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GENOME SEQUENCES

Near-Complete Genome Sequences of New Strains of *Nylanderia Fulva Virus 1* from *Solenopsis invicta*

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ABSTRACT *Nylanderia fulva virus 1* (NfV-1) is a single-stranded positive-sense RNA virus that infects the tawny crazy ant. Three near-complete genomes of NfV-1SI (92% to 94% nucleotide identity to reference strain NfV-1) found infecting the red imported fire ant were determined. The genomes have 10,904 to 10,918 nucleotides and include most of the coding region for the polyprotein.

Ants are among the most destructive invasive species. In recent years, viruses infecting invasive ants have been sought and identified. Among these are viruses infecting the red imported fire ant (RIFA), *Solenopsis invicta*, and viruses infecting the tawny crazy ant, *Nylanderia fulva* (1–3). A new virus isolated from *N. fulva*, *Nylanderia fulva virus 1* (NfV-1; genus *Nyfulvavirus*, family *Solinviviridae* [4]), has been described and proposed as a biopesticide; this virus is not native to North America (1, 5). *S. invicta* colonies may host multiple concurrent virus infections (6, 7), but NfV-1 has never been isolated from RIFA. Genome sequences of a novel NfV-1 strain, designated NfV-1SI because they were identified from RIFA samples rather than the reported host *N. fulva*, are reported here. Because the samples were from a small geographic location (three ant colonies from within a 20-acre pasture), the virus was assumed to be the same infectious species.

Ant colonies were collected from Washington County, Mississippi, in March 2016. The ant colonies were confirmed by a cuticular hydrocarbon profile to be *S. invicta* polygyne form and were maintained under standard laboratory conditions for genetics analyses (8–11). Polygyne *S. invicta* colonies are composed of hundreds to hundreds of thousands of sterile workers and two to dozens of queens. A colony that will tolerate only one queen is called monogyne, and the adults of these two colony forms, or social organization forms, differ in their cuticular hydrocarbon profiles (11, 12). The colony also contains juvenile insect stages—eggs, larvae, prepupae, and pupae. The collective term for all juvenile forms of ants is brood. The larvae of ants grow and develop through multiple stages called instars, separated by molts. *S. invicta* larvae have four instars. The pupa does not molt, but as it progresses toward the adult stage, the cuticle darkens (12, 13). Total RNA was extracted from six samples of ant brood from three laboratory colonies. Ants from two juvenile life stages were collected, fourth instar larvae and white pupae, all of which were workers (nonreproductives). For each RNA sample, 20 individuals were pooled and extracted using USB PrepEase (Affymetrix, Cleveland, OH, USA) and were quantitated with a NanoDrop spectrophotometer. Total RNA was sequenced by LC Sciences (Houston, TX, USA). The sequencing provider verified RNA quality with an Agilent Bioanalyzer. Libraries were prepared by the sequencing provider after ribosomal depletion using Ribo-Zero Gold according to the Illumina TruSeq stranded total RNA sample preparation guide. Sequencing was performed on the Illumina HiSeq 2000 platform in the 100-bp paired-end (PE) configuration according to the manufacturer's instructions. Cutadapt (14) was used to remove

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adapters and low-quality or unresolved bases. Sequence quality was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Six pairs of filtered (fastq) read files were provided. The three larva samples, A01, B02, and C03, generated 104.9 million, 99.5 million, and 84.8 million reads, respectively (10). Paired fastq files were processed individually using the assembly function of SeqMan NGen version 14.1 (DNAStar, Madison, WI, USA) mapping to virus genes. Each larva assembly was visualized with SeqMan Pro (DNAStar) and found to contain long contigs matching the genome of NfV-1. To obtain full-genome sequences, the six paired-end Illumina file sets were processed again with SeqMan NGen using the NCBI reference genome of NfV-1 (GenBank accession number NC_030651.1) as a mapping reference. Each larva sample (but not the pupa samples), when visualized with SeqMan Pro, yielded a nearly complete viral genome based on the reference as follows: sample A01 generated a 10,909-nucleotide (nt) sequence, sample B02 generated a 10,918-nt sequence, and sample C03 generated a 10,904-nt sequence. These sequences were, respectively, 94%, 94%, and 92% identical to the reference genome at the nucleotide level (15). The sequences generated by samples A01 and B02 included a start codon and a stop codon followed by a polyadenylation sequence. The sequence from C03 lacked this putative start codon, because it was shorter. Each sequence encoded a predicted polyprotein as follows: samples A01 and B02 lacked the initial 6 amino acids (aa) but included a methionine (initiation amino acid) corresponding to the seventh amino acid in the reference sequence translation. The predicted partial polyproteins were 3,608 aa for samples A01 and B02 and 3,607 aa for sample C03 and were 97% (A01), 97% (B02), and 95% (C03) identical (16) to the predicted polyprotein (NCBI protein accession number YP_009268643) encoded by the reference genome (GenBank accession number NC_030651.1). The first seven translated residues of the NfV-1 genome are MTSEKVM; thus, while the first methionine of the reference polyprotein was not present in any of the *S. invicta* sequences, the second methionine, amino acid 7, retained in two of the three sequences found in *S. invicta* larvae, could be the start codon for the polyprotein.

The three putative virus sequences obtained from the three larva samples were highly similar to one another; nucleotide sequences from samples A01 and B02 were 97% identical to each other and 94% identical to C03. Translated sequences from samples A01 and B02 were 99% identical to one another and 96% identical to C03 (15, 16).

The detection of virus in transcriptomes assembled from late instar larval *S. invicta* (but not pupae) was consistent with host infection characteristics similar to those published; NfV-1 detected in *N. fulva* exhibited significantly larger amounts in the larval stage (1). This is the first documented occurrence of NfV-1 outside of the initial reported host ant species.

Data availability. The three genome sequences described here have been deposited in GenBank under the accession numbers MG696804, MG696805, and MG696806. The raw sequences were deposited in GenBank as BioProject number PRJNA393960, BioSample numbers SAMN07345557 and SAMN07345558, and Sequence Read Archive (SRA) numbers SRR5868358 to SRR5868363.

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