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Molecular Identification of Synanthedonini Members (Lepidoptera: Sesiidae) Using Cytochrome Oxidase I

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ABSTRACT Many North American sesiid moths within *Synanthodonini* have been studied extensively because their feeding activity can cause detrimental economic and esthetic impacts to many commercially important ornamental and native plant species. Recent discoveries of nonnative clearwing moth pest introductions [e.g., *Synanthedon myopaeformis* (Borkh.)], reinforce the need for reliable and accurate molecular diagnostic tools that can be used by nontaxonomic experts, particularly when juvenile life stages are recovered from infested host-plant tissues. Cytochrome oxidase I (cox I) previously has been used to successfully identify species and resolve species complexes. In this study, the cox I phylogeny inferred from sequences generated from 21 species of sesiid moths classified within *Synanthedonini* confirms the close evolutionary relationship between sesiid species. As other authors have suggested in previous works, we observed that *Synanthedon rileyana* H. Edwards appears atypical for the genus, as it paired with *Carmenta bassiformis* (Walker) one node removed from, but not sister to, a large well-supported *Synanthedon*-rich clade. *Sannina uroceriformis* Walker and *Podosesia* Möschler were observed nested deeply within the aforementioned well-supported clade (posterior probability [PP] of clade = 100) comprised of all *Synanthedon* species sampled, except *S. rileyana*. Placement of these two taxa conflicts with results from previous morphological studies. These placements were immune from repeated attempts to delete perceived nearby long branches within the data set. Despite these few conflicts and overall low statistical support for most interspecific and higher relationships, our data suggest that all species examined possess unique genetic signatures that lend themselves to accurate identification of all life history stages of these clearwing pests.

KEY WORDS Lepidoptera, clearwing moth, barcoding, DNA fingerprinting, woodborer

The tribe Synanthedonini (Lepidopterea: Sesiidae) was established by Niculescu 1964 based on adult morphological characters. The taxonomic division was supported in a subsequent revision of Sesiidae by using larval characters (MacKay 1968). Later, Naumann (1971) included *Synanthedon* and other closely-related genera in a tribe he called *Aegeriini*, now considered a synonym of *Synanthedonini* (Bradley et al. 1972). *Synanthedonini* can be separated from all other North American tribes in Sesiidae by using wing venation. With over 87 species described, *Synanthedonini* is considered to be the most species-rich sesiid tribe in North America (Eichlin and Duckworth 1988). The economic importance attributed to many of its members has made these moth species subjects of research that has elucidated life-history details and effective methods of control (Solomon and Dix 1979). Immature stages (i.e., eggs, larvae, and pupae), which are often encountered after feeding injury on plants first is observed, present particular difficulties for professionals charged with pest control and regulatory action because morphological characters required for accurate species identification often do not exist for these life-stages, or may only be apparent to a taxonomic expert.

Larvae of several species within the genus *Synanthedon* feed on fruit trees as well as native and nonnative ornamental plants. For example, larval feeding by *S. exitiosa* (Say) and *S. pictipes* (Grote and Robinson), results in root, trunk, and branch damage that consequently reduces fruit yield and can kill host plants. Likewise, the dogwood borer, *S. scitula* (Harris), is an increasingly important pest management challenge in apple orchards, particularly where size-controlling rootstocks are used that result in borer-susceptible burr knots on stems, trunks, and graft unions (Leskey and Bergh 2005). In addition to apple and dogwood trees, the dogwood borer has perhaps the widest host plant range within *Synanthedon.* Its geographic distribution extends across the eastern United States and to disjunct populations in the west-

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ern states of Colorado and Washington (Meyer et al. 1988, Bergh et al. 2009, E. H. LaGasa, personal communication). In addition to nonnative clearwing moth introductions to North America during the past 150 yr, the recent introduction of *S. myopaeformis* (Borkh.) from Europe reinforces the need for accurate molecular diagnostic tools to aid with identification of clearwing pests, regardless of life stage (Eichlin and Duckworth 1988, Philip 2006).

Mitochondrial genes are helpful diagnostic tools frequently used to quickly and reliably identify pest species (Hebert et al. 2003, Armstrong and Ball 2005). Combining gene sequence data with traditional morphological models eliminates sole reliance for taxonomic differentiation by using morphological characters that are often obscured or lost entirely by rough handling, storage, and transport of specimens. Careful dissection of damaged adult clearwing specimens caught and preserved within pheromone traps can yield sufficient genetic material to allow subsequent species identification. Indeed, unique species-specific nucleotide arrangements within the mitochondrial genome have been used in several cases where morphological characters are either hard to discern or unavailable (Ball and Armstrong 2006, Nwilene et al. 2006, Foottit et al. 2008). High quality, multicopy mitochondrial DNA is much easier to obtain than genetic information contained within lower copy nuclear genes, thus enabling successful amplification even when specimens are decades old (Gilbert et al. 2007).

