Molecular Phylogeny of Sesiidae (Lepidoptera) Inferred From Mitochondrial DNA Sequences[1]

Jackie A. McKern,[3] Allen L. Szalanski,[2,3] Donn T. Johnson,[3] and Ashley P. G. Dowling[3]

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ABSTRACT Partial DNA sequence data from the mitochondrial DNA (mtDNA) cytochrome oxidase I and II genes were used to construct a molecular phylogeny based on representative species from 10 of the 20 genera of Sesiidae. Maximum likelihood, maximum parsimony, and Bayesian analysis were utilized. Sequencing of a 606-base pair region of the mtDNA cytochrome oxidase I (COI), tRNA leucine, and COII gene revealed 271 polymorphic sites among 20 species. Genetic variation ranged from 0.8 to 21.2% among species. Maximum parsimony, maximum likelihood, and Bayesian analysis do not support the recent synonmy of *Synansphecia* as *Pyropteran*. Maximum parsimony and maximum likelihood support the recent divergence of *Synanthedon pamphyla* from *Synanthedon culciformis,* which are almost identical morphologicaly. Maximum likelihood, parsimony, and Bayesian analysis do not support the inclusion of *Melittia cucurbitae* in the Sesiinae subfamily. All analysis support *Synanthedon* included in the Sesiinae subfamily. All analysis also give support for *Vitacea* and *Paranthrene* forming the subfamily Paranthrenini. This is the first attempt to resolve relationships within Sesiidae with molecular data. Sesiidae are a divergent order of Lepidoptera in which many relationships should be examined more closely. Future studies should investigate nucleur markers to further support relationships supported by molecular data.

KEY WORDS COI, COII, 18S, Sesiidae, Lepidoptera

Larvae of many species of Sesiidae, the clearwing moths, are important pests in commercial nurseries, urban landscapes, timber stands, vineyards, and orchards (Nielson 1978). They cause economic loss by larval boring in stems and roots of herbaceous and woody plants. Most species are univoltine (requiring one year for development), but some require more than one year to develop. Sesiid species in the genera *Podosesia* Möschler, *Paranthrene* Hübner and *Synanthedon* Hübner cause economic loss to commercial nurseries and timber producers in the United States (Solomon et al. 1982). If not controlled, the peachtree borer, *Synanthedon exitiosa* (Say), and the lesser peachtree borer, *S. pictipes* (Grote and Robinson) can destroy entire orchards of fruit trees. Several species in the genera *Vitacea* Engelhardt, *Melittia* Hübner and *Pennisetia* Dehne can cause serious losses to various crops (Solomon & Dix 1979).

Morphological classification of Sesiidae has undergone several revisions since the 1960s (Naumann 1971, Bradley et al. 1972, Bradley & Fletcher 1974,

[1]Accepted for publication 18 September 2008.
[2]Corresponding author (aszalan@uark.edu).
[3]Department of Entomology, University of Arkansas, Fayetteville, Arkansas, USA.

Duckworth & Eichlin 1974, 1977, Hepner & Duckworth 1981). Naumann (1971) proposed two subfamilies: Tinthiinae with the tribes Tinthiini and Pennisetiini and Sesiinae with the tribes Sesiini, Melittiini, Paranthrenini and Aegeriini. Bradley et al. (1972) and Bradley & Fletcher (1974) proposed a third subfamily, Paranthreninae, which holds tribes, Paranthrenini and Synanthedonini. Duckworth & Eichlin (1974, 1977), Heppner & Duckworth (1981), and Eichlin & Duckworth (1988) agree that the subfamily Paranthreninae should be recognized, but without the tribe Synanthedonini, which is placed in the subfamily Sesiinae. Lastuvka & Lastuvka (2001) argue that the anagenetic changes in the tribe Paranthrenini are not distinct enough to require the establishment of a separate subfamily and follow Naumann's (1971) classification including only two subfamilies.

Eichlin & Duckworth (1988) placed 86 of the 123 described species of Sesiidae from America north of Mexico in the Synanthedonini, which accounts for 70% of the fauna. The 41 species of *Synanthedon* are morphologically grouped on the basis of similarities in genitalia. The subgroupings of *Synanthedon* correspond to several genera that were recognized by previous workers (Engelhardt 1946, Naumann 1971). Duckworth & Eichlin (1977) were convinced that these taxa had no concordance with other sesiid genera and were only defined by a few genetalic features. These character states often overlap from one taxon to another, so these genera were placed under *Synanthedon*. A molecular phylogenetic analysis could give insight among relationships within this family and should especially focus on the genus *Synanthedon*.

