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Establishing Host-parasitoid Linkages among Sirex noctilio, Sirex nigricornis,

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Establishing host-parasitoid linkages among *Sirex noctilio, Sirex nigricornis*, and native Hymenopteran parasitoids using genetic markers

by

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Abstract

Sirex noctilio Fabricius. (Hymenoptera:Siricidae) is an invasive xylophagous woodwasp native to Eurasia and introduced to North America. Introduction of this invader in the southern hemisphere resulted in widespread economic damage of the pine industry, however, it is apparently exerting less harm to North American forests. This is possibly due to the presence of native parasitoids that attack *S. noctilio*. The purpose of this study was to identify parasitoids of *S. noctilio*, and the native *Sirex nigricornis* Fabricius based on DNA sequence analysis of larvae using cytochrome oxidase I, cytochrome b, and ribosomal large subunit genes. These sequences were used to evaluate sequence diversity within each genus and species, and examine spatial distributions of genotypes and host-specificity. Specimens were collected from sites in New York and Pennsylvania. Parasitoid larvae were first morphologically categorized as either *Ibalia* species or rhyssines (Ichneumonidae: Rhyssinae) based on size, mandible morphology, and body structure. Sequence diversity was analyzed and specimens assigned to a genus and a letter designating sequence type: *Ibalia* A-H, *Pseudorhyssa* A&B, *Megarhyssa* A-C and *Rhyssa* A-E. Interestingly, *Rhyssa* B was the only sequence type found in our native siricid, *S. nigricornis*, while all other genotypes were found only in *S. noctilio.* There was no pattern of site specificity for the species types in this study, suggesting parasitoids were not isolated to certain locations. Because several parasitoid types exhibited host specificity to *S. noctilio*, it appears biocontrol of this invasive woodwasp is already occurring on the landscape by several species.

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Introduction

Sirex noctilio Fabricius (Hymenoptera: Siricidae) is a xylophagous woodwasp native to Eurasia and north Africa, which has recently been introduced to North America. Although it is unclear how long *S. noctilio* has been in this region, its distribution across the continent suggests that it has been here for several years. While this insect is of major concern for pine plantations in the southern hemisphere (Hurley et al. 2007), it faces some native biotic resistance in North America which lessens the degree of harm this species has in northern forests (Foelker 2015). The United States Department of Agriculture (USDA) has classified *S. noctilio* as a recognized pest risk for North American pine forests for multiple decades (USDA-APHIS 2000). This status is mainly due to its economic impacts to pine plantations in the Southern Hemisphere. The first of these economically harmful invasions by *S. noctilio* was in New Zealand in about 1900, followed by Australia (1952), Uruguay (1980), Argentina (1985), Brazil (1988), South Africa (1994) and Chile (2000) (Hurley et al. 2007) In New York State, *S. noctilio* has been associated with weakened *Pinus sylvestris* and *Pinus resinosa* (Eager et al. 2011) but no serious impacts have been caused to the ecosystem (Dodds et al. 2010).

Various silvicultural practices have been used to minimize impacts of *S. noctilio*, including pruning trees outside the flight season of *S. noctilio* to avoid stress during this period, timely thinning to reduce competition, and removal of infested trees to eliminate the source population in the year (Hurley et al. 2007). In Australia, the main strategy for control of *S. noctilio* was to locate and destroy infested trees, a practice commonly used in invasive insect management; however, due to costs associated with this strategy, and the realization that *S. noctilio* could not be eradicated from Australia, pest managers turned to biocontrol as an economically and ecologically beneficial strategy (Hurley et. al. 2007).

Unlike in the plantation systems of the southern hemisphere, *S. noctilio* has faced a guild of native parasitoids associated with native siricids in North America, with some of the most common being *S. nigricornis* Fabricius, *S. cyaneus* Fabricius, *Urocerus albicornis* Fabricius , and *Xeris spectrum* L. (Schiff et al. 2006; Coyle et al. 2012; Barnes et al. 2014). Unfortunately, this family is often understudied due to a lack of economic impact in North American forest management (Belyea 1952).

