



OPEN *Sirex obesus* (Hymenoptera: Siricidae) as invasive pest in pine plantations in Brazil

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The genus *Sirex* (Hymenoptera: Siricidae) consists of 29 species including the Sirex Woodwasp, *Sirex noctilio*, which is the main insect pest of pine plantations in the Southern Hemisphere including Brazil. In 2023, a species of *Sirex* similar to *S. noctilio* was discovered in Southeastern Brazil infesting pine plantations and causing tree mortality of up to 40%. We definitively identified this species as *Sirex obesus* based on both morphological characters and DNA barcodes. It is a species indigenous to the Southwestern United States and Northern and Central Mexico with little information available regarding its biology and control. This is the first record of *S. obesus* in Brazil and the first record of the species outside of North America. We document details about *S. obesus* occurrence in Brazil, describe preliminary damage caused in pine plantations and provide a partial list of natural enemies.

Keywords Biological control, Invasive pest, *Pinus*, Woodwasp

Woodwasps in the family Siricidae, with 20 known genera and 124 species¹, predominantly occur in the Northern Hemisphere (North America, Europe, Northern Africa and Asia) where they are typically considered minor or secondary pests. When accidentally introduced to countries in the Southern Hemisphere where *Pinus* species have been introduced, the Sirex Woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae), has become the most significant economic pest of *Pinus* species causing up to 80% mortality in pine trees².

In South America several siricid species have been detected since the 1980's including *S. noctilio*, *Urocetus gigas*, *Urocetus flavicornis* and *Tremex fuscicornis*. Although the other siricids are not as destructive, since 1988, *S. noctilio* has spread across Brazil's main *Pinus* producing regions with high mortality rates of pine trees in southern Brazil during the 1990's^{3,4}.

Given the destructive potential of certain woodwasp species and the uncertainty surrounding which combinations of woodwasps and host trees leads to significant mortality, detecting and monitoring new woodwasps is of paramount importance to Brazilian silviculture. Herein, we report the discovery in July of 2023 of another exotic woodwasp in the state of São Paulo, Brazil.

Material and methods

On July 18, 2023, a technical inspection was carried out at the Estação Experimental de Ciências Florestais at ESALQ/USP in Itatinga, São Paulo, to investigate the mortality of several *Pinus* species and subspecies. Trees were inspected in August of 2023 for damage such as mortality, dripping resin or woodwasp-shaped (round) adult emergence holes in the trunk (Fig. 1). For each pine taxon, three sets of ten trees were examined.

The insect specimens collected at this time were damaged; therefore, adult wasps were reared for identification from wood billets in the laboratory at Botucatu, SP. Specimens were identified morphologically by inspection using the keys in Schiff et al.⁵ under magnification (Olympus SZX9 dissecting microscope). A total of 32 insects, including 15 females and 17 males, were sent to Dr. Nathan Schiff (USDA Forest Service, Stoneville, MS, USA) for taxonomic confirmation by molecular and morphological characters.

Ten adults were separated for mitochondrial DNA Barcode analysis following the protocol proposed by Schiff et al.⁵. DNA extraction was performed using the Q-Extract DNA Solution (Neobio) in combination with proteinase K (20 mg/ml). Universal primers targeting the Cytochrome Oxidase Subunit I (COI) gene were

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Fig. 1. Typical *Sirex obesus* damage: Hybrid *Pinus caribaea hondurensis* x *P. elliottii* log with emergence holes of *S. obesus* adults (left); 15-year-old plantation with high tree mortality (right). Buri, SP, Brazil.

employed, following Folmer et al.⁶. The primers used were: LCO1490 (forward) 5'-GGTCAACAAATCATAA AGATATTGG-3' and HC02198 (reverse) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR reaction was conducted in a final volume of 25 µl, containing 10X PCR buffer (Invitrogen), 3 mM MgCl₂ (25 mM) (Invitrogen), 0.4 mM dNTPs (25 mM) (Invitrogen), 10 pmol of each primer, 0.5 U of Platinum[®] Taq DNA Polymerase (5 U/µl) (Invitrogen), and 3 µl of extracted DNA.