Like many Lepidoptera, Sesiidae use sex pheromones released by the female. Several species can be attracted to the same sex attractant (Payne et al. 1973), and cross-attraction sometimes occurs between males and females of different species (Comeau & Roelofs 1973). In cases where pheromone differences are not premating isolation mechanisms, other mechanisms exist, such as adult emergence on alternate years or different times of season, mating at different vegetational hosts or strata and geographic seperation (Sanders 1971, Brown 1972). Knowledge of the genetic relationships of sesiid species may help clarify the observed specificity of sex pheromones and observed behaviors.

In order to resolve difficulties in the classification of the family based on morphology, we tested molecular techniques to construct phylogenetic hypotheses based on DNA markers. The mitochondrial cytochrome oxidase I and II genes have been used extensively to infer phylogenetic relationships in insect families such as Drosophilidae (Simon et al. 1994), Tephritidae (Smith et al. 2003), Rhinotermatidae (Austin et al. 2004) and various families of Lepidoptera (Brower 1994, Sperling & Hickey 1994, Landry et al. 1999, Lange et al. 2004) and might be useful and appropriate for phylogenetic reconstruction of the lepidopteran family Sesiidae. Animal mitochondrial genes are known to evolve more rapidly than nuclear genes and are therefore good markers to analyze relatively close relationships, such as species relationships within a genus.

The phylogenetic relationships among members of Sesiidae and the amount of genetic variation among species were determined by 3 techniques using: the DNA sequences of the COI and COII genes; and mitochondrial DNA (mtDNA) sequence data. These data resulted in the formulation of a hypothesis of relationships and evolutionary history among genera and species of the family Sesiidae.

Materials and Methods

Sesiids were collected from four locations in Arkansas: Carrol Co., Faulkner Co., Madison Co., and Washington Co. during 2005 (Table 1). The moths were caught using commercially available pheromone lures placed in Trécé Pherocon IC wing traps (Trécé Inc., Adair, OK). The following lures were used for sesiid capture (abbreviations refer to lure type): oak borer (OB), *Paranthrene simulans* (Grote) and lilac borer (LCB), *Podosesia syringae* (Harris) lures from Scentry (Billings, MT); dogwood borer (DWB), *Synanthedon scitula* (Harris); lilac borer (LB); grape root borer (GRB), *Vitacea polistiformis* (Harris); lesser peachtree

Table 1. Sesiidae collection data including: species, sample number, and collection site.

Species name	Sample or accession #	Collection site city, county, state, country
Pennisetia marginata	1, 150, 148, 149	Conway, Faulkner Co., AR, USA
Vitacea polistiformis	131, 134	Conway, Faulkner Co., AR, USA
Paranthrene simulans	4, 5, 6	Fayetteville, Washington Co., AR, USA
P. simulans	14, 15	Berryville, Carroll Co., AR, USA
P. simulans	19	Conway, Faulkner Co., AR, USA
P. simulans	43	Fayetteville, Washington Co., AR, USA
P. simulans	79	Hindsville, Madison Co., AR, USA
P. simulans	99	Conway, Faulkner Co., AR, USA
Synanthedon pictipes	10, 11	Berryville, Carroll Co., AR, USA
S. pictipes	46, 87	Fayetteville, Washington Co., AR, USA
S. exitiosa	22, 73	Conway, Faulkner Co., AR, USA
S. scitula	25, 37	Fayetteville, Washington Co., AR, USA
S. scitula	49, 50	Berryville, Carroll Co., AR, USA
S. rileyana	36, 128	Fayetteville, Washington Co., AR, USA
S. rileyana	56, 57	Berryville, Carroll Co., AR, USA
S. rileyana	64	Conway, Faulkner Co., AR, USA
S. culiciformis	AY304170	Russia
S. culiciformis	AY304168	Germany
S. pamphyla	AY304169	Turkey
S. spheciformis	AJ862900	Austria
Podosesia syringae	29	Conway, Faulkner Co., AR, USA
P. syringae	127	Conway, Faulkner Co., AR, USA
Melittia cucurbitae	32, 33	Fayetteville, Washington Co., AR, USA
M. cucurbitae	68, 69	Conway, Faulkner Co., AR, USA
M. cucurbitae	77, 81	Hindsville, Madison Co., AR, USA
Chamaesphecia tenthrediniformis	AJ862898	Spain
Bembecia ichneumoniformis	AJ862897	Austria
B. uroceriformis	AJ862893	Greece
B. psoraleae	AJ862898	Spain
B. lomatiaeformis	AJ862899	Greece
Pyropteron chrysidiforme	AJ862901	Italy
P. minianiforme	AJ862902	Greece
Synansphecia kautzi	AJ862903	Spain