Several native parasitoids in North America use *S. noctilio* as a host. This includes *Ibalia leucospoides ensiger* (Norton), *Rhyssa persuasoria* (L.), *Rhyssa lineolata* (Kirby), *Rhyssa crevieri* (Provancher), *Megarhyssa nortoni nortoni* (Cresson), and *Pseudorhyssa nigricornis* (Ratzeburg) (Long et al. 2009; Eager et al. 2011; Ryan et al. 2012a; Standley et al. 2012; Foelker et al. 2016). There has been thorough research on these parasitoids in the Southern Hemisphere invaded by *S. noctilio*, but little is known on the ecology, host breadth, and distribution in their native ranges across North America and Europe (Townes & Townes 1960; Hurley et al. 2007; Coyle & Gandhi 2012; Foelker et al. 2015).

Several species of native parasitoids have been released as biocontrol in countries where *S. noctilio* is exotic and have had variable effectiveness (Hurley et al. 2007). For example, levels of infestation in South Africa remain variable despite release of biological control agents. In the Western Cape province, populations of *S. noctilio* remain low, but in parts of the Eastern Cape and KwaZulu-Natal provinces, populations are increasing rapidly, as is the associated tree mortality. Similar variability is also observed between and within other southern hemisphere countries where *S. noctilio* has been introduced (Hurley et al. 2007). Classical biological control of *S. noctilio* using parasitoids has focused on four species of ichneumonid wasps (*Rhyssa persuasoria, R. lineolata,* and *Megarhyssa nortoni*), and the ibaliid wasp *I. leucospoides*. These

parasitoids, native to North America, can provide some natural control of *S. noctilio*, but are not distributed evenly enough across the landscape to be significantly useful (Eager et al. 2011). Where *S. noctilio* has invaded, the natural role of these parasitoids is not fully understood.

Genetic markers can be used to differentiate species when morphological characteristics are unreliable or not present. Accurate identification using genetic markers is critical in the assessment of their performance and suitability as biocontrol agents in biological control programs (USDA 2017). A DNA diagnostic test was recently developed to distinguish *S. noctilio* and *S. nigricornis*, and to determine if parasitoid larvae were either *Ibalia* species or Rhyssine (Foelker et al. 2016). Use of genetic markers is crucial to this study because identification of wood-boring insect larvae and their parasitoids often requires extended, time-consuming rearing efforts to obtain more easily identifiable adults, which may still present challenges using morphological identification keys (Standley et al. 2012).

In the case of parasitoid larvae, there are very few morphological species to differentiate *S. noctilio* larvae from the several other wood inhabiting parasitoids. Thus, genetic markers such as cytochrome oxidase I (COI), ribosomal DNA large subunit (LSU), and cytochrome b (*cytb*) genes have been used to differentiate insect species (Garcia-Robledo et al. 2013; Anslan & Tedersoo 2015)

The purpose of this study was to identify North American siricid parasitoids associated with *S. noctilio* infested trees based on DNA sequence analysis of larvae compared to known DNA sequences using COI, *cytb*, and LSU (defined above), evaluate the sequence diversity within each genus and species, and examine spatial distributions of genotypes and host specificity.

Methods

Insects were collected as part of an earlier study in 2013 and 2014 (Foelker et al. 2016), targeting two pine species (*P. resinosa* and *P. sylvestris*) exhibiting resinosis (a sign of attack by *Sirex* species) from 10 sites across New York and Pennsylvania (Fig. 1). Infested trees were felled, brought to State University of New York College of Environmental Science and Forestry in Syracuse, NY, and sections dissected using an electric log splitter (Ryobi Limited, Japan). Parasitoid larvae were identified as either *Ibalia* species or rhyssines based on size, mandible morphology, and body structure (Hocking 1968; Nieves-Aldrey et al. 2005).