Despite the economic importance of many species within the tribe, little is known about inter- or intraspecific genetic diversity among members of *Synanthedonini.* To date, mitochondrial sequences deposited in GenBank represent specimens from a limited geographical distribution in the United States and Turkey, or are either too broad or narrowly focused within the family to provide reliable analyses of species relationships within *Synanthedonini* (Kallies 2003, McKern and Szalanski 2008, McKern et al. 2008). Molecular diagnostics based on limited sampling may fail when individuals from more disjunct populations are analyzed. In addition, some generic relationships within the tribe *Synanthedonini* have been ambiguous because of overlapping and intermediate morphological characters. These morphological challenges have led some taxonomists in the past to erect genera, further dividing the tribe (Engelhardt 1946, MacKay 1968). For example some species of *Synanthedon* and *Carmenta* have been particularly difficult to place at the generic level.

The main objective of this research is to use a particularly informative region of cox I to provide an alternate but reliable means of identifying moths in the tribe *Synanthedonini* based upon analyses of 25 distinct North American clearwing species collected in disparate geographical areas, insofar as distribution allows, in addition to 29 comparable GenBank sequences available from specimens collected in the Palearctic region.

Materials and Methods

Taxon Sampling. Several well-documented clearwing moth pests were specifically targeted, including *S. exitiosa*, *S. pictipes*, *S. scitula*, *S. viburni* Engelhardt, and *S. fatifera* Hodges. Species-appropriate pheromone lures and light trapping were used to attract male moths of desired taxa (Table 1). Where possible, modified Multipher-1 traps (Bio-Contrôle Ste-Foy, QC, Canada) were located within habitats where host-plant resources were known to occur. Traps were mounted ≈1 m (3.3 ft.) high on stands sited in partial noontime shade. Approximately 200 ml of ethanol was placed in a reservoir that was attached to the trap funnel part with hot glue. Collection periods were for durations not to exceed 7 d between reservoir refills. Pheromone lures were replaced at 6–8-wk intervals. All moths were held and shipped to the University of Tennessee in Knoxville in vials containing 95% nondenatured ethanol. In the lab, species were identified using the descriptive keys of Eichlin and Duckworth (1988), then stored at −20°C until DNA was extracted. Specimen-collection data and the number of species from each site are reported (Table 1). Pinned voucher specimens have been placed in the Entomology and Plant Pathology insect museum collection at the University of Tennessee. Voucher specimens collected within the Great Smoky Mountain National Park have been included with the park's museum reference collection. DNA vouchers are stored at −20°C in J. K. Moulton's laboratory at the University of Tennessee.

DNA Extraction, Polymerase Chain Reaction Amplification, Sequencing, and Analyses. As specimens allowed, legs, head capsule, or thorax tissues were used to extract total DNA from individual moths by using a phenol-chloroform based method (Moulton and Wiegmann 2004). Polymerase chain reaction (PCR) was carried out using the Ex *Taq* Hot-start PCR Kit (TaKaRa Bio Inc., Shiga, Japan) by using the manufacturer recommendations for a 50-μl reaction. ≈700 bp of the cox I gene was amplified with the following forward and reverse primers:

5′-ATAATYGGRGGATTTGGWAAYTG and 3′-GTTARTCCNCCYACWGTRAA (J.K.M., unpublished data). Each reaction was performed with 1 μl of template DNA. After an initial 2 min denaturing step at 94°C, the following touchdown PCR was performed: four cycles of 30 s at 94°C, 20 s at 57°C, then 90 s at 72°C, followed by 14 cycles of 30 s at 94°C, 15 s at 53°C, then 90 s at 72°C, and finished with 33 cycles of 30 s at 94°C, 15 s at 47°C then 90 s at 72°C, and at 72°C for 7 min.