borer (LPTB), *Synanthedon pictipes* and greater peachtree borer (GPTB), *Synanthedon exitiosa* lures from Trécé Inc.; raspberry clear-wing borer (RCW), *Pennisetia hylaeiformis* (Laspeyres) and squash vine borer (SVB), *Melittia cucurbitae* lures from Pherobank (Wageningen, Netherlands); and raspberry crown borer (RCB), *Pennisetia marginata* (Harris) lure from IPM Tech. (Portland, OR).

Seven traps were located at the University of Arkansas Experiment Station (Fayetteville, Washington County, AR) with the following lures: OB, LCB, DWB, LB, RCW, GRB, SVB, and RCB. Five traps baited singly with the following lures were located at a commercial apple and peach orchard in Berryville, Carroll County, AR: LB, DWB, RCW, LPTB and GPTB. Nine traps baited singly with the following lures were located at a commercial apple, peach, and blackberry orchard in Conway, Faulkner County, AR: LCB, DWB, LB, RCW, GRB, SVB, RCB, GPTB, and LPTB. Two GRB lure baited traps were located at a commercial vineyard in Hindsville, Madison County, AR. Traps were placed in the field in May and checked weekly through September. After specimens were collected from traps, they were identified using morphological keys (Eichlin & Duckworth 1988), and stored in glass specimen tubes at −20°C until DNA extraction. Voucher specimens are deposited in the University of Arkansas Arthropod Museum, Fayetteville, AR.

DNA was extracted from the thoraces of individual specimens using the Puregene® DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 μL of Tris: EDTA (10 mm Tris-HCl, 1 mm EDTA, pH = 8.0) and stored at −20°C. Mitochondrial DNA PCR was conducted using primers C1-J-2797 (5′-CCTCGACGTTATTCAGATTACC-3′) (Simon et al. 1994) and C2-N-3400 (5′-TCAATATCATTGATGACCAAT-3′) (Taylor et al. 1997). These primers amplify approximately 606 bp of the mtDNA cytochrome oxidase I gene (COI), tRNA-leu and cytochrome oxidase II gene (COII). PCR reactions were conducted using 2 μL of the extracted DNA. The thermal cycler profile for this region of mtDNA gene consisted of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 45 s per Szalanski et al. (2000). Excess dNTP's and primers were removed and the amplified DNA concentrated using minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to the University of Arkansas Medical School Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Sequence data were deposited in GenBank accession numbers DQ205539 to DQ205573.

The sesiid mitochondrial COI/COII sequences were initially aligned using Clustal W (Thompson et al. 1994) and subsequently refined by eye using BioEdit 5.89 (Hall 1999). Only 606 bp unambiguously aligned positions were used for analyses. The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution. Mitochondrial DNA sequences from the following sesiids were added from GenBank (Table 1): *Synanthedon culiciformis* (Linnaeus), *S. pamphyla* (Kallies), *Chamaesphecia tenthrediniformis* (Denis and Schiffermüller), *Bembecia ichneumoniformis* (Denis and Schiffermüller), *B. uroceriformis* (Treitschke), *B. psoraleae* (Bartsch and Bettag), *B. lomatiaeformis* (Lederer), *Pyropteron chrysidiforme* (Esper), *P. minianiforme* (Freyer), *Synansphecia kautzi* (Reisser), and *Synanthedon spheciformis* (Denis and Schiffermüller). DNA sequences were aligned using Clustal W (Thompson et al. 1994).