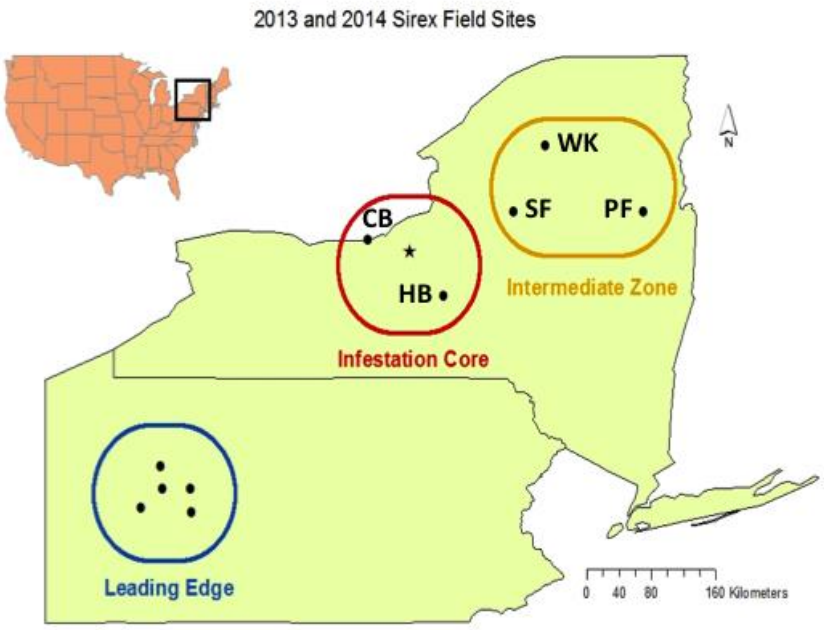

Figure 1. Forested stands where siricid infested trees were sourced from in summer 2013 and 2014. The star indicates the point of initial detection in North America, Fulton, NY. (Foelker et al. 2016)

Table 1. Site information for siricid larvae collected in sites across NY and PA in 2013 and 2014.

Site	State	Tree No.	Tree Species	# Larvae extracted
SF	NY	4	Scott's pine	2
		5	Scott's pine	17
		6	Scott's pine	3
		7	Scott's pine	2
		8	Scott's pine	18
CB	NY	3	Scott's pine	29
		4	Scott's pine	1
PF	NY	4	Scott's pine	3
		5	Scott's pine	14
HB	NY	4	Scott's pine	3
WK	NY	2	Scott's pine	1
		3	red pine	4
		5	red pine	2
V118	PA	1	red pine	22
S115	PA	1	red pine	3
P115	PA	2	red pine	2
		3	Scott's pine	2
		4	red pine	1
U118	PA	2	Scott's pine	4

Sirex larvae were identified as either *S. noctilio* or *S. nigricornis* as described by Foelker et al. (2016). For parasitoids, ~ 25 mg of tissue was excised from each larva. For adult parasitoids and pupae, the entire abdomen was crushed and used for extraction (Rougerie et al. 2011). Qiagen DNeasy Blood & Tissue kits (Qiagen Inc., Valencia, California) were used to extract DNA. Subsequent PCR was performed in 25 μL reaction volumes in Quick-Load® Taq 2X Master Mix (New England Biolabs, Ipswich, Massachusetts), using 0.5 μM forward and reverse primers and 2 μL of DNA. Primers targeted 3 genes: cytochrome oxidase I, cytochrome b, and the large subunit ribosomal DNA (Table 1). Amplifications for all genes were performed using a C1000™ Thermal Cycler (Bio-Rad Laboratories, Hercules, California) with initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 20s, 56 °C for 30 s and 68 °C for 60 s, and a final

extension at 68 °C for 5 min. All product amplification was evaluated by visual observation on a 1.5% agarose gel stained with Gel Red (Phenix Research Products, Candler, North Carolina).

Three genetic markers were used to differentiate parasitoid larvae, using the following primer sets (Table 2).

Table 2. Primer sets used to target 3 genes: cytochrome oxidase I, cytochrome b, and the large subunit ribosomal DNA.

Primer	**Locus**	**Primer sequence**	**Reference**
LCO1490	COI	GGT CAA CAA ATC ATA AAG ATA TTG G	Folmer 1994
HCO2198	COI	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer 1994
Cytb1F	*cytb*	TCT TTT TGA GGA GCW ACW GTW ATT AC	Belshaw and Quicke 1996
Cytb1R	*cytb*	AAT TGA ACG TAA AAT WGT RTA AGC AA	Belshaw and Quicke 1997
Ich28S-D2F	LSU	AGAGAGAGTTCAAGAGTACGTG	Belshaw and Quicke
28SD3A-R	LSU	TAGTTCACCATCTTTCGGGTC	Mardulyn and Whitfield

Positive samples were purified using the EZNA Gel Extraction Kit (Omega BioTek, Norcross, Georgia) and DNA quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Sequencing reactions were carried out using forward or reverse primers with the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1, using the ABI3730xl Genetic Analyzer (Applied Biosystems, Foster City, California). Resulting sequences were edited and analyzed using BioEdit (Hall, 1999). A BLAST search was conducted to check for matches with existing sequences in GenBank. Base pair differences were recorded in a table comparing sequence representatives of each type compared to the GenBank reference. Sequences were then cross-referenced with known sequences to confirm species IDs.