The amplification protocol was performed using 20 ng of DNA and consisted of an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. A final extension step was carried out at 72 °C for 7 min. The PCR products were visualized on an ultraviolet light transilluminator (Major Science) using a 1% agarose gel and a 100 bp molecular marker (Norgen). The amplified products were purified using magnetic beads (AMPure Agencourt) and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). The samples were sent to the Central Laboratory of IBTEC, UNESP, Botucatu, São Paulo, Brazil, for Sanger sequencing.

Species were identified by comparing sequences to those in a siricid database⁵ using Lasergene 15 by DNASTar software. Sequences from both directions were combined into contigs using Seqmanpro, aligned in Megalign using Clustal V and compared to generate a Neighbor-Joining tree based on P2K. The sequences were deposited in Genbank under the accession numbers PP790220, PP794013, PP794015 and PP794016.

Results and discussion

At the first inspections, the causal agent was initially assumed to be *S. noctilio*, given its established distribution in the state of São Paulo⁷, and the woodwasp-like damage of the trees (Fig. 1). However, there was cause to expect something unusual since in Brazil, *S. noctilio* is predominantly associated with loblolly pine, *Pinus taeda*, and many of the trees attacked were Caribbean species, subspecies and hybrids. The affected *Pinus* taxa included: *Pinus caribaea hondurensis*, *P. caribaea bahamensis*, *P. caribaea caribaea*, *P. maximinoi*, *P. tecunumanii*, and *P. taeda*.

After morphological examination of undamaged male and female woodwasps (Fig. 2) and DNA barcode analysis the species attacking these non-*P. taeda* pines was determined to be *Sirex obesus* Bradley, 1913. This species is native to North America, and found in the Southwestern United States, particularly in New Mexico and Arizona, as well as in Northern Mexico, near the U.S. border⁵, and in Aguascalientes in the central part of the country⁸. *Sirex obesus* females are very similar to those of *S. californicus*, another North American species to which they are most closely related but can be separated from it by the density of pits on the vertex and gena and

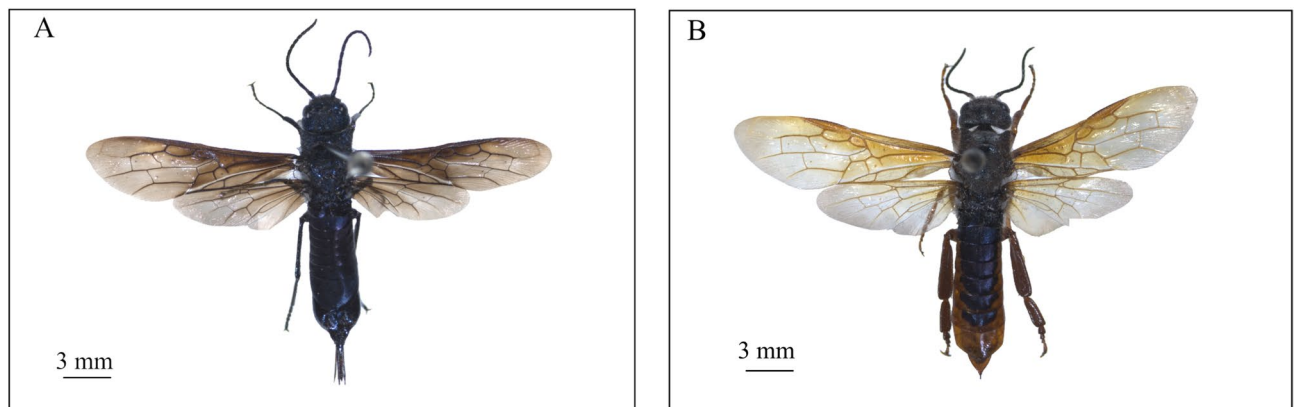


Fig. 2. *Sirex obesus* adults. (A) Female (left); (B) Male (right).