For model based phylogenetic analyses (i.e., Maximum Likelihood, Bayes) the best-fitting nucleotide substitution model was chosen according to the GTR+Γ model as selected from 64 different models using ModelTest v 3.7 (Posada & Crandall 1998) and PAUP* 4.0b10 (Swofford 2001). Phylogenetic analyses was conducted with maximum likelihood (ML) analysis using the best-fitting evolutionary model in PAUP*. Maximum likelihood bootstrapping was performed using stepwise addition (1000 replicates) to determine the reliability of obtained topologies. Phylogenetic trees were also obtained using Bayesian inference with the GTR+Γ model using MrBayes. There were 2 million generations with trees saved every 100 generations, and the split frequency distribution value used as a test for convergence of parameters was 25% to determine the number of trees discarded as burnin (5000). Unweighted parsimony (MP) analyses on the alignments were conducted using PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing data and 11 random addition sequences was used. A bootstrap test was used to test the reliability of trees (Felsenstein 1985). Parsimony bootstrap analysis included 1000 resamplings by using stepwise addition PAUP*. *Proserpinus clarkiae* (Boisduval) (Lepidoptera: Sphingidae), was used as the outgroup taxon.

Results

DNA sequencing of the mtDNA amplicon resulted in an average amplicon size of 606 bp. Nucleotide positions 1–220 were COI, 221–288 tRNA-leu, and 289–606 were COII. The aligned data matrix, including the outgroup taxon resulted in a total of 653 characters. Of these, 382 (59%) were fixed, 40 (6%) were phylogenetically uninformative, and 231 (35%) were phylogenetically informative.

The data set produced one most-parsimonious tree (Fig. 1) length = 811, CI = 0.446 as documented using a heuristic search in PAUP*, with 4 distinct groups. Maximum likelihood analysis recovered an optimal ML tree −ln likelihood = 6032 with nucleotide frequencies of A = 40%, C = 10%, G = 4% and T = 47%. Bootstrap ML analysis of the aligned sesiids and the outgroup taxon resulted in a consensus tree with many branches supported by values >50. The ML tree resolved four distinct groups similar to those found with the MP analysis. Bayesian analysis of the dataset also converged on four groups, although group 3 changed positions to share a node with group 1 (Fig. 2).

Pairwise Tajima Nei distances (Tajima & Nei 1984) within Sesiidae for mtDNA sequences ranged from 0.8% between *Synanthedon pamphyla* and *S. culiciformis* to 20.9% between *Pennisetia marginata* and *S. culiciformis* (Table 2). Within *Synanthedon*, genetic variation ranges from 0.8% between *S. pamphyla* and *S. culiciformis* to 11.9% between *S. rileyana* and *S. spheciformis*. Divergence between *P. marginata* and all other genera ranged between 14.1% (*Paranthrene simulans* and *Melittia cucurbitae*) to 21.2% (*Synanthedon spheciformis*). Divergence among *Bembecia* genera and other sesiid genera ranged from 8.9% (*Synansphecia kautzi*) to 19.8% (*Vitacea polistiformis*). *Vitacea polistiformis* divergence when compared to other sesiid genera ranged from 7.1% (*P. simulans*) to 19.8% (*Bembecia psoraleae*). *Melittia Cucurbitae* divergence when compared to other sesiid genera ranged from 12.5% (*P. simulans*) to 16.3% (*Synanthedon culiciformis*). *Chamaesphecia tenthrediniformis* divergence compared to other