Results

Sequence Diversity

Ibalias in this study exhibited moderate sequence diversity with a variance of 16 base pairs for COI (Table 3). There were 8 sequence representatives for *Ibalias* found in this study (A-H). *Pseudorhyssa* samples in this study exhibited low sequence diversity, varying by 4 base pairs for COI (Table 4). There were only 2 sequence representatives for *Pseudorhyssa* (A & B). *Megarhyssa* samples in this study exhibited low sequence diversity, varying by 2 basepairs for COI (Table 5). There were only 3 sequence representatives for *Megarhyssa* (A-C). *Rhyssa* samples in this study exhibited high sequence diversity varying by hundreds of basepairs (Table 6). There were 5 sequence representatives for *Rhyssa* found (A-E).

Table 3. Sequence representatives of *Ibalia* species by COI. Unique sequences are categorized by type (A-H), and samples used are noted. Best BLAST search results for each sequence type are shown. Nucleotide position determined relative to reference sequence (KJ814197).

			12	26	80	83	89	170	248	275	281	293	341	449	455	548	556	590
Type	**Sample**	**BLAST**																
Ibalia A	I61	*I. leucospoides*	A	A	C	C	T	T	T	C	A	G	T	T	T	C	G	C
Ibalia B	I68	*I. leucospoides*	G	A	A	C	T	T	T	C	A	G	C	T	C	C	G	C
Ibalia C	I92	*I. leucospoides*	G	A	A	T	C	T	C	C	A	G	C	T	C	C	G	C
Ibalia D	I51	*I. leucospoides*	A	A	A	C	C	T	T	T	A	G	C	T	T	C	G	C
Ibalia E	I43	*I. leucospoides*	A	A	A	C	C	T	T	C	A	T	C	T	T	C	G	C
Ibalia F	I111	*I. leucospoides*	A	A	A	C	C	T	T	C	A	G	C	T	T	C	G	C
Ibalia G	I59	*I. leucospoides*	A	A	A	C	C	T	C	C	A	G	C	T	T	C	G	C
Ibalia H	I64	*I. leucospoides*	G	A	A	C	C	T	T	C	A	G	C	T	C	C	G	C
KJ814197			G	T	T	T	T	A	T	T	G	G	C	C	T	T	A	T

Table 4. Sequence representatives of *Pseudorhyssa* by COI. Unique sequences are categorized by type (A-B), and samples used are noted. Best BLAST search results for each sequence type are shown. Nucleotide position determined relative to reference sequence (KR801358)

Type	**Sample**	**BLAST**	**283**	**404**	**413**	**425**
Pseudo A	R154	*P. nigricornis*	A	G	C	C
Pseudo B	R275	*P. nigricornis*	G	A	C	C
KR801358			G	A	T	T

Table 5. Sequence representatives of *Megarhyssa* by COI. Unique sequences are categorized by type (A-C), and samples used are noted. Best BLAST search results for each sequence type are shown. Nucleotide position determined relative to reference sequence (KM567806)

Type	**Sample**	**BLAST**	**280**	**551**
Megarhyssa A	R199	*Megarhyssa*	C	C
Megarhyssa B	R177	*Megarhyssa*	T	C
Megarhyssa C	R173	*Megarhyssa*	T	T
KM567806			C	C

Table 6. Sequence representatives of rhyssines by COI. Unique sequences are categorized by type (A-E), and samples used are noted. Best BLAST search results for each sequence type are shown. Nucleotide position determined relative to reference sequence (KR873979)

Type	**Sample**	**BLAST**	**4**	**13**	**19**	**25**	**28**	**31**	**40**	**42**	**46**	**49**	**55**	**58**	**61**	**63**	**64**	**67**	**70**	**73**
Rhyssa A	74	*R. howdenorum*	A	A	G	G	T	G	T	G	G	G	C	G	T	A	T	T	A	A
Rhyssa B	R271	*R. howdenorum*	A	A	A	A	T	A	A	G	G	A	C	A	G	G	A	T	T	T
Rhyssa C	R152	*R. howdenorum*	A	A	A	A	G	A	T	G	G	A	A	A	T	A	T	A	A	T
Rhyssa D	R128	*R. howdenorum*	G	A	A	A	T	A	T	G	A	A	T	A	T	G	A	T	T	G
Rhyssa E	R23	*R. howdenorum*	G	G	A	A	T	A	T	A	A	A	T	A	T	G	G	T	T	A
KR873979			A	A	G	G	T	G	T	G	G	G	C	G	T	A	T	T	A	A

Table 6 continued.