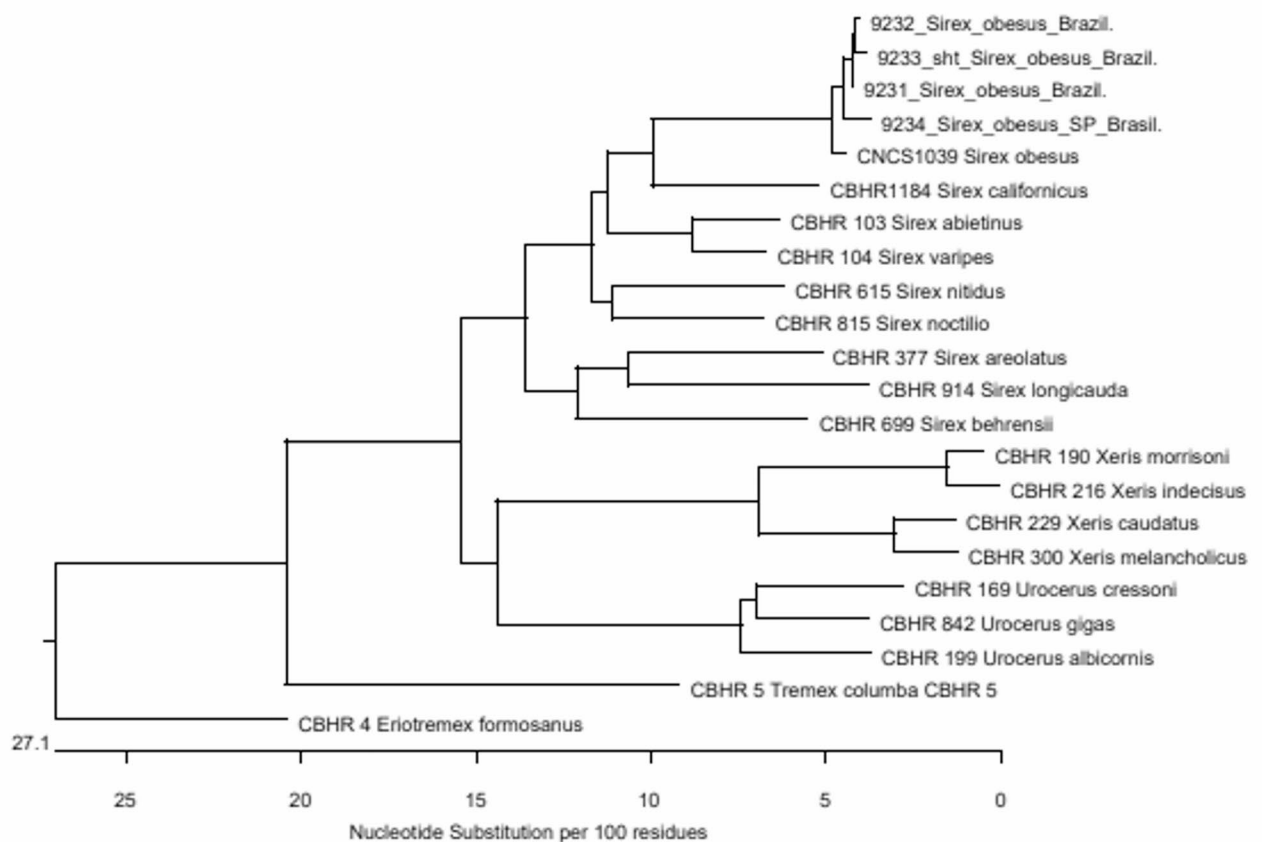


Fig. 3. Neighbor-joining tree of CO1 sequences demonstrates that 4 specimens from Brazil are most similar to a *Sirex obesus* sequence from North America.

the short metatarsomere 2. Males can be separated from closely related species by black antennae and yellowish tinted forewings⁵. Based on DNA barcodes several samples of the Brazilian species were almost identical to *S. obesus* from the United States and clearly distant from the next closest species, *S. californicus* (Fig. 3).