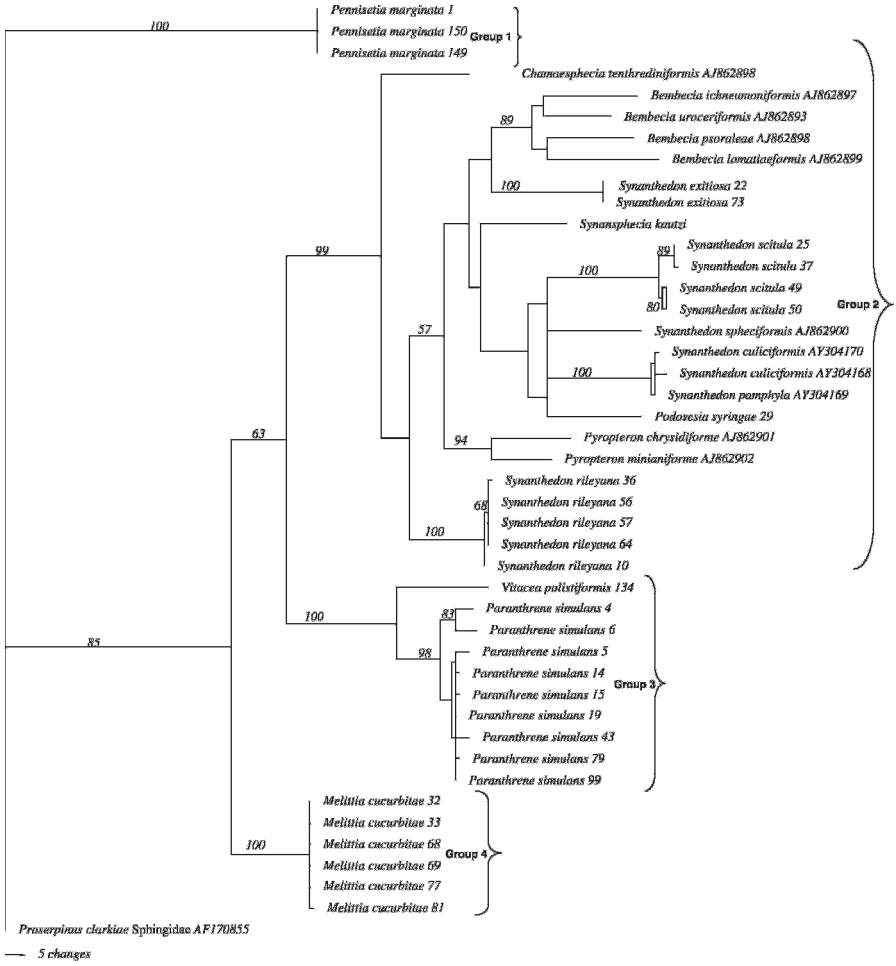

Fig. 1. Maximum parsimony phylogram of 10 genera of Sesiidae. Bootstrap values for 1000 replicates are listed above the branches supported at ≥50%.

sesiid genera ranged from 9.1% (*Synanthedon rileyana*) to 17.6% (*Pennisetia marginata*). *Pyropteron* divergence ranged from 7.9% (*Synansphecia kautzi*) to 19.2% (*P. marginata*) when compared to other genera of Sesiidae. *Synansphecia kautzi*'s divergence when compared to other sesiid genera ranged from 7.9% (*P. chrysidiforme*) to 18.2% (*P. marginata*). Pairwise Tajima Nei distances compared among Sesiids and the outgroup taxon *Proserpinus* ranged from 13.6 to 19.3%.

Discussion

This study represents the first attempt to address the phylogenetic relationships within the clearwing moth family Sesiidae at the molecular level. Most of the inferred relationships had strong quantitative support as determined by bootstrap analyses (Figs. 1 and 2). The relationships among genera inferred