Type	**Sample**	**BLAST**	**78**	**79**	**82**	**84**	**85**	**90**	**91**	**102**	**113**	**115**	**135**	**143**
Rhyssa A	74	*R. howdenorum*	A	A	A	G	A	T	T	T	G	G	T	A
Rhyssa B	R271	*R. howdenorum*	G	A	T	A	T	T	A	T	G	A	A	C
Rhyssa C	R152	*R. howdenorum*	A	A	T	A	A	C	T	T	G	G	A	A
Rhyssa D	R128	*R. howdenorum*	A	G	T	A	T	T	A	A	C	A	A	T
Rhyssa E	R23	*R. howdenorum*	A	A	T	G	T	T	A	T	G	G	A	A
KR873979			A	A	A	G	A	T	T	T	G	G	A	A

Diversity by Site

Species Diversity

Ibalia A, Ibalia F, Ibalia H, Rhyssa A, Rhyssa B, and *Pseudorhyssa A* occured widely across sites from NY and PA (Fig. 2 and 3). *Ibalia* B specimens were only found in *P. resinosa* at site V118 in PA. *Ibalia C, D,* and *E* were only found in *P. sylvestris* in western NY sites WK, SF, and PF (Fig. 1); *Ibalia G* was specific to NY as well, but exhibited more geographic breadth as it was found in *P. sylvestris* at CB (furthest west NY site) and PF (furthest east NY site). *Rhyssa C* (SF), *D* (SF, CB, PF, WK), and *E* (WK) were also only found in NY. No *Pseudorhyssa B* samples were matched with location information due to limited sampling information. *Megarhyssa A, B,* and *C* were only found in NY at CB. *Megarhyssa B* was also found in SF in NY. (Fig. 3 and Fig. 4).

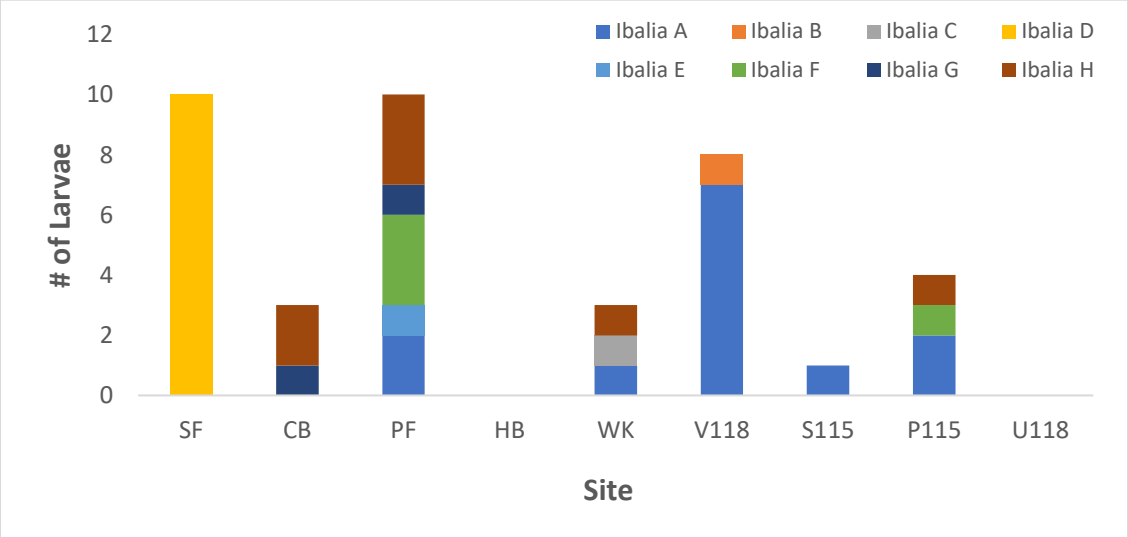

Figure 2. Diversity of *Ibalia* sequence types by sites across New York and Pennsylvania based on number of larvae collected at each site. Sites are defined in methods