Amplicons were electrophoresed and excised from agarose gels, then purified using silica spin columns. Purified PCR products served as templates for sequencing reactions by using the PCR primers. Templates were sequenced in both directions with BigDye v3.1 terminators (Applied Biosystems, Carlsbad, CA) in 1/8 or 1/16 reactions by using BetterBuffer (The Gel Company, San Francisco, CA). Dye terminator sequencing reactions were cleaned using Centri-sep purification columns (Princeton Separations, Adelphia, NJ), electrophoresed through a 6% polyacryl-

Table 1. Taxonomic authorities, geographic collection data, and pheromone lures used to trap clearwing moth adults for analyses

Species (taxonomic authority)	Collection location	Lure	GenBank accession no.
Melittia cucurbitae (Harris)	Ramsey County, MN (1)	PB-SVB (APTIV, Portland, OR)	HQ341467
Paranthrene simulans (Grote)	Knox County, TN (1)	GPTB (Trécé, Adair, OK)	HQ341460
Vitacea polistiformis (Harris)	Haywood County, NC (1)	Dogwood borer (Zhang et al. 2005)	HQ341435
Osminia ruficornis (Hy. Edwards)	Cherokee County, KS (3)	LPTB (Trécé, Adair, OK)	HQ341397
A. carolinensis	Knox County, TN (2)	L997 (Sentry Biologicals Inc., Billings, MT)	HQ341427
			HQ341456
C. bassiformis	Bourbon County, KS (1)	Dogwood borer (Zhang et al. 2005)	HQ341458
	Knox County, TN (1)		HQ341437
S. rileyana	Bourbon County, KS (1)	PB-SYVE (APTIV, Portland, OR)	HQ341408
	Knox County, TN (2)		HQ341413
	Anderson County, TN (1)		HQ341421
	Jefferson County, WV (2)		
	Henderson County, NC (1)		
S. tipuliformis	Hennepin County, MN (3)	PB-SYTI (APTIV, Portland, OR)	HQ341439
		CCWM (Trécé, Adair, OK)	
S. scitula	Ramsey County, MN (1)	Dogwood borer (Zhang et al. 2005)	HQ341399
	Ontario County, NY (4)		HQ341401
	Pearl River County, MS (2)		HQ341412
	Sevier County, TN (1)		HQ341418
	Knox County, TN (5)		HQ341419
	Fredrick County, VA (1)		HQ341420
	Bourbon County, KS (1)		HQ341422
	Henry County, IA (1)		HQ341433
	Peach County, GA (1)		HQ341443
	Warren County, TN (1)		HQ341444
	Jefferson County, WV (1)		HQ341446
			HQ341459
			HQ341469
			HQ341473
Synanthedon novaroensis (Hy. Edwards)	Thunder Bay, ON, Canada (2)	L103 (Sentry Biologicals Inc., Billings, MT)	HQ341406
Synanthedon rhododendri (Beutenmúller)	Knox County, TN (1)	GPTB (Trécé, Adair, OK)	HQ341396
	Sevier County, TN (2)		HQ341431
Synanthedon kathyae Duckworth and Echlin	Blount County, TN (2)	GPTB (Trécé, Adair, OK)	HQ341424
			HQ341451
Synanthedon sapygaeformis (Walker)	Dade County, FL (4)	LPTB (Trécé, Adair, OK)	HQ341410
			HQ341441
			HQ341464
Synanthedon fulvipes (Harris)	Thunder Bay, ON, Canada (2)	GPTB (Trécé, Adair, OK)	HQ341403
Synanthedon castaneae (Busck)	Haywood County, NC (3)	Raspberry crown borer (PheroTech, Inc., Delta, BC)	HQ341416
P. aureocincta	Dakota County, MN (4)	LILA (Trécé, Adair, OK)	HQ341428
P. syringae syringae	Anderson County, TN (1)	GPTB (Trécé, Adair, OK)	HQ341417
	Knox County, TN (2)	LILA (Trécé, Adair, OK)	HQ341455
P. syringae fraxini	Henry County, IA (3)	LILA (Trécé, Adair, OK)	HQ341442
(Color form of *P. syringae syringae*)	Sevier County, TN (1)		HQ341466
S. fatifera	Ramsey County, MN (2)	GPTB (Trécé, Adair, OK)	HQ341415
	Sevier County, TN (1)		HQ341431
	Anderson County, TN (2)		HQ341436
			HQ341447
S. viburni	MN: Ramsey County, MN (3)	L997 (Sentry Biologicals, Inc., Billings, MT)	HQ341395
	Hennepin County, MN (8)		HQ341407
	Wayne County, NY (4)		HQ341454
			HQ341461
			HQ341462
			HQ341472
S. acerrubri Engelhardt	Knox County, TN (5)	Dogwood (Zhang et al. 2005)	HQ341400
	Haywood County, NC (2)		HQ341405
	Hamilton County, OH (1)		HQ341420
			HQ341438
			HQ341453
S. exitiosa	Thunder Bay, ON, Canada (2)	GPTB (Trécé, Adair, OK)	HQ341393
	Peach County, GA (2)	L103 (Sentry Biologicals, Inc., Billings, MT)	HQ341404
	Cherokee County, KS (1)		HQ341411
	Bourbon County, KS (1)		HQ341414
	Sevier County, TN (2)		HQ341426
	Knox County, TN (2)		HQ341429
	Blount County, TN (1)		HQ341430
	Ramsey County, MN (1)		HQ341440
	Pearl River County, MS (2)		HQ341448
	Ontario County, NY (1)		HQ341449