A second woodwasp outbreak was observed on November 28, 2023, at a different location approximately 130 km from Itatinga, in Buri, SP. This farm had plantations of the pine hybrids *P. caribaea hondurensis* x *P. elliottii* and *P. caribaea hondurensis* x *P. tecunumanii*, which are used for resin production. Samples from this second site also were identified as *S. obesus* based on morphology and DNA barcoding. The combined results of the distribution surveys can be seen in Fig. 4.

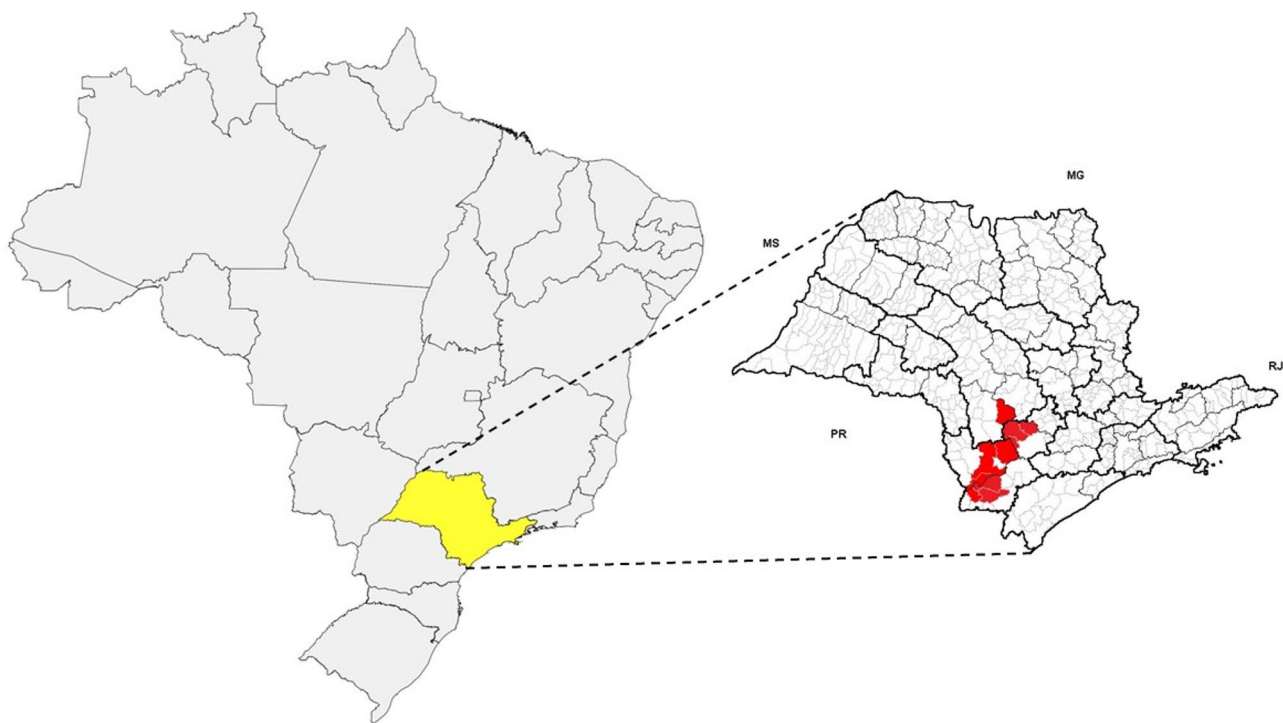


Fig. 4. Geographical distribution of *Sirex obesus* (Hymenoptera: Siricidae) in the State of São Paulo, Brazil.

