JHR 96: 937–947 (2023)
doi: 10.3897/jhr.96.112395
https://jhr.pensoft.net

SHORT COMMUNICATION

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

First record of the pine sawfly *Neodiprion warreni* (Hymenoptera, Diprionidae) in the state of Tennessee and on *Pinus virginiana*

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Academic editor: Marko Prous | Received 7 September 2023 | Accepted 11 October 2023 | Published 6 November 2023

https://zoobank.org/C3D5DEF4-8555-4168-8218-3EF565C527B5

Citation: Ridenbaugh RD, Glover AN, Linnen CR (2023) First record of the pine sawfly *Neodiprion warreni* (Hymenoptera, Diprionidae) in the state of Tennessee and on *Pinus virginiana*. Journal of Hymenoptera Research 96: 937–947. https://doi.org/10.3897/jhr.96.112395

Abstract

Pine sawflies in the genus *Neodiprion* Rohwer are widely distributed across the Northern Hemisphere and are pests of commercially important conifer trees. While sampling for *Neodiprion* species in eastern North America, two colonies of *Neodiprion warreni* Ross were discovered in Tennessee feeding on *Pinus virginiana* Mill. These are the first records of *N. warreni* in Tennessee and on the host *P. virginiana*. Here, we use a combination of larval and adult female morphology to confirm species identification. We also discuss two potential explanations for these observations: *N. warreni* was always present in Tennessee and feeding on *P. virginiana* but, until now, has gone unreported or these new records are attributable to a recent range expansion and host shift. We also discuss potential economic and evolutionary implications of range expansions and host shifts in plant-feeding insect pest species.

Keywords

Distribution, *Neodiprion*, new host, new record

* These authors contributed equally to this work.

Introduction

Pine sawflies in the genus *Neodiprion* Rohwer, 1918 (Hymenoptera, Diprionidae) are pests of pine trees and other conifers of commercial importance (Coppel and Benjamin 1965; Welch 1991; Darr et al. 2022; Davis et al. 2023). This genus consists of 50 species that, collectively, are widely distributed across the Northern Hemisphere and are found on a wide variety of conifer hosts in the family Pinaceae (Coppel and Benjamin 1965; Taeger et al. 2018; Davis et al. 2023). All parts of the *Neodiprion* life cycle are dependent upon their coniferous hosts. The adults meet and mate on the host. The mated female then uses her saw-like ovipositor to carve pockets into the needle tissue, where she deposits her eggs. The eggs develop in the needle tissue, hatch, and feed on the needles. After undergoing several molts, the larvae spin a fibrous cocoon either on or near the host tree. Inside the cocoon, pupation occurs, and the adult emerges from the cocoon to repeat the cycle (Dixon 2004). Although the sequence of life cycle events is the same for all *Neodiprion* species, there is variation among species in details for each stage of the life cycle that have implications for pest status (Davis et al. 2023). For example, species that spend the winter months as prepupae in cocoons and that tend to have multiple generations per year cause more damage to their hosts than species that spend the winter months as eggs in the needle tissue and that have only one generation per year (Kulman 1971; Lyytikäinen-Saarenmaa and Tomppo 2002).

Neodiprion warreni Ross, 1961 is a cocoon-overwintering pine sawfly that tends to have multiple generations per year (Wilkinson 1968; Dixon 2004). The previously published distribution of *N. warreni* includes Florida, Louisiana, Arkansas, and Georgia (Ross 1961; Wilkinson 1968; Baker 1972; Smith 1979; Linnen and Farrell 2007, 2010). Previously reported hosts of *N. warreni* include *Pinus echinata* Mill., *Pinus glabra* Walt., *Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg., and *Pinus taeda* L. (Ross 1961; Wilkinson 1968; Baker 1972; Smith 1979; Dixon 2004; Linnen and Farrell 2007, 2010), with one recorded outbreak on *P. echinata* in Union County, Arkansas in 1957 (Warren 1958). Here, we provide the first verified records of *N. warreni* in the state of Tennessee, the furthest northeast this species has been recorded, and on *Pinus virginiana* Mill., a newly reported host for this species. These new records could represent new information on an understudied species or could represent evidence of a recent range expansion and host shift. Thus, we also discuss potential implications of range expansions and host shifts.

