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Chromosome-level reference genome of X12, a highly virulent race of the soybean cyst nematode *Heterodera glycines*

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Abstract

Soybean cyst nematode (SCN, *Heterodera glycines*) is a major pest of soybean that is spreading across major soybean production regions worldwide. Increased SCN virulence has recently been observed in both the United States and China. However, no study has reported a genome assembly for *H. glycines* at the chromosome scale. Herein, the first chromosome-level reference genome of X12, an unusual SCN race with high infection ability, is presented. Using whole-genome shotgun (WGS) sequencing, Pacific Biosciences (PacBio) sequencing, Illumina paired-end sequencing, 10X Genomics linked reads and high-throughput chromatin conformation capture (Hi-C) genome scaffolding techniques, a 141.01-megabase (Mb) assembled genome was obtained with scaffold and contig N50 sizes of 16.27 Mb and 330.54 kilobases (kb), respectively. The assembly showed high integrity and quality, with over 90% of Illumina reads mapped to the genome. The assembly quality was evaluated using Core Eukaryotic Genes Mapping Approach and Benchmarking Universal Single-Copy Orthologs. A total of 11,882 genes were predicted using de novo, homolog and RNAseq data generated from eggs, second-stage juveniles (J2), third-stage juveniles (J3) and fourth-stage juveniles (J4) of X12, and 79.0% of homologous sequences were annotated in the genome. These high-quality X12 genome data will provide valuable resources for research in a broad range of areas, including fundamental nematode biology, SCN–plant interactions and co-evolution, and also contribute to the development of technology for overall SCN management.

KEYWORDS

chromosome scale, evolution, genome assembly, *Heterodera glycines*, Soybean cyst nematode, X12

1 | INTRODUCTION

Soybean cyst nematode (SCN) has become a major pest in soybean (*Glycine max* Merr.) worldwide, posing a serious threat to the sustainability of the soybean industry (Kim et al., 2016; Koenning & Wrather, 2010; Woo et al., 2014). SCN is estimated to cause annual yield losses of more than $1.2 billion in the United States (Koenning & Wrather, 2010) and more than $120 million in China (Wang, Zhao, & Chu, 2015). Moreover, increased virulence of SCN has been observed, and the dominant races appear to be shifting (Howland, Monnig, Mathesius, Nathan, & Mitchum, 2018; Hua et al., 2018; Lian et al., 2017). To some extent, this indicates that the ecological environment has caused evolution of nematode virulence, which is a serious threat to agro-ecology and agricultural production. Classifying SCN populations by the published race scheme (Riggs & Schmitt, 1988) or the HG type test (Niblack et al., 2002) based on their virulence phenotype involves assessing the reproductive potential of a given population on a set of soybean indicator lines. Currently, planting SCN-resistant cultivars is the primary method of controlling the nematode (Mitchum, 2016; Mitchum, Wrather, Heinz, Shannon, & Danekas, 2007; Niblack, Colgrove, Colgrove, & Bond, 2008). Because SCN-resistant cultivars can invoke a defence against SCN population, a solid understanding of SCN genome information is the basis for analysing the mechanisms underlying pathogenicity and breeding for new SCN-resistant cultivars (Gardner, Heinz, Wang, & Mitchum, 2017; Kadam et al., 2016; Patil et al., 2019).

Race X12 was isolated from a soybean field heavily infected by SCN in Shanxi Province, China. To date, this race is able to successfully parasitize all resistant soybean germplasm tested, including the four indicator lines of the race scheme (Peking, Pickett, PI88788 and PI90763) (Riggs & Schmitt, 1988), the seven indicator lines of the HG type test (Peking, PI88788, PI90763, PI437654, PI 209332, PI 89772 and PI548316) (Niblack et al., 2002) and ZDD2315, the most promising elite resistant germplasm from China. Indeed, ZDD2315 is resistant to all SCN populations identified thus far, except for the newly identified race X12 (Lian et al., 2017). PI437654 is another elite resistant germplasm from the United States that is vulnerable to few natural SCN populations (Donald & Young, 2004; Jiao et al., 2015). Accordingly, X12 is thought to express additional or new virulence factors compared with other races, and it constitutes a potentially serious threat to soybean production, especially in China. Overall, genetic and genomic information for X12 is crucial for understanding the evolution of SCN parasitism genes and breeding additional resistant cultivars. Genome sequencing of the free-living nematode *Caenorhabditis elegans* (The *C. elegans* sequencing consortium, 1998) and the parasitic nematodes *Meloidogyne hapla* (Opperman et al., 2008) and *Globodera rostochiensis* (Akker et al., 2016) has provided reference genomes that can be utilized for comparison with parasitic nematodes. The genetic map of SCN was reported in 2005 with 10 linkage groups (Atibalentja et al., 2005). Although a draft genome sequence for SCN was recently published with 738 contigs in the genome, these contigs were not successfully assembled into chromosomes (Masonbrink et al., 2019). Genome sequencing of the X12 race is extremely important for our understanding of SCN virulence genes.

In this study, PacBio sequencing, 10X Genomics sequencing and Hi-C were applied to assemble the genome of the newly reported SCN race X12. The sequence information provided in this study combined with other published nematode genomes will allow for comparative genomic approaches to study fundamental nematode biology, gene function, nematode parasitism and evolution. As plant–parasitic nematodes are among the most damaging and difficult-to-control agricultural pests, available genome sequences will help scientists meet the current and future worldwide demands for food and bioenergy by providing powerful information for the development of new control paradigms and by minimizing crop losses.

