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by

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Abstract

The European woodwasp, *Sirex noctilio*, is a wood-boring insect native to Eurasia and North Africa, which was accidentally introduced across the Southern Hemisphere where it caused extensive economic damage to conifer plantations. Discovered ten years ago in North America, *S. noctilio* competes directly with *Sirex nigricornis*, a native congener, through shared resources in host trees. The two species also share a suite of six parasitoids, which utilize both native and non-native species of woodwasps as hosts. To study these interactions, it is common practice to fell trees and split logs from which siricid larvae and their parasitoids are collected. It is exceedingly difficult to identify larval species due to their indistinct morphology. The objectives of this study were to: 1) differentiate between the native *S. nigricornis* and the non-native *S. noctilio* using PCR assays and 2) determine if such assays can be used to identify host species from a parasitoid's gut contents. DNA was extracted from 206 siricid larvae and 202 were identifiable as *S. noctilio* (154, 73%) or *S. nigricornis* (48, 23%) using PCR, illustrating the applicability of this technique to differentiate among *Sirex* species. Identification of species from DNA extracted from host cadavers associated with parasitoid larvae was also successful (17 of 21, 81%). However, identification of species from DNA extracted from gut contents of parasitoids was also successful (12 of 35, 34%). Being able to successfully identify trophic linkages associated with non-native species is important to understand how they will invade an area and their ecological impacts**.**

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Introduction

The coevolution of host-parasite interactions are a recognized but understudied power in ecology (Paterson and Piertney 2011). The interactions between hymenopteran parasitoids and xylophagus insects are an example and in particular, these may be overlooked, due to their cryptic and hard to document life cycles. Biologists can now employ molecular techniques to broaden the understanding of these relationships (Paterson and Peirtney 2011). For example, any life stage can be examined, whole insects are not needed for identification, and cryptic species can be discovered and targeted for further study.

The European woodwasp, *Sirex noctilio*, is a wood-boring insect native to Eurasia and North Africa, and was accidentally introduced across the Southern Hemisphere where it caused extensive economic damage to conifer plantations. Many of these conifer plantations consisted of species native to North America, such as the Monterey (*Pinus radiata*) and Loblolly Pines (*Pinus taeda*) (Hurley et al. 2007). In North America, *S. noctilio*, has been found in five states and two Canadian provinces (Dodds & deGroot 2012). In New York *S. noctilio* has been found in native Red Pine (*Pinus resinosa*) and introduced Scots Pine (*Pinus sylvestris*) (Hoebeke 2005). Due to the precedent that has been set in the Southern Hemisphere, the projected economic damage in the U.S. is estimated to exceed 8.2 to 10.7 billion in the southeastern and western pine plantations respectively (Borchert et al. 2007).

Sirex noctilio was discovered ten years ago in North America and competes directly with *Sirex nigroconis*, a native congener, through shared resources in host trees. The two species share a suite of six parasitoids, which utilize both native and non-native

species of woodwasps as hosts (Eager et al. 2011, Standley et al. 2012). These shared parasitoids include *Rhyssa lineolata, Rhyssa persuasoria, Megarhyssa nortoni nortoni, Ibalia leucospoides ensiger,* and *Pseudorhyssa nigricornis*. Determining the relationships between these parasitoids and their siricid hosts is difficult. A previous study by Eager et al. (2011) split logs into small sections from which larval parasitoids were reared to adults; however the siricid larvae cadavers could not be identified by morphology. The advancement of molecular techniques has allowed for these linkages to be elucidated by detecting host DNA in parasitoid gut contents (Rougerie et al. 2010).

The objectives of this study were to develop PCR assays to differentiate between the native *S. nigircornis* and non-native *S. noctilio*. It will then be determined if these assays can be used to identify host species from a parasitoid's gut contents. Elucidating these connections will provide a clearer ecological picture of these host-parasite interactions.

