Nematode Genome Announcement: Draft Genome of *Meloidogyne chitwoodi*, an Economically Important Pest of Potato in the Pacific Northwest

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Abstract

Meloidogyne chitwoodi is one of the most devastating pests of potato in the U.S. Pacific Northwest (PNW). Nematode-infected tubers develop external as well as internal defects, making the potato tubers unmarketable, and resulting in economic losses. Draft genome assemblies of three *M. chitwoodi* genotypes—race 1, race 2 and race 1 pathotype Roza—were generated using Illumina and PacBio Sequel RS II sequencing. The final assemblies consist of 30, 39, and 38 polished contigs for race 1, race 2 and race 1 pathotype Roza, respectively, with average N₅₀ of 2.37 Mb and average assembled genome size of approximately 47.41 Mb. On average, 10,508 genes were annotated for each genome. Benchmarking universal single-copy ortholog (BUSCO) analysis indicated that 69.80% of the BUSCOs were complete whereas 68.80, 0.93, and 12.67% were single copy, duplicated, and fragmented, respectively. These highly contiguous genomes will enrich resources to study potato–nematode interactions and enhance breeding efforts to develop nematode-resistant potato varieties for the PNW.

Potato is one of the major crops of the Pacific Northwest (PNW) region of the United States. Idaho, Oregon, and Washington account for approximately 65% of the processing and the fresh market potato production in the United States. *Meloidogyne chitwoodi* Golden et al., commonly known as the Columbia root-knot nematode (CRKN), is an important soil-borne, sedentary endoparasite of potato. It was first reported in the Columbia basin in 1980s and, since then, has become a quarantined pest of potato. Apart from potato, *M. chitwoodi* can also infect tomato, sugar beet, wheat, and corn (Santo et al. 1980). It has a short life cycle (23 to 25 days), resulting in rapid population density build-up over a single cropping season. Second-stage juveniles, the only infective stage in its life cycle, enter the host root tissue and establish feeding sites in the inner cortex region, where they undergo three additional molts and become adult males or females. Mature females produce 800 to 1,000 eggs and lay them in a gelatinous sac on the outer surface of the roots. The peculiarity of nematode infestation in the potato crop is that, in addition to roots, CRKN infects potato tubers.

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*The e-Xtra logo stands for “electronic extra” and indicates that a supplementary table and supplementary figure are published online.

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Infected tubers develop internal and external defects, making them unsuitable for fresh market as well as the processing industry. At present, nematode populations are mostly controlled by soil fumigation before the planting season and in-season application of nematicides. Commercial potato varieties resistant to *M. chitwoodi* are unavailable.

Previously, alfalfa was used as a rotation crop to control *M. chitwoodi* populations because the nematode could not reproduce on it (Santo et al. 1980) but, in 1985, one population was found to reproduce on 54 alfalfa accessions and was designated as Alfalfa race or race 2. The populations that did not reproduce on alfalfa were designated as race 1 (Santo and Pinkerton 1985). A later population distribution study in the PNW showed that both race 1 and race 2 coexisted in the majority of potato fields in the region (Pinkerton et al. 1987). Both race 1 and race 2 are unable to reproduce on a diploid wild potato species, *Solanum bulbocastanum* clone SB22. However, a newly emerged race 1 population identified in Prosser, WA could reproduce on *S. bulbocastanum* (SB22). This resistance-breaking population was designated as the pathotype of race 1 (Roza) (Mojtahedi et al. 2007).

Sequencing of nematode genomes could offer new tools to study genomics, evolution, and parasitism aspects of the pest. Nematode genomes are relatively small; the size typically ranges between 50 and 250 Mb and for *Meloidogyne* spp. and the genome size varies between approximately 47 and 51 Mb (Leroy et al. 2003). Genomes of economically important root-knot nematode species have been sequenced in the recent past using various sequencing platforms (Abad et al. 2008; Blanc-Mathieu et al. 2017; Koutsovoulos et al. 2020; Lunt et al. 2014; Opperman et al. 2008; Phan et al. 2020; Sato et al. 2018; Somvanshi et al. 2018; Susić et al. 2020; Szitenberg et al. 2017) (Table 1).