2 | MATERIALS AND METHODS

2.1 | Selection of individuals for sequencing

The genomes of *Heterodera glycines* are challenging to sequence and assemble because these animals are dioecious with exceptionally high levels of population heterozygosity (Masonbrink et al., 2019; reviewed by Jones et al., 2013). To assemble this highly heterozygous population, *H. glycines* race X12 was first purified using ZDD2315, an elite resistant soybean germplasm in China (Lu, Gai, Zheng, & Li, 2006) with high resistance to all races detected in the SCN survey in Huang-Huai Valleys (Lian et al., 2016), except for race X12, to which it is highly susceptible. X12 (Hg type 1.2.3.4.5.6.7) was grown on ZDD2315 in a

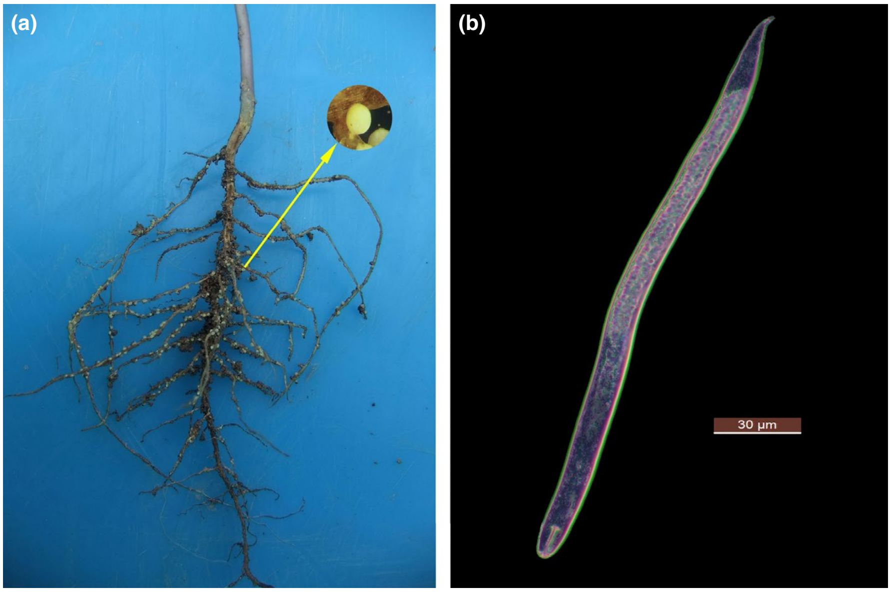


FIGURE 1 The combination of cysts on soybean roots (a) and micrograph of *Heterodera glycines* (soybean cyst nematode) second-stage juvenile (J2) (b) [Colour figure can be viewed at wileyonlinelibrary.com]

greenhouse at HeNan Academy of Agricultural Science. The starting culture was a single cyst selected from the X12 population, which was bulked for eight generations on ZDD2315 planted in steam-pasteurized soil and grown with approximately 16 daylight hours at 28°C. The X12 cysts used for genome sequencing were cultivated from soil infected with the 8th-generation purified X12 population.

2.2 | DNA/RNA isolation

The forms of *H. glycines* include cysts (Figure 1a) and early (Figure 1b) (second-stage juveniles, J2), middle (third-stage juveniles, J3) and late (fourth-stage juveniles, J4) life stages (as reviewed by Jones et al., 2013). Cyst nematodes moult to J2 in the egg; J2 is the dormant stage of the life cycle. J2, J3 and J4 are similar in morphology. Specimens for the four life stages of SCN were isolated from the X12 population. J2 were collected, followed by collection of J3 and J4 at 3, 8 and 15 days postinfection according to standard nematological methods (De Boer, Yan, Smant, Davis, & Baum, 1998). The samples were purified by sucrose gradient centrifugation (De Boer et al., 1996). Genomic DNA was extracted from approximately 20,000 eggs using MasterPure Complete DNA Purification Kit, and total RNA was extracted from 10,000 eggs or 300 J2/ J3/ J4 using Exiqon miRCURY RNA Isolation Kit.

2.3 | Genome sequencing

The DNA extracted from *H. glycines* was used for genome sequencing and sheared with a sonication device (Bioruptor Pico) for paired-end library construction. Libraries with 350 base pair (bp) insert sizes were produced according to the instructions provided in the ×TEN Illumina Library Preparation Kit. The Illumina HiSeq ×TEN platform was used to generate 13.65-gigabyte (Gb) whole-genome sequencing data, and the clean reads obtained from this process were employed for subsequent analyses. Construction of a 10X Genomics library produced 31.32-Gb sequencing data. For PacBio library construction, *H. glycines* genomic DNA was sheared to ~20 kb, and filtered fragments were converted into the proprietary 9 SMRTbell library using PacBio DNA Template Preparation Kit. In total, 28.48 Gb of quality-filtered data was obtained from PacBio sequencing.

2.4 | Genome assembly and quality control

The genome size was estimated based on the *k*-mer spectrum of de novo data. All raw reads from the PacBio platform were aligned to each other using ‘daligner’ executed using the mail script of the FALCON (v0.7) assembler. Overlapping reads and raw subreads were processed to generate consensus sequences, and error correction of the assembly was performed using the consensus-calling algorithm Quiver (smrtlink_5.0.7). The paired-end clean reads from the Illumina platform were further corrected using Pilon (v1.22), and the reads obtained after strict error correction were further used for the subsequent scaffolding.

The 10X Genomics scaffold extension was performed using fragScaff (v140324.1) software, in which the linked reads generated using the 10X genomic library were aligned to the consensus sequence of the PacBio assembly. To obtain the superscaffold, only the consensus sequence with linked-read support was used for assembly.

To assess the accuracy of the assembled X12 genome, a small fragment library was selected for comparison of the assembled genome using BWA software (v0.7.8) (Burton et al., 2013; Li & Durbin, 2009; Rao et al., 2014; Yaffe & Tanay, 2011). Core Eukaryotic Genes Mapping Approach (CEGMA: http://korflab.ucdavis.edu/dataseda/cegma/; Parra, Bradnam, & Korf, 2007) analysis was performed to assess the completeness and continuity of the SCN genome (X12 race) assembly, along with six additional published genomes, based on a core eukaryotic gene (CEG) library with 248 conserved genes. In addition, the assembly was evaluated with Benchmarking Universal Single-Copy Orthologs (BUSCO: http://busco.ezlab.org/; Simao, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015).

2.5 | Repeat prediction

LTR_FINDER (v1.0.7), RepeatScout (v1.0.5) and RepeatModeler (v1.0.3) were used for de novo identification of repeat elements and for generating a repeat element database. This database was used in RepeatMasker (v4.07) to predict repeat elements. Putative repeats were further filtered on the basis of copy number.