Table 1. Continued

Species (taxonomic authority)	Collection location	Lure	GenBank accession no.
	Bourbon County, KS (1)		HQ341434
	Thunder Bay, ON, Canada (1)		HQ341445
	Sevier County, TN (1)		HQ341450
	Henry County, IA (1)		HQ341457
			HQ341463
			HQ341468
			HQ341470
S. pictipes	Knox County, TN (4)	LPTB (Trécé, Adair, OK)	HQ341402
Synanthedon pyri (Harris)	Ontario County, NY (2)	PB-GRB (APTIV, Portland, OR)	HQ341425
	Lake County, OH (3)		HQ341452
	Montgomery County, MD (1)		HQ341465
			HQ341471
Synanthedon acerni (Clemens)	Marion County, GA (3)	Came to light trap	HQ341409
S. uroceriformis	Anderson County, TN (2)	Raspberry crown borer (PheroTech, Inc., Delta, British Columbia)	HQ341394
	Knox County, TN (2)		HQ341398
			HQ341423

Within collection location, number in parenthesis indicates tally of individual specimens by species analyzed from each location.

amide gel using a BaseStation-100 DNA Sequencer (Bio-Rad, Hercules, CA), and analyzed using Cartographer 1.2.7 software. Sequences from opposing strands were reconciled and verified for accuracy by using Sequencher 4.2.2. All sequences were deposited in GenBank (Table 1).

Sequence Analysis. Alignment of sequences was straightforward. The optimal evolutionary model for the data were GTR + I + G based upon the Akaike's information criterion as calculated by Modeltest 3.7 (Posada and Crandall 1998). Bayesian analysis was performed using Mr. Bayes 3.1 (Huelsenbeck and Ronquist 2001) with 2.5 million iterations performed. Tracer 1.5 (Rambaut and Drummond 2004) was used for visual inspection of the point where log likelihood became stationary. Trees sampled before this point were discarded as burn-in. The remaining trees of two simultaneous runs were included in PP calculations.

A sequence from the 2-yr cycle moth *Choristoneura biennis* (Freeman) (Lepidoptera: Tortricidae) (GenBank DQ792587), was chosen as the distal outgroup and ones from five sesiid moth species belonging to tribes outside of *Synanthedonini* served as proximal outgroups (Fig. 1; Table 1). To bolster sampling within *Synanthedonini*, sequences from several species from the Palearctic genera *Pyropteron* Newman, *Chamaesphecia* Spuler, and *Bembecia* Hübner were added from GenBank, as were a few Palearctic species of *Synanthedon.*

Results

Data from cox I sequences strongly support, with a PP of 100, the monophyly of *Synanthedonini.* Among all sampled species, *Alcathoe carolinensis* Engelhardt likewise is strongly supported as the basal-most species of *Synanthedonini. Carmenta bassiformis* (Walker), *Synanthedon rileyana* (H. Edwards), and the Palearctic genera *Pyropteron* Newman, *Chamaesphecia* Spuler, and *Bembecia* Hübner form a weakly supported sister group to a well-supported (100% PP) *Synanthedon*-rich clade that also contains *Podosesia* and *Sannina.* This *Synanthedon*-rich clade includes all *Synanthedon* species sampled, with the exception *S. rileyana.* Although node support is weak within this largely *Synanthedon* clade, our inference does not support distinct generic status for *Podosesia* and *Sannina.* Visualization and subsequent systematic removal of putative long branches in the data set had no effect upon placement of these two genera. Intraspecific sequence divergence among the Nearctic species sampled ranged from zero (several instances) to nearly 5% (between *S. exitiosa* and *S. scitula*).

