ABSTRACT

Title of Thesis: MOLECULAR PHYLOGENETIC ANALYSIS OF THE HAWKMOTHS (LEPIDOPTERA: BOMBYCOIDEA: SPHINGIDAE) AND THE EVOLUTION OF THE SPHINGID PROBOSCIS

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A molecular phylogenetic analysis of the hawkmoths was conducted using five protein-coding nuclear genes for 131 sphingid ingroups and eleven bombycoid outgroups. The study utilizes 6,793 bp of cDNA from *CAD*, *DDC*, *EF-1α*, *period*, and *wingless*. Genes were analyzed separately and in combination. Results from the combined simultaneous analysis corroborated many previously postulated sets of relationships based on larval, pupal, and adult morphological characters, but also uncovered many novel relationships. Application of parsimony and maximum likelihood optimality criteria led to the recovery of monophyletic Macroglossinae, Sphinginae, Acherontiini, Ambulycini, Philampelini, and Choerocampina. The most likely tree and the most parsimonious trees recovered the following relationships among subfamilies: Macroglossinae + (Sphinginae + Smerinthinae). Monophyly of the Sphinginae was corroborated with strong support in all analyses, as well as for the sister-group relationship of the paraphyletic Sphingulini + Sphinginae. A reconstruction of ancestral

states reveals that the short, non-feeding proboscis was the ancestral condition in the family. The nectar-feeding proboscis independently arose multiple times, but was subsequently lost at least three times. This thesis also includes a supplementary section in which the five gene dataset was combined with the barcoding region of the mitochondrial COI gene. The purpose of the supplementary section was to tentatively explore the effect of combining the COI barcoding region for available sphingid taxa to a larger dataset with greater character sampling.

MOLECULAR PHYLOGENETIC ANALYSIS OF THE HAWKMOTHS (LEPIDOPTERA: BOMBYCOIDEA: SPHINGIDAE) AND THE EVOLUTION OF THE SPHINGID PROBOSCIS

By

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"The 'saturniid' [non-feeding] strategy exhibited by many Smerinthini would appear to be the plesiomorphic biology for Sphingidae, while the 'typical' [nectar feeding] strategy … would be apomorphic within the family. The interesting problem now is to resolve phylogenetic relationships of the family in order to determine the possible evolutionary pathways by which this latter strategy could have developed"

(Kitching and Cadiou, 2000: 9)

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CHAPTER 1

PHYLOGENETIC ANALYSIS OF THE HAWKMOTHS: EVIDENCE FROM FIVE PROTEIN CODING NUCLEAR GENES (LEPIDOPTERA: SPHINGIDAE)

1.1. INTRODUCTION

Hawkmoths are one of the most conspicuous groups of moths, and they are found on every continent except Antarctica (Rothschild and Jordan, 1903; Kitching and Cadiou, 2000). Sphingids are models for studies on biochemistry (Willis *et al.*, 1995; Wink and Theile, 2002; Bowers, 2003), functional morphology (Eaton, 1971; 1988), nutritional ecology (Slansky, 1993), physiology (e.g., Liu *et al.*, 1998; Göpfert *et al.*, 2002; Kelber *et al.*, 2003; Wannenmacher and Wasserthal, 2003; Davidowitz and Nijhout, 2004; Davis and Hildebrand, 2006), plant-insect interactions (e.g., Jackson, 1990; Osier *et al.*, 1996; Kessler and Baldwin, 2002; Agosta and Janzen, 2005), pollination biology (e.g., Gregory, 1963-1964; Nilsson *et al.*, 1985; 1987; Haber and Frankie, 1989; 1992; Wasserthal, 1997; Nilsson, 1998; Raguso and Willis, 2002), biogeography (Beck *et al.*, 2006c), population genetics (Hundsdoerfer and Wink, 2006) and developmental genetics (Song and Gilbert, 1994; Jindra *et al.*, 1997; Jochova *et al.*, 1997). Due to their large size, sphingids have also been the focal group in faunistic studies to assess habitat quality for conservation (Beck *et al.*, 2006a). Some species are agricultural pests (Winder, 1976; Coffelt and Schultz, 1990; Bellotti *et al.*, 1992; 1993), and some are biological control agents (Batra, 1984). Recently, sphingids have become a model group to study taxonomic species boundaries with the onset of DNA barcoding (Janzen *et al.*, 2005; Hajibabaei *et al.*, 2006). Despite their conspicuous nature and their role in a wide range of biological systems, a robust phylogenetic analysis of hawkmoth genera has not been conducted.

Phylogenetic analyses of hawkmoths have generally focused within a tribe or lower (e.g., Acherontiini (Kitching, 2002; 2003), Ambulycini [Kitching, unpublished data, 1993], *Hyles* (Derzhavets, 1993; Hundsdoerfer *et al.*, 2005a; 2005b)), and relationships of

many sphingid taxa still remain largely unknown. The only published modern phylogenetic analysis on higher relationships within the family was the preliminary molecular analysis of Regier *et al.* (2001) which included two genes and fourteen species.

Sphingidae include 201 genera and almost 1400 species classified into three subfamilies: Macroglossinae, Smerinthinae, and Sphinginae (Kitching and Cadiou, 2000). Morphological studies on Bombycoidea strongly support the monophyly of the Sphingidae (Minet, 1994; Lemaire and Minet, 1999). Although several putative apomorphies have been proposed for each subfamily (Minet, 1994), monophyly remains speculative for nearly all tribes and subtribes, particularly that of the Smerinthinae. Smerinthines have been noted for sharing life history strategies such as non-feeding mouthparts with another macrolepidopteran family, the Saturniidae (Janzen, 1984), and was predicted to be the basal subfamily of Sphingidae (Kitching and Cadiou, 2000).

There have been several efforts to reconstruct relationships of Sphingidae using morphology (e.g., Rothschild and Jordan, 1903; Nakamura, 1976; Kitching, 2000; Figs. 1-3), but delimiting and coding adult and immature characters in a modern cladistic context has been challenging (Kitching, pers. com.). In their monumental revision on Sphingidae, Rothschild and Jordan (1903) classified hawkmoths into two groups, the "Sphingidae Semanophorae" and the "Sphingidae Asemanophorae", which roughly corresponds to Kitching and Cadiou's (2000) Macroglossinae and (Smerinthinae + Sphinginae), respectively (Fig. 1). Although Rothschild and Jordan's study came decades before the development of modern cladistic methodology (Hennig, 1950; 1965; 1966), Rothschild and Jordan presented a "tree" of all sphingid genera known at the time, and these genera were grouped according to shared morphological structures. Rothschild and

Jordan separated sphingids into Semanophorae and Asemanophorae based on one morphological feature: presence or absence of a patch of short sensory hairs (microtrichia) on the inner surface of the first segment of the labial palp (Rothschild and Jordan, 1903). It was later discovered that spiracular furrows on the pupa also supports the division of the family into these two groups (Mosher, 1918). Rothschild and Jordan assigned five subfamilies to the Sphingidae: Acherotiinae, Ambulycinae, Choerocampinae, Philampelinae, and Sesiinae. Their revision formed the foundation for sphingid classification, and for the following several decades, studies on sphingid classification mainly revised particular aspects of their work.

Janse (1932) lowered the taxonomic rank of Rothschild and Jordan's subfamilies to tribes, and treated Semanophorae and Asemanophorae as subfamilies (Semanophorinae and Asemanophorinae). Carcasson (1968) replaced Rothschild and Jordan's "Sesiinae" with Dilophonotini, and "Sesiicae" with Aellopodes. Hodges (1971) formally changed Semanophorinae and Asemanophorinae to Macroglossinae and Sphinginae after the type genus of each subfamily. Hodges also rejected many of Carcasson's names because they were not based on any available generic name.

In a series of papers based on larval, pupal, and adult morphology, Nakamura (1976; 1977; 1978) reclassified particular sphingid tribes, and presented relationships of Japanese sphingid genera based on characteristics of the larva and pupa (Fig. 2). The tree he presented was not based on a modern cladistic analysis, but Smerinthini and Sphingini were thought to be sister-groups, and the remaining tribes forming a group which roughly corresponds to Macroglossinae *sensu* Kitching and Cadiou (2000).

Grouping Sphingidae into two subfamilies was generally accepted until the work of Minet (1994). Minet separated Sphinginae *sensu lato* into Smerinthinae and Sphinginae *sensu stricto* in part because it was believed that Sphinginae *s. l.* may be paraphyletic. Specifically, the labial palp character which Rothschild and Jordan first described cannot be used to describe a group because it was based on the *absence* of the microtrichial patch. Furthermore, this patch is also known in other bombycoid families, and was therefore thought to render Sphinginae *s. l.* paraphyletic (Lemaire and Minet, 1999).

The most recent major contribution to sphingid classification was the revision of (Kitching and Cadiou, 2000). In addition to stabilizing sphingid taxonomy and classification, Kitching and Cadiou also proposed provisional relationships of sphingids based on some unpublished morphological analyses. Unlike Rothschild and Jordan (1903) and Nakamura (1976), Kitching and Cadiou tentatively placed the Smerinthinae at the base of the family, and Sphinginae and Macroglossinae as more derived (Fig. 3). Within the Smerinthinae, Smerinthini was thought to be paraphyletic. However, their interpretation was "provisional and subject to change" (Kitching and Cadiou, 2000: 16), and I therefore consider their proposed phylogenetic relationships tentative.

Recent molecular phylogenetic analyses of Sphingidae (Regier *et al.*, 2001; Mignault, 2003) based on elongation factor-1α (EF-1a, Cho *et al.*, 1995), and dopa-decarboxylase (DDC, Fang *et al.*, 1997), preliminarily tested Kitching and Cadiou's classification. Regier *et al.* (2001) found comparable information content and no significant conflict in signal between 1,240 bp of *EF-1α* and 709 bp of *DDC* across fourteen sphingids, and conducted combined analyses of the two genes under parsimony

(MP) and likelihood (ML) optimality criteria. The phylogeny which they presented (Fig. 4) was similar to the morphological interpretation of Rothschild and Jordan (1903) and Nakamura (1976), as relationships among subfamilies were: Macroglossinae + (Smerinthinae + Sphinginae). However, less than 15% of all recognized sphingid genera were included in their analysis, and three key tribes, Acherontiini, Ambulycini, and Sphingulini, were not included.

Mignault (2003) increased taxon sampling to 45 sphingid genera (Table 1), and also conducted a combined analysis of the two genes with MP and ML. The MP analysis resulted in a monophyletic Sphinginae, a strongly supported Sphingulini + Sphinginae, and a weakly supported monophyletic Macroglossinae (Fig. 5). Smerinthines were basal and paraphyletic with respect to the rest of the Sphingidae. The ML analysis (Fig. 6) resulted in subfamily relationships identical to Regier *et al.* (2001), excluding one sample which was labeled an acherontiine, but had part of its sequence switched with a macroglossine (see Figs 5, 6, figure legend). Ambulycini, Philampelini, and two tribes in the Dilophonotini (Dilophonotina and Hemarina), were monophyletic. Monophyly of Sphingulini was not tested, as Mignault's study only included *Hopliocnema brachycera* (Lower) from this tribe. Due to computational limitations, Mignault (2003) did not calculate bootstrap support values for his ML analysis.

The current study builds upon the works of Regier *et al.* (2001) and Mignault (2003) increasing both taxon and gene sampling to test the monophyly of the Sphingidae, phylogenetic placement of subfamilies, tribes and subtribes, as classified in the revision of Kitching and Cadiou (2000). Although inclusion of morphological characters often has a significant and positive effect on phylogenetic signal in combined analyses (Baker *et al.*,

1998; Wahlberg *et al.*, 2005; Wortley and Scotland, 2006), the current study does not include morphological characters as a coded morphological character set for the Sphingidae is as yet unavailable, and combined, simultaneous analysis of morphology and molecules must therefore be the focus of a future study.

Taxon sampling was increased because inclusion of additional sphingid genera is necessary to understand further the relationships of subtribes and genera. Taxa were also added because increasing taxon sampling has been shown to break long branches (Hillis, 1996; Graybeal, 1998); but see also Mitchell, 2000), and reduce phylogenetic error for both MP and ML optimality criteria (Zwickl and Hillis, 2002). Biases such as long-branch attraction (LBA, originally termed *long-edge attraction* (Hendy and Penny, 1989)) have been argued to be a drawback of parsimony in particular (Felsenstein, 1978), but it has been shown that ML can also suffer from long branch effects if the model is misspecified (Gaut and Lewis, 1995; Chang, 1996; Zhang *et al.*, 2006), or prone to long-branch repulsion in particular situations (Siddall, 1998; Siddall and Whiting, 1999). The current study explores the effect of LBA as it pertains to the sphingid dataset presented here.

Additional genes were sequenced because increasing taxa while keeping gene number constant may lead to a decrease in accuracy due to introducing new long branches (Poe and Swofford, 1999), or reducing the relative amount of characters to resolve the newly added taxa (Kim, 1998; Bininda-Emonds *et al.*, 2001). Multiple evolutionarily independent genes were chosen to reduce inherent biases which may be confined to a particular gene, and because combining characters from different genes tends to improve phylogenetic accuracy and support (e.g., Cummings *et al.*, 1995; Otto *et*

al., 1996; Zardoya and Meyer, 1996; Cummings *et al.*, ; Yoder and Irwin, 1999; Mitchell *et al.*, 2000; Rokas *et al.*, 2003; Rokas and Carroll, 2005).

Specifically, this study utilizes 6,793 bp combined from five protein coding nuclear genes: *EF-1α* (EF-1a, Cho *et al.*, 1995), *DDC* (DDC, Fang *et al.*, 1997), *CAD* (Moulton and Wiegmann, 2003), *period* (Regier *et al.*, 1998), and *wingless* (Brower and DeSalle, 1998). The five nuclear gene dataset was also supplemented with a 658 bp region of the mitochondrial Cytochrome-Oxidase-1 (COI) gene for 69 ingroup taxa for which this gene was available (Supplementary Table S1). The main purpose for adding COI was to include additional taxa which were not represented in the nuclear gene dataset, and to test the effect of adding a small mitochondrial gene to a much larger nuclear gene dataset.

1.2. Materials and Methods

1.2.1. Taxon Sampling

The current study includes 106 sphingid genera (131 ingroup species), from all subfamilies, tribes and subtribes recognized in Kitching and Cadiou (Table 2). I included as many genera as possible, given the availability and quality of samples. Several genera were represented by more than one species. Specimens were collected on all continents, with help from many international collectors (see Acknowledgments).

Outgroup choice was based on the classification of Bombycoidea proposed by Minet (1991; 1994; Lemaire and Minet, 1999), in which nine families, including the Sphingidae, were arranged into putative monophyletic groups. According to Minet, the Sphingidae is the sister group to Brahmaeidae + Lemoniidae, with Cartheidae being the

closest relative of the clade consisting of the Brahmaeidae, Lemoniidae, and Sphingidae (see Fig. 71 of Minet, 1994). A recent molecular analysis indicates very different relationships of Bombycoidea (Regier, in prep.). For this reason, eleven outgroups from eight different bombycoid families were included in the current study (Table 2). The primary purpose of this study was to test the monophyly of Sphingidae, and resolve relationships between sphingid genera. Due to limited space available on each page, outgroup relationships are excluded from all figures except figure 12.

1.2.2. Gene Sampling

Many recent studies on the molecular systematics of Lepidoptera have focused on a few selected genes (e.g., *COI*, *EF-1α*, *wingless*). Protein-coding nuclear genes have been successfully utilized in the Bombycoidea (Regier *et al.*, 2000; 2001; 2002; 2005), and it is unfortunate that protein-coding nuclear genes are not further utilized in molecular phylogenetic studies of Lepidoptera. Such genes are minimally exploited, due mainly to the difficulty in developing primers and the ease of using genes that easily amplify (Cummings and Meyer, 2005). It is hoped that this study will help facilitate the use of some of these protein-coding nuclear genes to the larger community of lepidopteran molecular systematists.

This study included 2929 bp of *CAD* (46F-1028R), 1282 bp of *DDC* (1.2F-7.5sR), 1228 bp of *EF-1α* (30F-41.2R), 951 bp of *period* (197sF-532sR), and 403 bp of *wingless* (wg1-wg2a). Primer sequences of these five genes are listed in Table 3. Sequences will be assigned accession numbers and submitted to GenBank prior to publication of this manuscript. See Supplementary information for sequence information of COI.

1.2.3. Nucleic acid extraction and RT-PCR

Nucleic acid extractions were performed using the head or prothorax of each specimen to reduce the possibility of contamination, and to retrieve high concentrations of nuclear genes. In particular cases, a leg of a specimen was used because the rest of the body was unavailable (e.g., *Aleuron chloroptera, Deidamia inscriptum*). Nucleic acid extractions were conducted with the Promega SV Total RNA Isolation System (Promega, 2004), with slight protocol modifications (exclusion of part IV. E. steps 4, 5) to permit extraction of both genomic DNA and RNA. All specimens and extractions are stored at -85°C in the University of Maryland Lepidoptera molecular collection.

Selective amplification of gene coding regions (e.g., mRNA) on the genomic whole nucleic acid extraction was conducted using the reverse transcription polymerase chain reaction (RT-PCR) to avoid introns, and because RT-PCR has yielded better results than DNA-PCR in experiments conducted previously in the Regier Lab (Regier, 2006). The RT reaction solution (10 μL) was made with the following ingredients: 2 μL 25 mM $MgCl_2$, 1 μL GeneAmp 10X PCR Buffer II, 0.5 μL Reverse Transcriptase (50 units/μL), 0.5 μL RNase Inhibitor (20 units/μL; all obtained from Applied Biosystems), 2 μL 10 mM dNTPs, 1.25 μL primer, 1.75 μL deionized DEPC-treated H_2O, and 1.0 μL purified extract. Reactions were conducted on a MJ Research DNA Engine Peltier Thermal Cycler (PTC-200) pre-cooled to 4°C and incubated at 42°C for 35 minutes, followed by 99°C for 5 minutes.

An individual PCR reaction consisted of a 10 μL RT reaction, and 40 μL of the following ingredients: 3 μL 25mM $MgCl_2$, 4 μL GeneAmp 10X PCR Buffer II, 31.25 μL

deionized DEPC-treated H_2O, 0.5 µL AmpliTaq (50 units/µL, Clontech), 1.25 µL primer. Touchdown thermal cycling (Don *et al.*, 1991) was employed in the amplification of in vitro synthesized cDNA. For the first 25X cycles, annealing temperature was iteratively decreased by 0.4°C per cycle, while extension time was iteratively increased by 2 seconds per cycle. After 25X touchdown cycles, traditional 3-step PCR at a standard annealing temperature was conducted for an additional 13 cycles, increasing the extension time by 3 seconds each cycle. Thermal cycling was completed with a final extension at 72°C for 10 minutes.

PCR products were visualized using agarose gel electrophoresis and ethidium bromide staining. Double stranded amplification products were isolated from agarose gels, and isolated bands were melted at 70°C for 8 minutes, before 1 mL of Promega, Wizard PCR Preps DNA Purification Resin was added to each sample and cooled at 25°C for 2 minutes. Samples were placed on 20-channel Vac-Man Laboratory vacuum manifolds and washed with 80% isopropanol to purify products. For all genes except *wingless*, products were reamplified using PCR and nested primers to improve yield and ensure clean results. For fragments with weak amplification, products were gel isolated and purified a third time and both strands were directly sequenced from M13 sites at the 5' end of all primers.

1.2.4. Data matrix construction

Sequence chromatograms were checked for accuracy and contigs were edited and assembled with the Staden GAP4 software package (Staden *et al.*, 2000). Sequence alignments were conducted manually using the Genetic Data Environment (GDE)

software (Smith *et al.*, 1994). Manual alignments were employed as RT-PCR yields few introns and alignment has been proven straightforward (Regier, 2006). For each gene, a data matrix was constructed in GDE and saved as a Nexus-formatted text file.

Single gene matrices were sequentially combined to create a matrix of five genes using the "New Matrix Merge" command in Winclada (Nixon, 2002). Simultaneous analyses were conducted because novel relationships may be uncovered through the combination of multiple partitions (Chippindale and Wiens, 1994), and because they provide the greatest possible explanatory power over other consensus methods (Farris, 1983; Nixon and Carpenter, 1996). However, parsimony analyses with lots of missing data can lead to a plethora of most-parsimonious trees (Nixon and Wheeler, 1992; Wilkinson, 1995; 2003), and support values can be sensitive to missing data (Makovicky, 2000; Wilkinson, 2003; Brower, 2006). Simply for the purpose of testing for the relative contribution of each gene towards the MP topology, a smaller 99-taxon dataset was constructed by removing all taxa that had missing data for over half of any partition. A dataset without third codon positions (nt3) was also created. Third positions were deleted in Winclada (Nixon, 2002) with following the commands: "Analyze/Moleculoid/Select third positions FROM CURSOR; delete selected chars".

1.2.5. Phylogenetic analyses

Phylogenetic analyses were conducted under MP and ML optimality criteria. MP analyses were conducted using Winclada (Nixon, 2002) and NONA (Goloboff, 1999). Heuristic searches were computed with the following commands: "hold 1000" (sets the maximum number of trees to be stored in memory as 1000), "hold/100" (sets 100 trees to

be retained during each replication), "mult*100" (generates a Wagner tree based on a randomized taxon order and conducts SPR, where branches are clipped and reattached to the tree in all possible positions), "max*" (conducts TBR, where each clipped branch is reattached in all possible positions and re-rooted at each possible attachment point). Congruence between multiple most-parsimonious cladograms (MPCs) was assessed using a strict consensus tree (Sokal and Rohlf, 1981). Branch support was evaluated using the bootstrap (Felsenstein, 1985), Bremer Support (BS; Bremer, 1988; 1994), and Partitioned Bremer Support (PBS; Baker and DeSalle, 1997; Baker *et al.*, 1998).

MP bootstrap values were computed in NONA (Goloboff, 1999) with 500 replications, 100 search replications (mult*100), and holding 10 starting trees (hold/10). BS and PBS values were calculated in TreeRot (Sorenson, 1999) and the subsequent command files executed in PAUP* (Swofford, 2002) for both all-taxon and 99-taxon datasets, and each analysis was repeated. PBS values were calculated to estimate congruence between data partitions and to test the relative contribution of each gene to each node. A metric for measuring congruence between partitioned datasets, here called the relative congruence index (RCI) was calculated by dividing the total PBS value by the number of parsimony informative characters.

Models for maximum likelihood (ML) were chosen using the Akaike Information Criterion (AIC, Akaike, 1973) implemented in Modeltest (Posada and Crandall, 1998). MrMTgui (Nuin, 2007) was used as an interface program to calculate the best model for each gene. In all cases (including COI), the best model was determined to be the general-time-reversible substitution model (Lanave *et al.*, 1984; Rodriguez *et al.*, 1990), with a gamma distribution and invariant sites (GTR+Γ+I).

All ML analyses were conducted in Garli ver. 0.951 (Zwickl, 2006). Garli uses a genetic algorithm as described by Lewis (1998) which involves the evolution of a population of solutions, each which encodes a tree topology, branch lengths, and model parameters to search for the optimal solution (Zwickl, 2006). Fitness is assigned to each individual based on its log likelihood score, and fitness is recalculated after random mutations are applied to individuals. Individuals with the highest log likelihood (-*lnL*) values are kept as parents for the next generation, and this process is repeated until a higher log likelihood score cannot be obtained.

A random starting tree was chosen in Garli, and none of the Garli default settings were changed except for the number of generations to termination, which was doubled to improve the search for the most optimal solution (genthreshfortopoterm = 20,000). To further assure the search for best tree, the search process was repeated eight times for each ML analysis. To assess the relative contribution of each gene toward the five-gene simultaneous ML analysis, I conducted single gene ML analyses and analyses that included four genes in all combinations. For each ML bootstrap analysis, 500 bootstrap replications were conducted (bootstrapreps = 500), and the bootstrap value for each clade was examined in PAUP*4b10 (Swofford, 2002). All analyses presented in this study were rooted with *Macrothylacia rubi* (L.), and computed on a Windows PC platform with 3.0 GHz, Opteron 175 dual processors.

1.3. RESULTS

1.3.1 Sequence amplification success and base composition

Sequencing reactions were successful for over 80% of taxa for each gene. *Wingless* was the most successful, with 132 successful sequencing reactions, while *period* was the least successful (Table 2). Base frequencies were approximately equal in all genes except *CAD* and *wingless*. *CAD* showed a slight A-T bias, while *wingless* showed a G-C bias (Table 4). Similar biases were documented in studies that have utilized *CAD* or *wingless* (e.g., Brower and DeSalle, 1998; Moulton and Wiegmann, 2003; Nazari *et al.*, 2007). The percentage of parsimony-informative characters per gene (within Sphingidae) was highest for *period* (55.9%), and lowest for *EF-1α* (25.4%; Table 5). For each gene, third codon positions had the greatest number of parsimony-informative characters, and nt3 was close to saturation for all genes except *EF-1α*.

1.3.2. Phylogenetic analyses

The MP simultaneous analysis included 3044 parsimony-informative characters, and the analysis yielded 12 MPCs (L = 43023, CI = 0.15, RI = 0.53; Fig. 7). The MP analysis recovered a monophyletic Sphingidae (bootstrap = 100%; from hereon bootstrap values indicated by percentages only), monophyletic Sphinginae (99%) and a poorly supported monophyletic Macroglossinae (51%). Monophyletic tribes and subtribes recovered include: Acherontiini, Ambulycini, Choerocampina, Hemarina, and Philampelini (all with ≥ 94%; summarized in Table 6). The clade comprising Sphinginae + paraphyletic Smerinthinae was recovered (87%, node 2), and Sphinginae and

paraphyletic Sphingulini was well supported (98%, node 7). Results from individual analyses are presented as supplementary material (Figs. S1-S10).

Bremer support values were calculated to estimate support for each node in the MP five gene simultaneous analysis. PBS was calculated to determine the relative contribution of each gene to each node in the overall topology, which may not be obvious from separate analyses of each dataset. Within a combined-gene analysis framework, a positive PBS value for a particular node and a particular gene indicates support from that particular partition, while negative values indicate negative contribution from that gene for that particular node. A PBS score of zero indicate the indifference of that partition to that particular node (Baker and DeSalle, 1997; Baker *et al.*, 1998; Gatsey *et al.*, 1999; Gatsey and Arctander, 2000). Interestingly, PBS values calculated from the combined-gene analysis suggest that the largest gene, CAD, contributed the least when PBS values were summed over all nodes. The total summed PBS value of CAD was -1046.55, while the total PBS for DDC was 1880.08 (Table 6). This was unexpected, as the MP single gene analysis of CAD was overall fairly similar in topology to the tree resulting from the simultaneous analysis. When examined carefully, it was discovered that TreeRot was often assigning negative PBS values to clades all the members of which were not present in a particular partition. For instance, *Amplypterus mansoni* + *A. panopus* is well supported in the simultaneous analysis (bootstrap = 100%, Bremer Support = 37, node 66), but while the entire CAD sequence was available for *Amplypterus panopus* it was missing for *A. mansoni*. The PBS value for this node was positive for all genes except CAD, which received a PBS value of -28.85. It remains unclear if there is a calculation or implementation error in TreeRot, but the current results suggest that when TreeRot

calculates PBS values, negative PBS values are assigned to a clade that includes a taxon with considerable missing data and a taxon without missing data. Theoretically, however, the net contribution to the particular branch should be zero.

CAD is also the largest of all five genes analyzed (more than double the size of any of the other genes), and it is therefore possible that a greater negative contribution is being assigned to this partition because of its size. Strange values were also obtained when the analysis was repeated. Further investigation of the effect of missing data to PBS clearly is necessary.

The MP 99-taxon analysis (which excluded taxa with missing data > 50% for any partition) resulted in one MPC (Fig. 8). Overall, this tree was similar in topology to the strict consensus from the all-taxon analysis, but differed slightly in the position of several taxa. PBS values from the 99-taxon analysis demonstrate that all gene partitions contribute positively to the tree generated from the simultaneous analysis (Table 7). This analysis was repeated multiple times and similar results were obtained. A measure of the relative contribution of each gene was calculated by dividing the total PBS value by the number of parsimony informative characters. This index, here termed the Relative Contribution Index (RCI), was highest for *EF-1α*, and lowest for *wingless* (Table 8, for further details on this method see: Wahlberg *et al.*, 2005).

The ML simultaneous analysis resulted in a tree similar to the all-taxon MP strict consensus but support for many relationships was stronger (Fig. 9). Within the well supported Sphingidae (100%) monophyletic subfamilies included Sphinginae (100%) and Macroglossinae (97%), the latter which was poorly supported in the MP analysis. Monophyletic tribes and subtribes recovered include the Acherontiini (100%),

Ambulycini (100%), Choerocampina (88%), Hemarina (100%), and Philampelini (100%). As with the MP analysis, a monophyletic Sphinginae + paraphyletic Smerinthinae (88%, node 2) was recovered, and Sphingulini + Sphinginae was well supported (100%, node 7).

