A draft genome of *Steinernema diaprepesi*

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Abstract

Entomopathogenic nematodes within the genus *Steinernema* are used as biological control agents against significant agricultural pests. *Steinernema diaprepesi* is native to Florida and very effective in controlling citrus root weevil, a devastating pest of citrus, ornamental plants, and vegetables. Here, we present the draft genome of *Steinernema diaprepesi*, which is a valuable tool for understanding the efficacy of this nematode as a biological control agent.

Keywords

Entomopathogenic nematode, Biological control, Genomics.

Citrus root weevil, *Diaprepes abbreviates*, is an important pest of citrus, ornamental plants, and other vegetables in Florida and is spreading throughout southern Texas and southern California (Lapointe et al., 2007; Stuart et al., 2008; Cherry et al., 2011). Root weevil is polyphagous, its increasing geographical distribution has made them a subject of quarantine and eradication programs (Stuart et al., 2008; Campos-Herrera et al., 2015). Soil-applied halogenated hydrocarbons are effective against this insect, but have since been deregistered. Currently, there are no effective registered pesticides against this insect pest (Campos-Herrera et al., 2015). Studies on the use of biological control agent to manage this weevil has been of interest for the past couple of decades (Beavers et al., 1983; Shapiro et al., 2000) and as a result, the use of entomopathogenic nematodes as control agents was found to be effective on *D. abbreviates* larva (McCoy et al., 2002; Ali et al., 2010; Duncan et al., 2013). Entomopathogenic nematodes endemic to citrus growing regions in Florida include *Steinernema diaprepesi* and *S. khuongi* and their role in determining the distribution of root weevil is also evident (Nguyen and Duncan, 2002; Duncan et al., 2003; Stuart et al., 2008; Campos-Herrera et al., 2013; Stock et al., 2018).

The endemic entomopathogenic nematode *S. diaprepesi* is commercially applied to control citrus root weevil. As an obligate parasite, *S. diaprepesi* relies on the toxin produced by its symbiotic bacteria *Xenorhabdus doucetiae* to kill insect hosts (Goodrich-Blair and Clarke, 2007; Stock and Blair, 2008; Castillo et al., 2011). The genome of any organism is the basis of the biological, molecular, and cellular processes that are vital for development and reproduction as it encodes the entire inheritance message of living organisms. Improved understanding of the genome aid in the knowledge of complex gene networks, molecular mechanisms of underpinning symbiosis and pathogenicity, and also provides a foundation for engineering trait improvements (Bolger, Weisshaar, Scholz, Stein, Usadel and Mayer, 2014; Lu et al., 2016; Rodríguez-Leal et al., 2017). The full genome sequence of *X. doucetiae* is currently available and provides a resource for understanding the evolution of virulence genes in bacteria. However, there is little information about the genome of the nematode (Ogier et al., 2014).

In this study, the genome of *S. diaprepesi* was sequenced and assembled. This information will be very valuable to understand the mechanism of evolution, molecular processes that determine parasitism and symbiosis within this complex system followed by the genetic features that make this nematode more effective against citrus root weevil and the extent of their host range.

Nematode samples for genome sequencing were received from Dr. Duncan's lab at UF/IFAS Citrus Research and Education Center. To confirm the identity of nematodes, we sequenced the ITS region of the ribosomal DNA. The primers used were AB28: 5′-ATATGCTTAAGTTCAGCGGGT-3′ and TW81:

5´-GTTTCCGTAGGTGAACCTGC-3´ The protocol for DNA extraction and condition for Polymerase chain reaction (PCR) amplification (reaction and cycling condition) were followed as Hominick et al. (1997) (Stock et al., 2018). The sequences were queried at the NCBI nucleotide database utilizing megablast with other sequences available at the GenBank using the BLASTn similarity search program. Finally, nematode was confirmed as *S. diaprepesi* with 99.07% identity. Approximately 10,000 freshly collected infective juveniles (IJ) were surface sterilized. Sterilized nematodes were flash-frozen in Liquid nitrogen and thawed twice for DNA extraction. High molecular weight genomic DNA was extracted using a phenol-chloroform method (Donn et al., 2008). The DNA pellet was resuspended in 100 μl Tris-EDTA buffer. University of Florida's campus-wide Interdisciplinary Center for Biotechnology Research (ICBR) NextGen DNA Sequencing Core Facility (Gainesville, FL) performed library preparation and sequencing using MiSeq Illumina sequencing platform with 2X300v3 format.