2.6 | Gene structure prediction

For gene structure prediction, both de novo and homology-based approaches were combined to predict protein-coding genes in the SCN genome. For the former, gene sets from *Ascaris_suum* (wormbase. WBPS6), *Brugia malayi* (ensembl. metazoa.v32), *Caenorhabditis_briggsae* (ensembl.metazoa.v32), *C. elegans* (ensembl.metazoa.v32), *Drosophila melanogaster* (ensembl.metazoa.v32) and *Onchocerca volvulus* (ensembl.metazoa.v34) were used as queries to search against the SCN genome. For the de novo-based method, Augustus (v3.2.3), GlimmerHMM (v3.0.4), SNAP (v2013.11.29) and Genscan (v1.0) were employed as engines to predict gene models. The gene prediction results derived from both methods were merged using GLEAN to generate a consensus gene set.

2.7 | Functional annotation of protein-coding genes

Translated coding sequences were aligned to known databases such as Swiss-Prot (v20180824), Nr (v20180716), Pfam (v31.0), KEGG (v20160503) and InterPro (v5.31-70.0). We annotated all protein-coding genes identified in this study by retrieving functional terms according to Swiss-Prot, Nr, KEGG, InterPro, GO and Pfam.

2.8 | Chromosome preparation

Chromosome preparation was performed according to the method of Du et al. (2016). Briefly, eggs or J2 were collected and treated with colchicine (0.005 g/L) in 2-ml tubes for 3–5 hr before fixation in a solution of 3:1 ethanol:acetic acid (v/v) for 2–3 days. The samples were suspended in 45% acetic acid and squashed on a slide. After freezing at −70°C overnight, the slides that contained mitotic chromosomes were dehydrated in 100% ethanol followed by DAPI (4′-,6-diamidino-2-phenylindole) staining, and the chromosomes were observed and photographed under a fluorescence microscope using 450–490 nm excitation.

2.9 | Hi-C scaffolding of the assembly to the chromosome level

The Hi-C clean data were aligned to the primary assembly using BWA software. Only read pairs with both reads in the pair aligned to contigs were considered for scaffolding. The scaffolds (greater than 100 bp) were selected by Lachesis (v201701) to scaffold the assembly at the chromosome level.

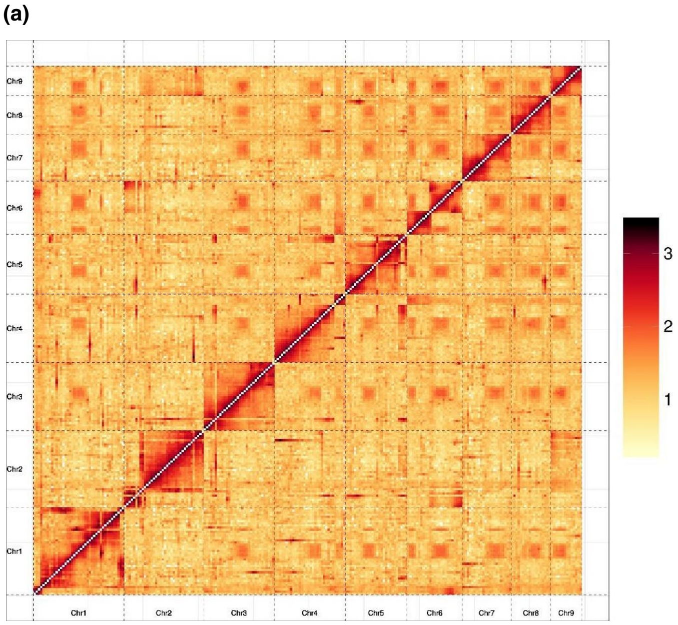
2.10 | Phylogenetic analysis and species divergence time estimation

To investigate the phylogenetic position of *H. glycines*, orthologous and paralogous groups from 11 species were assigned by OrthoMCL as follows: pig roundworm, *B. malayi*, *C. briggsae*, *C. elegans*, *D. melanogaster*, *Haemonchus contortus*, *Heterorhabditis bacteriophora*, *Loa loa*, *M. hapla*, *O. volvulus* and *Trichoplax adhaerens*. Orthologous groups that contained only one gene for each species were represented by the gene encoding the longest protein sequence. Genes encoding protein sequences shorter than 50 amino acids were filtered out to exclude putative fragmented genes. All-against-all blastP was applied to identify similarities among the filtered protein sequences in these species with an *E*-value cut-off of $1e^{-5}$. MUSCLE (Robert, 2004) with default parameters was used to generate a multiple sequence alignment of the protein sequences in each single-copy family. The alignments of each family were then concatenated to form a superalignment that was used for phylogenetic tree reconstruction using maximum-likelihood methods (Guindon et al., 2010; Yang & Rannala, 2012). Species divergence time was estimated using mcmctree (http://abacus.gene.ucl.ac.uk/software/paml.html) in the PAML software package. The correction time points were *T. adhaerens* and *D. melanogaster* (1147–713 million years ago), *D. melanogaster* and *M. hapla* (946–551 million years ago), and *M. hapla* and *C. elegans* (217.5–190.0 million years ago).

3 | RESULTS AND DISCUSSION

3.1 | Genome summary

Multiple libraries with different insert sizes were constructed from DNA extracted from the eggs of the purified X12 population. In total, 95.22 Gb of sequencing data was generated, of which 13.65 Gb (96.81X coverage) was produced from Illumina reads, 28.48 Gb (201.97X coverage) from PacBio reads, 31.32 Gb (222.13X coverage) from 10X Genomics linked-read libraries and 21.77 Gb (154.39X coverage) from the Hi-C library (Table S1). The assembled genome is estimated to be 141.01 Mb, with scaffold and contig N50 sizes of



(b)

Statistics of chromosome genome of X12 race

Sample ID	Contig		Scaffold	
	Size	Number	Size	Number
Total	140,322,532	889	141,354,287	267
Max	1,600,666	–	21,025,901	–
Number≥2000	–	845	–	224
N50	330,544	145	16,265,615	4
N60	283,960	191	14,483,173	5
N70	226,952	246	12,909,208	6
N80	178,857	315	9,809,787	8
N90	121,407	408	7,602,578	9

(c)

Statistics of chromosome length of X12 race

Cluster numbers	Number of contigs	Length of chromosome
chr1	73	21,025,901
chr2	49	185,539,096
chr3	58	13,924,437
chr4	85	16,265,615
chr5	56	14,483,173
chr6	48	14,483,173
chr7	46	11,423,398
chr8	39	9,809,787
chr9	31	7,602,578
Total	258	12,371,094