Methods

DNA Extraction and Purification

The following tissue types were examined: 206 siricid larvae, 35 cadavers, 46 gut contents from parasitoids. Approximately 25 mgs of tissue from insect larvae was cut into small pieces and put into a labeled screwcap vial and DNA was extracted using the DNeasy Blood and Tissue Kit following manufactorer's protocol (Qiagen Inc., Valencia, California) with the following modifications. For digestions, 30 µl of Proteinase K was used instead of 20 µl and for the final elution 200 µl of AE buffer was used.

Polymerase Chain Reaction (PCR)

PCR was performed in 25 µl reaction volumes with Quick-Load® Taq 2X Master Mix (New England Biolabs, Ipswitch, Massachusetts), 0.5 µM of each primer and 2 µl of template DNA. Amplification used primers Sirex2F (5'-GATTCTGACTCCTTCCTC-3') and Sirex1R. Amplifications were performed on a C1000™ Thermal Cylcer (BioRad Laboratories, Hercules, California) with initial denaturation 95C for 3 minutes then cycling 95C for 20 seconds, 56C for 30 seconds, 68C for one minute for a total of 34 times, and a final extension step at 68C for 5 minutes.

Nested PCR

For difficult to amplify template DNA (cadavers, gut contents) a nested PCR was used to maximize the likelihood of detection of *Sirex* spp. DNA. Conditions for nested PCR were the same as the standard PCR with the exception of the primers used. The first round used Sirex1F (5'-TCTATAAGAATTATTATTCGAAC-3') and Sirex1R (5'-GTTAAAAGTATAGTRATTGCTC-3') primers, the second used Snoc1F (5'-TAATTCAATTATCACAAGTCATG-3') and Snoc1R (5'-GAAATTACTAGTAAGATTGCG-3').

Restriction Digest

Restriction digests were performed using 25 µl reaction volumes containing: 1x CutSmart Buffer (New England Biolabs, Ipswitch, Massachusetts), 10 units of AluI enzyme (New England Biolabs, Ipswitch, Massachusetts), and 10 µl of PCR amplified sample. Digests were incubated at 37C for 2 hours followed by a heat kill step at 80°C for 20 minutes. The products of the PCR and restriction digestions were analyzed using a

1% agarose gel stained with GelRed (Phenix Research Products, Candler, North Carolina).

Sequencing

A subsample of amplified products were selected for confirmation of identity by DNA sequencing. For these, product amplification using Sirex2F/Sirex1R was evaluated by observation on a 1% agarose gel and the remainder of the sample purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, Georgia). DNA was quantified using a DNA spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Direct sequencing was performed using the primer Sirex2F. Reactions were carried out with the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI3730x1 Genetic Analyzer (Applied Biosystems, Foster City, California). Sequence identity was evaluated by GenBank BLAST search and alignment with our own database of *Sirex* species sequences.

Results

Amplification of DNA from *Sirex* species and restriction digest was used to differentiate between *S. noctilio* and *S. nigrcornis* was conducted for 206 siricid larvae. Of these 202 were identifiable as *S. noctilio* (154, 73%) or *S. nigricornis* (48, 23%) (Table 1). AluI restriction digest does not cut *S. noctilio* DNA, and produces 3 fragments (23, 150, 158 bp) from the 331 bp of *S. nigricornis* cytochrome oxidase gene (Figure 1).

