First Genomic Resource of the Columbia Lance Nematode *Hoplolaimus columbus*

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Abstract

The Columbia lance nematode *Hoplolaimus columbus* has been reported frequently from North America due to its negative impact on agricultural production. In this study, for the first time, we sequenced the whole genome of a female specimen by using whole-genome amplification and Illumina MiSeq. Data were de novo assembled to form scaffolds of 205.75 Mbp consisting of 118,374 contigs. The largest scaffold was 636,881 bp. Benchmarking Universal Single-Copy Orthologs completeness was 66.6% (eukaryotic dataset), and >8,000 unique genes were predicted by GeneMark-ES. In total, 61,855 protein sequences were predicted by AUGUSTUS, and 10,085 of them were annotated by PANNZER2 with at least one function. These data will provide valuable resources for studies focusing on pathogenicity and phylogenomics of plant-parasitic nematodes.

Genomic Resource Announcement

Lance nematodes (*Hoplolaimus* spp.) is a genus of soil-inhabiting plant parasites distributed around the world that includes >30 species (Ma et al. 2011, 2019; Sher 1963). *Hoplolaimus columbus*, also known as the Columbia lance nematode, was first reported in 1963 in Columbia, SC, and later was found in North Carolina, Georgia, Alabama, and Louisiana (Astudillo and Birchfield 1980; Gazaway and Armstrong 1994; Holguin et al. 2015, 2016; Koenning et al. 1999; Krusberg and Sasser 1956). Most of its close relatives are reported in Pakistan, India, China, and Japan (Siddiqi 2000). These lance nematodes have a distinct cephalic region and a well-developed stylet (Sher 1963) that enable them to be migratory ecto- and endoparasites. They can destroy cortex cells, which results in the extension of necrotic lesions (Krusberg and Sasser 1956; Lewis and Fassuliotis 1982). As a significant threat to agricultural plants in the southeastern United States, *H. columbus* can cause up to 25% production losses for cotton, and its damage on soybean production can be as high as 70% (Appel and Lewis 1984; Lewis et al. 1976; Mueller and Sanders 1987; Noe 1993). In spite of its importance to phytopathology, no genomic data are available yet. In this study, for the first time, we provide a genomic resource for an *H. columbus* female isolate from North America. In addition, a complete mitochondrial chromosome of *H. columbus* was de novo assembled using NOVOPlasty (Dierckxsens et al. 2017) and has been reported (Ma et al. 2020).

In April 2017, *H. columbus* specimens were collected from the Edisto Research Center in Blackville, SC. Specimens were extracted from soil samples (Jenkins 1964), then diagnosed and identified according to results of morphological observation and molecular methods (Bae et al. 2009; Handoo and Golden 1992; Ma et al. 2011). This species identification was later validated by aligning the assembled mitochondrial genome of the isolate with *COX1* sequences of *H. columbus* in GenBank (Ma et al. 2020). The whole genomic DNA was first extracted from a

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Keywords

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Table 1. Genome assembly information of *Hoplolaimus columbus* analyzed using QUAST^a

Sample information	<i>Hoplolaimus columbus</i>	
	Contigs	Scaffolds
Assembly metrics		
Number of contigs	118,374	117,809
Number of contigs ($\geq 1,000$ bp)	67,667	67,120
Number of contigs ($\geq 5,000$ bp)	5,653	5,759
Number of contigs ($\geq 1,0000$ bp)	862	923
Number of contigs ($\geq 25,000$ bp)	59	51
Number of contigs ($\geq 50,000$ bp)	40	32
Largest sequence	386,658	636,881
Total length	205,719,153	205,753,054
N ₅₀	2,321	2,341
N ₇₅	1,198	1,199
L ₅₀	22,274	21,829
L ₇₅	53,864	53,302
GC (%)	39.42	39.42
Mismatches		
Number of Ns	0	29,168
Number of Ns per 100 kbp	0	14.18
Predicted genes (GeneMark-ES)		
Number of predicted genes (unique)	–	8,242
Number of predicted genes (≥ 0 bp)	–	15,691 + 0 part
Number of predicted genes (≥ 300 bp)	–	9,303 + 0 part
Number of predicted genes ($\geq 1,500$ bp)	–	714 + 0 part
Number of predicted genes ($\geq 3,000$ bp)	–	76 + 0 part