In the present study, we extracted *M. chitwoodi* race 1, race 2, and race 1 (Roza) eggs from 3-month-old infected tomato plants (*S. lycopersicum* 'Rutgers') using the hypochlorite method (Hussey 1973). Genomic DNA was isolated using a silica-membrane-based nucleic acid purification kit QIAamp DNA Mini Kit (catalog number 51304; Qiagen) following the kit protocol. In total, 30,000 eggs yielded >2.5 µg of DNA, which was sent to the Center for Genome Research and Biocomputing (CGRB), Oregon State University, Corvallis, OR for Illumina sequencing. Two replicate samples of each nematode genotype were barcoded, and sequencing libraries were pooled prior to running on a single lane of a HiSeq3000 (2 × 150 bp). In parallel, libraries from each genotype were also run separately on a MiSeq instrument (2 × 350 bp).

HiSeq3000 generated an average of 36 billion reads, whereas MiSeq generated 7 million reads on an average for three of the genomes. The quality of the data were checked using FASTQC, and the adapters were filtered and sequences quality trimmed using Cutadapt v. 3.1. The per-base sequence quality of Illumina reads was above average (Supplementary Fig. S1). The microbial and mitochondrial genome contamination was checked during the sequence vetting process done by NCBI servers and removed before final genome submission. The *k*-mer-based estimation of the genome size was calculated using Jellyfish and GenomeScope (Marçais and Kingsford 2011; Vurture et al. 2017). Using the cleaned Illumina reads, first, Jellyfish was used to extract and count canonical *k*-mers at *k* = 21, 31, and 71 nucleotides. Subsequently, for each *k*-mer value, GenomeScope was used to estimate heterozygosity, haploid genome length, repeat content, unique length, and read error rate (Supplementary Table S1). The haploid genome size was estimated to be 43.03, 42.68, and 42.66 Mb for race 1, race 2, and Roza, respectively, at *k*-mer 21 (Supplementary Table S1), which is closer to the expected genome size recently reported for *M. graminicola* (i.e., 41.5 Mb) (Phan et al. 2020). *M. graminicola* is closely related to *M. chitwoodi* because both are placed in clade III based on their molecular phylogenetics.

Highly repetitive nematode genomes challenge the generation of high-quality sequence data using short-read Illumina sequencing platforms. In order to improve the genome contiguity, we complemented the Illumina assembly by resequencing three of the nematode genomes using PacBio Sequel RS II (Pacific Biosciences). This technology is highly efficient and results in longer read lengths, greater consensus accuracy, and a lower degree of sequencing bias (Nakano et al. 2017).

For good-quality, higher molecular weight nematode DNA extraction, approximately 30,000 *M. chitwoodi* eggs each of race 1, race 2, and race 1 (Roza) were sent to Amplicon Expression, and DNA was later sent to CGRB for long-read sequencing. Libraries were prepared using the SMRT bell library prep protocol (Pacific Biosciences), followed by DNA size