FIGURE 2 Chromatin conformation capture-based improved assembly *Heterodera glycines* genome. (a) Postclustering heat map showing the density of Hi-C interactions between scaffolds from the proximity-guided assembly. (b) Statistics of the completeness of the hybrid de novo assembly of X12 race genome. Listed are the assembled genome of ~141 Mb with scaffold and contig N50 size of 16.27 Mb and 330.54 Kb. Also listed in the table are the size and number of N60, N70, N80 and N90 of contigs and scaffolds. (c) Clustering of scaffolds using Hi-C data into pseudochromosome-scale scaffolds. Listed are the 258 scaffolds of total length ~12 Mb used for clustering. Also listed in the table are the cluster numbers, the number of contigs and the reference length of contigs [Colour figure can be viewed at wileyonlinelibrary.com]

16.27 Mb and 330.54 kb, respectively (Figure 2b). In addition, the sequencing results (*SCN_Lian*) were compared with the newly released sequencing results (*SCN_Masonbrink*) of 2019 (Masonbrink et al., 2019) and the genomes of the plant–parasitic nematode *M. hapla* (Opperman et al., 2008) and the free-living nematode *C. elegans* (The *C. elegans* sequencing consortium, 1998) (Table 1). The genome size of *SCN_Lian* is 141.01 Mb, which is almost identical to that of *SCN_Masonbrink*, at 123 Mb. Notably, *SCN_Masonbrink* did not assemble the genome of *H. glycines* at the chromosome scale, though *SCN_Lian* did. The BUSCO value of *SCN_Lian* is 53.4% compared with 72% for *SCN_Masonbrink*, but the BUSCO value of *SCN_Masonbrink* is ~54% when analysed using the nematode database and the genomic data supplied by Masonbrink et al. Therefore, there is little difference in assembly quality between the genomes of *SCN_Masonbrink* and *SCN_Lian*. The GC content of *SCN_Lian* (36.89%) is similar to that of *C. elegans* (35.4%), whereas *M. hapla* has an unusually low GC content of 27.4%. *SCN_Masonbrink* annotated 29,769 genes, and *SCN_Lian* annotated 11,882 genes.

The data quality control results are shown in Tables S2–S4 and Figures S1 and S2. The following was obtained for assessment of polymerase length distribution: read number of 2,080,111, with mean read length of 13,703 and read length N50 of 23,355. Insert size length distribution showed the following: read number of 2,080,111, with mean read length of 9,875 and read length N50 of 14,429. Assessment of subread length distribution revealed that the read number was 3,179,171, with mean read length of 8,948 and read length N50 of 12,988. According to BWA software, the mapping rate of all small fragment reads to the genome was approximately 90.72%, and the coverage rate was approximately 98.33% (Table S5); thus, the reads show good agreement with the assembled genome. After sorting chromosome coordinates, removing repeated sequences and performing single nucleotide polymorphism (SNP) calling for the BWA comparison results, 247,046 SNPs were obtained, with 0.213% SNP heterozygosity and 0.0024% SNP homozygosity based on SAMtools (http://samtools.sourceforge.net/) (Table S6); therefore, the genome assembly has high single-base accuracy. In addition, the GC content and average depth of the assembled genome were calculated and mapped using 10k Windows without repeated calculation. The results showed that the GC content is concentrated in a region encompassing 40% of the genome, without apparent separation, which showed that the genome was not contaminated by foreign sources (Table S7 and Figure S3).

The results of CEGMA analysis demonstrated that the assembly was complete, with mapping rate of 86.29% (a total of 214 genes) (Table S8). BUSCO evaluation results also indicated that the assembly result was complete, with 53.4% assembled complete single-copy genes of 978 homologous single-copy genes (Table S9). Remarkably, only 53.4% of the genes in the *H. glycines* assembly are single copy according to the BUSCO analysis, with 3.7% duplicated. For comparison, the BUSCO results for *SCN_Masonbrink* indicate that 56% of the genes in *H. glycines* are single copy, with 16% duplicated (Masonbrink et al., 2019).

Results of repeat prediction showed that the X12 genome contains 51.10% repeat sequences. Repetitive sequence statistics and classification results are shown in Tables S10 and S11 and Figure

TABLE 1 Comparison of *Heterodera glycines* genome statistics with other plant–parasitic nematodes *Meloidogyne hapla* and *Caenorhabditis elegans*

	H. glycines			
	SCN-Masonbrink	***SCN-Lian***	***M. hapla***	***C. elegans***
Sequencing material	Inbred population TN10 (Hg type 1.2.6.7)	Natural population X12 (Hg type 1.2.3.4.5.6.7)		
Genome size, Mb	123.85	141.01	54	100
Contigs, bp	738	889	3,452	N/A
Contig N50, Kb	304,130	330,544	N/A	N/A
Scaffolds	N/A	267	1,523	N/A
Scaffolds N50, bp	N/A	16,265,615	83,645	17,494,000
Assembled, bp	N/A	141,354,287	53,578,246	100,267,623
Sequence coverage, %	N/A	98.33	99.2	100
Per cent complete BUSCO, %	72	53.4	59	99.6
G+C, %	N/A	36.89	27.4	35.4
Annotated genes	29,769	11,882	14,420	20,060
Repeats numbers accounted for the genome, %	34	51.10	17	16.5
Identified SNPs	1,619,134	247,046	N/A	N/A
Chromosomes	NA	18	16	6
Chromosomes-level assembly	N/A	Yes	N/A	Yes

Abbreviation: SCN, soybean cyst nematode.