For all genes, nt3 provided the greatest amount of parsimony informative characters. Greater than 90% of the characters at the third codon position (nt3) were parsimony informative for all genes except *EF-1α* (Table 5). This suggests that nt3 may be saturated and causing unwanted "noise" which may affect the resulting tree (e.g., Mindell *et al.*, 1996; Naylor, 1997 #366, but see also Wenzel and Siddall, 1999). Exclusion of nt3 resulted in an MP tree that was considerably less resolved, with many uncertain deep relationships (Supplementary Fig. S11). The number of MPCs rose from twelve to over ten thousand when third positions were excluded. Support values after excluding nt3 were much lower than when all nucleotide positions were included (Table 6). When nt3 was excluded, high support (> 90%) was recovered for only 21 nodes, while the nt-all dataset recovered 76 nodes of high support. Similar results were recovered for the ML nt-12 analysis, which also resulted in lower bootstrap support compared to the ML simultaneous analysis (Table 9). Although exclusion of nt3 may be necessary at deeper levels (Mindell *et al.*, 1996; Naylor and Brown, 1997), inclusion of nt3 is essential for resolving some relationships within Sphingidae.

Since *EF-1α* was demonstrated as the most informative gene with the highest RCI value (Table 8), and because *EF-1α* was the slowest evolving gene among all five genes (based on the highest percentage of parsimony informative characters at the third codon position of *EF-1α*, Table 5), I examined whether third codon position characters of *EF-1α*

were contributing significantly to overall tree structure. I removed all third codon positions of *EF-1α* and reran the MP analysis. The result (Supplementary Fig. S12) shows that much of the phylogenetic information for deep nodes within the Sphingidae comes from the third position of *EF-1α*, indicating that its slower rate of molecular evolution is critical for the present study.

The contribution of each gene partition to the ML tree was assessed by excluding one of the five genes and comparing bootstrap support values for each node. Support for many deep divergences within the Sphingidae decreased when CAD was excluded (Table 9), but exclusion of each of the other four genes did not affect support values of deep nodes. Interestingly, exclusion of DDC revealed higher support for several nodes (e.g., nodes 12, 30, 37) that were not obtained when any other single gene was excluded. These results suggest that CAD is contributing substantially to deep divergences within Sphingidae, while DDC may contain strong conflicting signal for particular nodes. However, further analyses are required to examine the effect of gene size, as CAD is more than twice the size of any other gene included in this study.

1.4. Discussion

1.4.1 Monophyly of Sphingidae and basal divergences within the family

The present study reveals a novel hypothesis for sphingid relationships, which contain elements of prior studies. Results were most congruent with the molecular analyses of Regier *et al.* (2001) and Mignault (2003), although increased taxon sampling uncovered many well supported relationships among taxa that were not included in their studies. Simultaneous analysis under both optimality criteria yielded a monophyletic

Sphingidae (100% MPML) and the monophyly of the family is also corroborated by at least nine larval, pupal, and adult synapomorphies (Minet, 1994). Both MP and ML analyses recovered a monophyletic Macroglossinae (51% MP, 96% ML, node 3), and a clade comprising of a paraphyletic Smerinthinae + Sphinginae (87% MP; 97% ML, node 2). *Langia zenzeroides* was the most basal taxon within this clade (node 2), but the basal position of *L. zenzeroides* at node 2 was not recovered in any of the single gene analyses. This result indicates that the combination of multiple datasets can uncover relationships that are not present when partitions are analyzed independently, as previously demonstrated (Chippindale and Wiens, 1994).

Differences between MP and ML topologies lie mainly in the placement of several long-branched taxa (i.e., *Cypa decolor*, *Cautethia spuria*, *Neogurelca himachala,* and *Sphingonaepiopsis gorgoniades*). To explore the difference between the two topologies, different combinations of outgroups were initially excluded. In each MP exclusion analysis, one or more of the four sphingids with the longest terminal branches moved to the base of the Sphingidae. Instability in the position of particular ingroup taxa caused by exclusion of particular outgroups suggests that these long-branched ingroups may be attracted to certain outgroups via LBA when other outgroups are excluded. To determine whether LBA was a factor in the simultaneous analysis with all outgroups, the analysis was repeated without outgroups, and the ingroup topology was compared. Topologies were fundamentally very similar, the only difference was in the MP analysis, where *C. decolor* was recovered as the sister taxon to the clade consisting of the Smerinthinae + Sphinginae excluding *Langia*. These results suggest that LBA may be an

artifact in the MP analysis when particular outgroups are excluded, but inclusion of all outgroups simultaneously does not appear to affect ingroup relationships significantly.

1.4.2 Relationships within Smerinthinae and Sphinginae

Within Smerinthinae, the tribe Smerinthini was paraphyletic in all analyses conducted. Paraphyly of Smerinthini was predicted based on morphology (Kitching and Cadiou, 2000). Monophyletic Ambulycini + paraphyletic Smerinthini (excluding *Langia*) was recovered in the ML analysis (97%, node 6) but other deep divergences among the Smerinthinae remain speculative, as deep relationships of this group were typically characterized by short internal branch lengths and weak bootstrap support under both optimality criteria.

Several well-supported groups were recovered within Smerinthini. *Laothoe*, *Pachysphinx*, *Paonias*, and *Smerinthus* form a well supported monophyletic group (100% MPML, node 30 MP, 31 ML), and clades nested within this group are also relatively well supported (≥ 88% MP, ≥ 98% ML). Following Rothschild and Jordan (1903), Kitching and Cadiou (2000) predicted the monophyly of *Pachysphinx, Paonias,* and *Smerinthus*. *Laothoe* is widely distributed from Ireland across to China and its larva feeds on leaves of *Populus* and *Salix* (Pittaway, 1997-2006), the same larval hosts of the temperate New World *Pachysphinx* (Hodges, 1971). Unlike *Laothoe* and *Pachysphinx*, whose larval hosts are restricted to the Salicaceae, *Paonias* and *Smerinthus* are polyphagous, and feed on a variety of different hostplant families (Hodges, 1971). The current study suggests a larval host shift from monophagy to polyphagy in this particular clade, and the secondary development of the adult hindwing eyespot in *Paonias* and *Smerinthus*.

The clade containing *Amorpha, Mimas,* and *Phyllosphingia* was well supported (100% MPML, node 49 MP, 71 ML). Recovery of *Amorpha* + *Phyllosphingia* as sister genera corroborates evidence from larval and pupal morphology and larval hostplants being Juglandaceae (Pittaway and Kitching, 2006). Pupal morphology may also support the affinity of these two genera with *Mimas* (Kitching, pers. com.). Monophyly of the *Clanis*-group, including *Clanis*, *Afroclanis, Neoclanis*, and *Viriclanis* was also well supported (100% MPML, node 36 MP, 29 ML). *Clanis* is distributed in Asia, and the latter three are restricted to mainland Africa.

Kitching and Cadiou (2000) predicted the monophyly of *Daphnusa, Gynoeryx, Likoma,* and *Marumba* based on similar forewing line patterns and tarsal morphology. Although *Gynoeryx* was unobtainable for inclusion in the current study, *Daphnusa*, *Likoma*, and *Marumba* form a well supported monophyletic group (100% MPML, node 71 MP, 73 ML). The sister-group relationship of this "*Likoma*-group" with the *Polyptychus*-group" is well supported (94% MP, 99% ML). The *Polyptychus* group was represented in the current analysis by *Neopolyptychus, Polyptychus, Polyptychoides,* and *Pseudoclanis*, and its monophyly was also well supported (100% MPML). Although the pupal stage of *Chloroclanis* is unknown, all other genera in this clade (*Andriasa*, *Neopolyptychus*, *Polyptychoides*, *Polyptychus s.s.*, and *Pseudoclanis*) have a row of punctures near the anterior edge of some lateral segments of the pupa. However, this feature is not found in *Polyptychus andosa* or many other *Polyptychus*, suggesting that further division of *Polyptychus* is required (Ian J. Kitching, pers. com.).

Monophyly of Ambulycini was well supported (100% MPML). Within the tribe, two clades follow their geographic distribution: the Neotropical *Adhemarius* +

Protambulyx and the Old World *Ambulyx* + *Amplypterus*. A morphological analysis (Kitching unpublished data, 1993), places *Ambulyx* and *Amplypterus* as basal within the Ambulycini. It has been hypothesized, however, that some Old World Ambulycini (e.g., *Akbesia*, *Batocnema*, *Compsulyx*) may be more closely allied to the Neotropical species than *Ambulyx* + *Amplypterus* based similarities of the hindwing eyespot marking and a spinose gnathos in the male genitalia (Kitching and Cadiou, 2000). A future study incorporating more taxa and characters will test this hypothesis.

The current study strongly supports a sister-group relationship of the paraphyletic Sphingulini + monophyletic Sphinginae (98% ≥ MPML, node 7). Rothschild and Jordan (1903) placed seven genera in the Sphingulini as the sister group to the Sphinginae, but none of the other studies predicted the placement. Nakamura (1977) noted that both groups share a unique character: "[the] caudal end of [the] pupal eye piece [is] attached to the structure restricting the proximal margin of the maxilla" (Nakamura, 1977: 6). However, Nakamura did not place the Sphingulini as sister to the Sphinginae in any of his figures (Nakamura, 1976; 1977; 1978).

Monophyly of Sphingulini has remained uncertain, but morphology suggests that *Dolbina* and *Kentrochrysalis* are closely related (Rothschild and Jordan, 1903; Eitschberger and Zolotuhin, 1997; Kitching and Cadiou, 2000). The current analysis renders the tribe paraphyletic, as the Australian *Hopliocnema* is sister to the reciprocally monophyletic Sphinginae and *Dolbina* + *Kentrochrysalis*. This paraphyly is not unexpected as Sphingulini was diagnosed by Rothschild and Jordan (1903) solely on character reductions and absences.

Monophyly of Sphinginae was strongly supported (≥ 99% MPML), and relationships among taxa in the subfamily are nearly identical under both optimality criteria (difference being the polytomy of *Sphinx* in the MP strict consensus). Within the Sphinginae, Acherontiini is monophyletic (100% MPML). Acherontiini include five genera, four of which are included in the present study. A feature of the labial palp and three characters of the genitalia support the monophyly of the tribe (Kitching, 2002; 2003). Although *Callosphingia* could not be obtained for inclusion into the current study, relationships among *Acherontia*, *Agrius*, *Coelonia*, and *Megacorma* are congruent with Kitching's (2002; 2003) morphological analyses under equal weighting (see Fig. 22 in Kitching 2002).

Acherontiini and *Xanthopan* are known to share a unique hearing organ which is used to detect ultrasonic wavelengths of bats (1999a; Göpfert and Wasserthal, 1999b; 2002). These hearing organs differ slightly from ears found in the Choerocampina, although all sphingid hearing organs are composed of a specialized sound-receiving structure on the labral pilifer and labial palp (Göpfert *et al.*, 2002). Specifically, the pilifer of particular Acherontiini and *Xanthopan* lack a distinct distal lobe, the second palpal segment is deeply depressed without being swollen or lacking in hairs, and a scale plate that interacts with the pilifer is also present on the labial palpus (Göpfert *et al.*, 2002).

Kitching (2002) noted that Acherontiini also share similar pupal morphology with *Xanthopan* and the *Cocytius*-group (*Cocytius* + *Neococytius*). He therefore tested whether these long-tongued sphingines form a monophyletic group, but was unable to determine the exact placement of *Xanthopan* (Kitching, 2002), as different weighting schemes

placed the genus in different positions of the tree. The current study reveals that *Xanthopan* and the *Cocytius*-group are closely related (100% MPML, nodes 52 MP, 97 ML), and suggests two independent origins of the hearing organ in Sphinginae.

The monotypic *Dolba hyloeus* (Drury) was recovered within the speciose genus *Manduca* with very strong support (100% MPML). Rothschild and Jordan (1903) recognized *Dolba* as a genus separate from *Manduca* based mainly on the presence of "lashed eyes". However, where this character has been used in other groups, it has been found wanting as a synapomorphy (e.g., in Noctuidae 1984; Kitching, 1987) and it is certainly insufficient to form the basis of a genus. Although changing the classification of Sphingidae is not the immediate goal of this study, the molecular results indicate that the monotypic *Dolba* should be synonymized with *Manduca*.

The clade containing *Sphinx* is strongly supported (100% MPML, node 53 MP, 33 ML). Tuttle (in press) proposed to include 21 species of *Sphinx* in a newly resurrected genus, *Lintneria* Butler, based on larval and adult characters. Butler (1876) believed that the more rounded forewings of particular *Sphinx* species were substantially different, and therefore erected this genus over a century ago. Forbes (1911) noted the close similarity in the larva of *Lintneria* and *Sphinx*, but described the unique mesothoracic dorsal hump of *Lintneria* as the distinguishing feature. Despite these distinctive characters of *Lintneria*, subsequent studies generally placed these species in *Sphinx*. Recent larval rearing experiments and a closer examination of adults corroborate the previous findings, and it has been shown that that the first four instars of all members of *Lintneria* for which the larval stages are known have a large, fleshy, dorsal protuberance that angles anteriorly, and adults have unique wing markings that are typically not found in *Sphinx*

sensu stricto (Tuttle, in press). The present molecular analysis included two species, *S. istar* (Rothschild and Jordan) and *S. merops* Boisduval, which are proposed to be transferred to *Lintneria*. These two species have a more southerly distribution compared to the other *Sphinx* species in the present study, and together form and separate, well supported clade (100% MPML, node 77 MP, 125 ML).

The second strongly supported clade within *Sphinx* includes *S. caligineus*, *S. chersis* and *S. dollii*, together with *Isoparce cupressi*, and *Lapara coniferarum* (100% MPML). *Sphinx caligineus, S. dollii, I. cupressi* and *L. coniferarum* form a monophyletic group, and larvae of these species are unique in that they feed on conifers (Hodges, 1971). Furthermore, the proboscis of these conifer-feeding species is substantially reduced (see Chapter 2). Strong support for these two clades corroborates resurrection of *Lintneria* based on larval and adult morphology, and suggests that *Isoparce* and *Lapara* should be synonymized with *Sphinx*.

1.4.3 Relationships within Macroglossinae

A monophyletic Choerocampina was recovered (94% MP; 88% ML) in the paraphyletic tribe, Macroglossini. Choerocampines have a swollen, air-filled second labial palp segment that is devoid of hair or scales (Roeder and Treat, 1970), and monophyly of this subtribe was predicted (Kitching and Cadiou, 2000). Like the Acherontiini and *Xanthopan*, the second labial palp segment of Choerocampina is used as an 'ear' in combination with the pilifer to detect ultrasonic sounds emitted by echolocating bats (Roeder *et al.*, 1968). A recent study demonstrates that particular choerocampines produce ultrasound in response to bat calls, and these responses may be

used to startle the bat, jam biosonar, or warn the predator (Barber and Conner, submitted).

Choerocampines and hawkmoths in the Smerinthini, Sphingini, Sphingulini, and diurnal Macroglossina typically have genital stridulatory organs which are similar, but differ slightly in morphology (Kitching and Cadiou, 2000). The functional significance of these stridulatory organs remains unknown, but the organs are presumed to be used for courtship (Barber, submitted; Kitching and Cadiou, 2000; for an account of male hawkmoth using acoustic sounds in the vicinity of a female, see Mell [1922]). If stridulatory organs are used during courtship, members of the opposite sex must be able to recognize stridulatory stimuli. The primary function of the pilifer/labial palp ultrasonic hearing organ of Choerocampina may therefore be to detect these signals during mating, and its function to detect ultrasounds of bats may be a secondary gain which evolved thereafter (Kitching and Cadiou, 2000; Barber and Conner, submitted). The question of how and why stridulatory organs evolved in the Sphingidae will be the focus of a future study which includes additional physiological data and additional sampling of sphingid taxa.

Within Choerocampina, the Neotropical genus *Xylophanes* forms a monophyletic group, although this clade was not strongly supported (84% MP, 83% ML). In both MP and ML analyses, *Cechenena*, *Rhagastis* and *Theretra* together form a monophyletic group (80% MP, 90% ML). These three genera share similar eyespot patterns along the body of the larva, and were believed to be fairly closely related (Nakamura, 1976).

The Southeast Asian *Eupanacra* was recovered as the sister group of the Choerocampina, and *Eupanacra* + Choerocampina is well supported (96% MP, 99%

ML). Ancestral to this divergence is *Gnathothlibus*, and the relationship (*Gnathothlibus* (*Eupanacra* (Choerocampina))) is also well supported (100% MPML). The general biogeographical pattern within this clade suggests a dispersal event from the Old World (e.g., *Eupanacra, Gnathothlibus*) to the New World by the ancestor of *Xylophanes* and another by the ancestor of *Hyles*. Subsequently, there was a dispersal event back into the Old World (for further discussion on the biogeography of Hyles, see: Hundsdoerfer *et al.*, 2005a).

Macroglossina was polyphyletic, and the subtribe was separated into several fairly well-supported monophyletic groups. The clade comprising of the temperate *Proserpinus*-group (*Arctonotus, Euproserpinus, Proserpinus*) was well supported (100%, MPML, nodes 87 MP, 91 ML). All three genera share larval hostplants of the Onagraceae (Hodges, 1971), and these genera were thought to be morphologically closely related to each other and to *Amphion* (Rothschild and Jordan, 1903; Kitching and Cadiou, 2000). Interestingly, both analyses recovered *Pachygonidia* (Dilophonotini, here represented by *P. subhamata*) as the sister group to the *Proserpinus* group, although this relationship was not strongly supported (69% MP, 68% ML). However, inclusion of *Pachygonidia* within Macroglossina is fairly unambiguous (96% MP, 100% ML).

Two species of *Ampelophaga* were included in this study, *A. dolichoides* (Felder) and *A. rubiginosa* (Bremer & Grey). Based on adult morphology, Rothschild and Jordan (1903) included *Ampelophaga dolichoides* in *Ampelophaga* but they noted a close affinity between this genus and *Elibia*, in which they included only a single species, *E. dolichus* (they also included the second current species of *Elibia, E. linigera,* in *Ampelophaga*). Recently, the immature stages of *dolichoides* were discovered and an

apparent similarity with *Elibia* was revealed. Although the pupa of *dolichoides* lacks a free, jug-handle tongue-case found in *E. dolichus*, the species was tentatively transferred to *Elibia* (Pittaway and Kitching, 2006). Results from the present study corroborate the inclusion of *dolichoides* in *Elibia*, as it was recovered as the sister taxon to *E. dolichus* (89% MP, 90% ML). *Ampelophaga rubiginosa*, *Clarina*, and *Darapsa* are essentially congeneric (their male genital structures are essentially identical; Kitching, pers. com.), and their relationship (*Darapsa* (*Ampelophaga rubiginosa* + *Clarina*)) is well supported by molecules (100% MP, 99% ML) and morphology (Kitching and Cadiou, 2000).

Philampelini was represented by three species of *Eumorpha*. All species were recovered together in the same clade with strong support (100% MPML). Surprisingly, the dilophonotine genus *Enyo* was recovered as the sister group to *Eumorpha*, although not strongly supported in either analysis (67% MP, 53% ML). Philampelini also includes one other genus, *Tinostoma*, which is an endemic of the Hawaiian island of Kauai (Kitching and Cadiou, 2000). Unfortunately, *Tinostoma* is known from only a few specimens, and could not be obtained for inclusion in the current study.

The subtribe Dilophonotina is restricted to the New World and is predominantly Neotropical, and the larvae of many species feed on Vitaceae (Tuttle, in press, see also Table 11). Dilophonotina was paraphyletic in all analyses, but a well-supported monophyletic group exists within the tribe (100% MPML; node 20 MP, 26 ML). Monophyly of this clade was previously predicted based on morphology (Kitching and Cadiou, 2000). Monophyly of *Aellopos, Eupyrrhoglossum, Nyceryx,* and *Perigonia* is also well-supported (100% MPML), which is also corroborated by morphology (Kitching

and Cadiou, 2000). All subordinate relationships within this clade were well supported (≥ 96% MP, ≥ 99% ML).

Similarly, *Erinnyis*, *Hemeroplanes*, *Isognathus*, *Madoryx*, and *Pseudosphinx* form a well supported group (100% MPML). There is evidence that two dilophonotine genera, *Erinnyis* and *Pseudosphinx*, exhibit an acoustic response, although they are considerably less sensitive to acoustic signals than the choerocampines (Roeder, 1972). Unlike species with palp/pilifer acoustic hearing organs of the Choerocampina and Acherontiini, hearing organs of these two dilophonotine genera are not on the labial palp or pilifer, and their location remains unknown (Roeder, 1972).

The present study recovered a well supported Hemarina (100% MPML). This subtribe includes two diurnal clear-winged sphingid genera, *Cephonodes* and *Hemaris*, and monophyly of Hemarina was previously predicted based on morphology (Kitching and Cadiou, 2000), and there mounting evidence that these two genera may need to be sunk into one genus (Ian J. Kitching, pers. com.). Interestingly, the placement of Hemarina differs between MP and ML trees. In the MP tree, the clade composed of *Neogurelca* + *Sphingonaepiopsis* (75% MP, 99% ML) is the sister group of Hemarina, while this tribe is placed at the base of the Macroglossinae in the ML tree. Both Hemarina and the clade comprising of *Neogurelca* + *Sphingonaepiopsis* are long-branched taxa. In order to test whether MP resolution was caused by LBA between Hemarina and *Neogurelca* + *Sphingonaepiopsis*, the MP analysis was repeated without *Neogurelca* and *Sphingonaepiopsis*. When Hemarina were excluded, *Neogurelca* + *Sphingonaepiopsis* moved to the base of the Sphingidae. The shift in position of *Neogurelca* + *Sphingonaepiopsis* and Hemarina when either is removed provides

preliminary evidence for LBA between Hemarina and *Neogurelca* + *Sphingonaepiopsis* (see also Fig. 8 for position of Hemarina in the 99-taxon analysis which did not include *Neogurelca* and *Sphingonaepiopsis*).

1.5. Conclusion

The current molecular analysis corroborated many previously postulated sets of relationships based on larval, pupal, and adult morphological characters. However, it also uncovered many novel relationships. Molecular data strongly supports the monophyly of Sphinginae and its sister relationship to the paraphyletic Sphingulini, although many deep relationships within the paraphyletic Smerinthinae remain ambiguous. Additional genes and taxa may resolve the uncertainty within the Smerinthinae, as deep divergences within this subfamily are characterized by weak support and short internal branches. Monophyly of Macroglossinae was strongly supported in the ML analysis, although it was much lower in the MP analysis. MP was subject to LBA within Macroglossinae, and inclusion of additional closely-related sphingids, such as the yellow hindwinged species of *Temnora* which superficially resemble *Neogurelca* and may help break these long branches. The current study also demonstrated the utility of the five protein-coding genes for resolving relationships within a family of Lepidoptera, and the synergistic effects which are generated when independent genes are analyzed together in a simultaneous analysis.

The supplementary six gene analysis demonstrates that small amounts of data from the barcoding region of the COI gene can be useful in uncovering relationships when combined with a larger dataset with dense taxon and gene sampling. However,

considerable missing data can depress bootstrap and BS values, and the effect of missing data on PBS needs to be further explored. Understanding the effect of missing data on PBS is imperative, as many recent phylogenetic studies are implementing PBS with large taxon and gene sampling. Specifically, future studies should be conducted on a smaller dataset with fixed taxon and gene size, and missing data should be simulated to test the effect of missing data on PBS.

CHAPTER 2

EVOLUTION OF THE SPHINGID PROBOSCIS

2.1. INTRODUCTION

It has been well known that some hawkmoth adults have extremely long proboscises, while others have very short tongues and can be non-feeding (Rothschild and Jordan, 1903; Fleming, 1968; Miller, 1997b; Kitching and Cadiou, 2000). The evolution of the long tongue in particular sphingid species has been hypothesized as an example of coevolution between pollinating sphingids and flowers from which they extract nutrient-rich nectar (Darwin, 1862). Perhaps the most well-known example of this interaction is *Xanthopan morganii praedicta* Rothschild and Jordan and *Angraecum sesquipedale* Thouars in Madagascar (Kritsky, 1991). In *The origin of species*, Darwin (1859:202) stated, "As certain moths of Madagascar become larger through natural selection … as the proboscis alone was lengthened to obtain honey from … deep tubular flowers … the seedlings would generally inherit long nectaries; and so it would be in successive generations of the plant and of the moth." This model has been a classic example of a "coevolutionary race" (Darwin, 1862) whereby the evolution of increasing flower depth leads to the subsequent increase in hawkmoth tongue length.

Nilsson (1985; 1988; 1998) experimentally tested various predictions of Darwin's proposal and found support for this coevolutionary hypothesis. Wasserthal (1992; 1997; 1998) presented an alternative model in which the long-tongued sphingid has evolved to increase the distance from shallow flowers and allow for sideways hovering behavior in order to prevent being ambushed by predators which may be resting on the flower. Wasserthal's hypothesis is based on the fact that many long-tongued hawkmoths also feed on nectar from flowers with very short tubes. This "pollinator shift" hypothesis is a modification of the coevolutionary race model. Preadapted long-tongued sphingids shift

from feeding on flowers with short corollas to flowers that have long tubes, and the occurrence of long-tongued species on long-tubed flowers exerts selective pressure towards floral tube elongation.

Despite numerous competing hypotheses on the evolutionary process by which the sphingid tongue may have lengthened (‚Wasserthal, 1997 #163, see also: Janzen, 1984; Nilsson *et al.*, 1985; Miller, 1997b), very few studies report hawkmoth tongue length in a comprehensive manner, and even fewer studies examine nectar feeding records across the entire family. Miller (1997) compiled a list of tongue lengths for 152 sphingid species and stated, "Tongue shortening to 10 mm or less renders hawkmoths incapable of nectar foraging" (Miller, 1997: 11). Miller's decision to categorize functionality on tongue length was based on an anatomical study by Fleming (1968), in which internal cranial muscles of short and long-tongued sphingids were examined. Fleming presented measurements of fifteen hawkmoth species, and concluded that the reduction or absence of particular muscles renders short-tongued species to have non-functional proboscises. Contrary to Miller's statement, Fleming never explicitly stated that proboscis length shortening below 10 mm implies non-nectar feeding.

The discovery of muscle reduction in particular sphingids led Fleming (1968) to postulate that short-tongued sphingids, such as smerinthines, were derived from sphingids with longer tongues. The hypothesis that the long sphingid tongue is an ancestral condition dates back to Rothschild and Jordan's (1903) classification and treatment of sphingid relationships. Rothschild (1903) believed that long tongues are found in basal sphingids, and shorter tongues became "reduced in each derivation from [the] ancestral type" (Rothschild and Jordan, 1903; xcix). Recent coevolutionary hypotheses on long

tongue evolution in Sphingidae (e.g., Nilsson, 1988; Wasserthal, 1992; 1998; 1998) were based on Rothschild and Jordan's classification, and assumed that hawkmoths with long tongues were basal in the family. Nilsson (1998: 260) stated, "The world's most long-tongued species of hawkmoths, one of which is *X. morganii* are also among the largest and/or heaviest and most primitive …" Similarly, Wasserthal (1998 :459) asserted, "… extremely long tongues are old adaptations…" If Rothschild and Jordan's "retrogressive" hypothesis is correct, one would expect the ancestral condition of the sphingid tongue to be long, and derived sphingids to have shorter tongues.

Alternatively, Kitching (2000) predicted that smerinthines, with their short tongues, are basal in the family (see quotation on the second page of this thesis). In general, long-tongued hawkmoth species are found in the other two subfamilies, the Macroglossinae, and Sphinginae (Miller, 1997b; Lemaire and Minet, 1999; Kitching and Cadiou, 2000). Therefore, under Kitching and Cadiou's scenario, one would expect a general trend from short ancestral tongues to longer tongues in derived lineages. After examining tongue length across different taxonomic ranks in Sphingidae, Miller (1997a) also predicted that tongue length was ancestrally short and became longer over time.

Despite the competing hypotheses on proboscis length evolution, none of the aforementioned authors formally tested their hypothesis because a sphingid phylogeny was not available at the time. The only study which used modern phylogenetic methodology to understand tongue length evolution in Sphingidae was the study of Kitching (2002) on Acherontiini and several long-tongued, non-acheronitine sphingines. Kitching categorized tongue length according to the number of coils in the pupal tongue case, and conducted a rigorous cladistic analysis. Multiple weighting schemes were

implemented, and in all cases, nectar feeding hawkmoths with extremely long proboscises were never recovered as a monophyletic group, and the placement of *Xanthopan morganii* remained ambiguous.

To date, a phylogenetic study that examines nectar feeding and tongue length evolution across all sphingid subfamilies has never been conducted. The purpose of the present study is fourfold: (1) to examine and measure proboscis length across a broad taxonomic range of sphingids, (2) to compile a list of valid field nectar feeding records for sphingids included in the current study, (3) to determine if nectar feeding is strongly correlated to tongue length, and (3) to determine how the nectar feeding behavior evolved in the family.

