.283333
<i>wilmattae</i>	Vwilm10	15.09036667	-92.28638333
<i>wilmattae</i>	SC199	16.140094	-92.777981

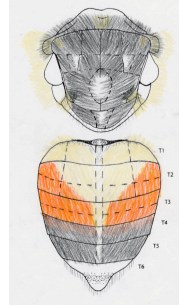
**Appendix D. Color pattern drawings.** Drawings by the author of *B. ephippiatus* and *B. wilmattae* color patterns. Drawings were used to determine morphological characters and character states for color pattern coding.



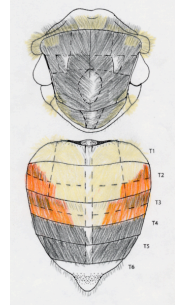
VEP03: Chihuahua, MX



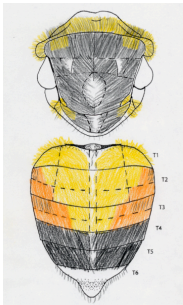
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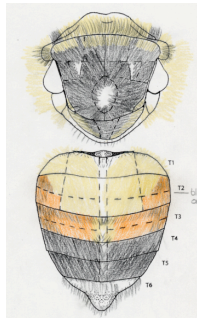
VEP05: Guerrero, MX



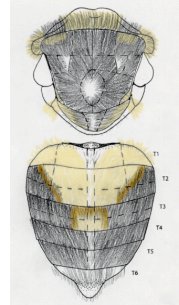
VEP07: Oaxaca, MX



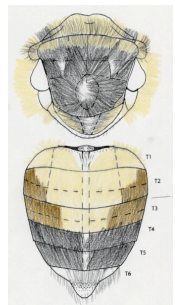
VEP17: Chiapas, MX



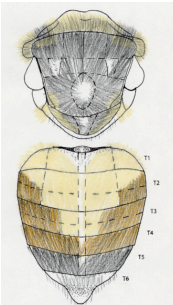
VEP44: R.B. El Uyuca, FM, HN



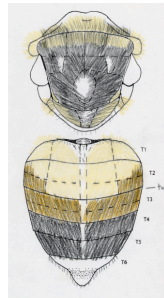
VEP45 R.B. El Uyuca, FM, HN



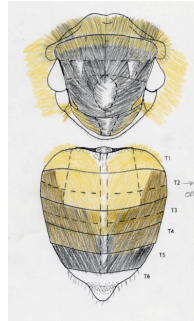
VEP46: Tatumbla, FM, HN



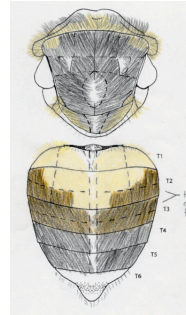
VEP47: Tatumbla, FM, HN



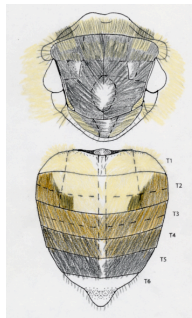
VEP23: Villa Mills, CR



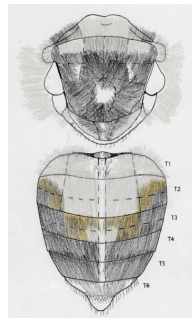
VEP15: Puntarenas, CR



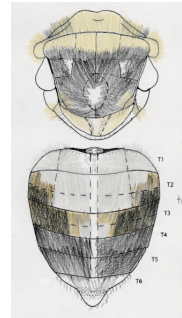
VEP09: Puntarenas, CR



VEP18: San Jose, CR



Vwilm04: Chiapas, MX



Vwilm05: Chiapas, MX