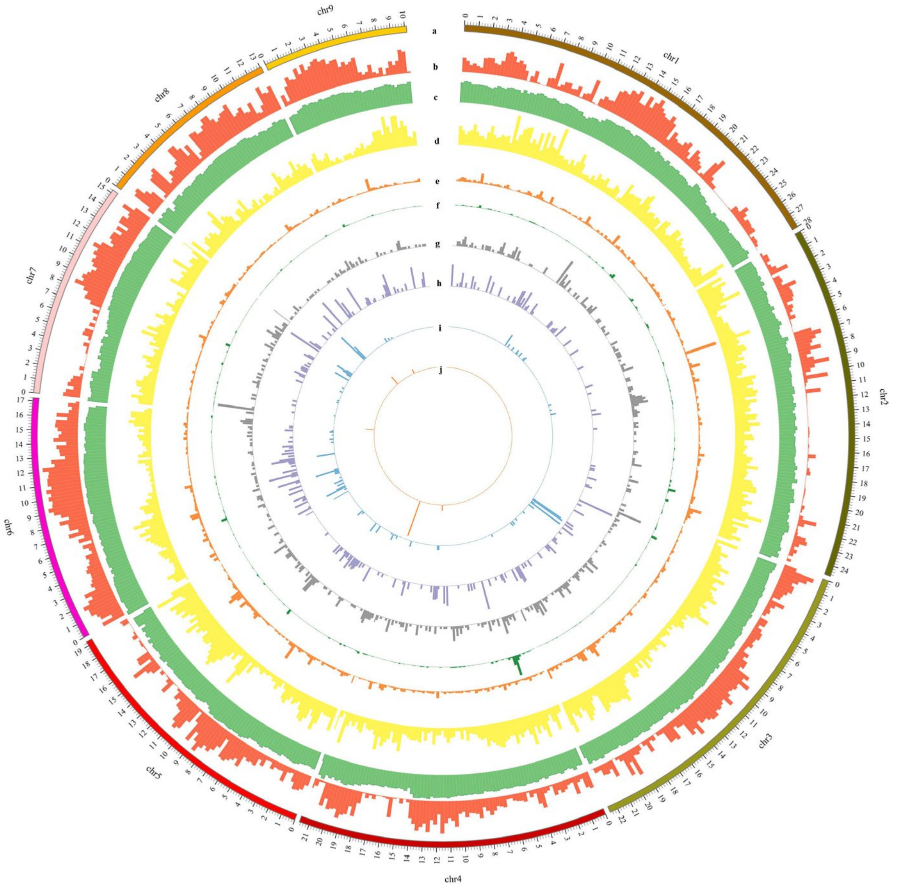


FIGURE 3 The genome characteristics of *Heterodera glycines*. Circos plot showing the genomic features. 1u = 40 kb, small scale means 5u and large scale means 25u. From outer to inner circles: Track a: nine chromosomes of the genome; Track b: gene distribution in nine chromosomes; Track c: GC content distribution in nine chromosomes; Track d: LTR distribution in nine chromosomes; Track e: LINE distribution in nine chromosomes; Track f: SINE distribution in nine chromosomes; Track g: tRNA located on chromosomes; Track h: miRNA located on chromosomes; Track i: snRNA located on chromosomes; Track j: rRNA located on chromosomes [Colour figure can be viewed at wileyonlinelibrary.com]

S4. The genome of *H. glycines* is diploid and consists of repeated sequences with higher nucleotide divergence (19.21%) than the genomes of *Meloidogyne* species, which are polyploid and consist of duplicated regions with low nucleotide divergence (~8%) (Abad et al., 2008; Blanc-Mathieu et al., 2017; Sato et al., 2018; Szitenberg et al., 2017).

Gene structure prediction was performed, and 11,882 protein-coding genes were predicted, with a mean of 1,233.92 bp of coding sequence (CDS) and 8.3 exons per gene (Table S12 and Figure S5). The transcript lengths of genes, CDSs, exons and introns of SCN are comparable to those of the genomes used for homology-based prediction (Table S13 and Figure S6). In addition, noncoding RNA genes were predicted in the SCN genome, including a total length of 17,688-bp ribosomal RNA (rRNA), 46,685-bp transfer RNA (tRNA), 39,375-bp microRNA (miRNA) and 21,549-bp snRNA genes (Table S14). Based on functional annotation of protein-coding genes, 64.5% (7,663), 76.5% (9,093), 60% (7,126), 70.7% (8,405), 49.1% (5,840) and 61.5% (7,303) of genes are annotated in Swiss-Prot, Nr, KEGG, InterPro, GO and Pfam, respectively. The four life stages of SCN were isolated and then mixed before sequencing for genome annotation. In total, 9,383 protein-coding genes (79.0%) with conserved functional motifs and functional terms were successfully annotated (Table S15 and Figure S7). The distribution of genes, GC contents, long terminal repeats (LTRs), long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs), tRNAs, miRNAs, snRNAs and rRNAs in X12's chromosomes are shown in Figure 3.

There are some differences regarding the results for *SCN_Lian* and *SCN_Masonbrink*, such as the number of annotated genes. The possible reasons are as follows. First, there were differences in the sequencing technologies used. For *SCN_Masonbrink*, PacBio long-read technology was mainly used, whereas combined Illumina short-read and PacBio long-read technologies were employed for *SCN_Lian*. Second, there were differences in the materials sequenced. The inbred population TN10 (Hg type 1.2.6.7) was used for *SCN_Masonbrink*, but the natural population X12 (Hg type 1.2.3.4.5.6.7), which is the most virulent SCN population identified to date, was utilized for *SCN_Lian*. The differences in the pathogenicity of these populations may also be judged from the differential proportions of S genes (50.4% and 45.3% in *SCN_Lian* and *SCN_Masonbrink*, respectively) and D genes (2.3% and 8.7% in *SCN_Lian* and *SCN_Masonbrink*, respectively) in the BUSCO results (Table 2). Third, different annotation methods were applied. Gene annotations were performed using Braker for *SCN_Masonbrink* with an unmasked assembly, which annotated 29,769 genes including 12,357 expressed repetitive elements and showed that the *H. glycines* genome has a significant number of repeats, at 34% of the genome. To prevent the number of genes from being too high, which can be caused by false positives from repeats during gene annotation, repeat masking before structure annotation was performed for *SCN_Lian*, as also conducted in many other studies

TABLE 2 Genomic statistics and Benchmarking Universal Single-Copy Orthologs analysis using nematode database