2.2. Materials and Methods

Four hundred and seventeen sphingid specimens were examined in the course of this study. Specimens were studied at the Smithsonian Institution Maryland Support Center (MSC) in Suitland, Maryland. Species which were not represented at the MSC were examined from alcohol specimens preserved in the University of Maryland Lepidoptera frozen collection at College Park, Maryland. Eleven specimens were examined at the Natural History Museum in London. For each species, up to five specimens were examined and a combination of males and females were chosen whenever possible. Because proboscis length may vary within species (Miller, 1997b; Kitching, 2002), I chose specimens that were collected from different seasons and from a wide geographic range whenever possible.

For each species examined, the proboscis was removed by gently pulling the coil from underneath the head of the moth with a pair of fine forceps. Two forceps were used to assure that the tongue was being separated at its base. When proboscises were short, the labial palp was gently pushed aside, and the tongue removed. All proboscises were placed in a pre-labeled glassine envelope. Each tongue was then placed in a 1 mL solution of 10% KOH and heated on a hot plate for 10 minutes. After heating, the proboscis was uncoiled and its length measured with a millimeter ruler, washed with 70% ethanol, and placed into a gelatin capsule and pinned on the bottom of the specimen. Proboscises removed from specimens in the UMD alcohol collection were placed in gelatin capsules in glassine envelopes.

In order to standardize for body size, the length of the forewing (FWL) was measured from each specimen. The right forewing was arbitrarily chosen, but whenever the right forewing was missing or damaged, the left forewing was measured. FWL was calculated by measuring the distance from the wing base to the farthest point on the forewing tip. FWL was preferred over body length because body length can be subject to biases arising from body compression or extension. Furthermore, FWL was chosen because this measure has been used to standardize body size in Sphingidae and other Lepidoptera (e.g., Loder *et al.*, 1998; Beck and Kitching, 2007), and because it is known to be strongly correlated with body weight in hawkmoths (Miller, 1997a).

Ancestral state conditions were determined using Mesquite Ver. 1.12 (Maddison and Maddison, 2006). A matrix of standard categorical data was created for one unordered character with three states: non-feeding (0), nectar feeding (1), nectar and bee-hive feeding (2). I define non-feeding species as species which do not feed on nectar from

flowers. Although there are occasional reports of sphingids feeding on water droplets (Kernbach, 1962; Pittaway, 1993), visiting mud puddles (Bänzinger, 1988; Büttiker *et al.*, 1996), drinking tear from mammalian eyes (Bänzinger, 1988), and probing decaying animal remains (Sbordoni and Forestiero, 1985), these records were excluded because they are considered anomalies which do not constitute the regular diet of hawkmoths. Species with average tongue lengths less than 1 mm were coded as non-feeding, and those without any biological information regarding feeding behavior were left as missing data. All statistical analyses were conducted with the JMP Ver. 6 statistical software (JMP, 2006). Character states were mapped onto the five gene ML sphingid tree (Chapter 1, Fig. 9).

2.3. RESULTS

2.3.1. Proboscis length and nectar feeding

Average tongue length measurements per sphingid species ranged from less than 1 mm (*Andriasa contraria, Hopliocnema brachycera*, *Marumba quercus*) to 211.8 mm (*Neococytius cluentius*; Table 10). In general, shortest tongues were recorded from Smerinthinae, and longest tongues were found in particular species of Sphinginae. However, some smerinthines had much longer tongues than others in the subfamily (e.g., all Ambulycini examined had tongue lengths 22.8 mm – 35.8 mm), and some sphingines had very short tongues (e.g., *Isoparce*, *Lapara*; 6.9 mm and 4.6 mm respectively). Tongue length also varied within each species; *Cocytius duponchel* had the greatest intraspecific tongue length variation, which ranged from 73 mm to 150 mm. Results from the present study are comparable to previous reports on hawkmoth tongue length (e.g.,

Gregory, 1963-1964; Bullock and Pescador, 1983; Haber and Frankie, 1989; Miller, 1997b).

Of the 131 sphingid species that were included in the present study, 61 had documented nectar-feeding records, 17 were reported in the literature to be non-feeding, and 32 remain unknown. A comparison of hawkmoth tongue length to known nectar feeding records shows that long-tongued species are generally nectar feeding, while short-tongued species are typically non-nectar feeding (Fig. 10). However, extrapolating functionality solely on the basis of length can be misleading, as there is an overlap in tongue length for nectar feeding and non-feeding species. Tongue lengths for nectar-feeding species were greater than 9.0 mm, while non-feeding species had tongue lengths less than 10 mm (Table 10; see also Fig. 10 for a comparison of average tongue lengths per species).

Comparison of tongue length to forewing length shows that the two variables are strongly correlated (Pearson's Correlation Coefficient = 0.571, $p < 0.0001$; Fig. 11), corroborating results from previous studies which correlated hawkmoth tongue length and forewing length (e.g., Bullock and Pescador, 1983; Haber and Frankie, 1989; Miller, 1997b). These results illustrate the general trend for small sphingids to have short tongues, and large sphingids have longer tongues. Actual and relative proboscis lengths both show a similar pattern when compared (Fig. 12).

2.3.2. Ancestral state reconstruction

Reconstruction of ancestral states reveals that the short, non-feeding tongue is the ancestral condition in Sphingidae. The nectar-feeding long tongue independently evolved at least three times in the family, but was subsequently lost at least three times (Fig. 12).

2.4. DISCUSSION

2.4.1. Evolution of nectar feeding in Sphingidae

The plesiomorphic condition of the short, non-feeding proboscis supports the hypothesis of Kitching and Cadiou (2000). Within the typically short-tongued Smerinthinae, there was a transition from non-feeding (*Langia zenzeroides*) to nectar feeding in the Ambulycini (but see also Rothschild and Jordan [1903] for a discussion on *Trogolegnum*). Although nectar feeding has not been documented for any species in the Smerinthini, *Afroclanis calcareus* and *Clanis bilineata* may feed on flowers, as tongue length of these two species are substantially longer than other species in the tribe (24.0 mm and 26.0 mm respectively, Table 10; see also Carcasson [1968], Miller [1997]). Presence of long-tongued smerinthines in the sister-clade to Ambulycini suggests that long tongues may predate to the ancestor of the Ambulycini, but increased taxon sampling and additional field observations are necessary to verify this hypothesis.

Species in the Sphingulini also have very short, non-feeding proboscises, and the sister-group relationship of the paraphyletic Sphingulini and monophyletic Sphinginae is well supported (see Chapter 1). Sphingines are predominantly nectar-feeding, and present study reveals a behavioral shift from non-nectar feeding to nectar feeding in the ancestor of this subfamily (Fig. 12).

The basal clade in the Sphinginae includes three genera, *Cocytius*, *Neococytius*, and *Xanthopan*, all which have extremely long tongues. *Xanthopan* is well known to feed on orchids in Africa (e.g., Darwin, 1862; Nilsson, 1988; Wasserthal, 1992; 1998), and the neotropical *Cocytius* has also been observed pollinating a long-tubed orchid, *Polyrrhiza lindenii* (Tuttle, in press) which has a corolla reaching 170 mm in length (Long and Lakela, 1971). Although we can only speculate the mechanism by which very long tongues evolved, strong support for the placement of *Xanthopan* in this clade suggests the possibility that the ancestor of *Cocytius*, *Neococytius* and *Xanthopan* may have coevolved with the flowers from which they feed. Preliminary morphological evidence supports the inclusion of the monotypic sphingid genus, *Amphimoea*, in this clade (Kitching and Cadiou, 2000). *Amphimoea walkeri* (Boisduval) also has a very long tongue which can reach 280 mm in length (Miller, 1997b). Unfortunately, too little is known about the biology of the species in these genera to assess whether they coevolved with long-tubed flowers.

Long tongues were also documented in the genus *Sphinx*. Average tongue length of *S. merops* and *S. istar* was 69.8 mm and 83.7 mm, respectively. However, proboscis length varied considerably in this genus, as the average tongue length of *Sphinx dollii* was only 9.0 mm. *Sphinx merops* and *S. istar* together form a well-supported clade (see Chapter 1) which is reciprocally monophyletic to the remaining *Sphinx* species. Correlation of tongue length to phylogeny reveals a geographic trend from long-tongued *Sphinx* species at low latitudes to shorter-tongued species which are distributed at higher latitudes. A similar latitudinal trend was documented across the Sphingidae (Miller, 1997b).

Reduction of tongue length also occurred within *Ceratomia*. Although the present analysis only included two species of *Ceratomia*, the two species sampled represent different feeding conditions within the genus. *Ceratomia catalpae* (7.6 mm) has lost its ability to feed on nectar (Fleming, 1968; Tuttle, in press), while *C. undulosa* (13.0 mm) still maintains the ability to feed (Fernald, 1884; Tuttle, in press). Observations on the natural history of these species are further supported by the fact that the proboscis extensor muscle is absent in *C. catalpae*, but present in *C. undulosa* (Fleming, 1968). According to Schmitt (1938), fully functional mouthparts must have at least two pairs of proboscis extensor muscles.

Fleming (1968) also discovered that the dilator muscles of the sucking pump are weaker and divided into two parts in some individuals of *C. catalpae*, while these muscles are moderately developed in *C. undulosa* (but less so than very long-tongued sphingids). When describing the muscles of *C. catalpae*, Fleming (1968:22) stated, "the tendency to lose functional feeding apparatus is more advanced than in *C. undulosa* … Possibly [*C. catalpae*] is presently in a state of losing these muscles, since some individuals have fewer and/or smaller muscles than others." Although not as prominent in *C. catalpae*, reduction of particular cranial muscles of *C. undulosa* compared to other sphingine species suggests that *C. undulosa* may also be in the process of losing its proboscis. Cranial muscle reduction may also be taking place in other taxa with tongues that are short but still functional (e.g., *Sphinx dollii*).

A secondary reduction of the proboscis also occurred within Acherontiini. *Acherontia* has a much shorter proboscis than any other taxon sampled within this tribe (Table 10). Like other species of *Acherontia*, *A. styx* feeds on honey from beehives

(Künckel d'Herculais, 1916; Pittaway, 1993), and an adult was observed to regurgitate honey when captured (Kitching, 2003). Based on a nearly perfect overlapping distribution, it is hypothesized that *A. styx* is a specialized cleptoparasite of *Apis cerana* (Kitching, 2003). Regardless of its host species, the tip of the proboscis of all *Acherontia* species is sharply pointed and modified to break capped honey cells in the hive (Kitching, 2003), but *Acherontia* still maintains its ability to feed on flowers (Tutt, 1904; Pittaway, 1993). The reduction in proboscis length and its unique morphological modifications were undoubtedly due to its close ecological association with *Apis*.

In Macroglossinae, tongue length varied from 4.3 mm to 85.3 mm (X-bar = 32.18). As the name suggests, all macroglossines were presumed to have long functional tongues (Lemaire and Minet, 1999), but a recent study on *Arctonotus lucidus* suggests otherwise (Rubinoff, 2001). The sister genus to the monotypic *Arctonotus* is *Proserpinus,* and the close affinity of these two genera is supported by molecules (Ch. 1, Fig. 9), morphology (Rothschild and Jordan, 1903), and larval hostplants on the Onagraceae (Hodges, 1971). *Proserpinus* includes six species (Pittaway, 1993), all of which are active as adults during the summer. *Arctonotus*, on the other hand, has a distribution that is restricted to localized habitats in Washington, Oregon and California, and is active mainly during cold months of the year (Hodges, 1971; Rubinoff, 2001) when flowers may not be readily available. Although there are certainly many alternative plausible hypotheses, I postulate that the fewer number of available flowers during adult activity times has led to a reduction in tongue length in this genus.

2.4.2. Nectar feeding and correlations with other life-history traits

Adult diet can influence various life-history traits, such as egg-production and longevity in Lepidoptera (e.g., Hill, 1989; Hainsworth *et al.*, 1991; Karlsson, 1994; Fischer and Fielder, 2001). Lepidoptera that obtain resources during the adult stage for egg production and upkeep have been termed *income breeders*, while species with rudimentary non-feeding proboscises have been termed *capital breeders* because they must obtain all nutrients from larval hostplants (e.g., Sibly and Calow, 1984; Boggs, 1992; Tammaru and Haukioja, 1996; Bonnet *et al.*, 1998), see also (Janzen, 1984; Beck *et al.*, 2006c).

Janzen (1984) compared life history strategies of non-feeding saturniids and with nectar-feeding hawkmoths. He concluded that saturniids generally lay eggs in large batches, are typically polyphagous, and tend to feed on conspicuous plants with chemical toxins. Similarly, smerinthines generally have a rudimentary non-feeding proboscis, and develop a greater number of mature eggs at eclosion (Miller, 1997b), which suggests at least in part, a saturniid-like *capital breeding* life history strategy (Kitching and Cadiou, 2000). On the other hand, hawkmoths in the Macroglossinae and Sphinginae have an *income breeding* life history strategy as they generally possess a strong ability to fly, typically feed on nectar, have fast development before adulthood, and are believed to be specialized feeders of particular larval hostplants with higher food quality (Janzen, 1984).

Miller (1997b) further developed this idea after documenting a trend towards long hawkmoth tongues at low latitudes. He hypothesized that sphingids in tropical regions require long tongues, as they need more energy to disperse to look for inconspicuous, non-persistent larval food plants in structurally complex habitats. On the contrary,

hawkmoth species distributed at higher latitudes do not need to fly a much, as their food plants are more conspicuous, and easy to find. This food-searching hypothesis (here called the FS-hypothesis) has never been formally tested. A comparison of larval hostplants across sphingid subfamilies reveals that many smerinthines feed on trees, while Macroglossines are typically feeders of shrubs or vines (Table 10, see also Janzen [1984], Miller [1997]).

If the FS-hypothesis is correct, species with very long tongues would be predicted to be monophagous, while there would be a trend towards polyphagy in non-feeding sphingids. A preliminary comparison between tongue length and larval hostplants reveals a much more complex pattern. Species with very long tongues are often distributed at low latitudes and are monophagous (e.g., *Cocytius, Xanthopan*), but there are also species which have long tongues that have a cosmopolitan distribution (e.g., *Agrius cingulata*). There are also many non-feeding, monophagous smerinthines that are found in tropical habitats (e.g., *Callambulyx tatarinovii*, *Cypa decolor*; Table 10). The question of local versus global scale must be addressed as well, as there may be localized exceptions to the overall pattern.

Furthermore, one would expect nectar-feeding sphingids to have larger range sizes if they are searching for nectar and larval hostplants. Although range size is not a direct measure of how much a moth can fly during its lifespan, a recent study reveals that all three sphingid subfamilies all have similar range sizes (Beck *et al.*, 2006b), and that adult feeding has little or no significant influence on range size (Beck and Kitching, 2007). Beck (2007) determined that polyphagy has the strongest influence on range size for sphingids, and their findings contradict the FS-hypothesis.

2.5. Conclusion

The present study was a preliminary step towards understanding proboscis length evolution in Sphingidae. Correlation of tongue length to phylogeny suggests that the ancestral condition of the sphingid tongue was short, and that nectar-feeding evolved independently at least three times within the family. Multiple independent losses from nectar-feeding to non-nectar feeding were also revealed in different subfamilies. Nectar feeding cannot be directly inferred from tongue length, nor can a value such as 10 mm be assigned to distinguish tongue functionality, as there is an overlap in length between nectar and non-nectar feeding tongues below 10 mm.

It is critical that future studies on sphingid phylogeny increase ingroup and outgroup sampling, as it is possible that "hidden signals" of life-history traits are present within particular clades for taxa not sampled. Future studies on hawkmoth tongue length evolution should also examine the amount of variation according to sex, geographic distribution, and seasonality. These additions, supplemented with further analysis of hostplant growth and secondary chemistry, may answer the question of whether sphingids developed long tongues to search for inconspicuous, non-persistent larval hostplants, and whether particular lineages subsequently gained very long proboscises due to tight ecological associations with particular flowers.

Table 1. Number of genes and sphingid taxa in Regier *et al.* (2001), Mignault (2003), and the current study. Classification is based on Kitching and Cadiou (2000). “Nuclear + COI” refers to the 6-gene simultaneous analysis in which available genbank COI sequences were combined with the five nuclear gene dataset generated from the current study (see supplementary materials for details on the 6-gene analysis).

	Regier *et al.* (2001)	Mignault (2003)	Kawahara (current study)	
			Nuclear Genes	Nuclear + COI
Genes	2	2	5	6
Sequence size (bp)	1949	2647	6793	7452
Taxa				
Subfamilies	3	3	3	3
Tribes	6	8	8	8
Subtribes	4	4	4	4
Genera	14	45	106	108
Species	14	64	131	163

Table 2. Sphingid species and outgroups included in this study. In total, 131 ingroup and 11 outgroup species were included. Taxa are listed alphabetically by subfamily. Numbers indicate the sequenced fragment of the gene. A solid line "—" indicates that the sequence could not be obtained for that particular gene, "Locality" refers to the locality of the specimen from where it was collected, "Voucher #" refers to the University of Maryland Molecular Collection specimen voucher number. Genbank numbers are listed in square brackets where available. *PER = period*, *WG = wingless*. Refer to Table S1 for a list of species with COI data.

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Macroglossinae								
Dilophonotini: Choerocampina								
Basiothia medea (Fabricius, 1781)	Mauritius: Mare de Vacoas	IJK-02-0005	2922	1282	1228	936	--	6368
Cechenena helops (Walker, 1856)	Malaysia: Pahang, Genting Highlands	AYK-04-0168	2929	1282	1228	951	403	6793
Cechenena subangustata Rothchild, 1920	Taiwan: Taiitung, Liyuan	AYK-04-0214	--	709	1228	--	403	2340
Chaerocina dohertyi Rothchild & Jordan, 1903	Kenya: Kakamega Nature Reserve	IJK-03-3166	2929	1282	1228	951	403	6793
Deilephila elpenor (Linnaeus, 1758)	England: Oxfordshire, Didcot	IJK-02-5866	2194	1282	1228	879	403	5986
Euchloron megaera (Linnaeus, 1758)	Kenya: Kakamega Nature Reserve	IJK-03-3155	1327	1282	1228	--	403	4240
Hippotion celerio (Linnaeus, 1758)	Czech Republic	IJK-02-5932	2929	1282	1228	951	--	6390
Hyles hippophaes (Esper, 1789)	France: Hautes Alpes, San Crépin	IJK-02-5817	--	1282	1228	--	403	2913
Hyles lineata (Fabricius, 1775)	USA: Arkansas, Scott Co, USA: Colorado: Arapahoe Co.	DCR-02-1881 RSP-96-0929	2929	1282	1228	951	403	6793
Pergesa acteus (Cramer, 1779)	Malaysia: Cameron Highlands Taiwan, Hualien, Chihkuhshan	AYK-04-0117 AYK-04-0231	2929	1282	1228	951	403	6793
Rhagastis mongoliana (Butler, [1876])	Japan: Shizuoka, Fujinomiya	AYK-04-2556	2929	940	1228	951	403	6451
Rhodafra marshalli Rothschild & Jordan, 1903	Tanzania: Kifulo Plateau	MF-05-0009	1591	544	1093	--	403	3631
Theretra alecto (Linnaeus, 1758)	Cyprus	IJK-02-5880	2929	1282	1228	951	403	6793
Theretra capensis (Linnaeus, 1764)	Tanzania: Rufiji River, Pwani	IJK-03-3184	2929	1282	1228	951	403	6793
Xylophanes chiron (Drury, 1773)	Costa Rica	IJK-02-5904	2929	1282	1228	951	--	6390
Xylophanes falco (Walker, 1856)	USA: Cochise Co., Copper Canyon	JPT-xx-0838	--	709	1228	--	--	1937

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Xylophanes porcus (Hübner, [1823])	Costa Rica: Guanacaste	DHJ-02-2369	2929	1282	1228	951	403	6793
Xylophanes tersa (Linnaeus, 1771)	USA: Arkansas, Scott Co, Waldron	DCR-02-1879	2929	1282	1228	951	403	6793
Dilophonotina								
Aellopos ceculus (Cramer, 1777)	Costa Rica: Guanacaste	DHJ-02-2399	2929	1282	1228	951	403	6793
Aellopos tantalus (Linnaeus, 1758)	USA: Florida: N. Key Largo	RSP-95-1070	--	708	1228	--	--	1936
Aleuron choloroptera (Perty, [1833])	Belize: Cayo District, Las Cuevas Research Station	AYK-04-0506	2929	1282	1228	951	403	6793
Callionima falcifera (Fabricius, 1775)	British Virgin Islands: Guana Island	RFD-96-0966	2929	1282	1228	951	403	6793
Cautethia spuria (Boisduval, [1875])	Mexico: San Luis Potosi, El Salto Falls	JKA-02-1668	2929	1282	1228	951	403	6793
Enyo ocypete (Linnaeus, 1758)	Kenya: Kakamega Nature Reserve	DHJ-02-2390	2929	1282	1228	951	403	6793
Erinnyis ello (Linnaeus, 1758)	USA: Arizona, Santa Cruz County	JPT-02-1542	2929	1282	1228	951	403	6793
Eupyrrhoglossum sagra (Poey, 1832)	Costa Rica: Guanacaste	DHJ-04-46898	2929	1282	1093	951	403	6658
Hemeroplanes ornatus Rothschild, 1894	Costa Rica: Heredia, La Selva	AYK-04-0003	2929	1282	1228	951	403	6793
Isognathus rimosa (Grote, 1865)	Mexico: El lobo, Queretaro Dominican Republic: Pedernales	JKA-02-1646 AYK-04-0348	2929	963	1228	951	403	6474
Kloneus babayaga Skinner, 1923	Costa Rica: Guanacaste	DHJ-04-2375	1591	--	1093	--	403	3087
Madoryx plutonius (Hübner, [1819])	Costa Rica: Heredia, La Selva	AYK-04-0029	2929	1282	1093	951	403	6658
Nyceryx magna (R. Felder, [1874])	Costa Rica: Guanacaste	DHJ-02-2378	2431	1282	1228	951	403	6793
Oryba kadeni (Schaufuss, 1870)	Costa Rica: Guanacaste	DHJ-04-55866	2194	1051	1093	951	403	5692
Pachygonidia subhamata (Walker, 1856)	Costa Rica: Guanacaste	DHJ-04-61221	2929	1282	1228	951	403	6793
Pachylia ficus (Linnaeus, 1758)	Mexico: El lobo, Queretaro	JKA-02-1644	2929	1282	1228	951	403	6793
Pachylioides resumens (Walker, 1856)	Costa Rica: Heredia, La Selva	AYK-04-0002	2929	1282	1228	951	403	6793
Perigonia ilus Boisduval, 1870	Ecuador: Manabi, SE El Anegado	WJK-03-2191	2929	1282	1228	951	403	6793
Pseudosphinx teretrio (Linnaeus, 1771)	Dominican Republic: Puerto Escondido	AYK-04-0334	2929	1282	1093	951	403	6658

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Unzela japix (Cramer, 1776)	Costa Rica: Guanacaste	DHJ-02-2296 DHJ-02-2376	1697	1282	1228	951	403	5561
Hemarina								
Cephonodes hylas (Linnaeus, 1771)	Costa Rica: Guanacaste	IJK-02-5931	2929	1282	1228	951	403	6793
Hemaris diffinis (Boisduval, 1836)	USA: Arkansas, Bred stock	DCR-02-1882	2929	1282	1228	951	403	6793
Hemaris diffinis (Boisduval, 1836)	USA: Maryland, Montgomery Co, Potomac	TPF-94-1450	2929	709	1228	--	403	5269
Macroglossini								
Acosmerycoides harterti (Rothschild, 1895)	Malaysia: Pahang, Genting Highlands	AYK-04-0200	2929	1282	1228	951	403	6793
Acosmeryx naga (Moore, [1858])	Japan: Yamanashi, Yamatomura	AYK-04-2570	2521	1051	499	951	403	5425
Ampeophaga dolichoides (R. Felder, [1874])	Malaysia: Pahang, Cameron Highlands	AYK-04-0121	2929	1051	1093	--	403	5476
Ampelophaga rubiginosa Bremer & Grey, 1853	Japan: Yamanashi, Yamatomura	AYK-04-2573	2929	940	1093	951	403	6316
Amphion floridensis Clark, 1920	Florida, Alachuca Co., Gainesville	JYM-05-0003	1591	544	1093	--	403	3631
Angonyx testacea (Walker, 1856)	Taiwan: Pingtung, Moutan	AYK-04-0243	2929	1025	1093	951	403	6401
Arctonotus lucidus (Boisduval, 1852)	USA: California, San Bernadino Co.	AYK-04-0065	2929	1282	1228	951	403	6793
Clarina kotschyi (Kollar, [1849])	Turkey: Alanya	IJK-04-0005	2200	1282	1228	899	403	6012
Daphnis nerii (Linnaeus, 1758)	France: bred stock	IJK-02-5810	2929	1051	1228	951	403	6562
Darapsa myron (Cramer, 1779)	USA, bred stock	IJK-02-5963	2885	940	1228	951	403	6407
Deidamia inscriptum (Harris, 1839)	Canada: Toronto	AYK-04-5756	2929	1051	1228	951	403	6562
Elibia dolichus (Westwood, 1847)	Malaysia: Pahang, Genting Highlands	AYK-04-0192	2194	1051	1228	951	403	5827
Enpinanga borneensis (Butler, 1879)	Malaysia: Kuala Lipis	AYK-04-0104	2194	1051	1228	951	403	5827
Eupanacra regularis (Butler, 1875)	Malaysia: Kuala Lipis	AYK-04-0103	850	1051	802	951	403	4057
Euproserpinus phaeton Grote & Robinson 1865	USA: California, San Diego Co., Ranchita	DR-05-9000	2929	1051	1093	951	403	6427
Gnathothlibus erotus (Cramer, 1777)	Malaysia: Pahang, Genting Highlands	AYK-04-0155	850	1051	1228	951	403	4483

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Macroglossum stellatarum (Linnaeus, 1758)	France: bred stock	IJK-02-5806	2929	1259	1228	951	403	6770
Neogurelca himachala (Butler, [1876])	China: Zhejiang	IJK-04-0004	2833	--	1228	951	403	5415
Nephele accentrifera (Palisot de Beauvois, [1821])	Kenya: Kakamega Nature Reserve	IJK-03-3161	2929	--	1228	951	403	5511
Proserpinus clarkiae (Boisduval, 1852)	Unknown	[AF170855]	--	--	995	--	--	995
Proserpinus terlooii Edwards 1875	USA: Arizona, Pima Co.	JBW-02-1513	2194	1038	1228	951	403	5814
Sphecodina abbottii (Swainson, 1821)	USA: Maryland, Cumberland Co., Lavale	AYK-04-0366	2929	1282	1228	951	403	6793
Sphingonaepiopsis gorgoniades (Hübner, [1819])	Ukrane	IJK-05-0003	1591	1051	1093	951	403	5089
Temnora eranga (Holland, 1889)	Kenya: Kakamega Nature Reserve	IJK-03-3157	1327	--	1228	951	403	3909
Philampelini								
Eumorpha achemon (Drury, 1773)	USA: Arizona, Santa Cruz Co.	JPT-02-1533	2929	1282	1228	--	403	5842
Eumorpha pandorus (Hübner, [1821])	USA: Indiana, Spring Mill State Park	CWM-95-0830	--	709	1228	--	--	1937
Eumorpha typhon (Klug, 1836)	USA: Arizona, Santa Cruz Co.	JBW-02-1504	--	1282	1228	--	--	2510
Smerinthinae								
Ambulicini								
Adhemarius daphne (Boisduval, 1875)	Mexico: San Luis Potosi, Tamazunchale	JKA-02-1642	2929	1282	1228	951	403	6793
Ambulyx schauffelbergeri Bremer & Grey, 1853	Japan: Yamanashi, Tosuka	AYK-04-2568	2929	1282	1228	--	403	5842
Amplypterus mansoni (Clark, 1924)	Taiwan: Nantou, Wujieh	AYK-04-0174	--	1051	1228	--	403	2682
Amplypterus panopus (Cramer, 1779)	Malaysia: Pahang, Cameron Highlands	AYK-04-0276	2929	1282	1093	951	403	6658
Protambulyx euryalus Rothschild & Jordan, 1903	Ecuador: Morona-Santiago Prov.	WJK-03-1945	2929	1282	1228	951	403	6793
Smerinthini								
Afroclanis calcareus (Rothschild & Jordan, 1907)	Tanzania: Udzunqua	MF-05-0005	2929	1051	1093	951	403	6427
Amorpha juglandis (J. E. Smith, 1797)	USA: Texas, Uvalde Co., Concan	CWB-02-1595	2929	1282	1228	951	403	6793