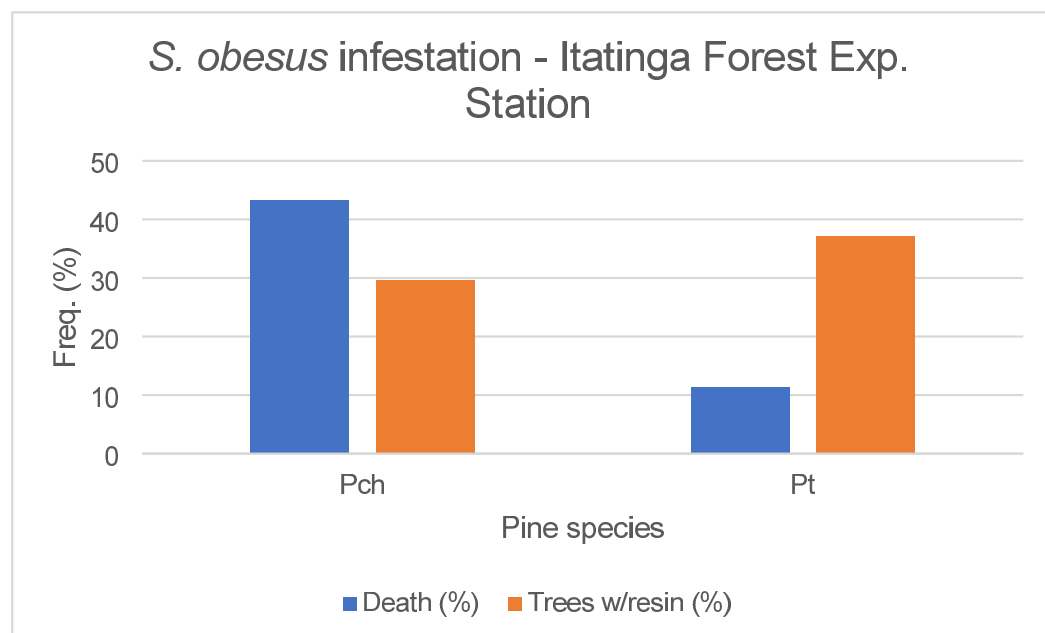


Fig. 5. Observed *Sirex obesus* damage on blocks with two *Pinus* species. Itatinga, SP, Brazil, July 18th, 2023. Pch: *P. caribaea hondurensis*; Pt: *P. taeda*.

It's not possible to determine whether these two outbreaks are related, as the initial point of *S. obesus* establishment in Brazil is unknown, making it difficult to track its dispersal. In Brazil, *S. obesus* has been detected in the state of São Paulo, located in the southeastern region, in plantations across 14 municipalities. There is potential for its expansion to the southern region, where the main *Pinus* plantations are located.

At this time, damage could only be estimated through some preliminary sampling (Fig. 5). One of the most significantly impacted species was *P. caribaea hondurensis* with, in the worst cases, an average mortality reaching as high as 43%. In contrast, loblolly pine (*P. taeda*) was much less impacted, averaging 11% mortality.

Unfortunately, the mortality statistics alone do not adequately describe the extent of the damage. In addition to the trees that have already died, 30% of *P.c. hondurensis* trees are dripping resin, a consequence of woodwasp oviposition. Therefore, the eggs will hatch presumably within the next year, and the woodwasp borers will kill those trees. This would increase total mortality from 43% to approximately 73% ($43 + 30 = 73\%$). Likewise, for *P. taeda*, the current mortality rate of 11% could rise to 49% as an additional 38% of trees succumb. Following this logic, these areas could experience complete tree mortality within a few years.

As this is the first South American population of a poorly known species, we include some descriptive statistics and biological information. Females of *S. obesus* ($n = 207$) collected in Itatinga and Buri have an average length of 20.8 mm (ranging from 12.5 to 31.7 mm) and wingspan of approximately 30.7 mm while males ($n = 252$) measure 16.6 mm on average (ranging from 12.5 to 26.6 mm) and a wingspan of 26.2 mm.

Females are recognized by their overall dark body coloration, thin and dark hind legs, and a long, developed ovipositor protected by a pair of sheaths. The last abdominal segment contains a triangular structure called a cornus. According to Schiff et al.⁵ females of *S. obesus* are very similar to those of *S. californicus* and can be easily confused. Females of *S. obesus* have a black body, legs, antennae, and palps with metallic blue reflections (Fig. 2).