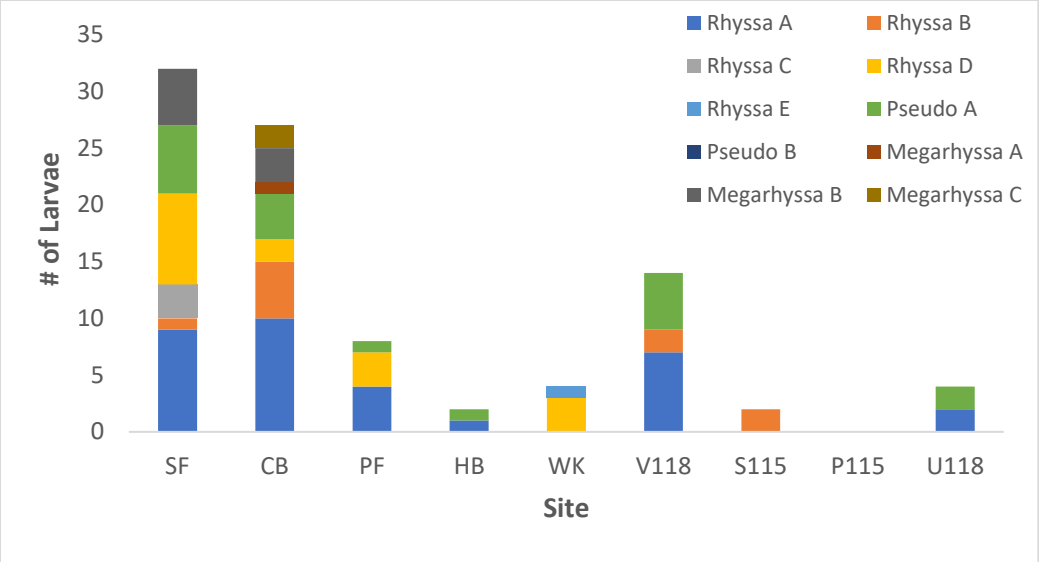

Figure 3. Diversity of rhyssine sequence types by site across New York and Pennsylvania based on number of larvae collected at each site. Sites are defined in methods.

Genus Diversity by Site

Ibalia samples occurred in all sites except one in PA (Site P115). *Ibalia* species made up the majority of samples found in five of nine sites across NY and PA (SF at 50%, CB at 56.7%, WK at 57.1%, S115 at 66.7%, and U118 at 50%). *Ibalia* occurred at the highest percentage in PA (66.7% at S115). *Rhyssa* were found at all sites except one in NY (HB) and one in PA (U118). *Rhyssa* made up the majority of samples in one site in NY (52.9% at PF) and one site in PA (80% at P115). *Pseudorhyssa* occurred in all sites except one in NY (WK) and one in PA (S115). *Pseudorhyssa* make up the majority in only two sites, one in NY (66.7% in HB) and one in PA (50% in U118). Lastly, *Megarhyssa* were only found in two sites in NY (SF and CB) and occurred at 11.9% and 20%, respectively (Table 7).

Table 7. Percent of each parasitoid genus by site (as defined in methods)

Site	% *Ibalia*	% *Rhyssa*	% *Psuedorhyssa*	% *Megarhyssa*
SF	50.0%	23.8%	14.3%	11.9%
CB	56.7%	10.0%	13.3%	20.0%
PF	41.2%	52.9%	5.9%	0.0%
HB	33.3%	0.0%	66.7%	0.0%
WK	57.1%	42.9%	0.0%	0.0%
V118	40.9%	36.4%	22.7%	0.0%
S115	66.7%	33.3%	0.0%	0.0%
P115	0.0%	80.0%	20.0%	0.0%
U118	50.0%	0.0%	50.0%	0.0%

Diversity by Tree Species

Larvae from both *P. resinosa and P. sylvestris* from NY and PA were collected for this study. *Ibalia B* and *Ibalia C* were only found in *P. resinosa* specimens while *Ibalia E* and *Ibalia G* were found only in *P. sylvestris.* All *Rhyssa* sequence types except *Rhyssa B* showed specificity for *P. sylvestris. Pseudorhyssa A* were found in both pine species while *Pseudorhyssa B* were only found in *P. sylvestris.* All *Megarhyssa* types were found only in *P. sylvestris* (Table 8). Neither *S. nigricornis* nor *S. noctilio* appeared to exhibit tree preference (Table 9). Unfortunately, four *S. noctilio* samples did not have tree information associated with them.