^a GenBank BioProject accession number PRJNA659263, GenBank BioSample number SAMN15902582, GenBank Sequence Read Archive number SRR12516078, and accession number JAEHOM000000000.

single nematode (female) using a Sigma Extract-N-Amp kit (XNAT2) (Sigma-Aldrich) with an optimized extraction protocol (Ma et al. 2011), then was amplified to obtain an adequate quantity using an Illustra Ready-To-Go GenomiPhi V3 DNA Amplification kit (GE Healthcare). Three replications of whole genomic DNA amplification were performed, DNA concentration in each step was checked using a Qubit fluorometer (Invitrogen), and the one with the highest DNA concentration was selected for library preparation using the Nextera XT kit (Illumina). The library quality was checked using a Bioanalyzer 2100 (Agilent Technologies), then sequenced on the Illumina MiSeq platform using the v3 kit chemistry.

The sequencing generated 40,837,196 paired-end (PE) 300-bp reads for *H. columbus*. Raw reads were deposited into the NCBI Sequence Read Archive (SRA) with the accession number SRR12516078. The NCBI-SRA taxonomy analysis identified 49.79% of raw reads as bacteria, which may be potential contamination from nematode intestine. Raw reads were trimmed using Trimmomatic 0.36 (Bolger et al. 2014) to clean adapters. Phred score threshold was 30. The quality of trimmed reads was checked using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and synchronized using Fastq-pair (Edwards and Edwards 2019). Those trimmed high-quality PE reads then were de novo assembled using SPAdes (3.14.1) (Nurk et al. 2013). Abyss-Sealer (Paulino et al. 2015) was used for gap-closing and scaffolding. The assembly was analyzed on the QUAST webserver (<http://cab.cc.spbu.ru/quast/>) (Gurevich et al. 2013) (Table 1).

The total size of assembled scaffolds was 205.75 Mbp. The longest scaffold was 636,881 bp, N₅₀ was 2,341 bp, and GC content was 39.42%. GeneMark-ES (Lomsadze et al. 2005) on the QUAST webserver predicted 8,242 unique genes. The Benchmarking Universal Single-Copy Orthologs (BUSCO 3.02) analysis (Seppey et al. 2019) was performed on the webserver GenomeQC (<https://genomeqc.maizegdb.org/>) (Manchanda et al. 2020) with the eukaryote_odb9 dataset. The BUSCO results found 66.6% of BUSCO genes in the assembly, including 41.2% complete genes (38.6% single copy + 2.6% duplicated copy) and 25.4% fragmented genes. AUGUSTUS analysis (Stanke and Morgenstern 2005) on WebAUGUSTUS Service (<http://bioinf.uni-greifswald.de/webaugustus/>) predicted 61,855 protein sequences referring to the database of *Caenorhabditis*. The protein sequences were then analyzed on PANNZER2 (<http://ekhidna2.biocenter.helsinki.fi/sanspanz/>) (Törönen et al. 2018) for functional annotation and gene ontology analysis, resulting in 10,058 protein sequences annotated with at least one function. The assembly scaffolds >2,000 bp have been processed and filtered by NCBI and deposited into GenBank.

Data Deposition

Raw reads from the MiSeq platform have been deposited in the NCBI Sequence Read Archive with the accession number PRJNA659263. The accession number of deposited assembled scaffolds is JAEHOM000000000 in GenBank. The mitochondrial chromosome sequence has been deposited in NCBI GenBank with accession number MH657221.

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