Discussion

The inferred cox I phylogeny obtained in this study successfully grouped all individuals according to their morphological-based identifications. Unique sequence data from clearwing species can provide rapid and accurate identification of all life stages, offering a proactive alternative to monitoring and control of these pests both in the United States and internationally, where nonnative insect introductions invoke significant economic and esthetic concerns. In addition, species with overlapping preferences for key host plants are particularly difficult to identify when detected as immature larvae. For example, *S. viburni* shares the same affinity for *Viburnum* spp. host plants as *S. fatifera.* A chance introduction of *S. andrenaeformis* (Laspeyres), a Palearctic viburnum pest, could make species identification of larvae even more difficult. Similarly, *S. scitula* has an extremely broad host-plant range that overlaps with many other sympatric clearwing species. This overlap makes identification of larvae less certain when samples are collected from an infested host plant. Taxonomic keys for the immature stages of many species are not available and fewer still are qualified taxonomists to positively identify specimens.

Practical use of gene sequence data for species identification first requires an informative gene at the tax-

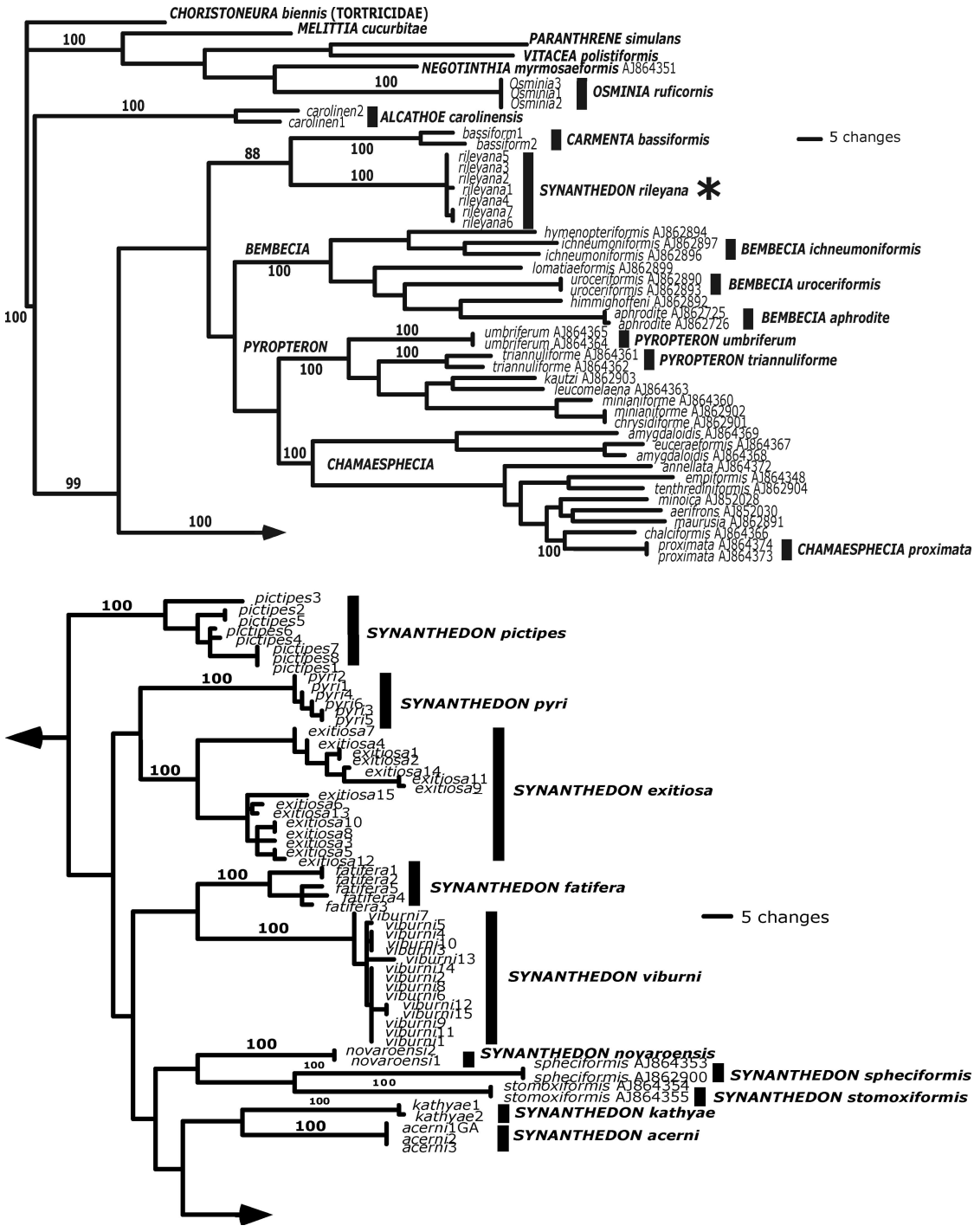

Fig. 1. Inferred phylogeny of Synanthedonini based on the mitochondrial gene cox I. Accession numbers of species taken from GenBank are shown. Supporting posterior probabilities are given at each node.