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Andriasa contraria Walker, 1856	Kenya: Kakamega Nature Reserve	IJK-03-3153	2929	--	--	951	403	4283
Callambulyx tatarinovii (Bremer & Grey, 1853)	Japan: Shizuoka, Fujinomiya, Kitayama	AYK-04-2581	2929	940	1228	951	403	6451
Chloroclanis virescens (Butler, 1882)	Kenya: Kakamega Nature Reserve	IJK-03-3160	2929	1282	1228	951	403	6793
Clanis bilineata (Walker, 1866)	Taiwan: Nantou Pref., Puli	AYK-04-0184	2929	1282	1093	951	403	6658
Cypa decolor (Walker, 1856)	Malaysia: Cameron Highlands	AYK-04-0110	2929	1282	1228	951	403	6793
Daphnusa ocellaris Walker, 1856	Malaysia: Pahang, Fraser's Hill	AYK-04-0240	2929	--	1228	--	403	4560
Langia zenzeroides Moore, 1872	Japan: Nagano, Iida, Kamikawaji	AYK-04-2580	2929	1282	1093	951	403	6658
Laothoe populi (Linnaeus, 1758)	England: London	IJK-02-0012	2929	1282	1228	951	403	6793
Likoma apicalis Rothschild & Jordan, 1903	Tanzania: Mbega	MF-05-0008	2929	1051	1093	951	403	6427
Marumba quercus ([Denis & Schiffermuller], 1775)	France: nr. Toulon	IJK-02-0118	2929	719	1228	951	403	6230
Mimas tiliae (Linnaeus, 1758)	England: London, Raynes Park	IJK-02-5836	2929	1038	1228	951	403	6549
Neoclanis basalis (Walker, 1866)	Tanzania: Morogoro, 1 km E. Mikumi	IJK-03-3225	1314	709	802	951	403	4179
Neopolyptychus compar (Rothschild & Jordan, 1903)	Tanzania: Usumbara Mts.	IJK-03-3207	2929	1282	1228	951	403	6793
Pachysphinx modesta (Harris, 1839)	USA: Colorado, Denver	[AF234573]	--	--	1228	--	--	1228
Pachysphinx occidentalis (Edwards, 1875)	USA: Arizona, Santa Cruz Co.	JPT-02-1528	2929	1282	1228	951	403	6793
Paonias excaecata (J. E. Smith, 1797)	USA: West Virginia, Mathias	[AF234572]	--	--	1228	--	--	1228
Paonias myops (J. E. Smith, 1797)	USA: Arizona, Santa Cruz Co. USA: Michigan, Barry Co.	JPT-02-1540 MCN-03-1796	2194	1282	1228	951	403	6793
Parum colligata (Walker, 1856)	Taiwan: Nantou, Wujieh	AYK-04-0182	2194	1051	802	951	403	5401
Phyllosphingia dissimilis (Bremer, 1861)	Taiwan: Wujieh	AYK-04-0176	2929	1051	1228	951	403	6562
Polyptychoides digitatus (Karsch, 1891)	Kenya: Kakamega Nature Reserve	IJK-03-3159	2929	1282	1228	951	403	6793
Polyptychus andosa (Walker, 1856)	Tanzania: Usumbara Mts.	IJK-03-3199	2194	1282	1228	951	403	6058
Pseudoclanis postica (Walker, 1956)	South Africa	IJK-02-5839	2929	1282	1228	951	403	6793

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Smerinthus cerisyi Kirby, 1837	USA: Wisconsin, Portage Co,	[AF234576] [AF234595]	--	1282	1228	--	--	2510
Smerinthus saliceti (Boisduval, [1875]	USA: Arizona, Pima Co.	JBW-02-1511	2929	1282	1228	951	403	6793
Viriclanis kingstoni Aarvik, 1999	Tanzania: Uzungura	MF-05-0010	2929	1051	1093	--	403	5476
Sphingulini								
Dolbina tancrei Staudinger, 1887	Japan: Yamanashi, Motosu-Shitsugen	AYK-04-2557	2929	1282	1228	951	403	6793
Hopliocnema brachycera (Lower, 1897)	Australia: Northern Territory	MJM-96-0232	2929	1282	1228	891	403	6733
Kentrochrysalis consimilis Rothschild & Jordan, 1903	Japan: Yamanashi, Yamatomura	AYK-04-2571	2929	1282	1228	951	403	6793
Sphinginae								
Acherontiini								
Acherontia styx Westwood, 1847	Philippines: Palawan, Bataraza	IJK-02-5989	2929	1282	1228	951	403	6793
Agrius cingulata (Fabricius, 1775)	USA: Georgia, Gilmer Co.	WJK-02-1941	2502	1282	1228	951	403	6366
Coelonia fulvinotata (Butler, 1875)	Zimbabwe bred stock	IJK-02-5816	2194	544	1228	--	403	4369
Megacorma obliqua (Walker, 1856)	Malaysia: Rte. 4 btwn Gerik and Jeli	AYK-04-0154	2521	1051	1093	951	403	6019
Sphingini								
Ceratomia amyntor (Geyer, [1835])	USA: Colorado, Adams Co., Barr Lake	RSP-96-0932	--	1051	1228	--	--	2279
Ceratomia catalpae (Boisduval, [1875])	USA: Arkansas, Scott Co., Waldron	DCR-02-1870	2929	1282	1228	951	403	6793
Cocytius duponchel (Poey, 1832)	Costa Rica: Guanacaste	DHJ-02-2359	2929	1282	1228	951	403	6793
Dolba hyloeus (Drury, 1773)	USA: Texas, San Antonio	RSP-02-1575	2929	1282	1228	951	403	6793
Dovania poecila Rothschild & Jordan, 1903	Kenya: Kakamega Nature Reserve	IJK-03-3169	2929	--	--	951	403	4283
Euryglottis dognini Rothschild, 1896	Ecuador: Morona-Santiago Province	WJK-03-2891	2909	1282	1228	951	403	6773
Isoparce cupressi (Boisduval, [1875])	USA: North Carolina, Craven Co.	JBS-05-0001	2194	1051	1093	951	403	5692
Lapara coniferarum (J. E. Smith, 1797)	USA: Georgia Gordon Co., Calhoun	JKA-02-1670	2929	1282	1228	951	403	6793

Ingroup Taxa	Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Macropoliana natalensis (Butler, 1875)	USA: Arizona, Santa Cruz Co.	IJK-03-3211	2929	1282	--	951	403	5565
Manduca florestan (Stoll, 1782)	Tanzania: Usumbara Mts, Amani	JPT-02-1529	--	1282	1093	--	403	2778
Manduca muscosa (Rothschild & Jordan, 1903)	USA: Pima Co., Santa Rita Mts.	JBW-02-1508	--	1282	1228	--	403	2913
Manduca quinquemaculatus (Haworth, 1803)	USA: Arkansas, Scott Co., Waldron	DCR-02-1876	2929	1113	1228	951	403	6624
Manduca sexta (Linnaeus, 1763)	Argentina: Cordoba Prov., Cuesta Blanca	"Mqui"	2929	1282	1228	951	403	6793
Meganoton analis (R. Felder, [1874])	Japan: Shizuoka, Fujinomiya	AYK-04-2579	2929	1282	1093	951	403	6658
Neococytius cluentius (Cramer, 1775)	Ecuador: Pichincha Prov., Tinalandia	WJK-03-1949	2929	1282	1228	951	403	6793
Paratrea plebja (Fabricius, 1777)	USA: Georgia, Gilmer Co., Ellijay	WJK-02-1939	2929	1282	1228	951	403	6793
Psilogramma increta (Walker, [1865])	Philippines: Palawan, Bataraza	IJK-02-5988	2929	1282	1228	951	403	6793
Sphinx caligineus (Butler, 1877)	China: Beijing	IJK-04-0012	2929	1282	1228	951	403	6793
Sphinx chersis (Hübner, [1823])	USA: Arizona, Santa Cruz Co., Pena Blanca Lake	JPT-xx-0839	--	709	1228	--	--	1937
Sphinx dollii Neumoegen, 1881	USA: Santa Cruz Co., Patagonia Mts.	JPT-02-1532	2929	1282	1228	951	403	6793
Sphinx istar (Rothschild & Jordan, 1903)	USA: Texas, Uvalde Co., Concan	CWB-02-1591	2929	1282	1228	951	403	6793
Sphinx kalmiae J. E. Smith, 1797	USA: Georgia, Gilmer Co., Ellijay	WJK-02-1938	2929	1282	1228	951	403	6793
Sphinx merops Boisduval, 1870	Ecuador: Manabi Prov., El Anegado	WJK-03-2198	2929	1282	802	951	403	6367
Xanthopan morganii (Walker, 1856)	Tanzania: Usumbara Mts, Amani	IJK-03-3209	2929	--	--	951	403	4283

Outgroup Taxa			Locality	Voucher #	*CAD* 2929bp	*DDC* 1282bp	*EF-1α* 1228bp	*PER* 951bp	*WG* 403bp	Total 6793bp
Bombycidae	Bombycinae	*Bombyx mori* (Linnaeus, 1758)	Unknown	UNK-90-0062/ UNK-90-0063	2929	1282	1228	891	403	6895
Bombycidae	Phiditiinae	*Phiditia sp.*	Brazil: Amazonas, Manaus	RWH-96-0916	2929	1282	1228	--	403	5842
Bombycidae	Primostictinae	*Oberthueria formosibia* (Matsumura, 1927)	Taiwan, Taitung, Lijia	AYK-04-0824	2909	--	--	--	403	3312
Brahmeidae		*Acanthobrahmaea europaea* (Hartig, 1963)	Italy	RSP-95-0990	2929	1051	1228	891	403	6502
Carthaeidae		*Carthaea saturnioides* Walker, 1858	Australia: Western Australia	TB-03-2177	2929	--	--	--	403	3332
Endromidae		*Endromis versicolora* (Linnaeus, 1758)	Germany, Roding	"Eversicol"	2194	1282	1228	891	403	5998
Eupterotidae		*Apha aequalis* (Felder, 1874)	Japan: Yamanashi, Motosu-Shitsugen	AYK-04-2506	2909	--	--	--	403	3312
Lasiocampidae		*Macrothylacia rubi* (Linnaeus, 1758)	Spain, Barcelona Prov., Gurb	"Mrubi"	2929	1282	1228	891	403	6733
Lemoniidae		*Lemonia dumi* (Linnaeus, 1761)	Sweden: Svartbacken, Handen, Stockholm	IJK-94-0551	2929	1282	1228	874	403	6716
Mirinidae		*Mirina christophi* (Staudinger, 1887)	South Korea: Taeweon SA, Jirisan	RXR-02-0511	2929	--	--	--	403	3332
Saturniidae	Cercophaninae	*Janiodes sp.*	Ecuador: Morona Santiago, 48km W Gualaquiza	"Jcer"	2929	1282	1228	951	403	6793

Table 3. List of genes and primers used in this study. “F” and “R” at the end of primer names refer to the forward and reverse primer. Primer names follow Regier (2006).

Gene	Primer name	Sequence (5’-3’)
CAD (2929 bp)	46F	GTNGTNTTYCARACNGGNATGGT
	295nF	TAYGGYAAYMGNGGNCAYAA
	309R	TCNACNGCRAANCCRTGRTTYTG
	350R	RTGYTCNGGRTGRAAYTG
	496F	CARACNGCNYTNAAYTGYGG
	576R	TCNTCYTCRTTRTTNGCRAA
	582F	TTYGCNAAYAAYGARGANGA
	606nR	ACNACYTCRTAYTCNACYTCYTTCCA
	267fin3R	TTYTCCATRTTRCANAC
	743nF	GGNGTNACNACNGCNTGYTTYGARCC
	782R	GCYTTYTGRAANGCYTCYTCRAA
	1028R	TTRTTNGGNARYTGNCCNCCCAT
DDC (1282 bp)	1.2F	GARAAYATYAGAGAYAGRCARGT
	1.7sF	GCHTGYATYGGITTYWCNTGGAT
	1.8R	CATNACNACYTCIARYTCIGTRCA
	1.9sR	CATYTGRCCBARCCARTCIADCAT
	3.2sF	TGGYTICAYGTIGAYGCNGCNTAYGC
	3.3sF	TTYAAYTTYAAYCCNCAYAARTGG
	3.3sR	CCAYTTRTGNGGRTTRAARTTRAA
	4sR	GGDATYTGCCARTGHCKRTARTC
	7.5sR	TCCCANGANACRTGVATRTC
EF-1α (1228 bp)	30F	CAYATYAAYATHGTSGTIATHGG
	45.71F	GTNGSNGTIAAYAARATGGA
	52F	CARGAYGTNTAYAARATHGG
	52R	CCDATYTTRTANACRTCYTG
	53.5R	ATRTGVGMIGTRTGRCARTC
	41.21R	TGYCTCATRTCDCGVACRGCRAA
period (968 bp)	197sF	GGNTTYCCNAARGAYATGTGG
	341sF	TAYYTNGGNTAYYTNCCNCARGA
	397nR	GACCANGGRTTDAYRAA
	532sR	TCRTTRTARTTNARYTGRTTRTA
wingless (403 bp)	Wg1aF	GARTGYAARTGYCAYGGYATGTCTGG
	Wg2aR	ACTICGCARCACCARTGGAATGTRCA

Table 4. Empirical base frequencies and GTR+*Γ*+*I* model parameters of the five genes in the ML analyses. Base frequencies were fairly equal except for *CAD* and *wingless*. *Γ* = α estimated shape parameter, *I* = proportion of invariant sites.

Gene Partition	Empirical Base Frequencies (%)				Substitution Rates						*Γ*	*I*
	A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T		
CAD	33.2	17.5	21.3	28.0	1.352	6.628	1.014	1.600	7.765	1.000	0.927	0.470
DDC	25.2	22.4	25.4	27.0	1.467	6.178	1.396	1.161	6.251	1.000	1.046	0.492
EF-1α	24.8	29.3	25.3	20.5	1.685	10.241	3.369	1.872	17.764	1.000	0.791	0.616
period	27.4	24.8	25.4	22.4	1.782	5.596	1.859	2.274	8.844	1.000	0.765	0.227
wingless	20.3	31.6	32.0	16.1	1.553	5.846	2.081	0.550	5.581	1.000	0.928	0.490

Table 5. Summary of characters by gene and codon position. Outgroups were excluded for these calculations.

Gene Partition:	*CAD*				*DDC*				*EF-1α*			
Codon Position	All	nt1	nt2	nt3	All	nt1	nt2	nt3	All	nt1	nt2	nt3
Total Characters	2929	976	976	977	1282	427	427	428	1228	427	427	428
Constant	1511	673	812	26	680	296	366	18	838	374	390	74
Uninformative	132	71	52	9	53	32	18	3	78	16	10	52
Informative	1286	232	112	942	549	99	43	407	312	19	9	284
% Informative	43.9%	23.8%	11.5%	96.4%	42.8%	23.2%	10.1%	95.1%	25.4%	4.6%	2.2%	69.3%
Gene Partition:	*period*				*wingless*				*5 Genes*			
Codon Position	All	nt1	nt2	nt3	All	nt1	nt2	nt3	All	nt1	nt2	nt3
Total Characters	951	317	317	317	403	134	134	135	6793	2268	2268	2268
Constant	288	12	162	5	221	99	118	4	3538	1568	1847	128
Uninformative	131	62	65	4	20	8	9	3	414	189	157	71
Informative	532	13	90	308	162	27	7	128	2841	511	264	2069
% Informative	55.9%	42.3%	28.4%	97.2%	40.2%	20.1%	5.2%	94.8%	41.8%	22.5%	11.6%	91.2%

Table 6. Bootstrap, Bremer support (BS), and Partitioned Bremer Support (PBS) values for the MP five gene simultaneous analysis with 142 taxa. "nt-12" refers to an analysis which only included first and second codon positions. *, bootstrap values < 50%.

		Bootstrap		BS	Partitioned Bremer Support (PBS)				
Clade	**Node**	**All-nt**	**nt-12**	**Total**	***CAD***	***DDC***	***EF-1α***	***Per***	***Wg***
Sphingidae	**1**	**100**	**88**	**41**	**1.36**	**-3.90**	**40.99**	**-1.47**	**4.03**
Smerinthinae + Sphinginae	**2**	**74**	*	**2**	**-24.64**	**-8.90**	**20.99**	**4.53**	**10.03**
Macroglossinae	**3**	*	*	**1**	**-26.52**	**33.42**	**0.37**	**-6.71**	**0.45**
	4	74	*	2	-24.64	-8.90	20.99	4.53	10.03
	5	*	*	1	-24.78	21.11	4.06	-1.44	2.06
	6	*	*	1	-27.14	36.60	-0.51	-7.97	0.03
Sphingulini + Sphinginae	**7**	**98**	**52**	**17**	**-6.64**	**-1.90**	**15.99**	**-5.47**	**15.03**
	8	*	*	1	-26.75	33.72	0.40	-6.72	0.35
	9	100	54	29	-9.64	4.10	21.99	2.53	10.03
	10	79	*	14	-49.14	48.10	22.99	-7.97	0.03
Sphinginae	**11**	**99**	*	**22**	**-11.64**	**11.10**	**5.99**	**4.03**	**12.53**
	12	*	*	5	5.96	-1.70	1.39	10.53	11.17
Smerinthini + Ambulycini (excl. *Langia* and *Cypa*)	**13**	**69**	*	**3**	**-35.64**	**45.10**	**-1.01**	**-5.47**	**0.03**
	14	*	*	6	-34.14	37.60	3.49	-3.47	2.52
	15	96	*	13	1.82	3.11	-0.04	5.07	3.04
	16	81	*	7	2.36	2.60	0.49	1.53	0.03
	17	*	*	4	-34.64	38.10	-8.01	5.53	3.03
	18	100	*	19	-14.14	36.60	-0.51	-7.97	5.03
	19	*	*	2	-24.97	16.76	9.32	-1.14	2.03
	20	100	77	42	-1.64	15.10	13.99	0.53	14.03
	21	77	*	11	9.22	-8.78	2.94	3.56	4.06
	22	*	*	1	-27.14	36.60	-0.51	-7.97	0.03
	23	*	*	4	-34.64	38.10	-8.01	5.53	3.03
	24	94	*	29	-15.10	15.45	5.93	10.49	12.23

	25	100	*	24	-20.99	22.62	17.81	1.88	2.68
	26	84	*	7	-25.64	7.10	12.99	3.53	9.03
	27	54	*	2	-25.64	5.60	14.99	1.53	5.53
	28	100	*	44	-10.97	33.76	10.32	3.86	7.03
	29	*	*	1	-27.14	36.60	-0.51	-7.97	0.03
	30	100	96	40	-25.64	7.10	44.99	6.03	7.53
	31	100	98	44	-10.64	29.10	5.99	11.53	8.03
	32	92	*	13	-21.97	23.76	8.32	0.86	2.03
	33	*	*	1	-18.64	4.60	18.99	-4.47	0.53
	34	96	*	18	3.36	-12.90	8.99	7.53	11.03
	35	99	61	21	6.36	-0.90	-2.01	11.53	6.03
	36	100	76	30	-5.64	-5.90	16.99	13.53	11.03
Ambulycini	**37**	**100**	**97**	**67**	**0.36**	**42.10**	**8.99**	**6.53**	**9.03**
	38	100	*	26	-27.64	21.10	29.99	-0.47	3.03
	39	100	95	47	30.10	5.93	-0.04	11.01	-0.02
	40	89	*	8	4.86	-0.90	0.99	4.03	-0.97
	41	100	79	52	6.11	30.22	4.61	5.78	5.28
	42	*	*	4	-31.64	1.10	19.99	6.53	8.03
	43	*	*	3	-27.64	0.10	12.99	8.53	9.03
	44	69	*	6	3.41	-12.07	3.44	7.07	4.16
	45	97	*	18	-26.31	5.10	11.65	15.53	12.03
Choerocampina	**46**	**94**	*****	**12**	**0.60**	**-17.17**	**7.78**	**11.75**	**9.03**
	47	99	65	15	-14.64	16.60	1.49	9.03	2.53
	48	*	82	3	-26.21	18.24	3.13	1.10	6.74
	49	100	98	73	14.78	27.89	4.94	18.44	6.94
	50	88	*	8	-19.59	24.40	0.59	0.58	2.02
	51	*	*	4	-34.64	38.10	-8.01	5.53	3.03
	52	100	98	48	18.02	15.76	0.99	10.86	2.36
	53	100	77	57	1.61	41.97	4.86	4.28	4.28
	54	100	*	12	-24.95	20.26	14.32	-1.16	3.52
Acherontiini	**55**	**100**	**82**	**47**	**16.78**	**7.78**	**21.06**	**-1.56**	**2.94**

	56	100	98	46	-35.97	41.10	31.99	2.19	6.69
	57	100	76	64	5.36	24.10	11.99	16.53	6.03
	58	67	*	6	1.36	-7.90	3.99	6.53	2.03
	59	100	*	15	-22.64	5.10	32.99	-3.47	3.03
Hemarina	**60**	**100**	**99**	**43**	**-24.73**	**18.06**	**35.98**	**4.09**	**9.59**
	61	63	*	2	4.36	-3.40	-1.01	2.03	0.03
	62	100	*	14	-2.29	5.90	10.49	-5.22	5.12
	63	100	*	22	-21.81	4.14	25.09	4.54	10.04
	64	*	53	1	-27.64	37.10	0.99	-7.97	-1.47
	65	93	61	24	-17.28	31.87	4.47	-3.16	8.10
	66	100	*	37	-28.85	35.79	11.94	3.84	14.28
	67	100	100	70	23.86	-5.40	15.99	32.53	3.03
	68	100	*	19	-0.04	0.04	19.00	-0.01	0.00
	69	97	*	5	-17.79	23.99	4.00	-5.23	0.03
	70	100	*	17	-0.14	5.60	11.50	0.03	0.03
	71	100	95	41	-5.64	25.60	11.49	0.03	9.53
	72	100	85	27	28.82	0.11	-3.04	0.07	1.04
	73	82	*	12	12.72	4.17	-2.00	0.06	-2.94
	74	83	*	12	-36.64	48.10	-2.01	4.53	-1.97
	75	100	100	99	46.86	29.10	8.99	11.03	3.03
	76	100	79	33	11.36	13.10	4.99	2.53	1.03
	77	100	99	73	31.11	14.89	14.61	8.11	4.28
	78	100	73	7	-28.39	42.72	0.11	-7.72	0.28
	79	*	*	0	-1.25	0.63	0.13	0.25	0.25
	80	100	67	12	-18.89	5.22	27.61	-4.22	2.28
	81	98	93	7	-9.64	3.43	3.99	4.53	4.69
	82	100	*	41	23.76	9.22	2.96	1.01	4.04
	83	89	57	15	-13.64	11.10	16.99	-3.47	4.03
	84	100	70	18	-0.14	10.10	7.99	0.03	0.03
	85	100	96	56	10.24	28.46	12.92	-0.43	4.82
Philampelini	**86**	**100**	**90**	**31**	**-11.14**	**25.60**	**16.49**	**-0.97**	**1.03**

87	99	*	8	-0.14	0.10	7.99	0.03	0.03
88	*	*	9	-24.14	17.35	10.99	7.03	-2.22
89	99	59	27	13.22	11.61	0.00	1.11	1.06
90	80	*	4	-25.64	21.10	8.99	-2.47	2.03
91	86	*	7	-15.18	22.87	3.97	-4.94	0.29
92	97	60	6	-0.16	1.10	4.98	0.05	0.04
93	100	71	28	-19.66	33.80	11.45	-2.61	5.02
94	100	98	31	0.05	18.83	12.06	0.05	0.00
95	100	97	168	62.92	46.55	17.44	27.53	13.57
96	94	*	9	-12.09	22.24	-2.07	-3.02	3.94
97	100	77	24	-10.64	21.10	6.99	-1.47	8.03
98	96	86	10	5.82	-14.43	2.96	10.60	5.04
99	99	79	11	-24.64	-2.90	23.99	4.53	10.03
100	*	73	1	-2.14	-14.40	3.99	7.53	6.03
101	100	99	37	11.36	8.10	2.99	11.53	3.03
102	100	*	88	-19.12	24.16	79.49	1.95	1.51
103	100	*	15	0.36	5.10	8.99	0.53	0.03
104	100	*	15	-13.19	39.68	-1.57	-7.98	-1.93
105	98	*	7	-0.12	0.11	6.98	0.01	0.02
106	75	69	3	-14.64	13.10	9.99	-6.47	1.03
107	100	99	49	-16.64	-1.90	57.99	2.53	7.03
108	*	*	5	5.92	-1.64	1.33	10.49	-11.10
109	*	*	5	5.96	-1.70	1.39	10.53	-11.17
110	100	100	100	49.86	29.10	10.49	3.53	7.03
111	89	*	10	-4.17	0.78	4.01	4.66	4.72
112	100	*	21	17.86	5.10	-3.01	2.03	-0.97
113	99	*	5	0.10	3.49	0.97	0.00	0.44
114	77	*	6	-24.14	35.60	2.49	-7.98	0.03
115	*	*	2	-20.33	25.78	2.21	-6.12	0.45
116	100	*	16	-0.11	10.95	5.02	0.10	0.03
117	84	*	6	4.86	6.10	-0.01	-4.97	0.03

	118	100	81	38	3.36	7.76	9.99	11.86	5.03
	119	*	*	2	-24.78	22.22	3.94	-1.44	2.06
	120	100	75	14	-34.64	41.10	-2.01	5.53	4.03
	121	83	*	2	-22.64	7.10	17.99	-3.47	3.03
	122	100	99	54	11.61	25.35	9.99	3.03	4.03
	123	54	*	1	-0.14	-1.90	3.49	0.03	-0.47
	124	100	52	9	-7.64	2.77	13.99	-1.14	1.03
	125	96	*	6	-25.66	24.97	6.55	-1.91	2.05
TOTAL				**2760**	**-1046.55**	**1880.08**	**1184.36**	**300.81**	**464.26**

Table 7. Bootstrap, Bremer support (BS) and Partitioned Bremer Support (PBS) values for the MP 99-taxon analysis without missing data. “nt-12” refers to an analysis which only included first and second codon positions.

		Bootstrap		BS	PBS				
Clade	**Node**	**All-nt**	**nt-12**	**Total**	***CAD***	***DDC***	***EF-1α***	***Per***	***Wg***
Sphingidae	**1**	**100**	**100**	**142**	**54**	**44**	**14**	**21.5**	**8.5**
Smerinthinae + Sphinginae	**2**	**79**	**75**	**11**	**18**	**5**	**-7**	**-7**	**2**
Macroglossinae	**3**	**70**	**56**	**17**	**14**	**4**	**-7**	**5**	**1**
Smerinthinae + Sphinginae (- *Langia*)	**4**	**87**	**91**	**32**	**34**	**25**	**-2**	**-33**	**8**
Macroglossinae (-Hemarina)	**5**	**63**	*	**12**	**0**	**8**	**2**	**3**	**-1**
Smerinthini + Ambulycini (- *Langia*)	**6**	**89**	**68**	**29**	**17.7**	**-4.7**	**-2.3**	**12**	**6.3**
Sphingulini + Sphinginae	**7**	**98**	**98**	**42**	**23**	**27**	**-1**	**-18**	**11**
	8	79	*	16	9	-1	3	2	3
	9	77	67	20	20	14	-5	-8	-1
	10	53	*	7	10	-5	-5	8	-1
	11	99	96	22	18	6	1	-4	1
	12	66	*	4	3	4	1	-3	-1
	13	*	*	5	4	-10	-3	13	1
Sphinginae	**14**	**100**	**85**	**37**	**21.5**	**5**	**-1**	**6**	**5.5**
	15	*	*	9	-2	-11	3	15	4
	16	*	*	1	-1	4	0	-1	-1
	17	75	*	5	2	1	0	2	0
	18	100	74	46	21	8.7	1.7	16.2	-1.5
	19	100	74	41	14	16	1	7	3
	20	91	61	27	8	11.5	6	-1.5	3
	21	100	87	64	34.4	11.4	2.2	12.2	3.8
	22	80	*	12	7	-4.5	2	3.5	4

	23	62	*	8	-5	9	6	-4	2
	24	*	*	4	2	-5.5	0	5	2.5
	25	98	58	14	2	0	-3	10	5
	26	99	97	47	14	8	4	13	8
	27	94	*	11	7	6	-2	0	0
	28	100	100	72	27	19	11	9	6
	29	*	*	3	4	9	-2	-3	-5
	30	66	*	2	0	9	-2	-2	-3
	31	78	*	8	-2	6	-4	6	2
Choerocampina	**32**	**91**	**65**	**10**	**0**	**-2**	**3**	**6**	**3**
	33	100	95	39	25	9	4	3	-2
	34	100	100	154	46	6	18	67	17
	35	80	*	4	4	-1	1	1	-1
	36	100	99	40	20	5	-2	16	1
	37	100	98	43	25	9	0	6	3
	38	*	*	2	-1	-13	3	7	6
	39	*	*	2	-1	-13	3	7	6
	40	99	59	28	8	-3	8	10	5
	41	67	*	4	1	-6	0	7	2
	42	93	*	12	4	4	3	-3	4
Ambulycini	**43**	**100**	**100**	**90**	**34**	**20**	**6**	**26**	**4**
	44	*	*	5	4	-10	-3	13	1
	45	100	100	72	23	5	11	22	11
	46	*	*	3	4	-2	3	-1	-1
	47	100	99	40	14	1	-2	24	3
	48	100	100	58	25	12	5	14	2
	49	*	*	1	5	-3	1	-1	-1
Acherontiini	**50**	**100**	**88**	**45**	**35**	**4**	**3**	**3**	**0**

	51	100	100	112	43	22	4	41	2
	52	100	73	64	40	11	3	10	0
	53	56	*	3	-6	3	4	0	2
	54	99	90	26	10	3.5	3.5	6.5	2.5
	55	*	*	1	1	0	0	0	0
	56	100	97	36	14	14	1	7	0
	57	94	58	27	15	11	3	1	-3
	58	*	*	5	4	-10	-3	13	1
	59	100	75	62	23	12	3	17	7
	60	100	99	68	25	9	14	23	-3
	61	99	100	46	29	15	-4	-6.5	12.5
	62	72	*	9	9.5	4	1	1	-6.5
	63	69	88	5	-1.5	1.5	-2	5.5	1.5
	64	100	100	101	52.5	30	9	5.5	4
	65	100	100	101	51	24	9	14	3
	66	100	82	40	25	5	5	2	3
	67	100	100	61	29	12	8	12	0
	68	100	100	67	35	12	9	8	3
	69	*	*	1	5	-3	1	-1	-1
	70	*	*	2	4.5	-2.5	0.3	0.8	-1
	71	100	54	44	30	10	2	0	2
	72	97	57	17	14	5	0	-3	1
Hemarina	**73**	**100**	**100**	**154**	**96**	**46**	**5**	**4**	**3**
	74	92	88	9	6	-13	2	10	4
	75	100	77	34	16.3	0.7	3.7	7.7	5.7
	76	*	73	2	1	-11	2.5	6	3.5
	77	100	99	55	33	11	2	5	4
	78	100	100	122	61	16	10	28	7

	79	98	*	14	14	4	-1	-1	-2
	80	72	*	4	0	0	5	-1	0
	81	*	*	2	2.5	-3.5	0	3	0
	82	100	99	37	12	7	3	12	3
	83	100	100	89	42.5	-1	13	22.5	12
	84	98	71	20	-9.8	16	2	10.3	1.5
	85	80	*	20	20	14	-5	-8	-1
	86	100	95	41	11	6	6	14	4
	87	100	88	40	15	3	8	13	1
	88	99	93	18	3	1	5	8	1
	89	66	*	4	10	0.7	-2	-3.3	-1.3
TOTAL				**2985**	**1439.1**	**526.3**	**206.6**	**598.9**	**214.5**

Table 8. Calculation of the relative contribution index (RCI) for the 99-taxon dataset.