Table 8. Sequence type distribution by tree species.

Sequence type	***Pinus sylvestris***	***Pinus resinosa***
Ibalia A	13	18
Ibalia B	0	1
Ibalia C	0	1
Ibalia D	13	3
Ibalia E	1	0
Ibalia F	3	1
Ibalia G	2	0
Ibalia H	4	1
Rhyssa A	16	0
Rhyssa B	6	4
Rhyssa C	3	0
Rhyssa D	11	0
Rhyssa E	1	0
Pseudo A	15	5
Pseudo B	1	0
Megarhyssa A	1	0
Megarhyssa B	8	0
Megarhyssa C	2	0

Table 9. *Sirex* woodwasp species distribution by tree species.

Species	***Pinus sylvestris***	***Pinus resinosa***
S. nigricornis	3	4
S. noctilio	96	30

Host Specificity

Rhyssa B was the only sequence type to exhibit host specificity to *S. nigricornis* (n = 6), while all other genotypes preferentially parasitized *S. noctilio* (n = 130) (Table 10). No other parasitoid sequence types were found in *S. nigricornis.* Six *S. nigricornis* larvae were parasatized by *Rhyssa* B.

Table 10. Parasitoid-host relationship based on sequence type.

Sequence Type	***S. noctilio***	***S. nigricornis***
Ibalia A	13	-
Ibalia B	1	-
Ibalia C	1	-
Ibalia D	10	-
Ibalia E	1	-
Ibalia F	4	-
Ibalia G	2	-
Ibalia H	6	-
Rhyssa A	33	-
Rhyssa B	-	6
Rhyssa C	3	-
Rhyssa D	16	-
Rhyssa E	1	-
Pseudo A	22	-
Pseudo B	6	-
Megarhyssa A	1	-
Megarhyssa B	8	-
Megarhyssa C	2	-

Discussion

The goal of this study was to identify siricid parasitoids based on DNA sequence analysis of larvae compared to known DNA sequences found in GenBank using COI, *cytb*, and LSU, evaluate the sequence diversity within each genus and species, and examine spatial distributions of genotypes and host specificity.

Moderate to high genetic diversity was found in all genera studied. Rhyssines exhibited the most genetic diversity of all native parasitoids studied while *Megarhyssa* species exhibited the least diversity. *Ibalia* A, F, H, *Rhyssa* A, B, and *Pseudorhyssa* A genotypes occurred widely across NY and PA. Several *Ibalia* (C, D, E, G), *Rhyssa* (C, D, E), and *Megarhyssa* (A, B, C) genotypes are found only in NY while *Ibalia* B specimens are only found in PA. Parasitoid genotype study appeared to exhibit regional specificity, but some were exclusive to certain locations, occurring only in one site in one state. For example, *Ibalia* D and *Megarhyssa* A were found in only one site in NY. Parasitoid genotypes also showed specificity for which trees they oviposited in as well, e.g., several *Ibalia* sequence types were only found in *P. resinosa*, while several sequence types of *Pseudorhyssa* were only found in *P. sylvestris.* All *Rhyssa* sequence types, except *Rhyssa* B, showed specificity for *P. sylvestris. Rhyssa* B was the only genotype to exhibit host specificity to the native *S. nigricornis,* however, this could be an artifact of sample size, as only six parasitoids of *S. nigricornis* were collected.

Rhyssa B was also the only sequence type found in both *P. resinosa* and *P. sylvestris.* This could indicate different habitat preferences in the parasitoids. Because several sequence types exhibited host specificity to the invasive woodwasp, it appears biocontrol of the invasive *S. noctilio* is already occurring on the landscape by several species, but perhaps through further

research, a single species most effective/efficient for biocontrol could be determined, reared in large numbers, and released.