onomic level of interest that can be amplified reliably across taxa. Primer availability, ease of amplification, and lack of introns all make the mitochondrial genome a practical choice for quick and reliable species identifications. Cytochrome oxidase I (cox I) is one of several mitochondrial genes for which amplification is relatively straightforward and primers are readily available (Simon et al. 1994). Indeed, all Nearctic species included in this study possess a unique genetic signature within this genetic region regardless of geo-

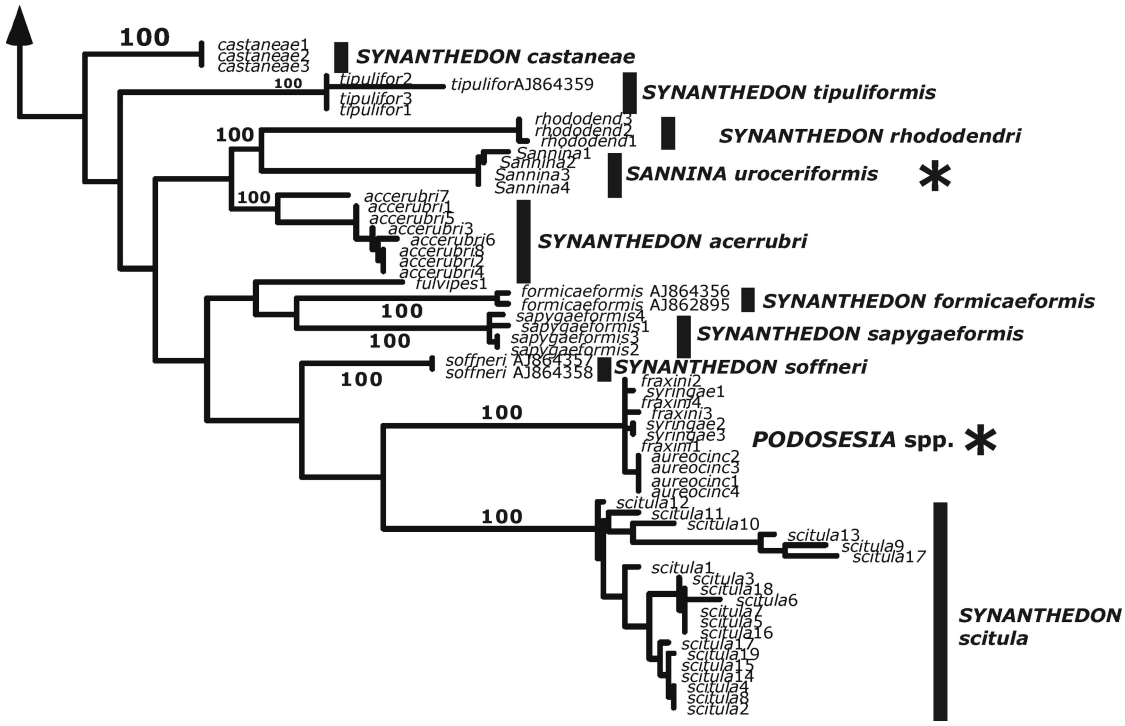

Fig. 1. (Continued).

graphic separation. For example, sequence data from the European *S. tipuliformis* (Clerck) match its North American counterpart almost exactly. Although cox I seems unable to distinguish some Palearctic taxa included in the analysis, this may be because of collection of too few specimens of closely related taxa. If the same species specificity of cox I sequences can be demonstrated for other clearwing species, cox I sequences would be an invaluable tool not just for regulatory authorities who monitor pests at entry points into the United States, but also as a lab diagnostic to assist pest management professionals working in urban landscapes, nurseries, and orchards who may have little taxonomic expertise.