Methods

Collection and species identification

On September 13, 2022, two separate colonies of *N. warreni* were found in Tennessee by authors ANG and RDR. The first colony of approximately 30–50 mid-late instars was found in Knoxville, Tennessee (36.0050, -83.7782) and was assigned the colony ID "AG170". The second colony of approximately 15 early-mid instars was found in Crossville, Tennessee (35.9293, -84.9127) and was assigned the colony ID "AG171". Colony

AG170 was preliminarily identified in the field as *N. warreni* based on larval pigmentation and photographed using an Apple iPhone X (Fig. 1). Because complete larval pigmentation pattern was not yet developed, a preliminary species identification of colony AG171 was not made in the field. Both colonies were determined to be feeding on mature *P. virginiana* based on needle and bark morphology (Petrides and Wehr 1998). To confirm species identity, both colonies were collected and brought back to the lab by clipping the branch containing the feeding larval colony and placing the clipping in a labeled paper bag.

To confirm the species identity of both colonies, we first photographed a single larva from colony AG170 using a Canon EOS Rebel T6 with a Canon EF 100 mm Macro lens (Fig. 2A, B) and used a *Neodiprion* key based on larval morphology (Davis et al.

Figure 1. *Neodiprion warreni* colony feeding on *Pinus virginiana*. A *Neodiprion warreni* Ross colony (colony ID: AG170) found in Knoxville, Tennessee feeding on a *Pinus virginiana* Mill. tree.

2023). We later used this key to confirm colony AG171 as *N. warreni* on September 22, 2022, once larvae had developed a diagnostic pigmentation pattern. Because adult female coloration and ovipositor morphology is also diagnostic (Ross 1961), we also reared the larvae of both colonies to adulthood using standard lab protocols (Harper et al. 2016; Bendall et al. 2017). Upon emergence from their cocoons, a single adult female from colony AG170 was photographed using a Canon EOS Rebel T6 with a Canon EF 100 mm Macro lens (Fig. 3A, B). From the AG170 colony two adult females and one male were preserved in 100% ethanol for pinning and ovipositor mounting. An ovipositor was dissected from one of the females, mounted, and photographed using a Zeiss DiscoveryV8 stereomicroscope with an Axiocam 105 color camera and ZEN lite 2012 Software (Carl Zeiss Microscopy, LLC Thornwood, NY) after Bendall et al. (2017). The AG171 colony was heavily parasitized and as such we were unable to rear *Neodiprion* adults for preservation. All pinned specimens are stored at the University of Kentucky (**UKIC**).

Figure 2. *Neodiprion warreni* larva. The lateral (**A**) and dorsal (**B**) view of a *Neodiprion warreni* Ross larva (colony ID: AG170) collected from Knoxville, Tennessee.