Gene partition	Characters	Parsimony informative	PBS total per gene (from Table 7)	RCI
CAD	2929	1315 (45%)	1439.1	0.91
DDC	1282	575 (45%)	526.3	1.09
EF-1α	1228	337 (27%)	206.6	1.63
period	951	562 (59%)	598.9	0.94
wingless	403	172 (43%)	214.5	0.80

Table 9. Node recovery and bootstrap support values with 5-gene, all combinations of 4-gene sub datasets. ↓ = BP value at least 20% less than results from exclusion of any other single gene for this node; ↑ = BP value at least 20% greater than results from exclusion of any other single gene for this node. Clades of interest are in bold. * = bootstrap values < 50%, but in some cases bootstrap values < 50% are shown for comparison.

		5 Genes		4 Genes				
Clade	**Node**	**All-nt**	**nt-12**	***-CAD***	***-DDC***	***-EF1α*** [a]	***-per***	***-wg***
Sphingidae	**1**	**100**	**99**	**↓66**	**100**	**100**	**100**	**100**
Smerinthinae + Sphinginae	**2**	**88**	*	**64**	**72**	**85**	**89**	**90**
Macroglossinae	**3**	**96**	*	**↓59**	**94**	**98**	**96**	**96**
Smerinthinae + Sphinginae (-*Langia*)	**4**	**99**	**50**	**↓75**	**98**	**99**	**96**	**98**
Macroglossinae (-Hemarina)	**5**	*	*	*****	*	*	*	*
	6	99	59	↓37	98	99	96	98
Sphingulini + Sphinginae	**7**	**100**	**56**	**98**	**100**	**100**	**100**	**100**
	8	73	*	↓4	83	85	84	65
Sphinginae	**9**	**100**	**55**	**↓70**	**99**	**100**	**100**	**100**
	10	69	*	81	↓41	67	78	71
	11	100	*	99	100	100	100	100
	12	83	*	↓2	↑99	71	69	79
	13	*	*	*	*	*	*	*
	14	*	*	*	*	*	*	*
	15	88	*	97	51	92	60	86
	16	68	*	63	67	↓37	76	73
	17	*	*	*	*	*	*	*
	18	100	*	93	100	100	100	100
Hemarina	**19**	**100**	*****	**99**	**100**	**100**	**100**	**100**
	20	73	*	↓23	76	57	67	64
	21	100	*	99	100	100	100	100

	22	*	*	*	*	*	*	*
	23	*	*	*	*	*	*	*
	24	100	57	98	100	100	99	100
	25	100	81	100	100	100	100	100
	26	100	*	98	99	100	100	100
	27	*	*	*	57	*	*	*
	28	96	*	95	98	92	↓58	89
	29	100	91	98	100	100	100	100
	30	56	*	13	↑73	33	31	49
	31	100	96	100	100	100	100	100
	32	99	70	73	99	100	98	98
	33	100	77	100	100	100	100	100
	34	91	*	66	68	91	93	93
	35	85	*	64	56	86	90	83
	36	70	*	*	*	78	71	83
	37	*	*	49	↑70	31	25	26
Choerocampina	**38**	**88**	*****	**94**	**75**	**61**	**46**	**77**
Ambuylcini	**39**	**100**	**95**	**99**	**99**	**100**	**100**	**100**
	40	100	*	100	100	100	100	100
	41	100	100	96	100	100	100	100
	42	100	76	100	89	100	100	100
	43	81	*	↓44	77	72	78	84
	44	56	*	*	50	52	*	53
	45	56	*	54	*	57	*	*
	46	59	*	46	↓17	68	58	71
	47	100	*	97	100	100	100	100
	48	100	*	95	73	100	100	99
	49	100	94	98	100	100	100	100

	50	*	*	59	*	58	*	*
	51	100	58	99	97	99	100	100
Acherontiini	**52**	**100**	**86**	**98**	**100**	**100**	**100**	**100**
	53	100	*	100	100	100	100	100
	54	*	*	55	*	*	*	*
	55	53	*	65	28	16	59	55
	56	100	*	97	99	99	99	99
	57	86	*	↓18	95	94	68	90
	58	100	*	100	98	100	100	100
	59	69	*	65	↓7	86	47	68
	60	*	*	55	*	*	*	*
	61	100	*	98	100	100	100	100
	62	68	*	*	62	77	56	61
	63	100	*	100	100	100	100	100
	64	90	*	96	86	↓43	80	90
	65	87	*	73	94	75	81	89
	66	100	*	99	99	100	99	99
	67	66	*	*	*	64	57	61
	68	79	*	*	53	70	97	82
	69	100	78	97	100	100	100	100
	70	79	83	97	↓27	89	62	80
	71	100	99	97	100	100	100	100
	72	98	*	99	96	100	↓69	95
	73	100	97	98	100	100	100	100
	74	*	*	*	*	*	*	*
	75	*	*	*	*	*	*	*
	76	100	94	99	84	98	100	100
	77	99	74	75	95	100	99	98

	78	100	*	99	100	100	100	100
	79	100	*	99	100	100	100	100
Philampelini	**80**	**100**	*****	**100**	**99**	**100**	**100**	**100**
	81	99	*	99	83	99	78	97
	82	53	*	1	69	20	57	57
	83	98	*	↓43	94	100	100	99
	84	100	*	100	97	97	100	100
	85	100	*	100	96	100	100	100
	86	68	*	59	*	61	68	75
	87	98	65	96	62	98	98	100
	88	100	62	100	99	100	100	100
	89	100	100	99	100	100	100	100
	90	100	89	100	100	--	100	100
	91	99	*	99	99	--	97	98
	92	100	*	100	100	100	100	100
	93	100	95	↓42	100	100	100	100
	94	69	*	61	*	68	*	83
	95	56	*	56	*	53	*	78
	96	100	100	100	100	100	100	100
	97	100	99	100	100	100	100	100
	98	*	*	*	*	*	*	*
	99	100	63	99	100	98	100	100
	100	100	81	100	100	100	100	100
	101	100	97	100	100	100	100	100
	102	100	*	100	100	100	100	100
	103	93	*	60	93	89	91	87
	104	100	*	100	100	100	100	100
	105	100	*	98	100	100	100	100

106	100	*	89	100	100	99	100
107	99	*	100	98	99	99	100
108	*	*	*	*	*	*	*
109	100	*	99	100	100	100	100
110	100	*	100	100	94	100	100
111	99	*	70	97	100	100	100
112	100	*	99	99	--	99	98
113	*	*	*	*	*	*	*
114	100	*	96	100	100	100	100
115	90	*	61	84	88	93	87
116	100	*	100	100	100	100	100
117	*	*	*	*	*	*	*
118	100	*	100	100	100	100	100
119	55	*	52	↓30	70	56	58
120	100	*	100	100	100	100	100
121	68	*	70	↓24	87	65	52
122	98	*	92	96	71	99	92
123	93	*	93	86	65	95	94
124	100	88	95	100	100	100	100
125	100	100	100	100	100	100	100
126	98	55	98	98	↓49	97	96
127	100	97	100	100	100	100	100
128	100	*	97	100	100	100	100
129	99	*	80	99	97	97	99
130	61	*	*	66	55	*	72

[a]Three taxa (*Proserpinus clarkiae*, *Pachysphinx modesta*, *Paonias excaecata*) were removed from the four gene bootstrap analysis without *EF-1α* because these taxa included data for *EF-1α* only. Inclusion would result in missing data for all remaining gene partitions for the three taxa. Nodes 90, 91, and 112 were therefore collapsed.

Table 10. List of sphingid species and their average proboscis and forewing lengths. “Nectar feeding” indicates whether nectar feeding has been reported for the particular species. An asterisk “*” indicates a behavior predicted from tongue length.

		Proboscis Length				Forewing Length					
Taxon		$\overline{X}$		SD	(*N*)	$\overline{X}$		SD	(*N*)	Nectar Feeding	Reference
Macroglossinae	*Aellopos ceculus*	18.0	±	0.8	(4)	23.8	±	3.1	(4)	Yes*	
Dilophonotini	*Aellopos tantalus*	18.3	±	1.0	(4)	27.0	±	2.2	(4)	Yes	(Tuttle, in press)
Dilophontina	*Aleuron chloroptera*	31.3	±	1.5	(4)	31.8	±	2.1	(4)	Yes*	
	Callionima falcifera	20.5	±	0.9	(3)	37.3	±	2.3	(3)	Yes*	(Haber and Frankie, 1989)
	Cautethia spuria	13.3	±	1.8	(2)	18.0	±	1.4	(2)	Yes	(Haber and Frankie, 1989)
	Enyo ocypete	25.8	±	1.8	(5)	27.6	±	1.1	(5)	Yes*	(Haber and Frankie, 1989)
	Erinnyis ello	37.3	±	3.3	(4)	43.5	±	4.0	(4)	Yes	(Fleming, 1970)
	Eupyrrhoglossum sagra	21.2	±	0.8	(3)	26.3	±	1.2	(3)	Yes	(Haber and Frankie, 1982)
	Hemeroplanes ornatus	43.3	±	4.5	(3)	42.3	±	0.6	(3)	Yes*	
	Isognathus rimosa	29.2	±	2.8	(3)	34.7	±	4.0	(3)	Yes	(Oehlke, 2007)
	Kloneus babayaga	61.0			(1)	49.0			(1)	Yes*	
	Madoryx plutonius	45.7	±	5.5	(3)	41.7	±	0.6	(3)	Yes	(Oliveira, 1996)
	Nyceryx magna	21.0	±	1.0	(3)	31.0	±	0.0	(3)	Yes*	
	Oryba kadeni	29.0	±	2.8	(2)	53.5	±	7.8	(2)	Yes*	
	Pachygonidia subhamata	31.0	±	0.0	(2)	31.5	±	0.7	(2)	Yes*	
	Pachylia ficus	50.0	±	2.4	(4)	59.5	±	3.5	(4)	Yes	(Tuttle, in press)
	Pachylioides resumens	28.7	±	0.6	(3)	43.7	±	2.3	(3)	Yes*	(Haber and Frankie, 1989)
	Perigonia ilus	16.7	±	0.6	(3)	24.7	±	0.6	(3)	Yes	(Haber and Frankie, 1982)
	Pseudosphinx teretrio	45.5	±	1.3	(4)	65.5	±	10.4	(4)	Yes	(Tuttle, in press)
	Unzela japix	22.3	±	2.1	(4)	19.3	±	1.7	(4)	Yes*	
Hemarina	*Cephonodes hylas*	21.3	±	1.5	(4)	29.5	±	0.6	(4)	Yes	(Esaki *et al.*, 1958)
	Hemaris diffinis	17.3	±	1.2	(3)	20.3	±	1.5	(3)	Yes	(Fleming, 1970)
Macroglossini	*Basiothia medea*	24.4	±	2.3	(5)	23.0	±	1.4	(5)	Yes	(Owen, 1972)
Choerocampina	*Cechenena helops*	78.0	±	1.0	(3)	50.0	±	1.0	(3)	Yes*	
	Cechenena subangustata	50.7	±	3.1	(3)	44.2	±	2.6	(3)	Yes*	
	Chaerocina dohertyi	44.0			(1)	49.0			(1)	Yes*	
	Deilephila elpenor	23.0	±	1.9	(5)	29.7	±	1.4	(5)	Yes	(Wahlgren, 1941)

	Euchloron megaera	39.8	±	1.9	(4)	45.3	±	3.0	(4)	Yes	(Wasserthal, 1997)
	Hippotion celerio	38.4	±	3.0	(5)	34.0	±	2.3	(5)	Yes	(Pittaway, 1993)
	Hyles hippophaes	25.3	±	1.0	(5)	32.8	±	1.3	(5)	Yes	(Pittaway, 1993)
	Hyles lineata	29.0	±	4.1	(4)	31.0	±	2.7	(4)	Yes	(Wahlgren, 1941)
	Pergesa acteus	80.3	±	3.2	(4)	31.5	±	1.7	(4)	Yes*	
	Rhagastis mongoliana	28.3	±	1.5	(3)	29.3	±	0.6	(3)	Yes*	
	Rhodafra marshalli	27.0			(1)	24.0			(1)	Yes*	
	Theretra alecto	54.0	±	2.9	(4)	40.3	±	2.6	(4)	Yes	(Eisikowitch and Galil, 1971)
	Theretra capensis	32.7	±	3.1	(3)	39.7	±	6.4	(3)	Yes*	
	Xylophanes chiron	50.0	±	1.7	(3)	36.7	±	1.5	(3)	Yes	(Haber, 1984)
	Xylophanes falco	33.7	±	2.5	(3)	39.7	±	4.0	(3)	Yes	(Hodges, 1971)
	Xylophanes porcus	28.3	±	0.6	(3)	33.7	±	3.1	(3)	Yes	(Haber, 1984)
	Xylophanes tersa	30.8	±	1.9	(4)	30.0	±	1.8	(4)	Yes	(Gregory, 1963-1964)
Macroglossina	*Acosmerycoides harterti*	29.3	±	1.5	(4)	38.8	±	1.3	(4)	Yes*	
	Acosmeryx naga	37.3	±	1.5	(3)	46.7	±	1.2	(3)	Yes*	
	Ampelophaga dolichoides	35.5	±	0.7	(2)	42.0	±	2.8	(2)	Yes*	
	Ampelophaga rubiginosa	31.0	±	2.2	(4)	41.5	±	2.6	(4)	Yes	(Pittaway and Kitching, 2006)
	Amphion floridensis	19.2	±	0.8	(5)	25.2	±	1.3	(5)	Yes	(Fleming, 1970)
	Angonyx testacea	23.3	±	0.6	(3)	30.7	±	3.5	(3)	Yes	(Bell and Scott, 1937)
	Arctonotus lucidus	4.3	±	1.3	(4)	21.8	±	0.5	(4)	No[1]	(Rubinoff, 2001)
	Clarina kotschyi	--				--				Yes	(Pittaway, 1993)
	Daphnis nerii	42.0	±	2.0	(3)	46.7	±	3.5	(3)	Yes	(Pittaway, 1993)
	Darapsa myron	15.8	±	2.1	(4)	28.0	±	1.8	(4)	Yes	(Fleming, 1970)
	Deidamia inscriptum	17.0	±	1.2	(4)	24.3	±	1.5	(4)	Yes	(Fleming, 1970)
	Elibia dolichus	85.3	±	3.2	(3)	59.3	±	5.1	(3)	Yes*	
	Enpinanga borneensis	22.3	±	0.6	(3)	23.7	±	1.2	(3)	Yes*	
	Eupanacra regularis	30.0			(1)	28.0			(1)	Yes*	
	Euproserpinus phaeton	11.5	±	1.3	(3)	15.3	±	1.5	(3)	Yes	(Tuttle, in press)
	Gnathothlibus erotus	42.3	±	3.1	(3)	36.0	±	1.0	(3)	Yes*	
	Macroglossum stellarum	27.8	±	1.0	(4)	22.0	±	1.4	(4)	Yes	(Wahlgren, 1941)
	Neogurelca himachala	22.0			(1)	19.0			(1)	Yes	(Pittaway and Kitching, 2006)
	Nephele accentifera	54.7	±	5.5	(3)	35.3	±	2.5	(3)	Yes	(Owen, 1972)
	Proserpinus clarkiae	15.8	±	1.0	(4)	17.8	±	1.5	(4)	Yes	(Hodges, 1971)

	Proserpinus terlooii	24.0			(1)	18.0			(1)	Yes	(Hodges, 1971)
	Sphecodina abbottii	20.8	±	1.0	(4)	30.8	±	2.5	(4)	Yes	(Fleming, 1970)
	Sphingonaepiopsis gorgoniades	11.5	±	2.1	(2)	11.5	±	0.7	(2)	Yes	(Pittaway, 1993)
	Temnora eranga	16.0	±	0.0	(2)	17.0	±	0.0	(2)	Yes*	
Philampelini	*Eumorpha achemon*	39.3	±	1.7	(4)	40.5	±	1.9	(4)	Yes	(Gregory, 1963-1964)
	Eumorpha pandorus	42.3	±	2.1	(3)	50.0	±	1.0	(3)	Yes	(Fleming, 1970)
	Eumorpha typhon	49.0	±	1.0	(3)	59.7	±	1.5	(3)	Yes	(Tuttle, in press)
Smerinthinae	*Adhemarius daphne*	30.3	±	1.7	(4)	52.5	±	5.8	(4)	Yes	(Haber, 1984)
Ambulycini	*Ambulyx schauffelbergeri*	22.8	±	1.0	(3)	43.3	±	1.5	(3)	Yes	(Bell and Scott, 1937)
	Amplypterus mansoni	29.0	±	1.4	(2)	56.5	±	3.5	(2)	Yes*	
	Amplypterus panopus	35.8	±	3.4	(4)	62.3	±	2.5	(4)	Yes	Jan Beck (pers. com.)
	Protambulyx euryalus	31.0	±	2.0	(3)	51.0	±	1.7	(3)	Yes*	
Smerinthini	*Afroclanis calcareus*	24.0			(1)	30.0			(1)	Yes*	
	Amorpha juglandis	3.6	±	0.9	(4)	32.0	±	5.4	(4)	No	(Fleming, 1968)
	Andriasa contraria	0.5	±	0.0	(3)	27.3	±	1.2	(3)	No*	
	Callambulyx tatarinovii	5.5	±	1.7	(5)	32.2	±	1.5	(5)	No	(Pittaway, 1997-2006)
	Chloroclanis virescens	5.5	±	0.7	(2)	31.0	±	0.0	(2)	No*	
	Clanis bilineata	26.0	±	1.6	(4)	52.5	±	2.9	(4)	Yes*	
	Cypa uniformis	3.5	±	0.7	(2)	25.5	±	2.1	(2)	No*	
	Daphnusa ocellaris	3.7	±	0.6	(3)	42.0	±	8.7	(3)	No*	
	Langia zenzeroides	9.3	±	0.6	(3)	73.0	±	2.0	(3)	No	(Pittaway, 1997-2006)
	Laothoe populi	4.7	±	0.6	(3)	47.0	±	2.6	(3)	No	(Pittaway, 1993)
	Likoma apicalis	1.8	±	0.4	(2)	47.0	±	2.1	(2)	No*	
	Marumba quercus	0.5	±	0.0	(4)	45.0	±	4.9	(4)	No	(Pittaway, 1993)
	Mimas tiliae	4.4	±	0.5	(4)	31.3	±	2.8	(4)	No	(Pittaway, 1993)
	Neoclanis basalis	6.0			(1)	40.0			(1)	No*	
	Neopolyptychus compar	6.3	±	0.3	(2)	34.5	±	6.4	(2)	No*	
	Pachysphinx modesta	5.3	±	0.6	(3)	54.0	±	2.6	(3)	No	(Fleming, 1968)
	Pachysphinx occidentalis	5.0	±	0.8	(4)	62.0	±	5.6	(4)	No*	
	Paonias excaecata	4.7	±	0.6	(3)	34.7	±	2.5	(3)	No	(Fleming, 1968)
	Paonias myops	4.7	±	0.6	(3)	31.0	±	2.6	(3)	No	(Fleming, 1968)
	Parum colligata	6.3	±	0.6	(3)	36.0	±	1.7	(3)	No*	
	Phyllosphingia dissimilis	7.0	±	1.7	(3)	39.3	±	6.7	(3)	No	(Kitching and Cadiou, 2000)

	Polyptychoides digitatus	1.8	±	0.4	(2)	40.5	±	3.5	(2)	No*	
	Polyptychus andosa	9.0			(1)	30.0			(1)	Uncertain	
	Pseudoclanis postica	6.7	±	0.6	(3)	42.7	±	2.1	(3)	No*	
	Smerinthus cersyi	4.3	±	0.8	(3)	37.0	±	2.6	(3)	No	(Pittaway, 1993)
	Smerinthus saliceti	1.2	±	0.3	(3)	34.7	±	5.0	(3)	No	(Pittaway, 1993)
	Viriclanis kingstoni	5.0			(1)	25.0			(1)	No*	
Sphingulini	*Dolbina tancrei*	6.0	±	0.0	(2)	32.5	±	0.7	(2)	No*	
	Hopliocnema brachycera	0.5	±	0.0	(2)	18.0	±	1.4	(2)	No*	
	Kentrochrysalis consimilis	4.2	±	0.3	(3)	26.0	±	1.0	(3)	No	(Pittaway, 1993)
Sphinginae	*Acherontia styx*	18.8	±	1.9	(4)	48.8	±	2.9	(4)	Yes[2]	(Pittaway, 1993)
Acherontiini	*Agrius cingulata*	110.8	±	7.9	(4)	50.5	±	2.6	(4)	Yes	(Gregory, 1963-1964)
	Coelonia fulvinotata	98.5	±	16.8	(4)	46.8	±	4.3	(4)	Yes	(Wasserthal, 1992)
	Megacorma obliqua	135.0	±	7.1	(2)	52.5	±	2.1	(2)	Yes*	
Sphingini	*Ceratomia catalpae*	7.6	±	0.8	(4)	43.8	±	4.8	(4)	No	(Tuttle, in press), (Fleming, 1968)
	Cocytius duponchel	93.0	±	38.0	(4)	73.8	±	9.6	(4)	Yes	(Haber, 1984)
	Dolba hyloeus	36.0	±	4.3	(4)	28.3	±	2.9	(4)	Yes	(Hodges, 1971)
	Dovania poecila	29.0	±	1.4	(2)	29.0	±	1.4	(2)	Yes*	
	Euryglottis dognini	64.7	±	5.5	(3)	57.7	±	4.0	(3)	Yes*	
	Isoparce cupressi	6.9	±	0.3	(4)	31.5	±	3.7	(4)	No	(Dominick, 1973)
	Lapara coniferarum	4.6	±	1.3	(4)	25.0	±	0.0	(4)	No	(Maier *et al.*, 2004)
	Macropoliana natalensis	27.5	±	3.5	(2)	60.0	±	4.2	(2)	Yes*	
	Manduca florestan	63.0	±	9.5	(3)	53.7	±	7.6	(3)	Yes	(Haber, 1984)
	Manduca muscosa	67.3	±	2.2	(4)	51.3	±	2.4	(4)	Yes	(Opler *et al.*, 2007)
	Manduca quinquemaculatus	122.0	±	6.2	(4)	56.8	±	4.6	(4)	Yes	(Gregory, 1963-1964)
	Manduca sexta	83.0	±	18.8	(4)	52.8	±	5.2	(4)	Yes	(Gregory, 1963-1964)
	Meganoton analis	67.0	±	19.9	(3)	60.7	±	10.8	(3)	Yes*	
	Neococytius cluentius	211.8	±	34.4	(5)	74.2	±	10.2	(5)	Yes	(Oehlke, 2007)
	Paratrea plebja	47.5	±	1.0	(4)	31.3	±	2.4	(4)	Yes	(Hodges, 1971)
	Psilogramma increta	72.7	±	4.6	(3)	54.3	±	4.5	(3)	Yes*	
	Sphinx caligineus	16.0	±	2.6	(3)	30.3	±	3.1	(3)	Yes*	
	Sphinx chersis	57.0	±	1.4	(5)	52.8	±	2.0	(5)	Yes	(Gregory, 1963-1964)
	Sphinx dollii	9.0	±	0.0	(3)	26.0	±	1.0	(3)	Yes	(Gregory, 1963-1964), (Grant, 1983)

	Sphinx istar	83.7	±	6.1	(3)	54.0	±	3.0	(3)	Yes	(Fleming, 1970)
	Sphinx kalmiae	39.8	±	1.9	(4)	44.0	±	1.2	(4)	Yes	(Tuttle, in press)
	Sphinx merops	69.8	±	5.4	(4)	50.5	±	3.7	(4)	Yes	(Haber, 1984)
	Xanthopan morganii	186.8	±	34.9	(4)	63.5	±	5.7	(4)	Yes	(Nilsson *et al.*, 1985)
Outgroups	*Acanthobrahmaea europaea*										
	Apha aequalis	0.1	±	0.0	(4)					No	
	Bombyx mori	0.1	±	0.0	(5)					No	
	Carthaea saturnioides										
	Endromis versicolora	0.1	±	0.0	(5)					No	
	Janiodes sp.										
	Lemonia dumi	0.1	±	0.0	(5)					No	
	Macrothylacia rubi	0.1	±	0.0	(5)					No	
	Mirina christophi										
	Oberthueria formosibia										
	Phiditia sp.										

[1]Hodges (1971) originally stated that *Arctonotus lucidus* visits flowers during the day. However, Rubinoff (2001) experimentally tested whether adults can feed, and concluded that the mouthparts are too vestigial to feed on nectar from flowers.
[2]Known to feed honey from beehives (Künckel d'Herculais, 1916), but also known to visit flowers (Pittaway, 1993).

Table 11. Preliminary list of larval hostplant families for Sphingidae included in the current study. Plant classification follows (APG II, 2003). Unfortunately, notable publications on sphingid larval hostplant records (e.g., Robinson *et al.*, 2007) could not be included at the time when this table was constructed. Additional larval records will be subsequently added. For specific host plant species, refer to publications listed and the papers cited therein. "--" indicates that no hostplant record was found.