Males exhibit considerable variation in body size, characterized by an orange abdomen with a dark dorsal spot extending almost the entire length of the abdomen. *Sirex californicus* and *S. obesus* males share significant similarities, but they can be differentiated by the metallic blue spot located between tergites 1–7, on the dorsal region of the abdomen in *S. obesus*, while males of *S. californicus* have golden-yellow tergites. The hind legs are enlarged, especially the femur, tibia and basitarsus, and are reddish-brown in color (Fig. 2).

There is scarce information about the biology of *S. obesus*^{5,8}. Reproduction can be sexual, as occurs in other *Sirex* species, with a higher proportion of males to females (7:1) for the specimens collected in Buri and Guareí, SP. Preliminary data on the longevity of *S. obesus* adults in Brazil indicate that females live for approximately 5.7 days, while males have a lifespan of about 7.5 days ($n \sim 1600$).

After mating, females seek out the trunk of *Pinus* trees to begin oviposition, very similar to the behavior of females of *S. noctilio*, leaving resin spots along the trunk of the infested trees (Fig. 1).

The development cycle for *S. obesus* is unknown, but for many temperate woodwasp species it may take 1–3 years between oviposition and the emergence of adults⁵. Some species in the Southern Hemisphere may take less than 1 year and preliminary rearing results obtained in Brazil, suggest *S. obesus* could have 2 or 3 generations per year, based on adult emergence in *Pinus* logs maintained in cages.

Direct observation in Brazilian plantations included tree mortality, larvae making tunnels in the wood, adult emergence holes in the trunk (Fig. 1), and wood staining caused by wood-staining fungi (not shown).

Woodwasps kill trees by injecting a symbiotic wood decay fungus and a phytotoxic mucus into the tree when laying eggs. The toxin weakens the tree, allowing the fungus to spread, typically killing the tree in as little as 3–4 months after oviposition. *Sirex noctilio* uses the fungus *Amylostereum areolatum* whereas *S. obesus* in North America uses *Amylostereum chailletii*, a species that has not yet been confirmed in Brazil^{5,9}.

Wood boring insects are often transported to new places in woody packing material which also may harbor natural enemies. To date we have observed two species known to feed on woodwasps emerging from logs infested with *S. obesus*: *Ibalia leucospoides* (Hymenoptera: Ibalidae) and a species of *Schlettererius* (Hymenoptera: Stephanidae). Although these species by themselves are not known to effectively control woodwasps they could be part of a control program. We are conducting, so far unsuccessfully, surveys for the parasitic nematode, *Deladenus siricidicola* (Nematoda: Neotylenchidae), that has been used successfully in several Southern Hemisphere countries to control *S. noctilio*.

There is no information about the entry pathways of *S. obesus* in Brazil. The main hypothesis is that the pest may have arrived in packaging material (pallets), as technicians from the Ministry of Agriculture have found pallets without the heat treatment seal required for international trade (ISPM #15¹⁰) during some inspections (Wilcken, personal information).

Furthermore, if left uncontrolled, the pest may spread to other pine producing regions in Brazil and neighboring countries and cause serious economic impacts. Considering this is a species new to Brazil, studies on the biological characteristics of the insect and its symbiotic fungus are underway. At the same time, control strategies are being studied to contain the spread of the pest.

Data availability

The datasets generated that support the findings of this study are openly available in Genbank repository (<https://www.ncbi.nlm.nih.gov/genbank/>). Accession numbers: PP790220; PP794015; PP794013; PP794016.

Received: 20 November 2024; Accepted: 9 June 2025

Published online: 02 July 2025

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Acknowledgements

To Resinas Brasil and Estação Experimental de Ciências Florestais, ESALQ/USP for allowing us to collect insects in the field and for providing billets infested with insects during this study. The reviewers, who kindly contributed to the manuscript.

Author contributions

CFW conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by CFW, TAM, CHO, VRC, LAB, JAG, SRSW, ELF, NMS, MBC and MFR. The first draft of the manuscript was written by CFW and MFR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)”, “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Finance Code 001)” and “Programa Cooperativo sobre Proteção Florestal (PROTEF) do Instituto de Pesquisas e Estudos Florestais (IPEF)”.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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