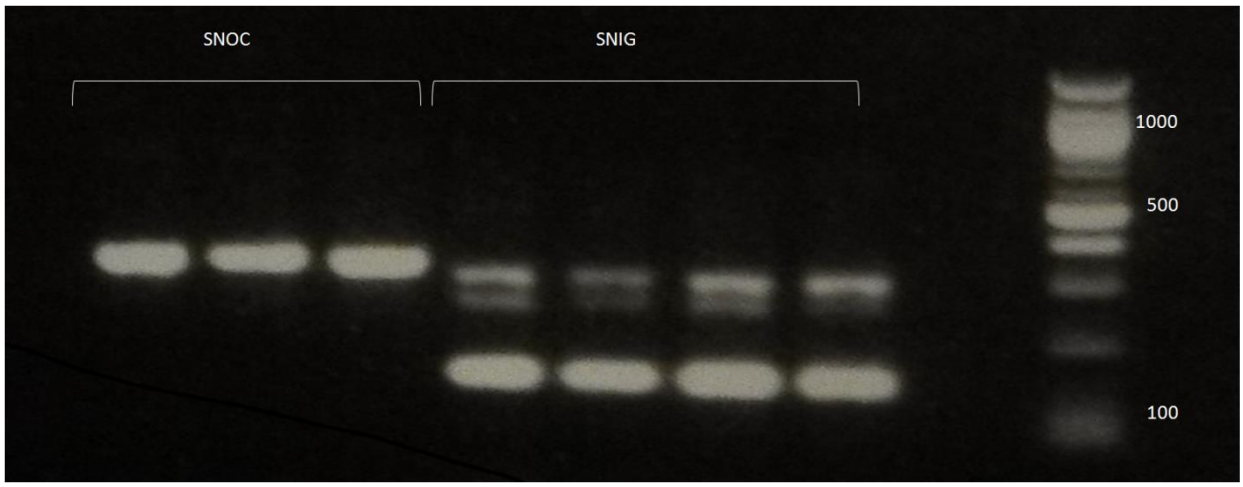

Figure 1. Gel electrophoresis showing amplified fragments with primers (Sirex 2F-Sirex1R) from *S. noctilio* (SNOC) and *S. nigricornis* (SNIG) from the same tree. Restriction digest cuts *S. nigricornis* at two locations.

Sequencing of a subsample (n=24) corroborated restriction fragment results. Identification of species from DNA extracted from cadavers associated with parasitoid larvae was also successful (17 of 21, 81%). However, identification of species from DNA extracted from gut contents of parasitoids was less successful (12 of 35, 34%).

Table 1. Sample sites and respective trees with the number of each species (*Sirex noctilio* and *Sirex nigricornis*) found in each tree.

Site	Tree	*S. noctilio*	*S. nigricornis*
P115	1	0	33 (100%)
P115	2	0	44 (100%)
P115	3	39(76%)	14(24%)
S115	4	9(100%)	0
S115	5	0	8(100%)
U115	6	0	24(100%)
V115	7	0	31(100%)

Of the seven trees sampled, only one was found to be inhabited by both *S. noctilio* and *S. nigricornis*. At this site the other two trees were found to only contain *S. nigricornis*. The other site where two trees were sampled one tree contained *S. noctilio* and the other contained *S. nigricornis*.

Discussion

The successful amplification of DNA from *Sirex* larva, cadavers, and parasitoid gut contents demonstrates how valuable molecular techniques can be when trying to identify cryptic species. These techniques can be employed to understand the trophic linkages between hosts and their parasites. The relationship between native species and their parasites is important to understand to be able to determine if host switching may occur from native to non-native species.

In other countries where *S. noctilio* has invaded native Hymenopteran have been released to attempt to control the population (Hurley et al. 2007). These control measures have been successful and could possibly be implemented in the North America. Biological control of *Sirex* through the use of parasitoids has focused on four ichneumonid wasps (*Rhyssa persuasoria, Rhyssa lineolata,* and *Megarhyssa nortoni*) and the ibaliid wasp, *Ibalia leucospoides*. The ichneumonid wasps attack late larvae while the ibaliid wasp attacks eggs and early instar larvae. These species are native to North America but do not have an even distribution (Eager et al. 2011).

In this study it appears that *S. noctilio* and *S. nigricornis* do not often inhabit the same tree. Of seven trees sampled only one was found to contain both species. There may

be a possibility that these two species compete with each other or that *S. noctilio* has not yet overtaken the niche of *S. nigricinis*. The relationship between these two species and specific species of parasitoids needs further studying to determine which parasitoids may parasitize *S. noctilio* more than *S. nigricornis*.

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