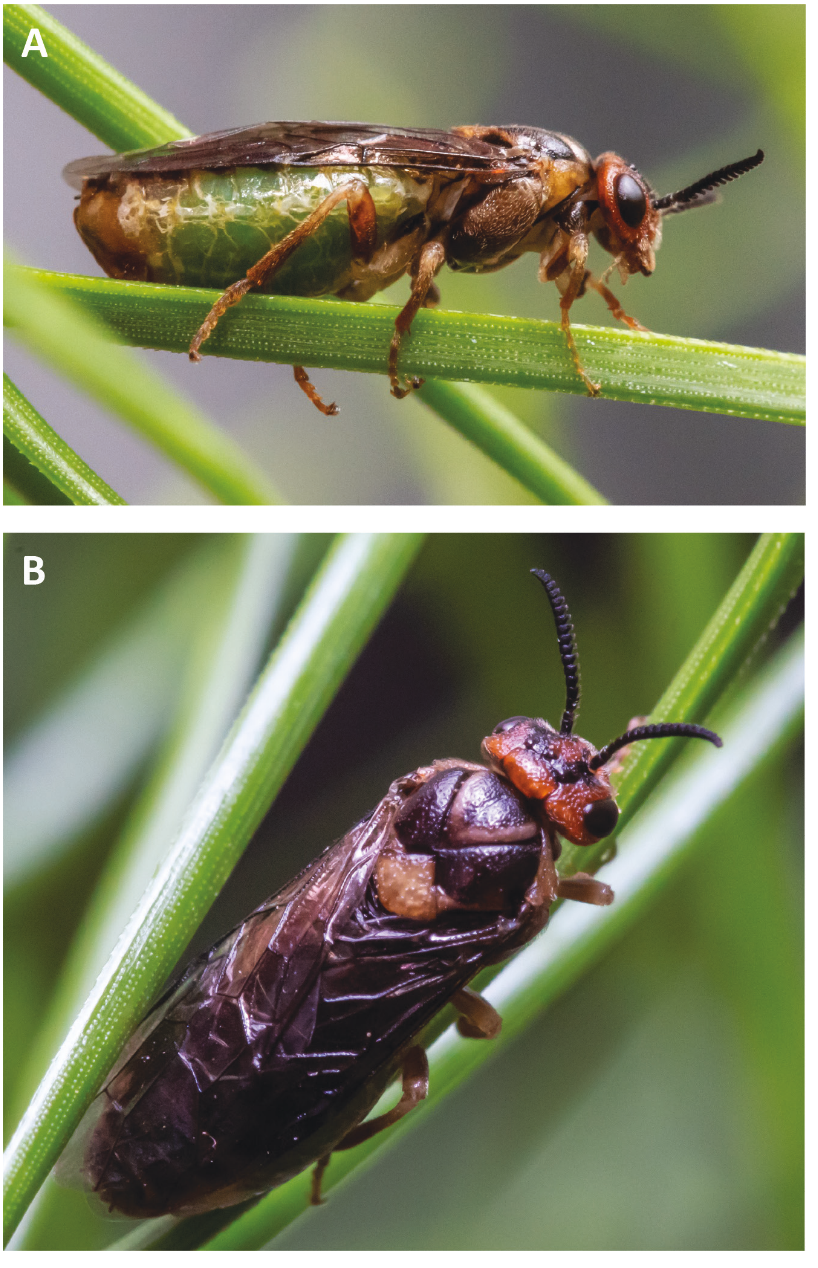

Figure 3. *Neodiprion warreni* adult female. The lateral (**A**) and dorsal (**B**) view of an adult *Neodiprion warreni* Ross female collected from Knoxville, Tennessee (colony ID: AG170).

Material deposited

Female (UKIC), "36.00496, -83.77818, 13-Sep-22, Knoxville, TN"; "AG170 ♀, NW23-V001, Ovipositor A"; "*Neodiprion warreni*, col. on *Pinus virginiana*, det. Ashleigh Glover", "UKIC_0056864". Female (UKIC) "36.00496, -83.77818, 13-

Sep-22, Knoxville, TN"; "AG170 ♀, NW23-V002"; "*Neodiprion warreni*, col. on *Pinus virginiana*, det. Ashleigh Glover", "UKIC_0056813". Male (UKIC), "36.00496, -83.77818, 13-Sep-22, Knoxville, TN"; "AG170 ♂, N. warreni; "*Neodiprion warreni*, col. on *Pinus virginiana*, det. Ashleigh Glover", "UKIC_0056720". Mounted Ovipositor (UKIC), "AG170, *Neodiprion warreni*, saw", "RDR, 06/06/23, A".

Neodiprion warreni distribution

To describe the current known distribution of *N. warreni*, we searched for the locations of all recorded *N. warreni* observations. These records were obtained from museum specimens (Linnen and Farrell 2010), research grade observations from iNaturalist (inaturalist.org), and our own collections (including the two new records above). The distribution map was created using the ggplot2 package (Wickham 2016) in R version 4.1.0 (R Core Team 2021). We also plotted the native range of *P. virginiana* on the map using the sf package (Pebesma 2018) to visualize the potential for further *N. warreni* range expansion. The *P. virginiana* shape files were downloaded from the USGS (https://web.archive.org/web/20170127093428/https://gec.cr.usgs.gov/data/little/).

Results

Using a *Neodiprion* key based on larval morphology (Davis et al. 2023), we confirmed that the two colonies collected on September 13, 2022 in Tennessee are *N. warreni*. Briefly, the larvae have a pale body, a completely black head, two black dorsal stripes, a row of thick black spots along each lateral side of the body that appear to bleed together, a black line above the prolegs, and a distinct black marking on the dorsal side of the last body segment (Fig. 2A, B). Additionally, adult female coloration and ovipositor morphology, as described by Ross (1961), further confirm that colony AG170 is *N. warreni*. The females are generally brownish in color, with an orange brown head (except for the eyes and antennae, which are black), a thorax that is a slightly paler brown with some darker areas on the dorsal side, an abdomen that contains some blackish areas and is greenish on the lateral side, and legs that are primarily light brown with a lighter straw coloration at the basal part of the tibiae (Fig. 3A, B). Finally, the ovipositors have unusually long and narrow lancets as well as numerous saw teeth that are unusually small, especially on annuli 2, 3, and 4 (Fig. 4), characteristics that are unlike other species in the *Neodiprion virginianus* complex to which this species belongs (Ross 1961).

Neodiprion warreni was previously reported as being distributed only in the southernmost parts of the United States (Georgia, Arkansas, Louisiana, and Florida; Ross 1961; Wilkinson 1968; Baker 1972; Smith 1979; Dixon 2004; Linnen and Farrell 2007, 2010). However, our collections in Tennessee provide evidence that the range of *N. warreni* extends further north than previously known (Fig. 5). Our Tennessee collections also provide evidence of a wider host range than has been reported: these collections are the first recorded occurrences of *N. warreni* using *P. virginiana* as a host.