Scientific name	Version	Genome size	Gene number	BUSCO genome
Caenorhabditis_elegans	ensembl.metazoa.v32	98M		C:98.6% (S:98.0%, D0.6%), F:0.8%, M:0.6%, *n*:982
Caenorhabditis_briggsae	ensembl.metazoa.v32	106M		C:97.7% (S:97.0%, D0.7%), F:1.5%, M:0.8%, *n*:982
Ascaris_suum	ensembl.metazoa.v32	265M		C:89.8% (S:88.0%, D1.8%), F:6.6%, M:3.6%, *n*:982
Brugia_malayi	wormbase.WBPS6	93M		C:96.6% (S:96.0%, D0.6%), F:2.4%, M:1.0%, *n*:982
Onchocerca_volvulus	ensembl.metazoa.v32	94M		C:97.6% (S:97.3%, D0.3%), F:1.7%, M:0.7%, *n*:982
Meloidogyne hapla		54M	14420	C:59.9% (S:58.7%, D1.2%), F:9.4%, M:30.7%, *n*:982
Meloidogyne incognita		184M	43718/45351	C:61.8% (S:25.8%, D36.0%), F:8.1%, M:30.1%, *n*:982
Heterodera glycines (SCN-Lian)		135M	11882	C:52.7% (S:50.4%, D2.3%), F:9.6%, M:37.7%, *n*:982
H. glycines (SCN-Masonbrink)		129M	29769	C:54.0% (S:45.3%, D8.7%), F:10.4%, M:35.6%, *n*:982

Abbreviation: SCN, soybean cyst nematode.

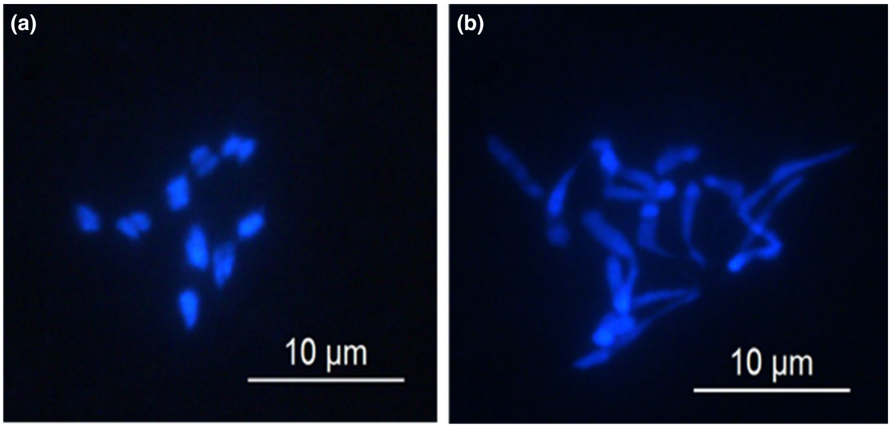


FIGURE 4 Observation of the chromosome of *Heterodera glycines* in meiosis under fluorescence microscope with 450–490 nm excitation light ($2n = 18$) [Colour figure can be viewed at wileyonlinelibrary.com]

(Xu et al., 2013; Zhang et al., 2019). To obtain more comprehensive and accurate repeat sequences, homologous sequence alignment and ab initio prediction were performed. Ultimately, 11,882 annotated genes and 51.10% nonredundant repeat sequences were obtained.

3.2 | Chromosome observation and Hi-C scaffolding

The chromosome number of *H. glycines* during meiosis was observed under a fluorescence microscope using 450–490 nm excitation ($2n = 18$) (Figure 4). The Illumina-based Hi-C data were remapped to the PacBio assembly, clustering into nine pseudomolecules using the Proximo Hi-C scaffolding pipeline (Figure 2a). The Hi-C scaffolding was able to anchor and order with high confidence all of the 258 scaffolds into nine pseudomolecules. The scaffold sizes ranged from 7.6 to 185 Mb with an N50 of 16.3 Mb (Figure 2c). The overall scaffolding rate was 91.2% (Table S16).

3.3 | Evolutionary analysis

A total of 25,535 gene family clusters were constructed. The genes used for gene family clustering in each species are shown in Table S17. In total, 482 single-copy gene families are common to all 12 species. The distribution of single-copy orthologs, multiple-copy orthologs, genes unique to *H. glycines* and other orthologs in different species is shown in Table S18. Protein sequences from the 482 single-copy gene families were used for phylogenetic tree reconstruction, and the estimation of divergence time was performed (Figure 5) with mcmctree software. Synteny diminished as phylogenetic relatedness declined, and our results showed that the divergence time between *H. glycines* and *M. hapla* is approximately 143.6 million years. Thus, the divergence of *H. glycines* preceded that of the model nematode *C. elegans*. Moreover, because plant parasitism is a lifestyle found in three different clades in the nematode tree of life, plant parasitism appeared at least three times independently during

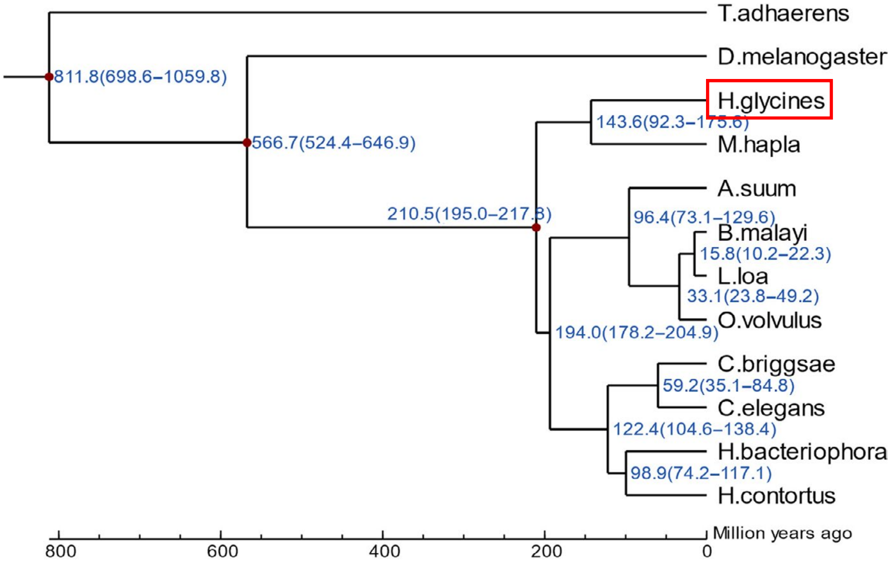


FIGURE 5 Phylogenetic relationships of species related to *Heterodera glycines*. Phylogenetic tree of the single-copy gene families coexistent in the 12 species representing the relatedness of each species. Red box surrounded denotes the sequenced species *H. glycines*. Node labels represent node ages [Colour figure can be viewed at wileyonlinelibrary.com]

the evolution of nematodes (Danchin & Perfus-Barbeoch, 2009). It was also inferred that plant parasites evolved from fungus-feeding nematodes, according to previous results that showed consistent coclustering of plant parasites with fungivorous species (Holterman et al., 2006).