Table 1. Summary of all root-knot nematode genomes sequenced thus far

Nematode species	Mode of reproduction	Occurrence	Isolate, strain, race, pathotype, genotype	Sequencing platform	Number of contigs per scaffold	Assembly size (Mb)	%GC	References	Resources
<i>Meloidogyne chitwoodi</i>	Facultative, meiotic parthenogenetic	Asia, Africa, North America, South America, Europe	PNW-race 1	HiSeq 3000, MiSeq and PacBio Sequel RS II	30	47.47	25	Present study	PRJNA666745
	—	—	PNW-race 2	—	39	46.98	24.9	—	—
	—	—	PNW-race 1-Roza	—	38	47.78	25	—	—
<i>M. graminicola</i>	Facultative, meiotic parthenogenetic	Asia, Africa, North America, South America, Europe	IARI strain	Illumina GALx	4,304	38.18	23.1	Somvanshi et al. 2018	NXFT00000000 , SAMN04041660
	—	—	Mg-VN18	Oxford Nanopore and Illumina	283	41.5	23.28	Phan et al. 2020	PRJNA615787, JABEBT000000000
<i>M. arenaria</i>	Obligatory, mitotic parthenogenetic	Asia, Africa, North America, Central America and Caribbean, South America, Europe, Oceania	A2-O	PacBio RS II platform	2,224	284.05	30	Sato et al. 2018	QEUI00000000
	—	—	HarA	Illumina	46,509	163.7	30.3	Szitenberg et al. 2017	PRJNA340324
	—	—	Guadeloupe	454, Illumina HiSeq 2000	26,196	258.07	29.8	Blanc-Mathieu et al. 2017	PRJEB871
<i>M. javanica</i>	Obligatory, mitotic parthenogenetic	Asia, Africa, North America, Central America and Caribbean, South America, Europe, Oceania	Avignon	454, Illumina HiSeq 2000	31,341	235.8	29.9	Blanc-Mathieu et al. 2017	PRJEB871
	—	—	VW4	Illumina	34,394	142.6	30.2	Szitenberg et al. 2017	PRJNA340324
<i>M. incognita</i>	Obligatory, mitotic parthenogenetic	Asia, Africa, North America, Central America and Caribbean, South America, Europe, Oceania	Morelos (INRA)	454, Illumina HiSeq 2000	12,091	183.53	29.8	Blanc-Mathieu et al. 2017	PRJEB871
	—	—	W1	Illumina	33,735	122	30.6	Szitenberg et al. 2017	PRJNA340324
	—	—	Morelos (INRA)	Sanger ABI3730X1 DNA Analyzer	2,817	86	31.4	Abad et al. 2008	CABB01000001
<i>M. floridensis</i>	Facultative, meiotic parthenogenetic	North America (Florida)	SJF1	Illumina	9,134	74.8	30.2	Szitenberg et al. 2017	PRJNA340324
			Isolate-5 (USA)	Illumina HiSeq2000	81,111	99.8	30	Lunt et al. 2014	ERP001338
<i>M. enterolobii</i>	Obligatory, mitotic parthenogenetic	Asia, Africa, North America, Central America and Caribbean, South America, Europe	Swiss pop	Illumina and PacBio RS II platform	4437	240	30	Koutsovoulos et al. 2020	https://meloidogyne.inrae.fr/
	—	—	L30	Illumina	46,090	162.3	30.2	Szitenberg et al. 2017	PRJNA340324
<i>M. hapla</i>	Facultative, meiotic parthenogenetic	Asia, Africa, North America, Central America and Caribbean, South America, Europe, Oceania	VW9	ABI3730, Megabase Sequence Analyzer	1,523	54	27.4	Opperman et al. 2008	SAMN02743742
<i>M. luci</i>	Obligatory, mitotic parthenogenetic	Asia, Europe (few occurrences), North America, South America	SI-Smartno-V13	PacBio RS II platform	327	209.16	30.2	Susić et al. 2020	ERS3574357

selection using the Blue Pippin system (Sage Science) to remove short insert templates. Subsequently, *M. chitwoodi* DNA was sequenced on PacBio Sequel RS II (Pacific Biosciences). Genomes were assembled using the SMRT analysis pipeline, v.2.3.0. Illumina reads were used to error correct PacBio data contigs using pilon v.1.22 (Walker et al. 2014). The final sequence assembly resulted in 30, 39, and 38 highly polished contigs for race 1,

Table 2. Summary of statistics of *Meloidogyne chitwoodi* race 1 (Mc1), race 2 (Mc27), and race 1 pathotype (Roza) sequenced genomes

Genotype	Assembly size (bp)	Maximum contig length (bp)	N ₅₀ contig length (bp)	Number of polished contigs	Estimated genome coverage (x)	Haploid genome size (Mb) ^a
Mc1	47,477,280	3,043,654	2,451,023	30	172.04	43.03
Mc27	46,924,610	3,043,255	2,317,798	39	84.34	42.68
Roza	47,730,107	2,942,887	2,363,161	38	168.05	42.66

^a Estimation (*k*-mer based) calculated using Jellyfish and GenomeScope (*k*-mer = 21 nucleotides).