Sphingid taxon	Hostplant family	Reference
MACROGLOSSINAE		
Macroglossini: Choerocampina		
Basiothia medea	Rubiaceae	(Kroon, 1999)
Cechenena helops	Vitaceae	(Inoue *et al.*, [1996] 1997)
Cechenena subangustata	--	
Chaerocina dohertyi	--	
Deilephila elpenor	Araceae Onagraceae Rubiaceae Vitaceae	(Pittaway and Kitching, 2006)
Enyo ocypete	Dilleniaceae Onagraceae Vitaceae	(Oehlke, 2007)
Euchloron megaera	Vitaceae	(Kroon, 1999)
Hippotion celerio	Amaranthaceae Araceae Bignoniaceae Blasminaceae Convolvulaceae Nyctaginaceae Oleaceae Onagraceae Polygonaceae Rubiaceae Scrophulariaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Hyles hippophaes	Elaeagnaceae	(Mell, 1922; Pittaway, 1993)

Hyles lineata	Asphodelaceae Nyctaginaceae Onagraceae Polygonaceae Portulaceae Rosaceae Rubiaceae Scrophulariaceae Solanaceae Ulmaceae Vitaceae	(Kroon, 1999; Opler *et al.*, 2007)
Pergesa acteus	Araceae Bignoniaceae Commeliaceae Leeaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Rhagastis mongoliana	Araceae Berberidaceae Blasminaceae Onagraceae Polygonaceae Rubiaceae Vitaceae	(Pittaway and Kitching, 2006)
Rhodafra marshalli	Dipsaceae	(Kroon, 1999)
Theretra alecto	Actinidaceae Dilleniaceae Rubiaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Theretra capensis	Vitaceae	(Mell, 1922; Kroon, 1999)
Xylophanes chiron	Rubiaceae	(Oehlke, 2007)
Xylophanes falco	Rubiaceae	(Opler *et al.*, 2007)
Xylophanes porcus	Rubiaceae	(Opler *et al.*, 2007)
Xylophanes tersa	Bignoniaceae Rubiaceae	(Opler *et al.*, 2007)

Dilophonotini: Dilophontina

Aellopos ceculus	Rubiaceae	(Mell, 1922; Oehlke, 2007)
Aellopos tantalus	Rubiaceae	(Opler *et al.*, 2007)
Aleuron chloroptera	Dilleniaceae	(Oehlke, 2007)
Callionima falcifera	Apocynaceae	(Oehlke, 2007)
Cautethia spuria	Rubiaceae	(Oehlke, 2007)
Erynnyis ello	Caricaceae Euphorbiaceae Myrtaceae Sapotaceae	(Opler *et al.*, 2007)
Eupyrrhoglossum sagra	Apocynaceae	(Mell, 1922; Opler *et al.*, 2007)
Hemeroplanes ornatus	Apocynaceae	(Oehlke, 2007)
Isognathus rimosa	Apocynaceae	(Opler *et al.*, 2007)
Kloneus babayaga	--	
Madoryx plutonius	Melastomataceae Onagraceae Vochysiaceae	(Mell, 1922; Oehlke, 2007)
Nyceryx magna	Rubiaceae	(Oehlke, 2007)
Oryba kadeni	Polygonaceae Rubiaceae	(Mell, 1922; Oehlke, 2007)
Pachygonidia subhamata	Vitaceae	(Oehlke, 2007)
Pachylia ficus	Moraceae	(Opler *et al.*, 2007)
Pachylioides resumens	Apocynaceae Moraceae	(Oehlke, 2007)
Perigonia ilus	Rubiaceae	(Oehlke, 2007)
Pseudosphinx teretrio	Apocynaceae	(Opler *et al.*, 2007)
Unzela japix	Dilleniaceae Vitaceae	(Mell, 1922; Oehlke, 2007)

Dilophonotini: Hemarina

Cephonodes hylas	Rubiaceae	(Inoue *et al.*, [1996] 1997; Pinhey, 1975; Kroon, 1999)
Hemaris diffinis	Apocynaceae Caprifoliaceae Rosaceae	(Opler *et al.*, 2007)

Macroglossini: Macroglossina

Acosmerycoides harterti	Actinidiaceae Vitaceae	(Pittaway and Kitching, 2006)
Acosmeryx naga	Actinidiaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Ampelophaga dolichoides	Vitaceae	(Pittaway and Kitching, 2006)
Ampelophaga rubiginosa	Saxifragaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Amphion floridensis	Solanaceae Vitaceae	(Opler *et al.*, 2007)
Angonyx testacea	Loganiaceae	(Inoue *et al.*, [1996] 1997)
Arctonotus lucidus	Onagraceae	(Opler *et al.*, 2007)
Clarina kotschyi	Vitaceae	(Pittaway, 1997-2006)
Daphnis nerii	Anacardiaceae Apocynaceae Rubiaceae Sapindaceae	(Inoue *et al.*, [1996] 1997; Kroon, 1999)
Darapsa myron	Caprifoliaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Deidamia inscriptum	Vitaceae	(Opler *et al.*, 2007)
Elibia dolichus	Actinidiaceae Leeaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Enpinanga borneensis	Dilleniaceae	(Inoue *et al.*, [1996] 1997)
Eupanacra regularis	Araceae	(Pittaway and Kitching, 2006)
Euproserpinus phaeton	Onagraceae	(Opler *et al.*, 2007)

Gnathothlibus erotus	Convolvulaceae Dilleniaceae Escalloniaceae Melastomataceae Rubiaceae Vitaceae	(Inoue *et al.*, [1996] 1997)
Macroglossum stellatarum	Rubiaceae	(Pittaway, 1997-2006)
Neogurelca himachala	Rubiaceae	(Bell and Scott, 1937)
Nephele accentifera	Moraceae	(Mell, 1922; Kroon, 1999)
Proserpinus clarkiae	Onagraceae	(Opler *et al.*, 2007)
Proserpinus terlooii	Nyctaginaceae	(Hodges, 1971)
Sphecodina abbottii	Vitaceae	(Opler *et al.*, 2007)
Sphingonaepiopsis gorgoniades	Rubiaceae	(Pittaway, 1997-2006)
Temnora eranga	--	
Philampelini		
Eumorpha achemon	Vitaceae	(Opler *et al.*, 2007)
Eumorpha pandorus	Vitaceae	(Opler *et al.*, 2007)
Eumorpha typhon	Vitaceae	(Oehlke, 2007)
SMERINTHINAE		
Ambulycini		
Adhemarius daphne	Lauraceae	(Oehlke, 2007)
Ambulyx schauffelbergeri	Anacardiaceae	(Inoue *et al.*, [1996] 1997)
		1997 #432;Mell, 1922 #430]
Amplypterus mansoni	--	
Amplypterus panopus	Anacardiaceae	(Inoue *et al.*, [1996] 1997)

	Bombaceae	
	Clusiaceae	
Protambulyx euryalus	Anacardiaceae	(Oehlke, 2007)
Smerinthini		
Afroclanis calcareus	Fabaceae	(Kroon, 1999)
Amorpha juglandis	Betulaceae Fagaceae Juglandaceae	(Opler *et al.*, 2007)
Andriasa contraria	Bignoniaceae	(Pinhey, 1975)
Callambulyx tatarinovii	Ulmaceae	(Pittaway, 1997-2006)
Chloroclanis virescens	--	
Clanis bilineata	Fabaceae	(Inoue *et al.*, [1996] 1997)
Cypa decolor	Fagaceae	(Inoue *et al.*, [1996] 1997)
Daphnusa ocellaris	Bombaceae Sapindaceae	(Inoue *et al.*, [1996] 1997)
Langia zenzeroides	Rosaceae	(Inoue *et al.*, [1996] 1997)
Laothoe populi	Salicaceae	(Pittaway, 1997-2006)
Likoma apicalis	Malvaceae	(Kroon, 1999)
Marumba quercus	Fagaceae	(Pittaway, 1997-2006)
Mimas tiliae	Betulaceae Fagaceae Juglandaceae Rosaceae Tiliaceae Ulmaceae	(Mell, 1922; Pittaway, 1997-2006)
Neoclanis basalis	--	
Neopolyptychus compar	Fabaceae Rubiaceae	(Kroon, 1999)

Pachysphinx modesta	Salicaceae	(Opler *et al.*, 2007)
Pachysphinx occidentalis	Salicaceae	(Opler *et al.*, 2007)
Paonias excaecata	Betulaceae Fagaceae Salicaceae Tiliaceae Rosaceae	(Opler *et al.*, 2007)
Paonias myops	Rosaceae Tiliaceae	(Opler *et al.*, 2007)
Parum colligata	Moraceae	(Inoue *et al.*, [1996] 1997)
Phyllosphingia dissimilis	Juglandaceae	(Inoue *et al.*, [1996] 1997)
Polyptychoides digitatus	--	
Polyptychus andosa	--	
Pseudoclanis postica	Loranthaceae Moraceae Ulmaceae	(Kroon, 1999)
Smerinthus cersyi	Salicaceae	(Opler *et al.*, 2007)
Smerinthus saliceti	Salicaceae	(Opler *et al.*, 2007)
Viriclanis kingstoni	--	

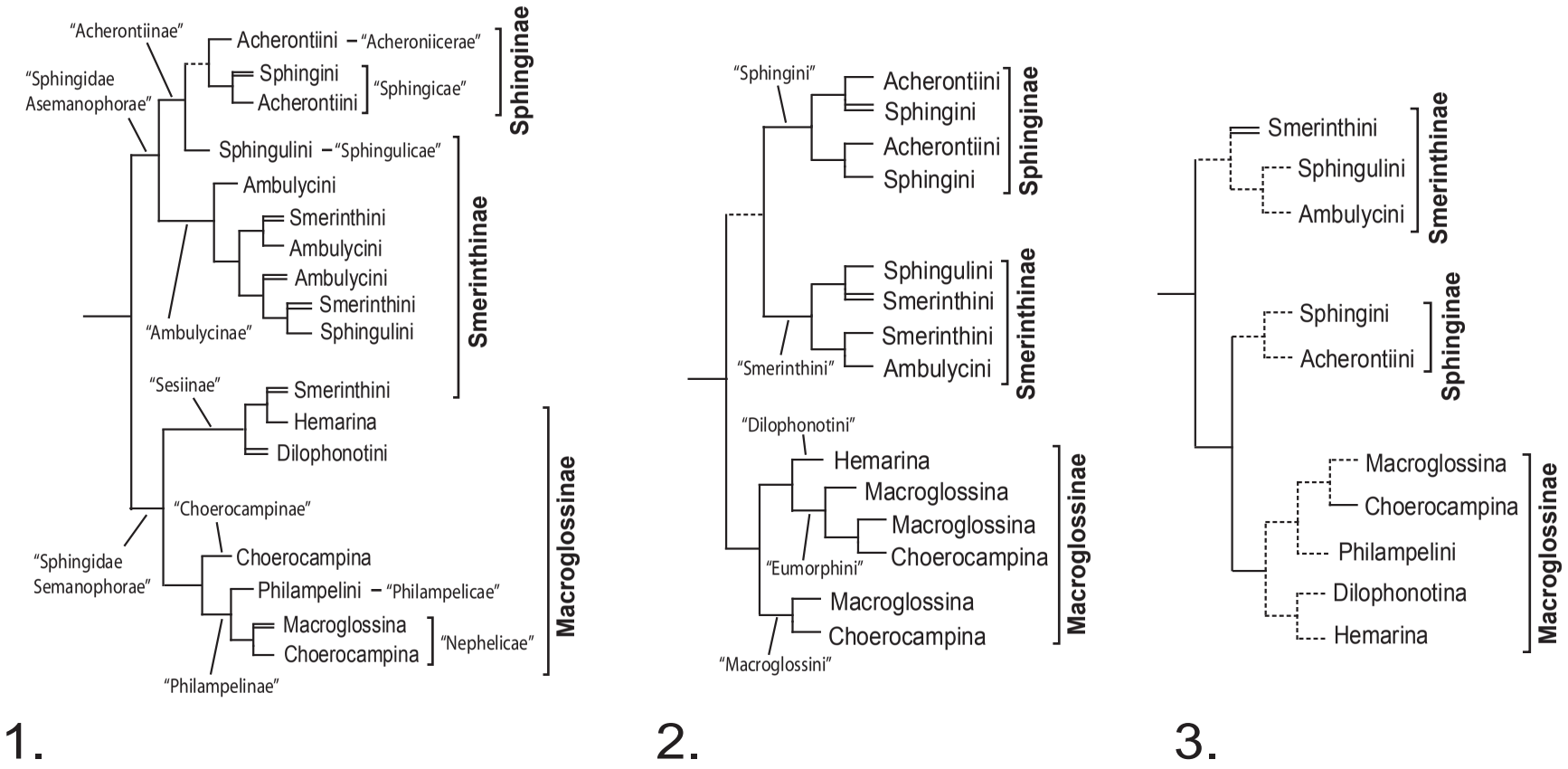
Sphingulini

Dolbina tancrei	Oleaceae	(Pittaway and Kitching, 2006)
Hopliocnema brachycera	--	
Kentrochrysalis consimilis	Oleaceae	(Pittaway and Kitching, 2006, Park *et al*., 1999).

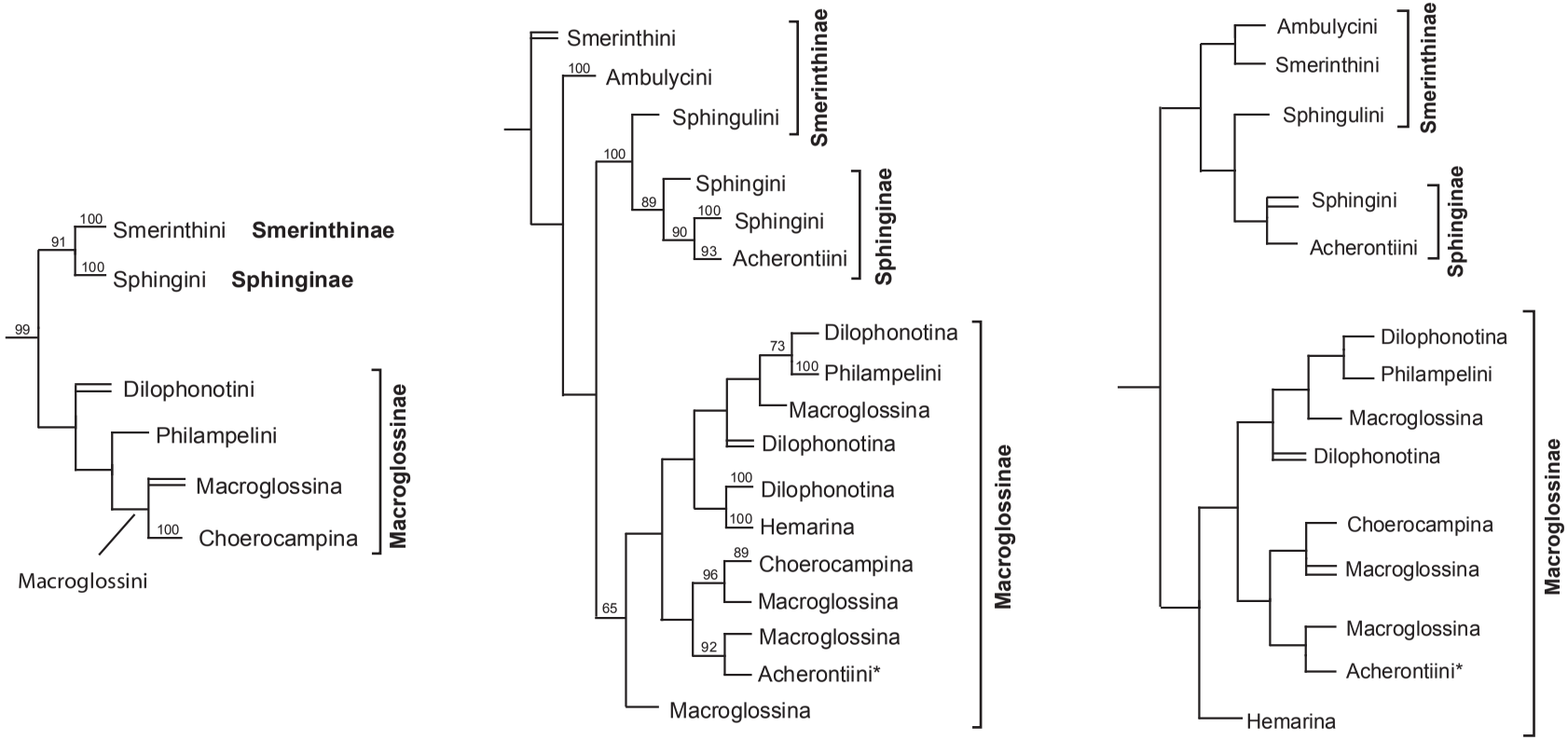
SPHINGINAE
Sphingini

Acherontia styx	Cucurbitaceae Fabaceae Oleaceae Solanaceae	(Inoue *et al.*, [1996] 1997)
Agrius cingulata	Convolvulaceae	(Pittaway, 1997-2006)
Coelonia fulvinotata	Asteraceae Bignoniaceae Boraginaceae Convolvulaceae Lamiaceae Solanaceae Verbenaceae	(Mell, 1922; Pinhey, 1975; Kroon, 1999)
Megacorma obliqua	--	
Ceratomia amyntor	Fagaceae Oleaceae Rosaceae	(Opler *et al.*, 2007)
Ceratomia catalpae	Bignoniaceae	(Opler *et al.*, 2007)
Cocytius duponchel	Annonaceae	(Opler *et al.*, 2007)
Dolba hyloeus	Annonaceae Aquifoliaceae Myricaceae	(Opler *et al.*, 2007)
Dovania poecila	--	
Euryglottis dognini	--	
Isoparce cupressi	Cupressaceae	(Opler *et al.*, 2007)
Lapara coniferarum	Pinaceae	(Opler *et al.*, 2007)
Macropoliana natalensis	Bignoniaceae Fabaceae Lamiaceae Oleaceae	(Carcasson, 1968; Pinhey, 1975; Kroon, 1999)
Manduca florestan	Bignoniaceae Verbenaceae	(Opler *et al.*, 2007)

Manduca muscosa	Asteraceae Verbenaceae	(Oehlke, 2007)
Manduca quinquemaculatus	Solanaceae	(Opler *et al.*, 2007)
Manduca sexta	Solanaceae	(Opler *et al.*, 2007)
Meganoton analis	Lauraceae	(Mell, 1922)
Neococytius cluentius	Annonaceae Piperaceae	(Mell, 1922; Opler *et al.*, 2007)
Paratrea plebja	Bignoniaceae Oleaceae Passifloriaceae	(Mell, 1922; Hodges, 1971)
Psilogramma increta	Caprofoliaceae Lamiaceae Oleaceae Pedaliaceae Scrophulariaceae Verbenaceae	(Inoue *et al.*, [1996] 1997)
Sphinx caligineus	Pinaceae	(Pittaway and Kitching, 2006)
Sphinx chersis	Oleaceae Salicaceae	(Opler *et al.*, 2007)
Sphinx dollii	Cupressaceae	(Opler *et al.*, 2007)
Sphinx istar	Lamiaceae	(Oehlke, 2007)
Sphinx kalmiae	Aquifoliaceae Caprifoliaceae Ericaceae Oleaceae Salicaceae	(Opler *et al.*, 2007)
Sphinx merops	Verbenaceae	(Oehlke, 2007)
Xanthopan morganii	Annonaceae	(Carcasson, 1968)



Figs. 1-3. Relationships of Sphingidae based on morphology. **1.** Adult morphology (Rothchild and Jordan, 1903). **2.** Larval and pupal morphology (Nakamura, 1976). **3.** Relationships inferred from Kitching and Cadiou (2000). Due to limited space, all terminals have been changed from species to Kitching and Cadiou's (2000) tribes and subtribes. A double-line leading to a terminal indicates paraphyly, a dotted line indicates uncertain relationships, and a name in quotations indicates higher groupings recognized by the particular study.



4. 5. 6.

Figs. 4-6. Relationships of Sphingidae based on molecules (*EF-1α* + *DDC*). **4.** MP cladogram of Regier *et al.* (2001). **5.** MP cladogram of Mignault (2003). **6.** ML tree of Mignault (2003). Bootstrap values were not calculated in Mignault's ML analysis due to compuational constraints. Trees were based on all nucleotide positions, numbers above branches indicate bootstrap values > 50%, double-lines leading to terminals indicate paraphyly. Due to limited space, terminals have been changed from species to Kitching and Cadiou's (2000) tribes and subtribes. *Mignault's analyses included a species in the Acherontiini, *Coelonia fulvinotata* (Butler), which had part of its DDC sequence accidentally switched with a species in the Macroglossina, *Macroglossum stellatarum* (L.).

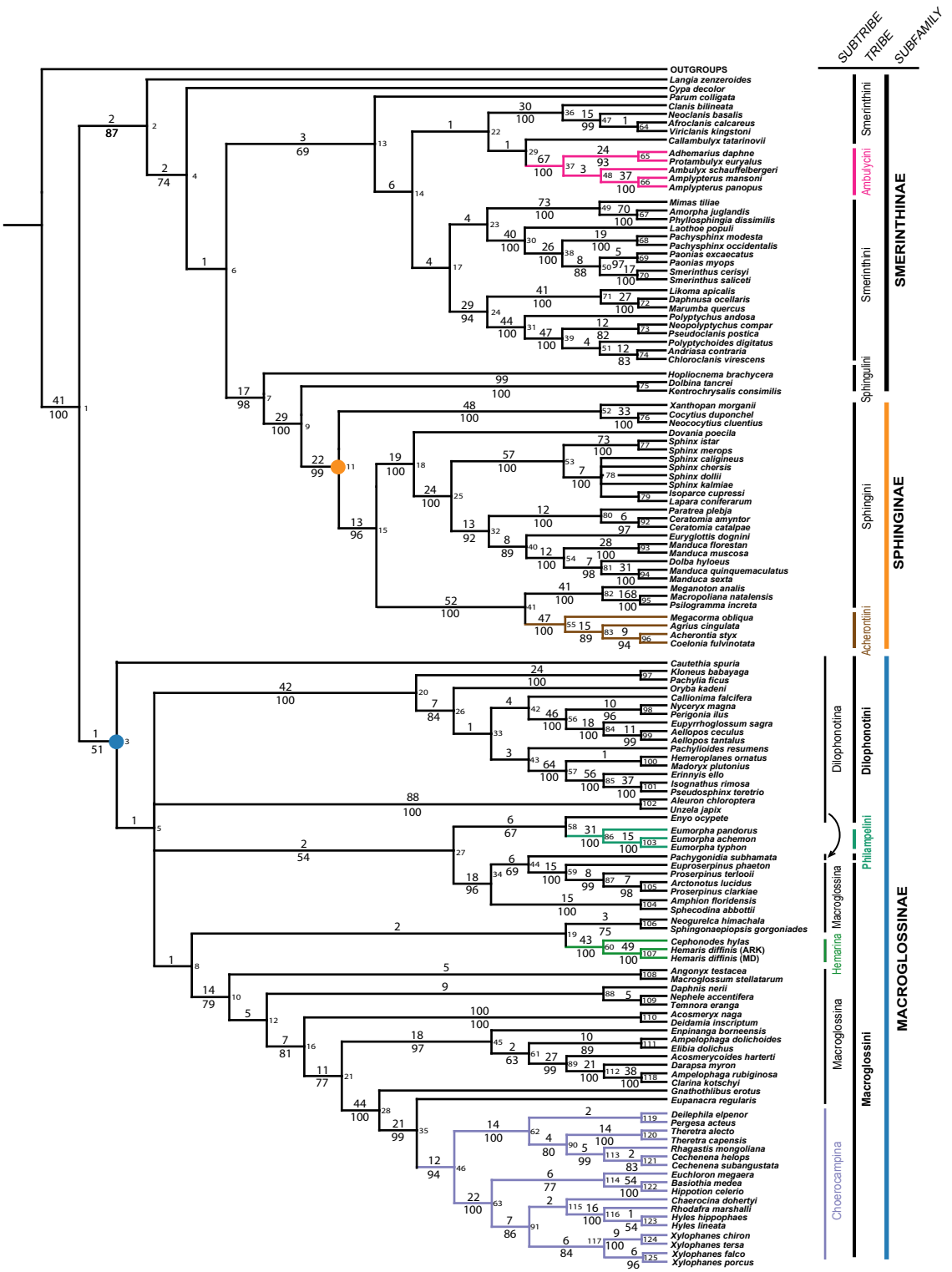


Fig. 7. Parsimony strict consensus of 12 MPTs (L=43023, CI=0.15, RI=0.53) based on the five gene simultaneous analysis. Bremer support indicated above branches, bootstrap (> 50%) indicated below. Nodes are labeled to the right of each internal branch. Colored dots indicate monophyletic subfamilies, colored clades indicate monophyletic tribes and subtribes recognized by Kitching and Cadiou (2000).

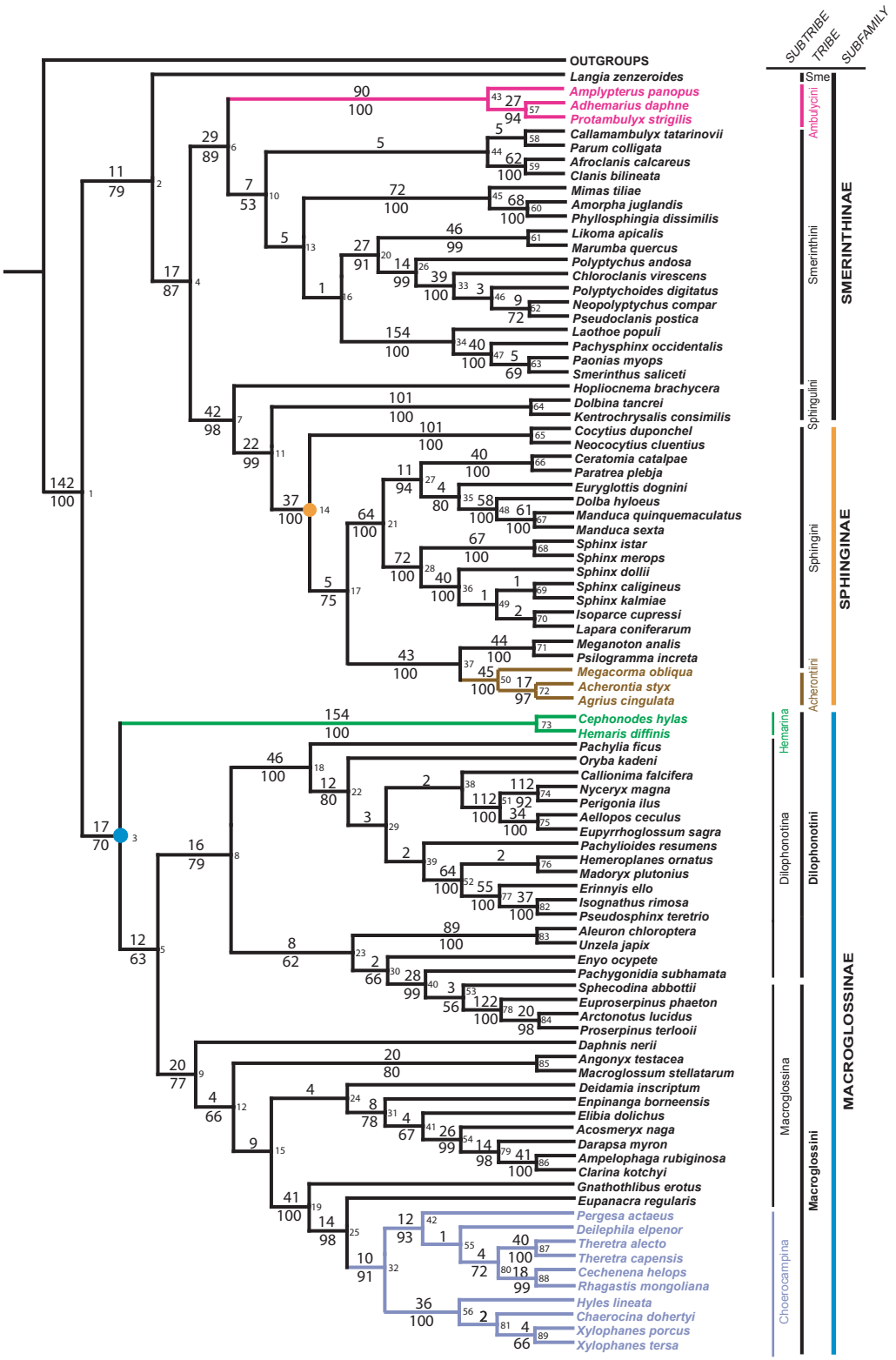


Fig. 8. The most parsimonius tree (L=36301, CI=0.17, RI=0.52) from the 99-taxon data matrix which excludes taxa with 50% missing data for any gene. Node numbers are indicated to the right of each node, Bremer Support above branches, Bootstrap support (> 50%) below.

Fig. 9. ML tree based on the five gene simultaneous analysis (*-lnL = 194121.6532*). Phylogram showing branch lengths on the right. Bootstrap (> 50%) indicated above each branch. Nodes are labeled to the right of each internal branch. Colored dots indicate monophyletic subfamilies, colored clades indicate monophyletic tribes and subtribes recognized by Kitching and Cadiou (2000).