Our phylogenetic analysis is based on a single mitochondrial gene, thus caution is warranted regarding any directed action toward reclassification based upon these results alone. Regardless, it is necessary to briefly consider discrepancies insofar as they help explain existing ambiguities in the literature and so possibly encourage future analyses of the specific taxa by using a more robust molecular approach. Unlike questions raised by our analysis (discussed below) about the generic designations of *Podosesia* and *Sannina*, which have not been questioned previously, generic placement of *S. rileyana* has been debated several times leading up to its current classification within *Synanthedon* (Engelhardt 1946, MacKay 1968, Duckworth and Eichlin 1977, Eichlin and Duckworth 1988). Our cox I phylogeny suggests *Synanthedon* species have evolved to use primarily woody host-plant tissues. Appearance of *S. rileyana* outside the *Synanthedon* clade suggests the need to reexamine its current generic placement.

The most current taxonomic position of *S. rileyana* has been accepted since the late 1980s (Eichlin and Duckworth 1988), but previously its placement within Synanthedonini was more fluid. *Synanthedon rileyana* has been placed in several different genera by taxonomists since its first description in 1881 (Duckworth and Eichlin 1977). Engelhardt 1946 included it in *Ramosia*, now synonymized with *Synanthedon*, based on wing venation. Later MacKay (1968) relegated *S. rileyana* to an unnamed genus that included four other species, based upon larval chaetotaxonomical characters. Eichlin and Duckworth (1988) assigned *S. rileyana* to *Synanthedon*, although noting its similarities to both *Carmenta* and *Synanthedon.* Designation of this species within *Synanthedon* appears to be based on the presence of straight crista sacculi on the valva of male genitalia, which is found in the majority of *Synanthedon* species (Fig. 2). Nevertheless, the female genitalia of *S. rileyana* have a mostly sclerotized ductus bursae and the close proximity of the ductus seminalis to the corpus bursae resembles closely the genitalic morphology of *Carmenta* females (Eichlin and Duckworth 1988) (Fig. 2). *Synanthedon rileyana* also infests an herbaceous host (i.e., *Solanum carolinense* L.), a trait not common among *Synanthedon* species (Eichlin and Duckworth 1988). Viewed in light of our results and the somewhat ambiguous prior placement within *Synanthedonini*, taxonomic placement of *S. rileyana* may need redressing.

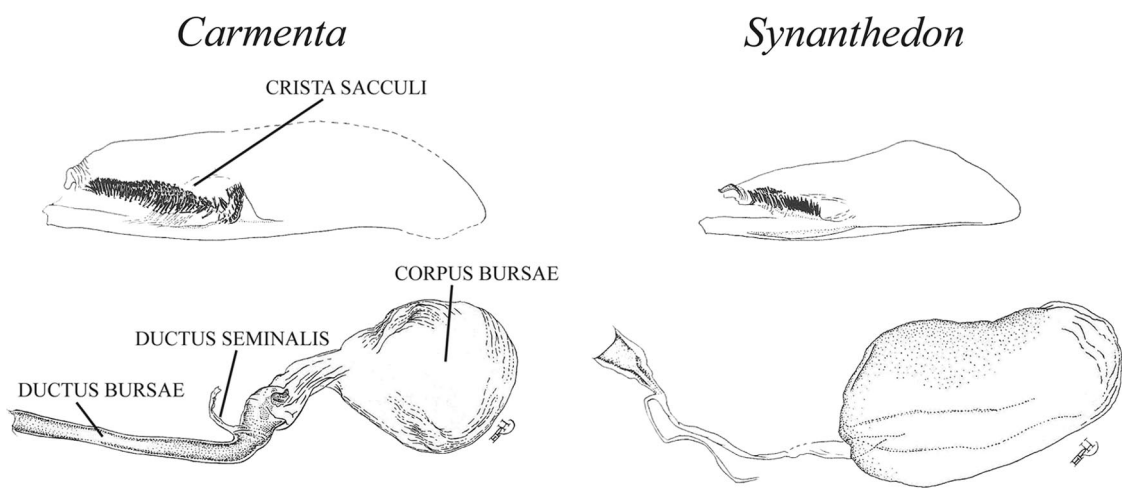

Fig. 2. Comparison of *Carmenta* and *Synanthedon* adult male (top) and female (bottom) genitalia. Illustrations modified with permission from Eichlin and Duckworth 1988. Illustrations rendered by Elaine R. S. Hodges.