Presence of such high genetic diversity among genera of parasitoids in this study could be due to parasitoids being introduced/reintroduced to North America when *S. noctilio* was introduced here. One explanation is that when raw, untreated lumber was shipped to and from the Southern Hemisphere, larvae of parasitoids released against *S. noctilio* in the country where the introduction came from may have come with *S. noctilio*. These parasitoids could have originally come from North America and/or they may be genera native to Europe that were released against *S. noctilio* in the Southern Hemisphere (Foelker et al., 2016).

The presence of so many native parasitoids which use this invasive as a host indicates built-in biological control agents and explains the pattern of lesser damage exhibited by this invasive in North America compared to the extensive damage caused to South American forests. (Dodds et al. 2010). *Sirex noctilio* faces a guild of native parasitoids in North America, including *I. leucospoides ensiger, R. persuasoria, R. lineolata, R. crevieri, M. nortoni nortoni,* and *P. nigricornis* (Eager et al. 2011, Standley et al. 2012, Foelker et al. 2016).

Several species of parasitoids native to North America have been released as biocontrol in countries where *S. noctilio* is exotic and have had variable effectiveness (Hurley et al., 2007). In areas where *S. noctilio* has become an economically important invasive, four genera of parasitoids (*Rhyssa, Megarhyssa, Ibalia,* and *Pseudorhyssa*) have been introduced for biological control (Standley et al 2012). These parasitoids, native to North America, provide control of *S. noctilio*, but are not distributed evenly enough across the landscape to be significantly useful (Eager et al. 2011). This study indicates these parasitoids, and many others in their genera, exhibit similar host-parasitoid linkages. Thus, we can hypothesize that *S. noctilio* is a low risk

invasive species in North America because of the presence of a diverse community of native parasitoids, which can use it as a host.

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Appendix

Appendix A. Diversity by site of all larvae, showing sequencing types per tree per site.

Site	State	Tree Number	Tree Species	# of Larvae extracted	Type
SF	NY	4	*Pinus sylvestris*	2	Rhyssa D (1) Megarhyssa B (1)
		5	*Pinus sylvestris*	17	Rhyssa B (1) Rhyssa A (2) Pseudo A (2) Megarhyssa B (2) Rhyssa D (5) Ibalia D (5)
		6	*Pinus sylvestris*	3	Pseudo A (2) Rhyssa A (1)
		7	*Pinus sylvestris*	2	Rhyssa A (2)
		8	*Pinus sylvestris*	18	Rhyssa D (2) Rhyssa C (3) Rhyssa A (4) Pseudo A (2) Megarhyssa B (2) Ibalia D (5)
CB	NY	3	*Pinus sylvestris*	29	Rhyssa B (5) Pseudo A (3) Ibalia G (1) Rhyssa D (2) Rhyssa A (10) Ibalia H (2) Megarhyssa C (2) Megarhyssa B (3)

					Megarhyssa A (1)
		4	*Pinus sylvestris*	1	Pseudo A (1)
PF	NY	4	*Pinus sylvestris*	3	Ibalia E (1) Ibalia F (2)
		5	*Pinus sylvestris*	14	Rhyssa A (4) Pseudo A (1) Ibalia F (1) Ibalia H (2) Ibalia A (2) Ibalia G (1) Rhyssa D (3)
HB	NY	4	*Pinus sylvestris*	3	Pseudo A (2) Rhyssa A (1)
WK	NY	2	*Pinus sylvestris*	1	Rhyssa E (1)
		3	*Pinus resinosa*	4	Ibalia H (1) Ibalia A (1) Ibalia C (1) Rhyssa D (1)
		5	*Pinus resinosa*	2	Rhyssa D (2)
V118	PA	1	*Pinus resinosa*	22	Rhyssa A (7) Pseudo A (5) Ibalia A (7) Rhyssa B (2) Ibalia B (1)
S115	PA	1	*Pinus resinosa*	3	Ibalia A (1) Rhyssa B (2)
P115	PA	2	*Pinus resinosa*	2	Ibalia A (1) Ibalia H (1)
		3	*Pinus sylvestris*	2	Pseudo B (1)

					Ibalia A (1)
		4	*Pinus resinosa*	1	Ibalia F (1)
U118	PA	2	*Pinus sylvestris*	4	Rhyssa A (2) Pseudo A (2)