4 | CONCLUSIONS

The chromosome-level reference genome sequence of X12, a notable race of the SCN *H. glycines*, has immediate and important implications for research on plant nematodes and also for broader biological studies. In total, approximately 95.22 Gb of sequencing data were generated using a combination of long- and short-read techniques. The assembled genome contains 267 scaffolds, with an N50 scaffold length of 16.27 Mb and a total length of 141.01 Mb. The assembly was estimated to contain 86.29% of core genes according to CEGMA analysis and 53.4% of complete single-copy genes. The assembled genome is more contiguous than is the previously published *H. glycines* genome (Masonbrink et al., 2019), with 1.2-fold more contigs and a 1.09-fold greater N50 contig length. The mapping rate for reads back to the assembled genome is approximately 90.72%. A total of 11,882 genes were predicted, assisted by RNA sequencing data, and 79.0% homologous sequences were annotated in the genome. This high-quality genome assembly of *H. glycines* will help to enable the identification of virulence-related genes.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Yun Lian and Weiguo Lu conceived and designed the experiments. He Wei, Chenfang Lei and Jinying Li collected the cyst samples and managed the data. Jinshe Wang performed the data analysis. Haichao Li collected the sequencing data. Jianqiu Guo collected the soil sample containing X12 from the soybean field. Pei Du participated in the chromosome preparation. Yongkang Wu, Shufeng Wang, Hui Zhang and Tingfeng Wang revised the manuscript.

DATA AVAILABILITY STATEMENT

The genomic (SRA917187) and transcriptomic sequence reads (SRA 917415) were deposited at the NCBI (National Center for Biotechnology Information) with BioProject Accession no.: PRJNA535374. The assembled genome and annotation results were available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.5b2m501. In addition, the supplementary figures and tables are provided in Supporting Information.

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REFERENCES

Abad, P., Gouzy, J., Aury, J. M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., … Wincker, P. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, *26*, 909–915.

Akker, S. E. D. A., Laetsch, D. R., Thorpe, P., Lilley, C. J., Danchin, E. G. J., Rocha, M. D., … Jones, J. T. (2016). The genome of the yellow potato cyst nematode, *Globodera rostochiensis*, reveals insights into the basis of parasitism and virulence. *Genome Biology*, *17*, 124.

Atibalentja, N., Bekal, S., Domier, L. L., Niblack, T. L., Noel, G. R., & Lambert, K. N. (2005). A genetic linkage map of the soybean cyst nematode *Heterodera glycines*. *Molecular Genetics and Genomics*, *273*, 273–281.

Blanc-Mathieu, R., Perfus-Barbeoch, L., Aury, J. M., Da Rocha, M., Gouzy, J., Sallet, E., … Danchin, E. G. J. (2017). Hybridization and polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes. *PLoS Genetics*, *13*, e1006777.

Burton, J. N., Adey, A., Patwardhan, R. P., Qiu, R., Kitzman, J. O., & Shendure, J. (2013). Chromosome-scale scaffolding of *de novo* genome assemblies based on chromatin interactions. *Nature Biotechnology*, *31*(12), 1119–1125. https://doi.org/10.1038/nbt.2727

Danchin, E. G. J., & Perfus-Barbeoch, L. (2009). The genome sequence of *Meloidogyne incognita* unveils mechanisms of adaptation to plant–parasitism in Metazoa. In P. Pierre (Ed.), *Evolutionary biology* (Chapter 17, pp. 287–302. Berlin, Heidelberg: Springer.

De Boer, J. M., Overmars, H. A., Pomp, H., Davis, E. L., Zilverentant, J. F., Goverse, A., … Schots, A. (1996). Production and characterization of monoclonal antibodies to antigens from second-stage juveniles of the potato cyst nematode, *Globodera rosto-chiensis*. *Fundamental and Applied Nematology*, *19*, 545–554.

De Boer, J., Yan, Y., Smant, G., Davis, E. L., & Baum, T. J. (1998). In-situ hybridization to messenger RNA in *Heterodera glycines*. *Journal of Nematology*, *30*, 309.

Donald, P., & Young, L. (2004). Characterization of two soybean cyst nematode populations that reproduce on PI 437654 source of resistance. *Journal of Nematology*, *36*, 315–316.

Du, P., Li, L. N., Zhang, Z. X., Liu, H., Qin, L. I., Huang, B. Y., … Zhang, X. Y. (2016). Chromosome painting of telomeric repeats reveals new evidence for genome evolution in peanut. *Journal of Integrative Agriculture*, *15*(11), 2488–2496.

Gardner, M., Heinz, R., Wang, J., & Mitchum, M. G. (2017). Genetics and adaptation of soybean cyst nematode to broad spectrum soybean resistance. *G3 (Bethesda)*, *7*(3), 835–841.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., … Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown Clades. *Molecular Biology and Evolution*, *23*(9), 1792–1800.

Howland, A., Monnig, N., Mathesius, J., Nathan, M., & Mitchum, M. G. (2018). Survey of *Heterodera glycines* population densities and virulence phenotypes during 2015–2016 in Missouri. *Plant Disease*, *102*(12), 2407–2410.

Hua, C., Li, C., Hu, Y., Mao, Y., You, J., Wang, M., … Wang, C. (2018). Identification of HG types of soybean cyst nematode *Heterodera glycines* and resistance screening on soybean genotypes in Northeast China. *Journal of Nematology*, *50*(1), 41–50.

Jiao, Y. Q., Vuong, T. D., Liu, Y., Meinhardt, C., Liu, Y., Joshi, T., … Nguyen, H. T. (2015). Identification and evaluation of quantitative trait loci underlying resistance to multiple HG types of soybean cyst nematode in soybean PI 437655. *TAG. Theoretical and Applied Genetics.*, *128*, 15–23.

Jones, J. T., Haegeman, A., Danchin, E. J., Gaur, H. S., Helder, J., Jones, M. G. K., … Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, *14*(9), 946–961.