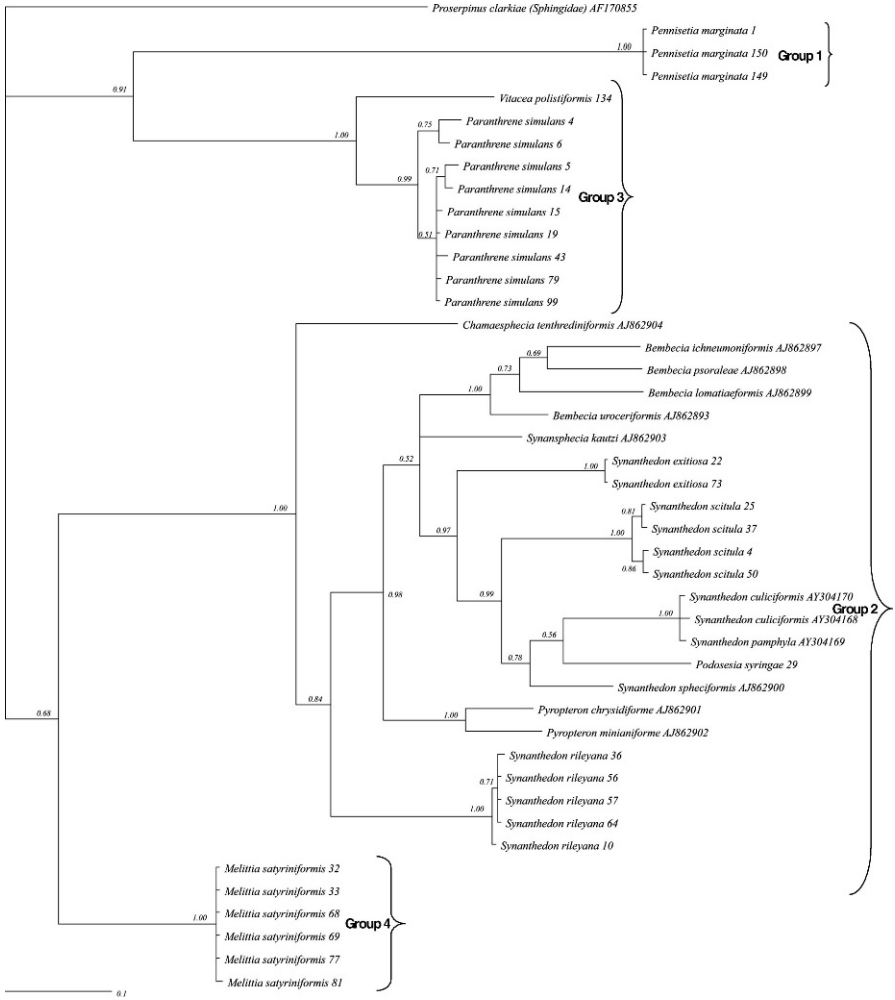

Fig. 2. Phylogram obtained by Bayesian analysis for 10 genera of Sesiidae. Posterior bootstrap values are listed above the branches supported at ≥50%.

from maximum parsimony and maximum likelihood analysis raised many questions about relationships among Sesiidae and gave support for many morphologicaly established relationships.

Four groups were resolved within Sesiidae. Group 1 consisted of sequences from a single species *Pennisettia marginata*. There was a clear delimination between group 1 and all other groups supported by MP, ML, and Bayesian analysis (Figs. 1 and 2). Maximum parsimony analysis did not include this group in monophyletically with the other groups (Fig. 1). This group is the most divergent compared to the other groups and its branching basal to other sesiids may be do to long branch attraction artifact (where the most divergent sequences tend to branch together) (Lartillot et al. 2007). Sequences from other *Pennisettia*

Table 2. Tajima-Nei pairwise distances within and among 20 species of Sesiidae.

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*P. marginata*	0.3																			
2	*C. tenthrediniformis*	17.6	–																		
3	*B. ichneumoniformis*	19.7	11.6	–																	
4	*B. uroceriformis*	19.6	11.4	7.5	–																
5	*B. psoraleae*	19.7	13.6	8.2	8.0	–															
6	*B. lomatiaeformis*	18.7	12.3	9.5	8.8	10.0	–														
7	*P. chrysidiforme*	19.0	11.6	10.0	10.1	12.5	12.6	–													
8	*P. minianiforme*	19.2	11.1	11.6	9.4	12.0	12.7	6.9	–												
9	*S. kautzi*	18.2	10.5	9.3	8.9	9.6	11.5	7.9	8.8	–											
10	*V. polistiformis*	16.4	16.3	18.0	18.0	19.8	19.1	15.1	16.3	16.6	–										
11	*P. simulans*	14.1	14.8	17.6	18.1	19.4	18.3	16.3	16.5	16.7	7.1	1.4									
12	*S. pictipes*	19.0	10.0	10.4	10.3	12.4	11.6	10.1	10.8	9.2	16.8	16.5	3.5								
13	*S. exitiosa*	17.7	10.1	11.2	10.3	11.7	11.7	11.1	11.1	10.0	17.8	16.3	8.0	0.0							
14	*S. scitula*	19.5	16.2	12.0	12.1	12.8	13.2	11.9	12.3	10.5	17.8	18.3	9.1	10.8	0.8						
15	*S. rileyana*	16.5	9.1	11.6	10.2	12.1	11.9	10.1	10.1	9.4	15.1	14.3	8.8	11.0	11.3	0.2					
16	*S. culiciformis*	20.9	10.6	12.0	10.8	12.2	14.8	11.3	12.8	9.7	18.8	18.8	10.7	10.3	11.1	10.9	0.9				
17	*S. pamphyla*	20.7	10.9	11.9	10.7	11.8	14.4	11.3	12.7	9.5	18.6	18.7	10.6	9.9	11.2	10.8	0.8	–			
18	*S. spheciformis*	21.2	12.1	11.9	11.3	12.4	13.3	12.4	11.6	11.2	18.5	18.7	9.1	9.1	10.3	11.9	9.4	9.4	–		
19	*P. syringae*	18.8	10.7	11.6	10.9	12.3	12.6	10.6	9.9	9.7	17.1	17.0	8.4	9.3	9.7	9.8	10.4	10.1	9.8	–	
20	*M. cucurbitae*	14.1	12.8	14.8	14.2	16.6	15.3	14.2	14.1	14.3	13.7	12.5	13.3	13.6	14.1	12.6	16.3	16.2	15.0	14.1	0.1