A total of ~22 million reads were generated, comprising 6.46 Gb using 300 bp paired-end sequencing. The sequence quality of the raw reads was analyzed using FastQC (Andrews, 2010). Quality trimming, read filtering, and removing adapter contamination were performed using Trimmomatic/0.36 (Bolger, Lohse and Usadel, 2014). Clean reads were subjected to De Novo assembly using the SPAdes/3.13.0 assembler (Bankevich et al., 2012) with Kmer size of 21, 33, 55, 77, 99, and 127. Assembly obtained from kmer 127 was used for downstream evaluation based on fewer contigs and higher N50. Preliminary genome assembly was likely contaminated with the symbiotic bacteria, fungal, and bacterial contaminants. To remove possible bacterial sequences, a sequence search using Blastn of all the contigs was conducted against the NCBI nucleotide database (with *E*-value cutoff <1e–05), and taxonomy was assigned to each contig. Each raw read was mapped to contigs using Bowtie2 (Langmead and Salzberg, 2012). Finally, the assembly was decontaminated using Blobtools v1.0 (Laetsch and Blaxter, 2017), which removed all bacterial contigs. Additionally, all contigs below 500 bp were removed from the final assembly. The quality of the draft assembly was determined by Quast (Gurevich et al., 2013). The draft genome presented here of *S. diaprepesi* contains 118 MB distributed among 35,545 contigs with contigs N50s of 11,474 bp and GC 45.01% with the longest contigs of 1,706,490 bp. There were zero N's per 100 kbp within this assembly. We assessed the genome for completeness using BUSCO (Simão et al., 2015). A total of 982 BUSCOs in the Nematoda dataset were used, and our draft genome of *S. diaprepesi* had a complete BUSCO score of 85%. Most of these genes are single-copy loci at 79.6%, with 5.4% complete and duplicated BUSCOs, 7.1% fragmented BUSCOs, and 7.9% missing BUSCOs. Prediction of protein-coding genes above 1,000 bp contigs was carried out by using GenMark-ES/4.33 tool (Borodovsky and McIninch, 1993), which predicted 15,094 genes (Table 1).

Due to the draft nature of this genome, it is incomplete, and we expect to see genome size variation between different isolates of the same nematode as *Steinernema feltiae* 82.5 Mb (Dillman et al., 2015) and 121.6 Mb (Fu et al., 2020). To confirm the genome size, checking for heterozygosity among the reads or using flow cytometry could provide a more accurate estimation. In its current state, this draft genome can provide support for comparative genomics of *Steinernema* nematodes, understand the evolution of genome network, genomic variation between different isolates, evolutionary process, and enable the functional genomics among entomo-pathogenic nematodes.

The Whole Genome Project of *S. diaprepesi* is deposited at GenBank under the accession number JAANPW000000000. All DNA sequence data are deposited in GenBank under Biosample No. SAMN14073714 Bio project No. PRJNA605202.

Table 1. Summary Statistics of the Assembly of *Steinernema diaprepesi*.

Assembly statistics	
Size (bp)	**118,329,602**
Number of contigs	35,545
Largest contigs (bp)	1,706,490
GC content (%)	45.01
N50 value (bp)	11,474
N's per 100 kbp	0
Number of predicted genes	15,094
Complete BUSCOs	835 (85%)
Complete and single-copy BUSCOs	782 (79.6%)
Complete and duplicated BUSCOs	53 (5.4%)
Fragmented BUSCOs	70 (7.1%)
Missing BUSCOs	77 (7.9%)

Acknowledgments

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