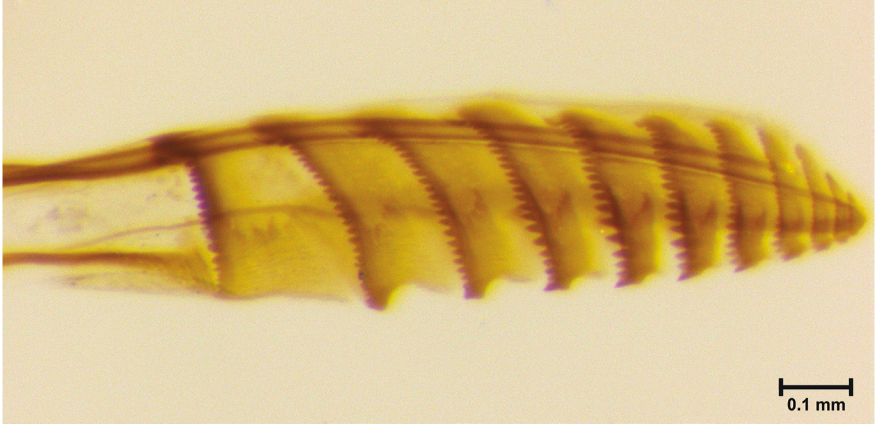

Figure 4. *Neodiprion warreni* female ovipositor. A mounted ovipositor from *Neodiprion warreni* Ross adult female individual "UKIC_0056864" (colony AG170) collected from Knoxville, Tennessee.

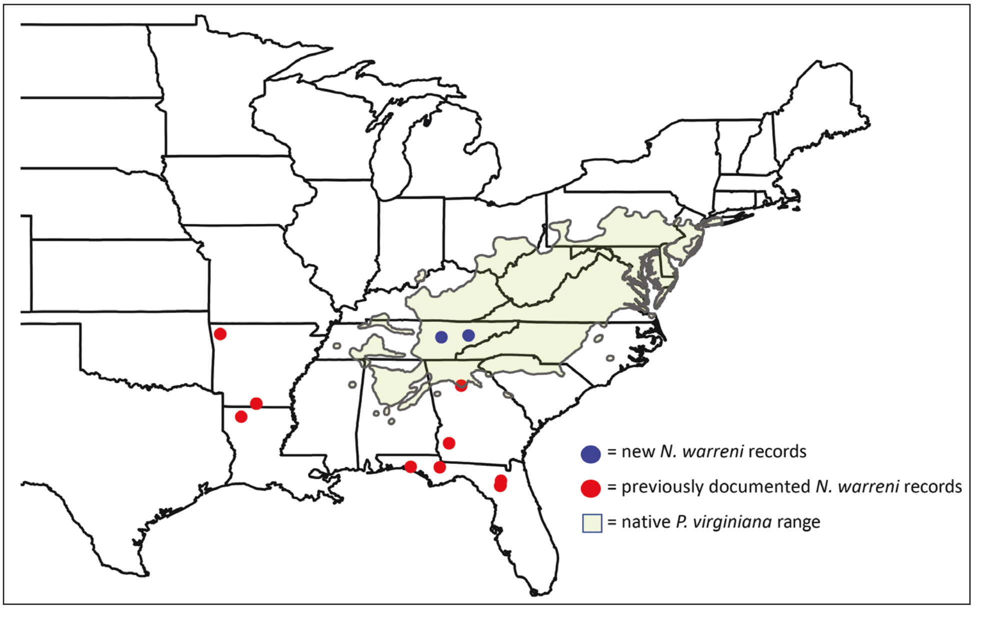

Figure 5. Currently known *Neodiprion warreni* distribution and native *Pinus virginiana* range. All recorded locations of *Neodiprion warreni* Ross observations in the eastern United States. Previously reported *N. warreni* observations are in red and the new recorded observations of *N. warreni* in this report are in blue. The native range of *Pinus virginiana* Mill. (newly reported host of *N. warreni*) is shown in green.