species should be added in the future, which may break the long-branch leading to this taxon and help get rid of possible long-branch attraction artifact. This divergence is also supported by pair-wise differences with divergence between *P. marginata* and all other genera ranging between 14.1% (*P. simulans* and *M. cucurbitae*) to 21.2% (*S. spheciformis*) (Table 2).

Pennisettia marginata has a narrow host range feeding on only *Rubus* species. *Rubus* is an ancient group of plants with fossils dating back to the Eocene (55.8–33.9 million years ago) (Devore & Pigg 2006). Since the host plants are quite ancient, it is not far fetched to believe that *P. marginata* could be a more historic genus than others in Sesiidae. A small amount of variation was found among the three *P. marginata* specimens with 0.3% divergence.

Group 2 included all genera grouped in the subfamily Sesiinae. This group was robustly supported in MP, ML, and Bayesian analysis (Figs. 1 and 2). Some relationships supported by high bootsrap values conflict with current morphological classification. Lastuvka & Lastuvka (2001) synonymized *Pyropteran* and *Synansphecia* because no clear distinguishing morphological characteristics can be found between these two genera. In all optimal trees *Synansphecia* and *Pyropteron* never branch together. It appears unlikely that they share a most recent common ancestor exclusive of all other taxa. Calculations of pair-wise genetic divergence between *S. kautzi* and *P. chrysidiforme* is 7.9% divergent, and *S. kautzi* and *P. minianiforme* is 8.8% divergent which gives support to the findings.

Group 2 is monophyletic and strongly supported and composed of 6 genera. This was reconstructed in MP, ML, and Bayesian analysis (Figs. 1 and 2). It is interesting to note that all *Chamaesphecia* species utilize a unique host plants in the *Euphorbia* genus (Lastuvka & Lastuvka 2001). No other sesiids represented in the data set feed on these host plants. Many of these plants have a sap that is very toxic to herbivores. This association could have caused coevolution of *C. tenthrediniformis* with its host plant, which could convolute phylogenetic analysis. From all trees *C. tenthrediniformis* is the most evolutionary divergent from all other taxa in group 2.

In group 2, the MP, ML, and Bayesian analysis did not resolve the relationship of *S. pamphyla* and *S. culiciformis* (Figs. 1 and 2). Divergence data supports that *S. pamphyla* is probably a synonym of *S. culiciformis* with only 0.8% divergence between the species (Table 2). If *S. pamphyla* is a distinct species within the *Synanthedon* genus it has less divergence among sister species than any other genera in this study, which is unlikely due to the large amount of genetic variation shown between all other species in the same genus. The two *S. culiciformis* sequences were found to be 0.9% divergent, which is slightly greater than 0.8% divergent when *S. culiciformis* is compared to *S. pamphyla*. Kallies (2003) found divergence between *S. pamphyla* and *S. culiciformis* to range between 0.8–1% using mtDNA.

Synanthedon culiciformis has been described as very similar to *S. pamphyla* with very similar genitalia. Although, external morphological differences have been found to exist with *S. pamphyla* having: a broader discal spot, smaller ETA of forewing, broader apical area, opaque cell between Cu1 and Cu2, absence of red scales at the forewing base, black labial palps, black legs, a different color of the abdomen, and larger size (Kallies 2003). Kallies (2003) applied a molecular clock to find out the corresponding age of the separation of *S. culiciformis* and *S.*

pamphyla, which was estimated at 300 to 500,000 y, which may explain the small number in divergence and unresolved molecular phylogeny.

