



Complete Genome Sequence of a New Isolate of *Solenopsis invicta virus 3* from *Solenopsis invicta* × *richteri* Hybrid Ants

Steven M. Valles,^a Jason B. Oliver,^b Karla M. Addesso^b

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA^a; Tennessee State University, Department of Agricultural and Environmental Sciences, Otis L. Floyd Nursery Research Center, McMinnville, Tennessee, USA^b

ABSTRACT *Solenopsis invicta virus 3* (SINV-3) is a positive-sense, single-stranded RNA virus that infects the red imported fire ant, *Solenopsis invicta* Buren. We report here the full genome (10,383 nucleotides) of an isolate infecting *Solenopsis invicta* × *richteri* hybrid ants, which we have identified as SINV-3 hybrid.

Solenopsis invicta virus 3 (SINV-3) is the type species of a new family of positive-sense, single-stranded RNA viruses named *Soliniviridae* (1). Initially, the monopartite genome was reported to encode two nonoverlapping open reading frames (ORFs) with the 5'-proximal ORF encoding proteins with identity to helicase, protease, and RNA-dependent RNA polymerase (RdRp), while the 3'-proximal ORF encoded proteins with identity to virus capsid proteins (2). However, it was later determined that the 3'-proximal ORF was expressed via a frameshifting mechanism and that the sequences encoding the structural proteins actually mapped to both ORF2 and the 3' end of ORF1 downstream of the sequence that encodes the RdRp (3). In addition, the structural proteins were found to be translated from both genomic and subgenomic RNA (3). Two isolates of the SINV-3 genome have been sequenced in their entirety, the DM isolate (2) from North American *Solenopsis invicta* (GenBank accession number FJ528584), and the SF isolate (4) taken from South American *S. invicta* (GenBank accession number GU017972).

During efforts to release SINV-3 (DM isolate) into *S. invicta* populations in Tennessee as a classical biological control agent, we discovered a unique isolate of the virus in the *S. invicta* × *richteri* hybridized host worker ant caste. Here, we report the genome sequence of the SINV-3 isolate, designated hybrid, from these hybridized ants.

Worker ants were collected from hybrid colonies on 17 April 2017 in a pasture near Ocoee, Tennessee, USA (35.136370 latitude, -84.712534 longitude). The ants were confirmed to be *S. invicta* × *richteri* hybrids by cuticular hydrocarbon and venom alkaloid analyses (5). Total RNA was extracted from worker ants with the TRIzol (Life Technologies, Inc.) method. Reverse transcriptase PCR was used to amplify 10 overlapping regions of the entire genome. The amplicons were gel purified, ligated into a pCR4-TOPO vector, transformed into TOP10-competent cells (Life Technologies, Inc.), and Sanger sequenced by MacroGen USA. The 5' and 3' termini of the genome were acquired by rapid amplification of the cDNA ends (RACE) using a gene-specific oligonucleotide primer and oligonucleotide dT primer for cDNA synthesis for the 5' and 3' termini, respectively. Assembly of the overlapping sequences was completed with Vector NTI software (Life Technologies, Inc.).

The SINV-3 hybrid genome is monopartite with two nonoverlapping ORFs, one at the 5' end (ORF1; nucleotides [nt] 92 to 7834) and one at the 3' end (nt 8,308 to 10,263), separated by an untranslated intergenic region (nt 7832 to 8307) and terminated in a polyadenylated sequence. An additional overlapping ORF (ORF2a; nt 8351 to 8827) was

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detected within ORF2. ORF1 yielded a polyprotein comprising 2,580 amino acids (aa) with a molecular mass of 299,050; ORF2 yielded a polyprotein of 651 aa with a molecular mass of 73,050; and ORF2a yielded a polyprotein of 158 aa with a molecular mass of 18,758. ORF1 contained conserved domains for the nonstructural proteins helicase (aa 396), protease (aa 1,379), and RNA-dependent RNA polymerase (aa 2,027), as well as a conserved domain for a structural protein (aa 2,305). Conserved domains for structural proteins were also recognized in ORF2. This sequence represents the third isolate of SINV-3 sequenced in entirety.

Accession number(s). The complete genome sequence of SINV-3 hybrid was deposited in GenBank under the accession number [MF797911](https://www.ncbi.nlm.nih.gov/nuccore/MF797911).

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