Kadam, S., Vuong, T. D., Qiu, D., Meinhardt, C. G., Song, L., Deshmukh, R., … Nguyen, H. T. (2016). Genomic-assisted phylogenetic analysis and marker development for next generation soybean cyst nematode resistance breeding. *Plant Science*, *242*, 342–350.

Kim, K. S., Vuong, T. D., Qiu, D., Robbins, R. T., Grover Shannon, J., Li, Z., & Nguyen, H. T. (2016). Advancements in breeding, genetics, and genomics for resistance to three nematode species in soybean. *Theoretical and Applied Genetics*, *129*(12), 2295–2311.

Koenning, S. R., & Wrather, J. A. (2010). Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Progress*, *11*(1), 5. https://doi.org/10.1094/PHP-2010-1122-01-RS

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

Lian, Y., Guo, J. Q., Li, H. C., Wu, Y. K., Wei, H., Wang, J. S., … Lu, W. G. (2017). A new race (X12) of soybean cyst nematode in China. *Journal of Nematology*, *49*(3), 321–326.

Lian, Y., Wang, J. S., Li, H. C., Wei, H., Li, J. Y., Wu, Y. K., … Lu, W. G. (2016). Race distribution of soybean cyst nematode in the main soybean producing area of Huang-Huai Rivers Valley. *Acta Agronomica Sinica*, *42*(10), 1479–1486.

Lu, W. G., Gai, J. Y., Zheng, Y. Z., & Li, W. D. (2006). Construction of a soybean genetic linkage map and mapping QTLs resistant to soybean cyst nematode (*Heterodera glycines Ichinohe*). *Acta Agronomica Sinica*, *32*(9), 1272–1279.

Masonbrink, R. E., Maier, T. R., Muppirala, U., Seetharam, A. S., Lord, E., Juvale, P. S., … Baum, T. J. (2019). The genome of the soybean cyst nematode (*Heterodera glycines*) reveals complex patterns of duplications involved in the evolution of parasitism genes. *BMC Genomics*, *20*, 119.

Mitchum, M. G. (2016). Soybean resistance to the soybean cyst nematode *Heterodera glycines*: An update. *Phytopathology*, *106*(12), 1444–1450.

Mitchum, M. G., Wrather, J. A., Heinz, R. D., Shannon, J. G., & Danekas, G. (2007). Variability in distribution and virulence phenotypes of *Heterodera glycines* in Missouri during 2005. *Plant Disease*, *91*, 1473–1476.

Niblack, T. L., Arelli, P. R., Noel, G. R., Opperman, C. H., Orf, J. H., Schmitt, D. P., … Tylka, G. L. (2002). A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *Journal of Nematology*, *34*, 279–288.

Niblack, T. L., Colgrove, A. L., Colgrove, K., & Bond, J. P. (2008). Shift in virulence of soybean cyst nematode is associated with use of resistance from PI 88788. *Plant Health Progress*, *9*(1), 29–https://doi.org/10.1094/PHP-2008-0118-01-RS

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., … Windham, E. (2008). Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences*, *105*, 14802–14807.

Parra, G., Bradnam, K., & Korf, I. (2007). CEGMA: A pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics*, *23*, 1061–1067.

Patil, G. B., Lakhssassi, N., Wan, J., Song, L., Zhou, Z., Klepadlo, M., … Nguyen, H. T. (2019). Whole genome re-sequencing reveals the impact of the interaction of copy number variants of the rhg-1 and Rhg4 genes on broad-based resistance to soybean cyst nematode. *Plant Biotechnology Journal*, *17*(8), 1595–1611.

Rao, S. S. P., Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., … Aiden, E. L. (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell*, *159*(7), 1665–1680.

Riggs, R. D., & Schmitt, D. P. (1988). Complete characterization of the race scheme for *Heterodera glycines*. *Journal of Nematology*, *20*, 392–395.

Robert, C. E. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Sato, K., Kadota, Y., Gan, P., Bino, T., Uehara, T., Yamaguchi, K., … Shirasu, K. (2018). High-quality genome sequence of the root-knot nematode *Meloidogyne arenaria* genotype A2-O. *Genome Announcements*, *6*(26), e00519-18.

Simao, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*, 3210–3212.

Szitenberg, A., Salazar-Jaramillo, L., Blok, V. C., Laetsch, D. R., Joseph, S., Williamson, V. M., … Lunt, D. H. (2017). Comparative genomics of apomictic root-knot nematodes: Hybridization, ploidy, and dynamic genome change. *Genome Biology and Evolution*, *9*, 2844–2861.

The C. elegans sequencing consortium. (1998). Genome sequence of the nematode *C. elegans:* A platform for investigating biology. *Science*, *282*, 2012–2018.

Wang, H. M., Zhao, H. H., & Chu, D. (2015). Genetic structure analysis of populations of the soybean cyst nematode, *Heterodera glycines*, from north China. *Nematology*, *17*, 591–600.

Woo, M. O., Beard, H., MacDonald, M. H., Brewer, E. P., Youssef, R. M., Kim, H., & Matthews, B. F. (2014). Manipulation of two α-endo-β-1,4-glucanase genes, *AtCel6* and *GmCel7*, reduces susceptibility to *Heterodera glycines* in soybean roots. *Molecular Plant Pathology*, *15*(9), 927–939.

Xu, Q., Chen, L. L., Ruan, X. A., Chen, D. J., Zhu, A. D., Chen, C. L., … Yijun Ruan, Y. J. (2013). The draft genome of sweet orange (*Citrus sinensis*). *Nature Genetics*, *45*(1), 59–67.

Yaffe, E., & Tanay, A. (2011). Probabilistic modeling of Hi-C contact maps eliminates systematic biases to characterize global chromosomal architecture. *Nature Genetics*, *43*(11), 1059–1065.

Yang, Z., & Rannala, B. (2012). Molecular phylogenetics: Principles and practice. *Nature Reviews Genetics*, *13*(5), 303–314. https://doi.org/10.1038/nrg3186

Zhang, X. J., Jianbo Yuan, J. B., Sun, Y. M., Li, S. H., Gao, Y., Yu, Y., … Jianhai Xiang, J. H. (2019). Penaeid shrimp genome provides insights into benthic adaptation and frequent molting. *Nature Communications*, *10*, 356

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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