Group 3 includes *Vitacea polistiformis* and *Paranthrene simulans*, which are both grouped within the subfamily Paranthreninae first established by Bradley et al. (1972) then modified by Duckworth & Eichlin (1974, 1977) and Heppner & Duckworth (1981). This relationship is supported by MP, ML, and Bayesian analysis (Figs. 1 and 2).

The position of group 3 varied depending on the method of phylogenetic analyses. The Paranthreninae are embedded in a clade with groups 2 and 4 in MP and ML trees while they strongly branch with *Pennisetia* (group 1), to the exclusion of groups 2 and 3 in Bayesian analyses. These branchings of MP and ML are supported by a morphologically-based classification scheme proposed by Lastuvka & Lastuvka (2001) (Fig. 1). Bayesian analysis supports the classification schemes proposed by Heppner, Eichlin & Duckworth (1988) in the establishment of a third subfamily Paranthreninae (Fig. 2). When divergence is considered *P. simulans* and *V. polistiformis* have a large amount of divergence compared to all other species in the other two subfamilies. This also gives support to the Paranthreninae subfamily.

Group 4 is represented by the single species *Melittia cucurbitae*, which is the only member of the tribe Melittiini in the subfamily Sesiinae (Figs. 1 and 2). Analysis of MP, ML, and Bayesian did not group *M. cucurbitae* within the other genera of the Sesiinae recovered in group 2 of all the analyses. This group branched basal to all other Sesiinae genera and may represent an early divergence. This species utilizes host plants in the family Cucurbitaceae, unlike other Sesiinae genera. In the subfamily Sesiinae, divergence ranged from12.5% (*M. cucurbitae*) to 19.8% (*B. psoraleae*). This is the greatest amount of divergence between tribes within the same subfamily thus supporting an earlier divergence.

Vitacea (group 3) and *Melittia* (group 2) share a node in the MP and ML analyses. Interestingly, these species respond to the same sex pheromones, (2E, 13Z)-2,13-octadecadien-1-ol acetate and (3Z, 13Z)-3,13-octadecadien-1-ol (Klun et al. 1990, Schwarz et al. 1983). This is supportive evidence that a common ancestor was recently shared. The divergence between these two species is 12.5%, which is the least amount of divergence between *Vitacea* and any other member outside of the Paranthrenini tribe (Table 2). The node shared by *Vitacea* and *Melittia* in MP and ML is also shared by the rest of the group 2 taxa, but the ancestral state could be a response to this pheromone with multiple pheromone switching in groups 3 and 4.

This study shows that Sesiidae have a large amount of genetic variation among species. Within *Synanthedon*, divergence ranges from 0.8–11.9% (Table 2). Landry et al. (1999) found that divergence among certain *Argyrotenia* species (Lepidoptera: Tortricidae), using the mitochondrial oxidase II gene, ranges from 2.6 to 9.3%. Sesiid divergence is slightly greater. Genetic divergence within *Coptotermes* termite species (Isoptera: Rhinotermitidae) ranges from 0.0–8.0% (Austin et al. 2004). Divergence within Sesiidae ranges from 0.8% between *S. pamphyla* and *S. culiciformis* to 21.2% between *P. marginata* and *S. spheciformis* (Table 2).

Host plant specificity could be leading to some of the variation and divergence found. Mitter & Futyuma (1978) studied the genetic consequences of feeding habits of some forest dwelling Lepidoptera and found that specialized feeders

(feeding on one family of host plants) have more genetic variation than generalized feeders (feeding on two or more families of host plants). Specialists could accumulate genetic variation due to local variation or lower migration rates in between environmental patches (Mitter & Futuyuma 1978).

Generalists could have a "homeostatic" mechanism that reduces the environmental variation perceived by loci (Mitter & Futuyuma 1978). If specialized species lacked this mechanism chemical changes and differences among host plants could maintain genetic variation that would not be seen in more generalized species. This hypothesis could account for the amount of genetic variation observed among sesiids.

Although many currently accepted morphologically-based classifications have been supported by our results, some taxa represented in this data set prompt as many questions as they answer. Sequence data from more genera and species of the Sesiinae subfamily, should be evaluated to strengthen or weaken molecular relationships. Future research should focus on resolving relationships within the entire family, and finding a nuclear marker or informative microsattellite loci. This study brings to light a wealth of interesting relationships to be studied within the group, especially those of each species with its host plant. A future study of mapping the host plant phylogenies on the sesiid phylogenies would be an interesting way to interpret relationships.

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