Data from our cox I phylogeny also do not support a more primitive origin for *Chamaesphecia* than other species in the same clade, as could be supposed (Naumann 1971). Male genitalia of *Chamaesphecia* species entirely lack the scopula androniales that typically is positioned above the distal end of the uncus, as is apparent in most other *Synanthedonini* members (Naumann 1971, Eichlin and Duckworth 1988). Instead, the uncus in male *Chamaesphecia* is crowned with simple sensory setae that probably serve the same function as sensory setae surrounding the scopula androniales in other members of the tribe (Naumann 1971, Eichlin and Duckworth 1988). The uncal character is more than likely a derived trait, and not plesiomorphic, as evidenced by the basal position within *Synanthedonini* of *Alcathoe carolinensis* Engelhardt, which has a distinct scopula androniales and not the reduced character state seen in *Chamaesphecia.* Regardless of is evolutionary placement; monophyly of *Chamaesphecia* is strongly supported by the mitochondrial data.

As indicated, perhaps the most striking contradiction of current clearwing taxonomy is presented by inclusion of *Sannina* and *Podosesia* with other *Synanthedon* species. Both *Sannina* and *Podosesia* species possess unique morphological characters that have helped justify their rank as separate genera (MacKay 1968, Naumann 1971, Eichlin and Duckworth 1988). Genitalia of *Sannina uroceriformis* males conform better to those of species within *Carmenta* than *Synanthedon* (Eichlin and Duckworth 1988).

The genus *Podosesia*, which includes the two species, *P. syringae* (Harris) and *P. aureocincta* (Purrington and Nielsen), appears firmly nested within *Synanthedon.* This same phenomenon was apparent also in McKern et al. (2008) who used a different mitochondrial sequence, although only a single specimen was sequenced. *Podosesia aureocincta* has been separated using slight differences in saccus morphology, as well as differing flight times, and sexual pheromones (Purrington and Nielsen 1979). Mating between the two species does produce viable offspring that exhibit intermediate forms of the genitalic trait used to separate the two (Purrington and Nielsen 1979).

Unlike *Podosesia* and the vast majority of clearwing species, the host range of dogwood borer, *S. scitula* (Harris), extends across many plant families. In addition, although the majority of studies argue for a univoltine life cycle, some have suggested the dogwood borer may be semivoltine or even multivoltine (Underhill 1935, Riedl et al. 1985, Snow et al. 1985, Solomon 1995). Bergh et al. (2009) reported that although dogwood borer larvae develop more rapidly in burr knot tissue, sustained flight activities observed in both orchards and urban landscapes across two growing seasons challenge previous assertions that flight peak bimodality can be explained by the host-plant tissues that larvae consume. Its wide range of host plant resources, differences in emergence peaks of generations within season, and inconclusive voltinism have raised questions about whether *S. scitula* may represent a species complex within the family. As the dogwood borer becomes an increasing economic threat to apple growers, it is important to understand if it is indeed part of a larger complex, particularly if some sibling species within the possible complex are pests, whereas others are not, so populations can be managed effectively (Bergh and Leskey 2003, Leskey and Bergh 2005). Evidence from our analysis of *S. scitula* individuals from both early and late seasonal flight peaks taken across their range points to a single monophyletic species within *Synanthedon*, dispelling the notion of a species complex and making *S. scitula* unique among sesiid moths for the breadth of its potential host plant range.

By contrast, both viburnum borer species *S. fatifera* and *S. viburni* are highly specialized. Adult specimens of these two species can be readily separated by the green metallic luster of the *S. viburni* abdomen versus

the duller color scales found on *S. fatifera.* Unfortunately, from a pest management perspective, correct identification of larval species is a much more daunting task requiring detailed knowledge of sesiid larval characters (MacKay 1968). Fortunately, cox I sequence data are sufficiently different between the two to distinguish them.

The inferred cox I phylogeny obtained in this study grouped all individuals of the multiply sampled Nearctic species examined as monophyletic, except for those belonging to the genus *Podosesia.* Genetic variability of partial cox I sequence analyses provides ample evidence for the monophyletic nature of Nearctic clearwing species included in this analysis. Unique sequence from clearwing species can provide rapid and accurate identification of all life stages, offering a proactive alternative to monitoring and control of these pests both in the United States and internationally, and wherever nonnative insect introductions are a concern.

The dearth of genetic data regarding sesiid species leaves much room for future molecular exploration of both *Synanthedonini* and Sesiidae as a whole. Future studies including nuclear genes are needed to fully elucidate evolutionary relationships of difficult taxa within the tribe. Identification of sesiids using cox I, as shown in this paper, appears to be a very effective method that can be used when only immature stages or damaged adult specimens are available. Still, there may be some species that because of recent speciation events are not amenable to this method and may require additional genes for positive identification (e.g., *Podosesia*).

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