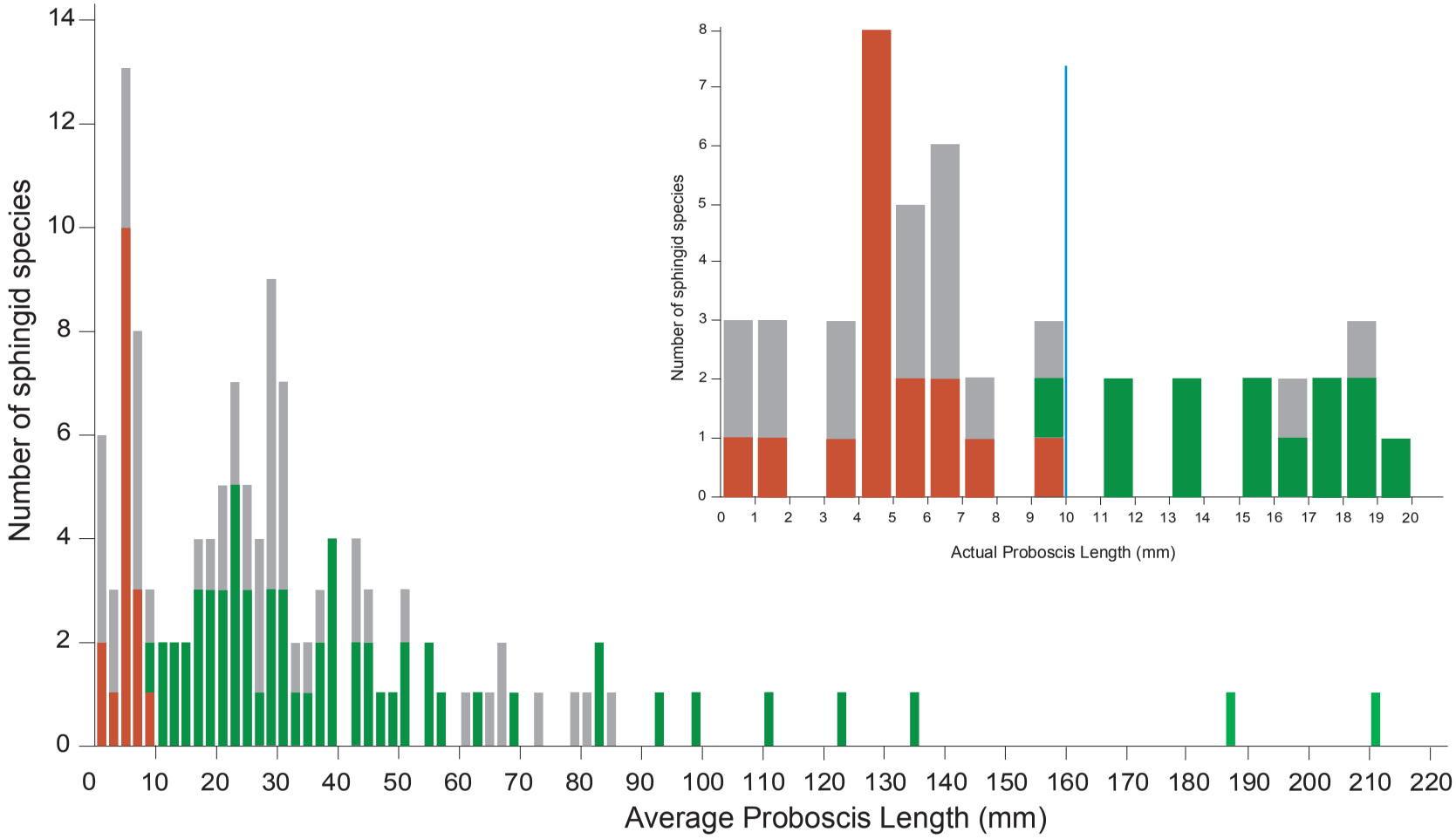


Fig. 10. Histogram showing the number of sphingid species relative to the average proboscis length for taxa included in the five gene analysis. The histogram on the upper right shows the pattern at a finer scale up to 20 mm. Red bars indicate non-feeding species, green bars indicate nectar-feeding species, grey bars represent species in which feeding remains unknown. The blue line denotes Miller's (1997) categorical division of nectar feeding and non-feeding sphingid taxa based solely on length.

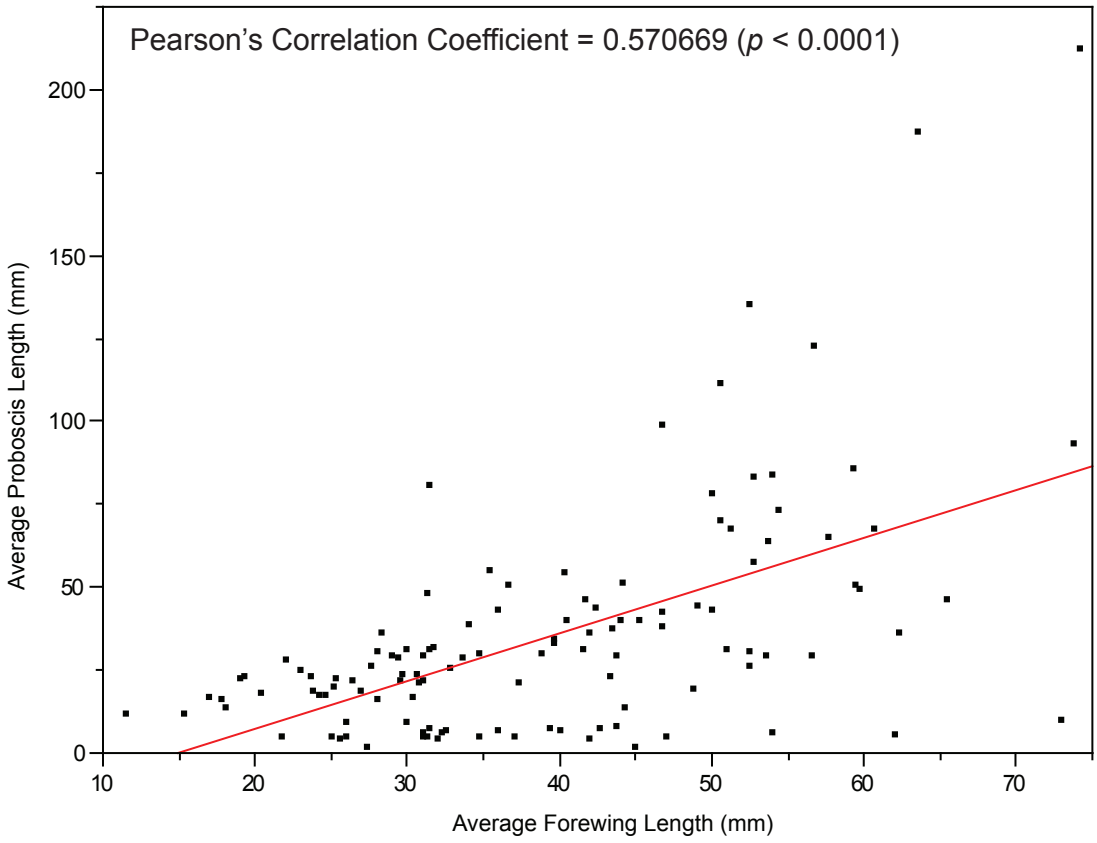


Fig. 11. Average proboscis length relative to average forewing length for each species.

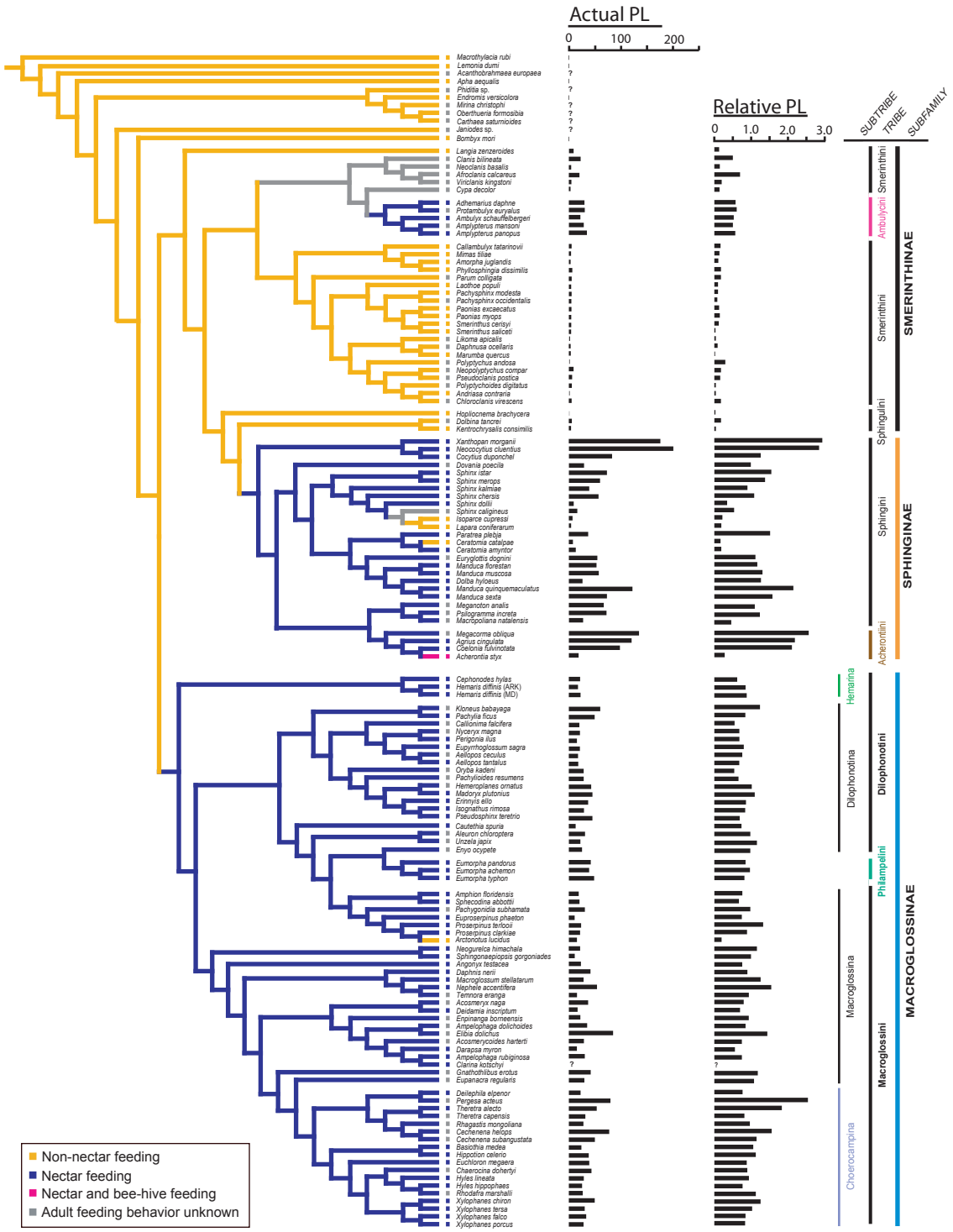


Fig. 12. Proboscis length mapped onto the ML five gene tree. *Actual PL* is the actual proboscis length averaged for each species; *Relative PL* is average proboscis length standardized for body size (proboscis length / forewing length).

SUPPLEMENTARY MATERIAL

SINGLE GENE ANALYSES

AND

SIX GENE SIMULTANEOUS ANALYSIS WITH COI

SUPPLEMENT 1

SINGLE GENE ANALYSES OF NUCLEAR PROTEIN CODING GENES

This section includes trees generated from single gene analyses for each of the five protein-coding nuclear genes. MP figures shown first (Figs. S1-S5), followed by ML figures (Figs. S6-S10).

Fig. S1. MP strict consensus of 3 MPTs based on the *CAD* gene (L=20202, CI=0.14, RI=0.53). Bootstrap values (> 50%) are indicated below branches.

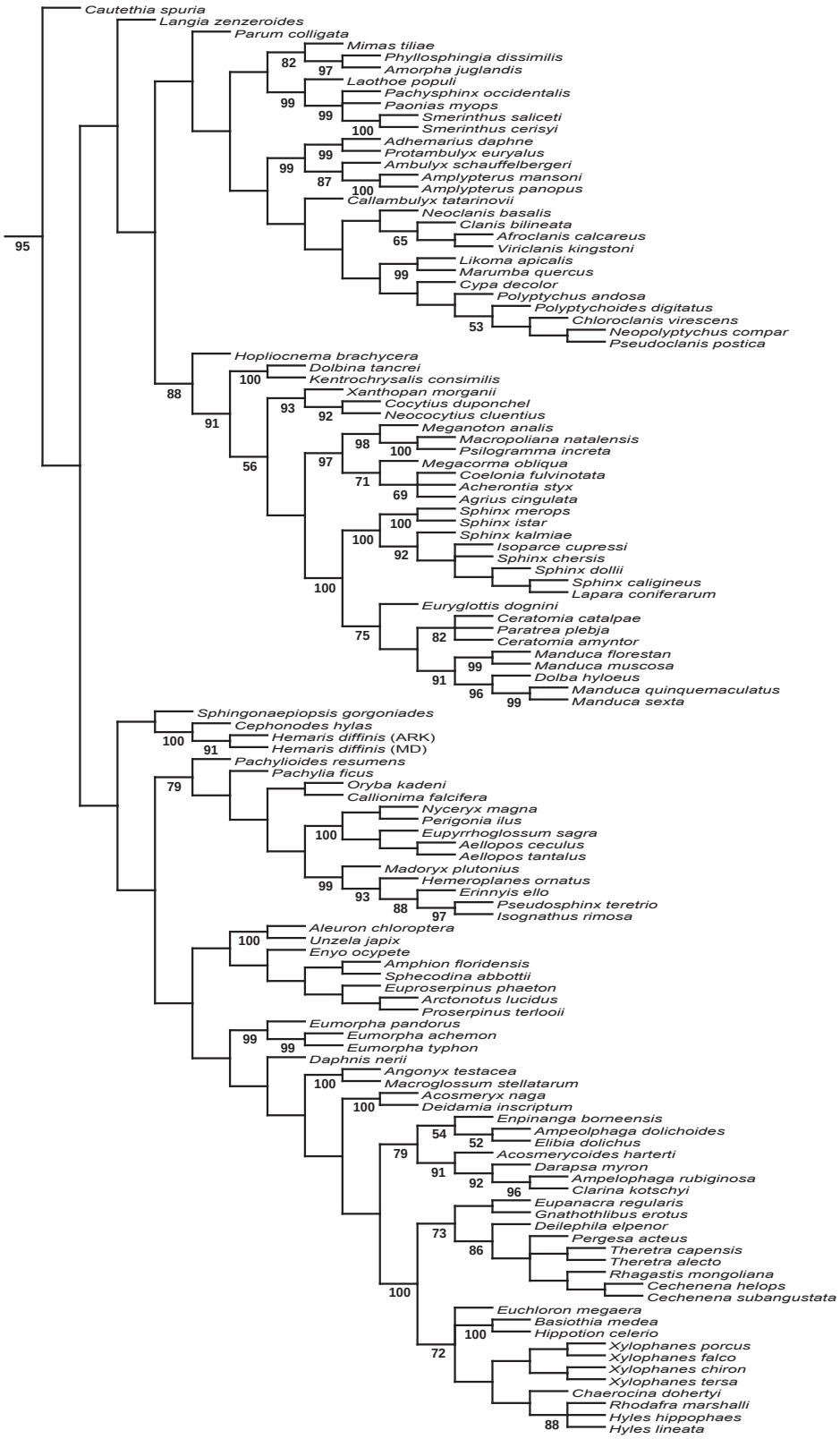


Fig. S2. MP strict consensus of 128 MPTs based on the *DDC* gene (L=8569, CI=0.14, RI=0.59). Bootstrap values (> 50%) are indicated below branches.

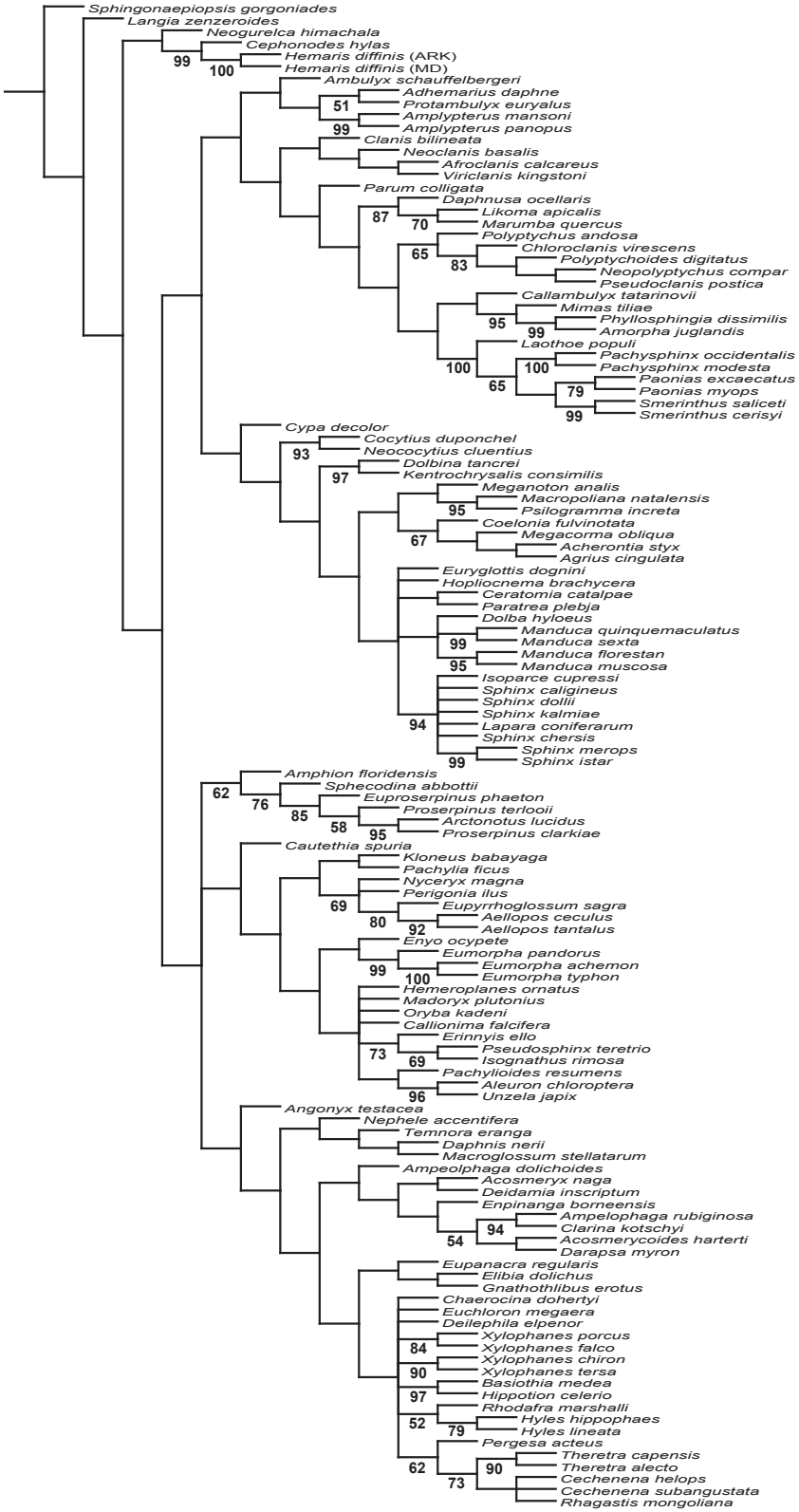


Fig. S3. MP strict consensus of 6384 MPTs based on the *EF-1α* gene (L=3755, CI=0.17, RI=0.51). Bootstrap values (> 50%) are indicated below branches.

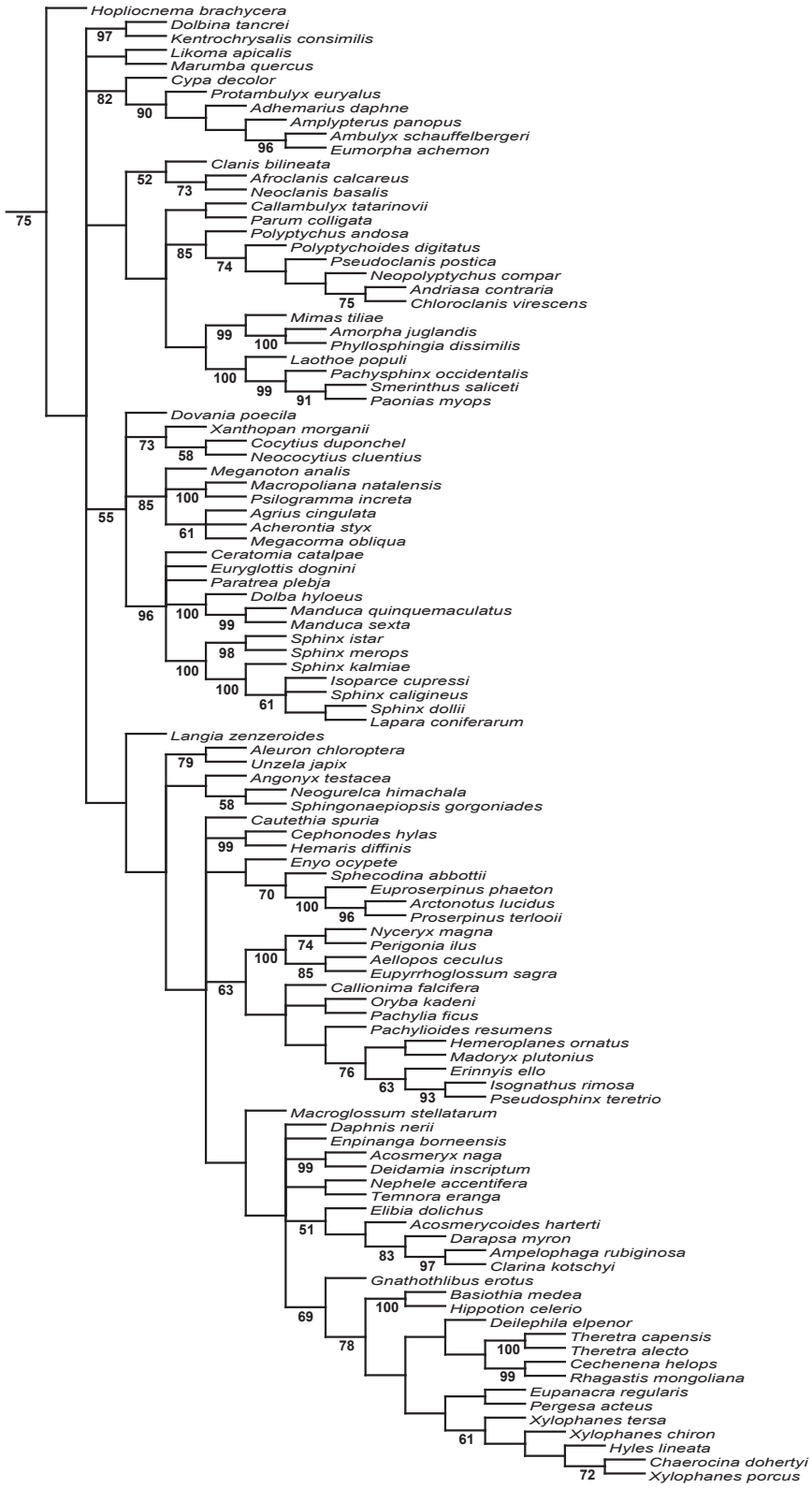


Fig. S4. MP strict consensus of 116 MPTs based on the period gene (L=9131, CI=0.15, RI=0.47). Bootstrap values (> 50%) are indicated below branches.

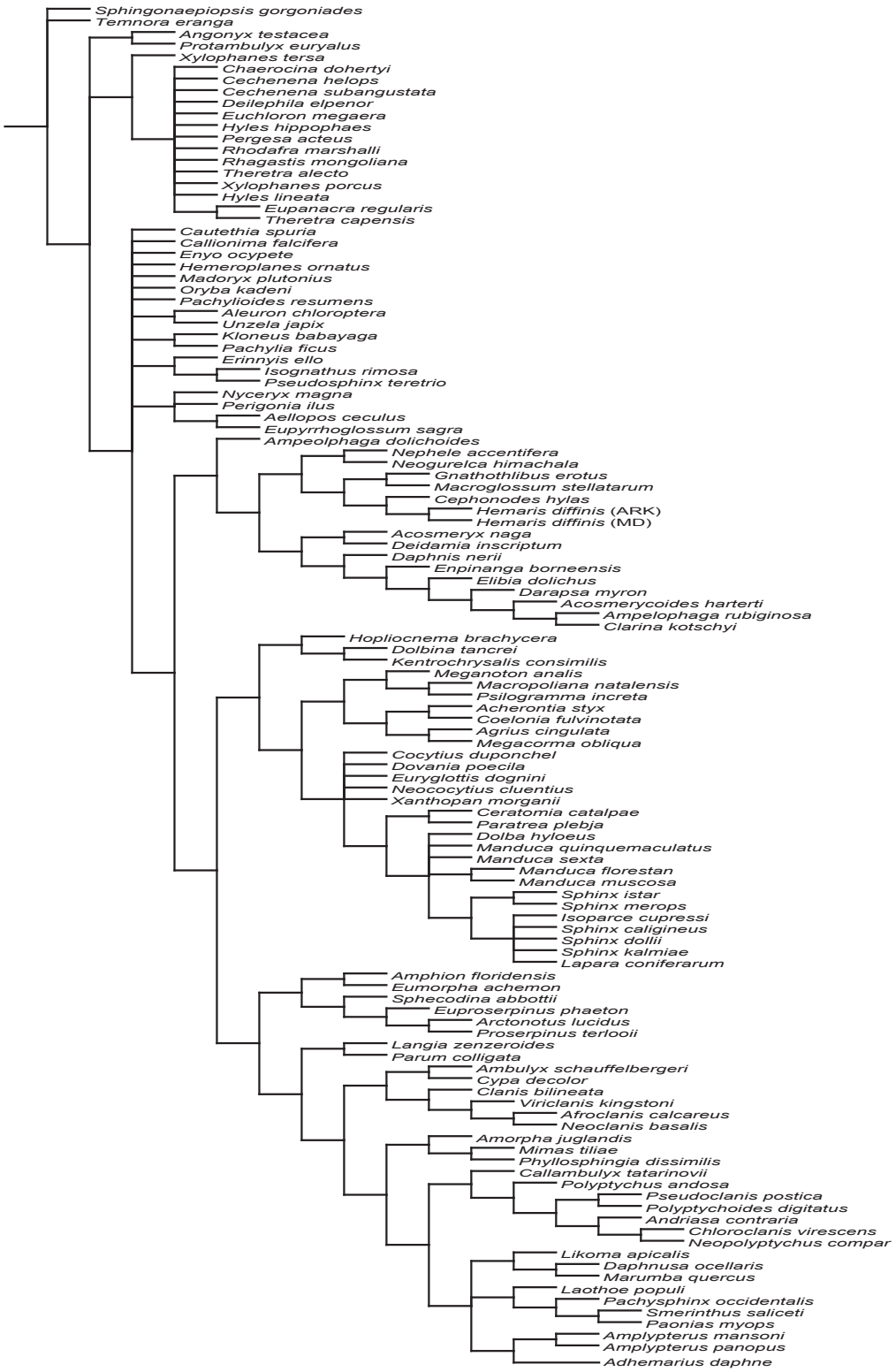


Fig. S5. MP strict consensus of 20000 MPTs based on the *wingless* gene (L=2346, CI=0.15, RI=0.48). Due to time constraints, the exact number of MPTs could not be calculated. Bootstrap values are therefore not shown.

Fig. S6. ML tree generated from the *CAD* gene (*-lnL* = 86356.14928). Bootstrap values (> 50%) are indicated below branches. The bar under "Sphingidae" indicates the branch length leading to the Sphingidae from the nearest outgroup (*Bombyx mori* L.).

Fig. S7. ML tree generated from the *DDC* gene (*-lnL* = 37127.65035). Bootstrap values (> 50%) are indicated below branches. The bar under “Sphingidae” indicates the branch length leading to the Sphingidae from the nearest outrgoup (*Bombyx mori* L.).

Fig. S8. ML tree generated from the *EF-1α* gene (*-lnL* = 18452.54643). Bootstrap values (> 50%) are indicated below branches. The bar under "Sphingidae" indicates the branch length leading to the Sphingidae from the nearest outgroup (*Bombyx mori* L.).

Fig. S9. ML tree generated from the *period* gene (*-lnL* = 38040.1666). Bootstrap values (> 50%) are indicated below branches. The bar under "Sphingidae" indicates the branch length leading to the Sphingidae from the nearest outgroup (*Bombyx mori* L.).

Fig. S10. ML tree generated from the *wingless* gene (*-lnL* = 10577.89695). Bootstrap values (> 50%) are indicated below branches. The bar under "Sphingidae" indicates the branch length leading to the Sphingidae from the nearest outrgoup (*Bombyx mori* L.).