Fig. 1. Summary of benchmarking universal single-copy ortholog (BUSCO) score analysis calculated for *Meloidogyne chitwoodi* race 1 (Mc1), race 2 (Mc27), and race 1 pathotype (Roza) sequenced genomes using the eukaryota database. *M. hapla* was used as a control and reference point.

Table 3. Benchmarking universal single-copy ortholog (BUSCO) score estimation of *Meloidogyne chitwoodi* race 1 (Mc1), race 2 (Mc27), and race 1 pathotype (Roza) sequenced genomes as compared with publicly available *M. hapla* genome^a

Name of the nematode	Complete BUSCOs (C) (%)	Complete and single-copy BUSCOs (S) (%)	Complete and duplicated BUSCOs (D) (%)	Fragmented BUSCOs (F) (%)	Missing BUSCOs (M) (%)
Mc1	68.6	68.2	0.4	12.9	18.5
Mc27	71.0	70.6	0.4	11.8	17.2
Roza	69.8	67.8	2.0	13.3	16.9
<i>M. hapla</i>	73.8	72.2	1.60	12.2	14.0

^a Scores were calculated using eukaryote odb10 dataset (BUSCO v5.0.0).

race 2, and race 1 (Roza), respectively. The sum of all contig lengths was 47.47 Mb for race 1 (*n* = 30 contig lengths), 46.98 Mb for race 2 (*n* = 39), and 47.78 Mb for race 1 (Roza) (*n* = 38) (Table 2). The largest contig length was 3.04 Mb for race 1, 3.04 Mb for race 2, and 2.94 Mb for race 1 (Roza). All three nematode genomes sequenced in the present study contained an average of 25% GC content.

Genome annotation was performed using the *M. hapla* protein dataset from wormBase and the SNAP training file prepared from the first round of annotation. The annotated gene numbers for race 1, race 2, and race 1 (Roza) were 10,441, 10,424, and 10,660, respectively. To check the completeness of the genomes, benchmarking universal single-copy ortholog (BUSCO) scores were calculated using the lineage dataset, eukaryota_odb10 (with number of species = 70). On average, three of the genomes showed 69.80% complete, 68.80% single copy, 0.93% duplicated, 12.67% fragmented, and 17.53% missing BUSCOs and are similar to the published *M. hapla* genome (Fig. 1; Table 3).

The whole-genome project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the BioProject number PRJNA666745. Three of the genomes can be accessed under accession numbers JACZZP000000000 (Mc1), JACZZO000000000 (Mc2), and JACZZN000000000 (Roza). Raw data files and the genome annotations are available at the Sathuvalli Lab website.

To the best of our knowledge, this is the first ever report of draft genomes of the *M. chitwoodi* races and pathotype prevalent in the PNW. The sequencing of highly contagious *M. chitwoodi* genomes will enable the identification of effectors involved in the susceptibility mechanism which, in turn, will enhance our knowledge of host–pathogen interactions. In addition, the sequence data could be used to design DNA-based markers for the identification and differentiation of *M. chitwoodi* races and pathotypes for diagnostic purposes. The availability of high-quality reference genomes for *M. chitwoodi* races and pathotypes will be a major step in understanding the nematode's evolution and diversification.

Author-Recommended Internet Resources

wormBase: <https://www.wormbase.org>

Sathuvalli Lab website: http://files.cgrb.oregonstate.edu/Sathuvalli_Lab/

Genome annotations: http://files.cgrb.oregonstate.edu/Sathuvalli_Lab/Meloidogyne_chitwoodi_GenomeAnnotation

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