Discussion

With the documentation of our new *N. warreni* records, two possibilities arise. One possibility is that *N. warreni* has always been present in Tennessee and feeding on *P. virginiana* but has gone unreported until now. Thus, our new records enhance our knowledge of the geographical and host range of an understudied species. Alternatively, our new records may be evidence of a recent range expansion and host shift for *N. warreni*. Currently, we do not have enough data to distinguish between these two possibilities. In general, range expansions of many organisms are becoming more common as humans continue to modify the environment. These range expansions can have important impacts on insect pest species, such as the ability for insect pest species to hybridize upon secondary contact (Grabenstein and Taylor 2018; Larson et al. 2019; Ottenburghs 2021). One potential outcome of hybridization is adaptive introgression, or the exchange of genetic material between lineages via backcrossing that confers an adaptive advantage (Payseur 2010; Pfennig et al. 2016). If hybridization results in adaptive introgression, pest population dynamics can quickly change so that they evade established integrative pest management strategies (Correa et al. 2019). It is currently unknown whether *N. warreni* is expanding its range and whether this will lead to hybridization between *N. warreni* and other *Neodiprion* species that they come into secondary contact with. However, in no-choice mating assays performed in the lab, *Neodiprion virginiana* Rowher males mated with *N. warreni* females (Glover and Linnen, unpublished data). Interestingly, Tennessee and North Carolina comprise the southernmost part of *N. virginiana*'s published range (Smith 1979; Dixon 2004). Thus, our finding of *N. warreni*'s presence in Tennessee is notable because it verifies that *N. warreni* and *N. virginiana* co-occur, providing an opportunity for hybridization between these two species in nature.

Another important finding of our Tennessee *N. warreni* collections is their use of *P. virginiana* as hosts. This finding can have important economic and evolutionary implications. From an economic perspective, *N. warreni* now represents another potential pest species of *P. virginiana*, which has commercial value as Christmas trees (Belanger and Bramlett 1975). Because *N. warreni* is a cocoon-overwintering species with the potential to have multiple generations per year (Wilkinson 1968; Dixon 2004), large, destructive outbreaks are possible (Kulman 1971). Thus, identification of this new *P. virginiana* insect pest is important for effective management. From an evolutionary perspective, host shifts have initiated speciation in several plant-feeding insect systems (Bush 1975; Berlocher and Feder 2002; Drès and Mallet 2002; Matsubayashi et al. 2010). When plant-feeding insects rely on their host plant for most or all of their life cycle (as is the case for *Neodiprion*), colonization of a new host plant can result in strong selection on traits required to utilize the new host (Forbes et al. 2017). If different traits are favored on different hosts, reproductive isolation can rapidly evolve between host-associated populations (Glover et al. 2023). It is unknown whether the finding of *N. warreni* on *P. virginiana* indicates a host shift, and if so, whether the shift onto *P. virginiana* will result in a speciation event in *N. warreni*. However, this finding of *N. warreni* on a newly reported host provides another opportunity to study the relationship between plant-feeding insects and their hosts. This information can help shed light on why plant-feeding insects are so unusually diverse (Mitter et al. 1988; Farrell 1998; Wiens et al. 2015; Vertacnik and Linnen 2017).

While more work is needed to determine the consequences of broader geographical and host ranges in *N. warreni*, the first step is knowledge of these ranges. In this regard, community involvement, such as via recording observations of *N. warreni* and its hosts on iNaturalist (inaturalist.org), is valuable for facilitating the documentation of current and changing distributions of *N. warreni* and other *Neodiprion* species. Notably, only one record on iNaturalist of *N. warreni* exists to date, and this observation was recorded by the authors in 2019. Therefore, this report provides a valuable foundation for future work to investigate the possibility and consequences of *N. warreni* range expansions and host shifts.

Acknowledgments

The author order was decided through a handicapping competition of five races at Churchill Downs the weekend of the 2023 Kentucky Derby and Oaks. The result of that competition is as follows: In the Grade 2 Edgewood Stakes Papilio (ANG) finished 4th, six lengths ahead of Preliminary (RDR) who finished 11th; In the Grade 1 Kentucky Oaks Pretty Mischievous (ANG) finished 1st, three lengths ahead of Wet Paint (RDR) who finished 4th; In the Grade 2 Pat Day Mile Stakes Echo Again (RDR) finished 6th beating Kangaroo Court (ANG) by twelve lengths, who finished 11th; In the Grade 2 American Turf Stakes Major Dude (RDR) finished 3rd ahead of Mo Stash (ANG) in 4th by a length; and in the Grade 1 Kentucky Derby Angel of Empire (RDR) finished in 3rd, beating 7th place Tapit Trice (ANG) by seven and three quarter lengths. ANG and RDR wish to thank the equine and human athletes who helped us decide which author was to be listed first. We would also like to thank members of the Linnen lab for help with insect rearing.

This work was funded by a USDA National Institute of Food and Agriculture Predoctoral Fellowship (2021-09497) to ANG and the National Science Foundation DEB-CAREER-1750946 to CRL.

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