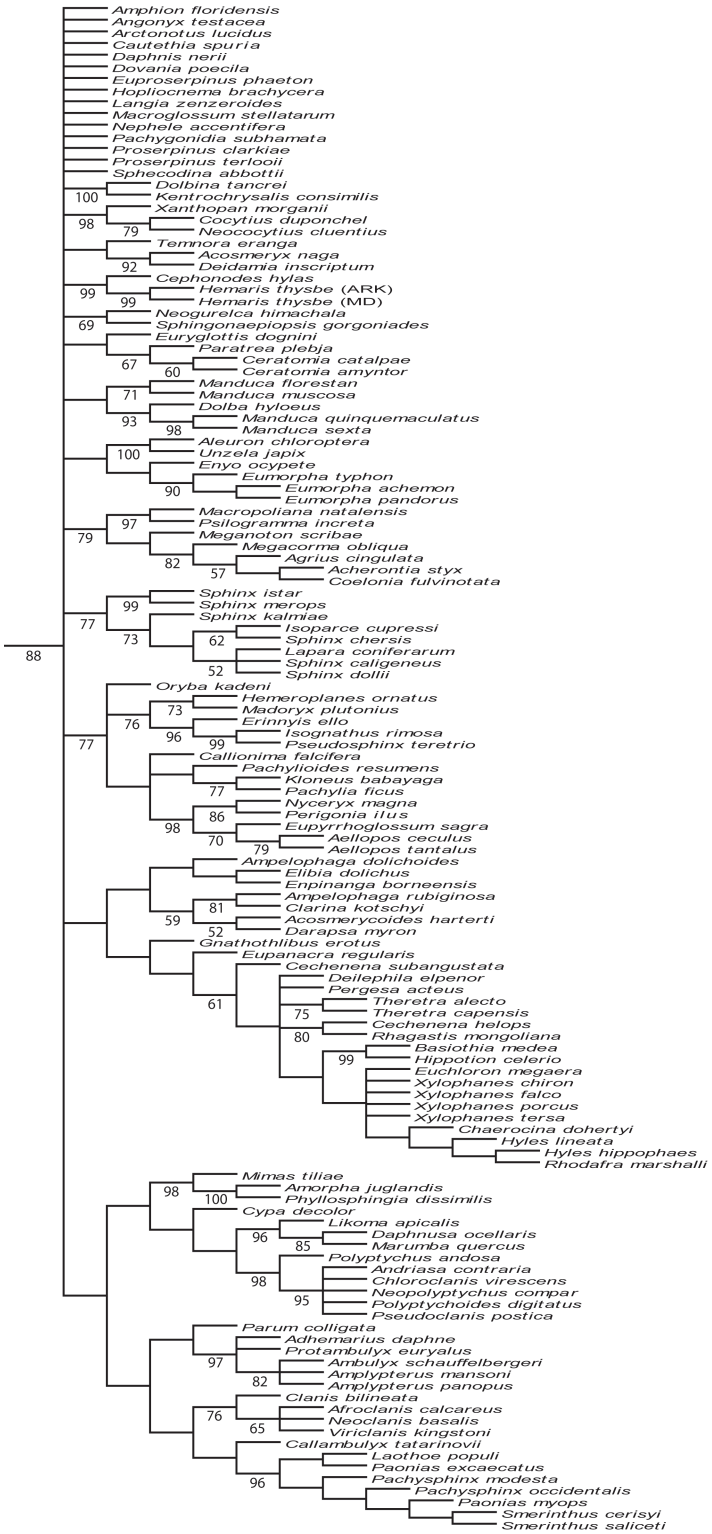


Fig. S11. MP strict consensus generated from the five gene dataset with third nucleotide postions removed (L = 6942, CI = 0.27, RI = 0.58). Bootstrap values (> 50%) are indicated below branches.

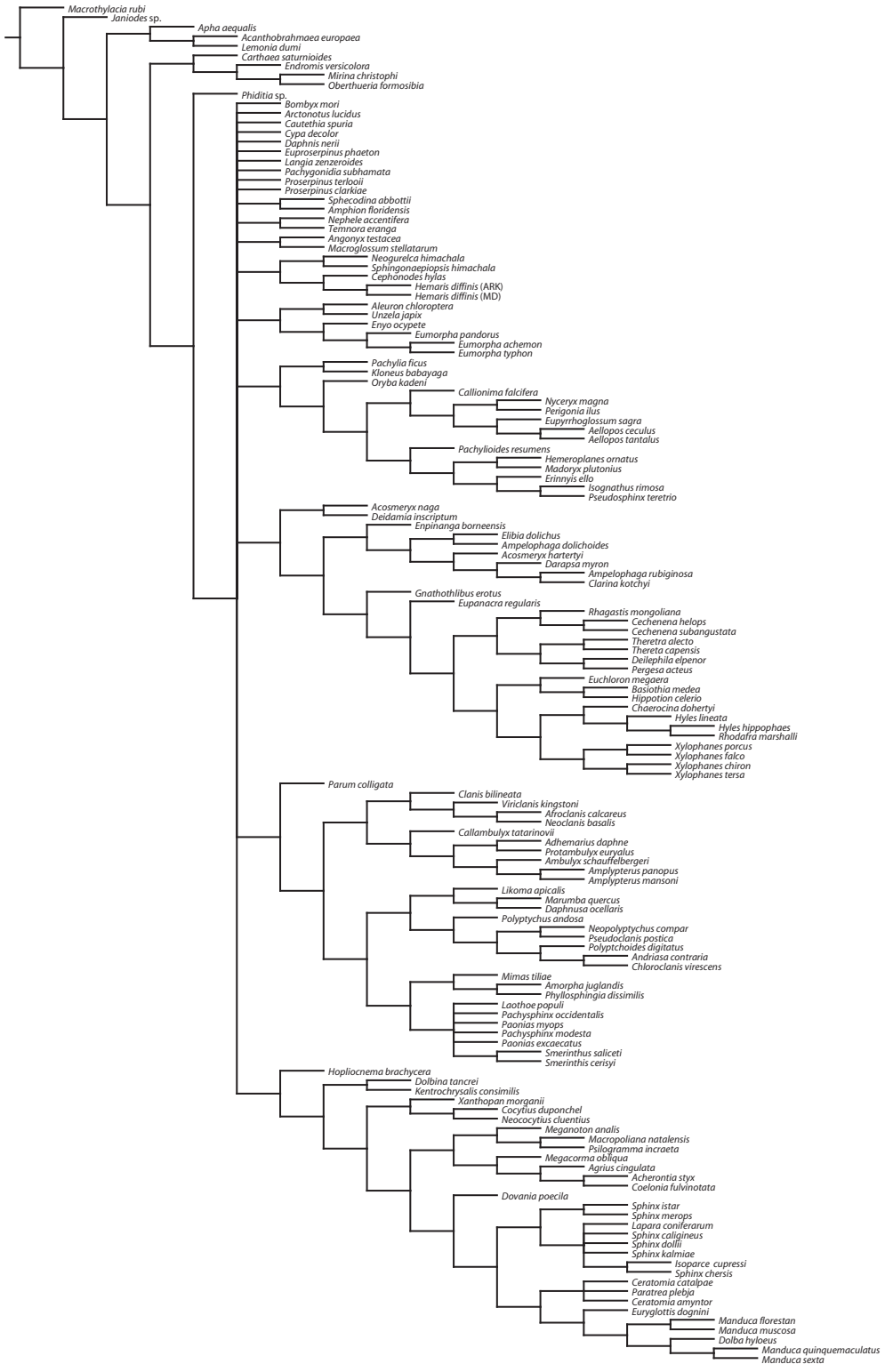


Fig. S12. MP strict consensus of 125 MPCs with nt3 of EF-1α excluded (L = 39586, CI = 0.14, RI = 0.53).

SUPPLEMENT 2

SIX GENE SIMULTANEOUS ANALYSIS WITH COI

Ideally, it is best to include as many taxa and as many data partitions as possible for any phylogenetic study. However, research funding and time are limiting factors. It is therefore essential to determine the best approach to tackle the particular phylogenetic question. Whether to choose more taxa or more genes has been an ongoing debate in phylogenetics (e.g., Mitchell *et al.*, 2000; Pollock *et al.*, 2002; Rokas and Carroll, 2005), but it has become evident that addition of both taxa and genes are imperative (Rokas, 2006). With the onset of the National Science Foundation's *Assembling the Tree of Life* (AToL) program, the goal for many large-scale phylogenomic projects has been to construct a robust tree with as many taxa as possible without loosing tree structure or getting strong support for incorrect relationships (Rokas, 2006).

One of the most commonly included genes in phylogenetic studies of arthropods is the COI gene, which has been shown to be useful for resolving recent divergences (e.g., Caterino *et al.*, 2000; Sperling, 2003; Braby *et al.*, 2006). A particular 648-bp region of the COI gene, known as the barcoding region (Hebert *et al.*, 2003; Savolainen *et al.*, 2005), has recently received attention as a tool to explore species boundaries between closely related taxa (e.g., Hebert *et al.*, 2003; 2004a; 2004b; Kress *et al.*, 2005). Numerous taxonomic projects have begun to compile COI barcodes for as many species as possible, which has resulted in the *Consortium for the Barcoding of Life* (CBOL), and online tools in aiding the acquisition, storage, analysis and publication of barcodes (Ratnasingham and Hebert, 2007). Despite the recent utility and availability of COI

barcodes, it remains unclear how much phylogenetic contribution the COI barcoding region can provide when combined with a much larger dataset.

This section was added as supplement to this thesis to explore the effect of combining the COI barcoding region for available sphingid taxa to a larger dataset which was sampled more thoroughly for five nuclear genes. A COI barcoding dataset was first constructed by downloading available sphingid COI sequences from Genbank in FASTA format. Each sequence was opened in BioEdit (Hall, 2001), and sequences were aligned with the COI sequence of *Bombyx mori* in ClustalW (Thompson *et al.*, 1994) and trimmed at both the 5' and 3' ends into a block which was 658 bp long. The COI matrix showed a strong A-T bias (31.8% A, 15.5% C, 14.5% G, 38.2% T). In total, 63 ingroup taxa included at least part of the COI sequence, 25 of which was represented solely by the COI sequence. A summary of the number of total characters for each taxon in the six gene matrix is listed in Table S1. Analyses were calculated for ML only, but will be subsequently explored under MP as well. The best model for COI was determined in Modeltest (Posada and Crandall, 1998) to be the GTR+Γ+I model. The six gene simultaneous analysis was conducted in Garli ver. 0.951 (Zwickl, 2006), and the number of generations to termination was increased from 10,000 to 100,000.

The six gene ML tree was fundamentally very similar to the five gene ML tree. In particular cases, taxa which were represented solely by the COI gene were recovered close to other species in its genus (Fig. S13). For instance, *Erinnyis crameri* (Schaus) included only 583 bp of the COI gene, but was nested within a clade which included three other species of *Erinnyis* (the clade also included *Phryxus*). *Erinnyis* and *Phryxus* were previously predicted to be sister genera – the only morphological difference

between the two genera is the scalloping of the forewing margin (Rothschild and Jordan, 1903). The COI barcoding region for *Phryxus* was available for only 291 bp, and this result suggests that the COI may be accurately placing these taxa. Three additional species in the genus *Hyles* were also included in the analysis, and the relationship between species in the genus were (*H. lineata* (((*H. hippophaes* + *H. euphorbiae*) + (*H. gallii* + *H. nicaea*))). This result is congruent with a previous study on *Hyles* which used three genes (Hundsdoerfer *et al.*, 2005a).

Without further in-depth analyses, it is difficult to assess whether these taxa are being correctly placed on the tree. It was also discovered that it is critical to have all taxa in the analysis sampled for COI, as placement of taxa can be influenced by whether the barcoding region was sampled for those particular taxa (e.g., taxa which are only represented by COI may be placed at the base of the tree because other closely related members do not include COI). Furthermore, results indicate that branch support values which were high in the five gene analysis can be depressed when considerable missing data are added (Fig. 12). As more sphingid barcoding sequences become available, I hope to incorporate additional data in order to explore the utility of the COI barcoding region in simultaneous analyses and test the effect of missing data on branch support values.

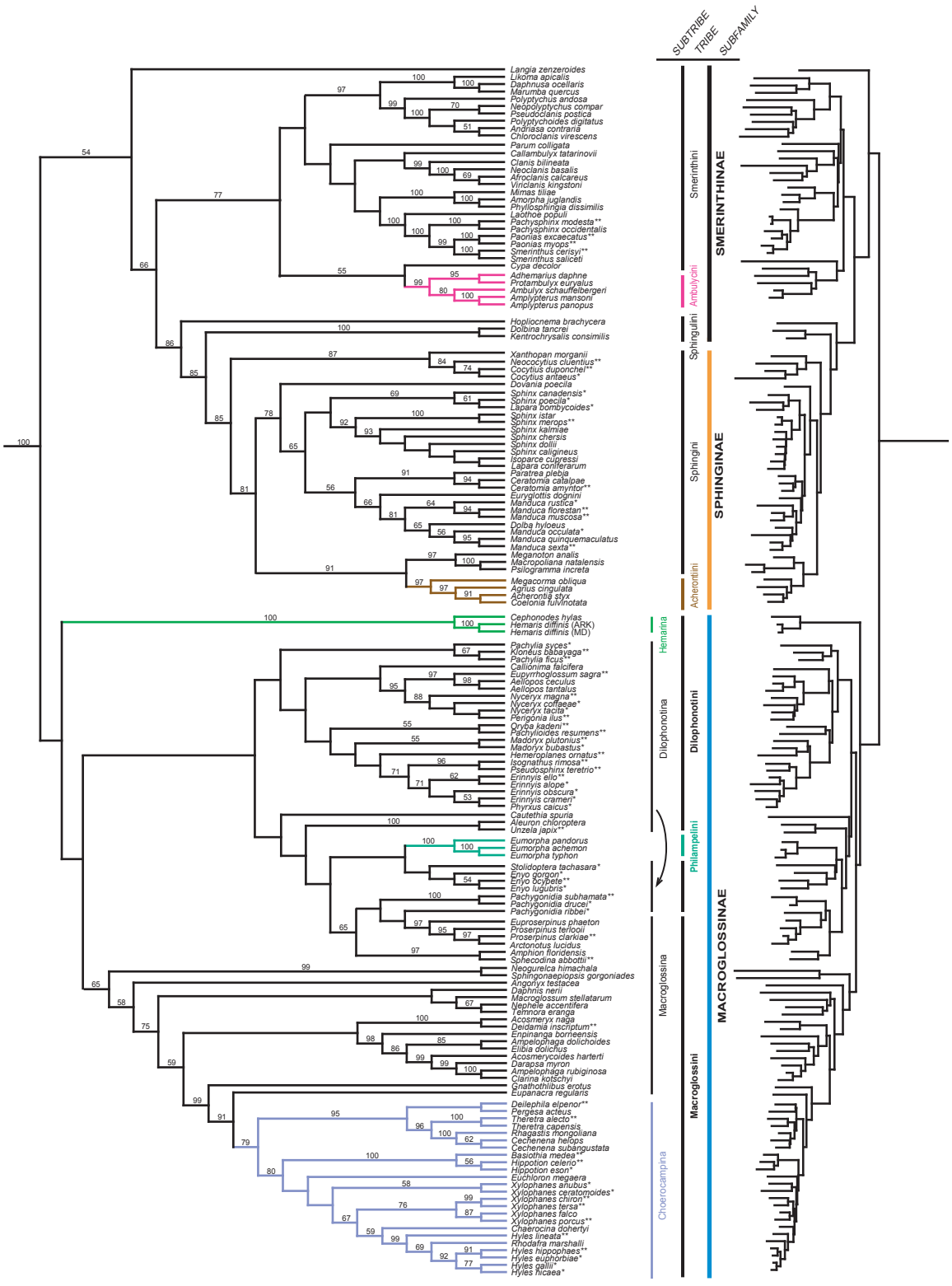


Fig. S13. ML tree (*-lnL* = -203427.4046) of the six gene simultaneous analysis with COI(*GTR+Γ+I*). Branch lengths shown on right side of figure. Bootstrap support (> 50%) below each branch. * = taxa which were sampled for COI only; ** = taxa which were sampled for both COI and at least one of the five nuclear genes.

Table S1. Sphingid species and outgroups which were included in the six gene analysis with COI. In total, 156 ingroup and 11 outgroup species were included (167 species total). Taxa are listed alphabetically by subfamily. " * " refers to taxa which are represented by the COI sequence only. "All Genes" refers to a combination of the five nuclear genes plus COI.

Ingroup Taxa		**COI Genbank Acc.#**	**COI (658 bp)**		**All Genes (7451 bp)**
Macroglossinae					
Dilophonotini					
Choerocampina	*Basiothia medea* (Fabricius, 1781)	AJ749414	658		7027
	Cechenena helops (Walker, 1856)	--	--		6793
	Cechenena subangustata Rothchild, 1920	--	--		2340
	Chaerocina dohertyi Rothchild & Jordan, 1903	--	--		6793
	Deilephila elpenor (Linnaeus, 1758)	AJ749419	658		6645
	Euchloron megaera (Linnaeus, 1758)	--	--		4240
	Hippotion celerio (Linnaeus, 1758)	AJ749424	658		7049
	Hippotion eson (Cramer, 1779)	AJ749421	658	*	658
	Hyles euphorbiae (Linnaeus, 1758)	AJ749516	658	*	658
	Hyles gallii (Rottemburg, 1775)	AJ749580	658	*	658
	Hyles hippophaes (Esper, 1789)	AJ749452	658		3572
	Hyles lineata (Fabricius, 1775)	AJ749436	658		7452
	Hyles nicaea (von Prunner, 1798)	AJ749444	658	*	658
	Pergesa acteus (Cramer, 1779)	--	--		6793
	Rhagastis mongoliana (Butler, [1876])	--	--		6451
	Rhodafra marshalli Rothschild & Jordan, 1903	--	--		3631
	Theretra alecto (Linnaeus, 1758)	AJ749419	658		7452
	Theretra capensis (Linnaeus, 1764)	--	--		6793

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)		All Genes (7451 bp)
	Xylophanes anubus (Cramer, 1777)	DQ276588	658	*	658
	Xylophanes ceratomoides (Grote & Robinson, 1867)	DQ276592	658	*	658
	Xylophanes chiron (Drury, 1773)	DQ276607	629		7019
	Xylophanes falco (Walker, 1856)	--	--		1937
	Xylophanes porcus (Hübner, [1823])	AJ749417	640		7433
	Xylophanes tersa (Linnaeus, 1771)	DQ276815	629		7422
Dilophonotina	*Aellopos ceculus* (Cramer, 1777)	--	--		6793
	Aellopos tantalus (Linnaeus, 1758)	--	--		1936
	Aleuron choloroptera (Perty, [1833])	--	--		6793
	Callionima falcifera (Fabricius, 1775)	--	--		6793
	Cautethia spuria (Boisduval, [1875])	--	--		6793
	Enyo gorgon (Cramer, 1777)	DQ276070	658	*	658
	Enyo lugubris (Linnaeus, 1771)	DQ276082	658	*	658
	Enyo ocypete (Linnaeus, 1758)	DQ276101	658		7452
	Erinnyis alope (Drury, 1773)	DQ276108	658	*	658
	Erinnyis crameri (Schaus, 1898)	DQ276110	583	*	583
	Erinnyis ello (Linnaeus, 1758)	DQ276125	658		7452
	Erinnyis obscura (Fabricius, 1775)	DQ276127	599	*	599
	Eupyrrhoglossum sagra (Poey, 1832)	DQ276232	658		7317
	Hemeroplanes ornatus Rothschild, 1894	DQ276233	658		7452
	Isognathus rimosa (Grote, 1865)	DQ276246	658		7133

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)		All Genes (7451 bp)
	Kloneus babayaga Skinner, 1923	DQ276250	658		3746
	Madoryx bubastus (Cramer, 1777)	DQ276251	658	*	658
	Madoryx plutonius (Hübner, [1819])	DQ276256	658		7317
	Nyceryx coffaeae (Walker, 1856)	DQ276376	658	*	658
	Nyceryx magna (R. Felder, [1874])	DQ276382	658		6954
	Nyceryx tacita (Druce, 1888)	DQ276389	658	*	658
	Oryba kadeni (Schaufuss, 1870)	DQ276418	658		6351
	Pachygonidia drucei (Rothchild & Jordan, 1903)	DQ276419	658	*	658
	Pachygonidia ribbei (Druce, 1881)	DQ276430	658	*	658
	Pachygonidia subhamata (Walker, 1856)	DQ276435	658		7452
	Pachylia ficus (Linnaeus, 1758)	DQ276451	658		7452
	Pachylia syces (Hübner, [1819])	DQ276466	658	*	658
	Pachylioides resumens (Walker, 1856)	DQ276475	658		7452
	Perigonia ilus Boisduval, 1870	DQ276496	658		7452
	Phryxus caicus (Cramer 1777)	DQ276509	291	*	291
	Pseudosphinx teretrio (Linnaeus, 1771)	DQ276521	658		7317
	Stolidoptera tachasara (Druce 1888)	DQ276524	658	*	658
	Unzela japix (Cramer, 1776)	DQ276539	658		6220
Hemarina	*Cephonodes hylas* (Linnaeus, 1771)	--	--		6793
	Hemaris diffinis (Boisduval, 1836)	--	--		6793
	Hemaris diffinis (Boisduval, 1836)	--	--		5269

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)	All Genes (7451 bp)
Macroglossini	*Acosmerycoides harterti* (Rothschild, 1895)	--	--	6793
	Acosmeryx naga (Moore, [1858])	--	--	5425
	Ampeophaga dolichoides (R. Felder, [1874])	--	--	5476
	Ampelophaga rubiginosa Bremer & Grey, 1853	--	--	6316
	Amphion floridensis Clark, 1920	--	--	3631
	Angonyx testacea (Walker, 1856)	--	--	6401
	Arctonotus lucidus (Boisduval, 1852)	--	--	6793
	Clarina kotschyi (Kollar, [1849])	--	--	6012
	Daphnis nerii (Linnaeus, 1758)	--	--	6562
	Darapsa myron (Cramer, 1779)	--	--	6407
	Deidamia inscriptum (Harris, 1839)	AF549805	618	7180
	Elibia dolichus (Westwood, 1847)	--	--	5827
	Enpinanga borneensis (Butler, 1879)	--	--	5827
	Eupanacra regularis (Butler, 1875)	--	--	4057
	Euproserpinus phaeton Grote & Robinson 1865	--	--	6427
	Gnathothlibus erotus (Cramer, 1777)	--	--	4483
	Macroglossum stellatarum (Linnaeus, 1758)	--	--	6770
	Neogurelca himachala (Butler, [1876])	--	--	5415
	Nephele accentrifera (Palisot de Beauvois, [1821])	--	--	5511
	Proserpinus clarkiae (Boisduval, 1852)	AF173394	658	7452
	Proserpinus terlooii Edwards 1875	--	--	5814
	Sphecodina abbottii (Swainson, 1821)	AF549799	618	7411

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)	All Genes (7451 bp)
	Sphingonaepiopsis gorgoniades (Hübner, [1819])	--	--	5089
	Temnora eranga (Holland, 1889)	--	--	3909
Philampelini	*Eumorpha achemon* (Drury, 1773)	--	--	5842
	Eumorpha pandorus (Hübner, [1821])	--	--	1937
	Eumorpha typhon (Klug, 1836)	--	--	2510
Smerinthinae				
Ambulicini	*Adhemarius daphne* (Boisduval, 1875)	--	--	6793
	Ambulyx schauffelbergeri Bremer & Grey, 1853	--	--	5842
	Amplypterus mansoni (Clark, 1924)	--	--	2682
	Amplypterus panopus (Cramer, 1779)	--	--	6658
	Protambulyx euryalus Rothschild & Jordan, 1903	--	--	6793
Smerinthini	*Afroclanis calcareus* (Rothschild & Jordan, 1907)	--	--	6427
	Amorpha juglandis (J. E. Smith, 1797)	--	--	6793
	Andriasa contraria Walker, 1856	--	--	4283
	Callambulyx tatarinovii (Bremer & Grey, 1853)	--	--	6451
	Chloroclanis virescens (Butler, 1882)	--	--	6793
	Clanis bilineata (Walker, 1866)	--	--	6658
	Cypa decolor (Walker, 1856)	--	--	6793
	Daphnusa ocellaris Walker, 1856	--	--	4560
	Langia zenzeroides Moore, 1872	--	--	6658

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)	All Genes (7451 bp)
	Laothoe populi (Linnaeus, 1758)	--	--	6793
	Likoma apicalis Rothschild & Jordan, 1903	--	--	6427
	Marumba quercus ([Denis & Schiffermuller], 1775)	--	--	6230
	Mimas tiliae (Linnaeus, 1758)	--	--	6549
	Neoclanis basalis (Walker, 1866)	--	--	4179
	Neopolyptychus compar (Rothschild & Jordan, 1903)	--	--	6793
	Pachysphinx modesta (Harris, 1839)	DQ276521	618	7452
	Pachysphinx occidentalis (Edwards, 1875)	--	--	6793
	Paonias excaecata (J. E. Smith, 1797)	AF549800	618	7452
	Paonias myops (J. E. Smith, 1797)	AF549798	618	6676
	Parum colligata (Walker, 1856)	--	--	5401
	Phyllosphingia dissimilis (Bremer, 1861)	--	--	6562
	Polyptychoides digitatus (Karsch, 1891)	--	--	6793
	Polyptychus andosa (Walker, 1856)	--	--	6058
	Pseudoclanis postica (Walker, 1956)	--	--	6793
	Smerinthus cerisyi Kirby, 1837	AF549799	618	7452
	Smerinthus saliceti (Boisduval, [1875]	--	--	5476
	Viriclanis kingstoni Aarvik, 1999	--	--	6793
Sphingulini	*Dolbina tancrei* Staudinger, 1887	--	--	6793
	Hopliocnema brachycera (Lower, 1897)	--	--	6733
	Kentrochrysalis consimilis Rothschild & Jordan, 1903	--	--	6793

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)		All Genes (7451 bp)
Sphinginae					
Acherontiini	*Acherontia styx* Westwood, 1847	--	--		6793
	Agrius cingulata (Fabricius, 1775)	--	--		6366
	Coelonia fulvinotata (Butler, 1875)	--	--		4369
	Megacorma obliqua (Walker, 1856)	--	--		6019
Sphingini	*Ceratomia amyntor* (Geyer, [1835])	AF549807	618		1669
	Ceratomia catalpae (Boisduval, [1875])	--	--		6793
	Cocytius duponchel (Poey, 1832)	DQ276040	658		7452
	Cocytius antaeus (Drury, 1773)	DQ276024	658	*	658
	Dolba hyloeus (Drury, 1773)	--	--		6793
	Dovania poecila Rothschild & Jordan, 1903	--	--		4283
	Euryglottis dognini Rothschild, 1896	--	--		6773
	Isoparce cupressi (Boisduval ,[1875])	--	--		5692
	Lapara bombycoides Walker, 1856	AF549802	617	*	617
	Lapara coniferarum (J. E. Smith, 1797)	--	--		6793
	Macropoliana natalensis (Butler, 1875)	--	--		5565
	Manduca florestan (Stoll, 1782)	DQ276294	658		3437
	Manduca muscosa (Rothschild & Jordan, 1903)	DQ276310	658		3572
	Manduca occulta (Rothchild & Jordan, 1903)	DQ276321	658	*	658
	Manduca quinquemaculatus (Haworth, 1803)	--	--		6624
	Manduca rustica (Fabricius, 1775)	DQ276345	658	*	658

Ingroup Taxa		COI Genbank Acc.#	COI (658 bp)		All Genes (7451 bp)
	Manduca sexta (Linnaeus, 1763)	U09843	658		7452
	Meganoton analis (R. Felder, [1874])	--	--		6658
	Neococytius cluentius (Cramer, 1775)	DQ276367	658		7452
	Paratrea plebja (Fabricius, 1777)	--	--		6793
	Psilogramma increta (Walker, [1865])	--	--		6793
	Sphinx caligineus (Butler, 1877)	--	--		6793
	Sphinx canadensis Boisduval, [1875]	AF549806	617	*	617
	Sphinx chersis (Hübner, [1823])	--	--		1937
	Sphinx dollii Neumoegen, 1881	--	--		6793
	Sphinx istar (Rothschild & Jordan, 1903)	--	--		6793
	Sphinx kalmiae J. E. Smith, 1797	--	--		6793
	Sphinx merops Boisduval, 1870	DQ276522	658		7026
	Spinx poecila Stephens, 1828	AF549801	617	*	617
	Xanthopan morganii (Walker, 1856)	--	--		4283

Outgroups	**COI Genbank Acc.#**	**COI (658 bp)**	**All Genes (7451 bp)**
Bombycidae: Bombycinae: Bombycini: *Bombyx mori* (Linnaeus 1758)	AF027953	557	7452
Phiditiinae: *Phiditia sp.*	--	--	5842
Primostictinae: *Oberthueria formosibia* (Matsumura 1927)	--	--	3312
Brahmeidae: *Acanthobrahmaea europaea* (Hartig 1963)	--	--	6502
Carthaeidae: *Carthaea saturnioides* Walker 1858	--	--	3332
Endromidae: *Endromis versicolora* (Linnaeus 1758)	--	--	5998
Eupterotidae: *Apha aequalis* (Felder 1874)	--	--	3312
Lasiocampidae: *Macrothylacia rubi* (Linnaeus, 1758)	--	--	6733
Lemoniidae: *Lemonia dumi* (Linnaeus 1761)	--	--	6716
Mirinidae: *Mirina christophi* (Staudinger 1887)	--	--	3332
Saturniidae: Cercophaninae: *Janiodes sp.*	--	--	6793

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