



## High-Quality Genome Sequence of the Root-Knot Nematode *Meloidogyne arenaria* Genotype A2-O

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**ABSTRACT** Root-knot nematodes (*Meloidogyne* spp.) cause serious damage to many crops globally. We report the high-quality genome sequence of *Meloidogyne arenaria* genotype A2-O. The genome assembly of *M. arenaria* A2-O is composed of 2,224 contigs with an  $N_{50}$  contig length of 204,551 bp and a total assembly length of 284.05 Mb.

Plant-parasitic nematodes are some of the most agriculturally important pests, causing estimated global losses of \$80 billion per year (1). Among plant-parasitic nematodes, mitotic parthenogenetic root-knot nematodes (RKNs) (i.e., *Meloidogyne incognita, Meloidogyne arenaria,* and *Meloidogyne javanica*) are obligatory parasites which are remarkably widespread geographically (1, 2). These asexual RKNs have a broader host range and are more devastating than sexual species of RKNs (3). RKNs invade roots and induce redifferentiation of root cells to form "giant cells," which serve as a specialized nutrient source for the parasites. The nematodes develop into adult females which lay eggs in a gelatinous matrix on the root surface.

Several genomes of agriculturally important RKN species have been sequenced using short-read sequencers (4-8). The genomes of asexual Meloidogyne species are polyploid and consist of duplicated regions with a high nucleotide divergence (~8%) (4, 7, 8). Moreover, the genomes of asexual *Meloidogyne* species contain more transposable elements (TEs) than the sexual Meloidogyne hapla genome. These features might confer genomic plasticity and functional divergence between gene copies in the absence of sex and meiosis. However, these genomic features make it technically difficult to generate contiguous assemblies using short reads. In fact, the reported genome assemblies of the asexual Meloidogyne species, such as M. incognita, M. arenaria, and M. javanica, are highly fragmented compared to the M. hapla genome assembly (5, 7). To overcome this problem, we applied single-molecule real-time (SMRT) sequencing technology with the PacBio RS II platform (Pacific Biosciences, CA, USA) to sequence the genome of M. arenaria genotype A2-O, isolated in Izu Oshima Island in Japan (9). For the preparation of genomic DNA, infective second-stage juveniles of M. arenaria A2-O hatched from sterilized eggs were collected by sucrose flotation. Collected nematodes were ground down, and their genomic DNA was extracted using Genomic-tips (Qiagen, Hilden, Germany). The SMRTbell template prep kit 1.0 (Pacific Biosciences) was used to prepare 20-kb insert PacBio libraries. Then, size selection was performed with a 15-kb cutoff using Blue Pippin (Sage Science, MA, USA).

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AMERICAN SOCIETY FOR MICROBIOLOGY We generated an approximately 60-fold whole-genome shotgun sequence using P6-C4 chemistry on the PacBio RS II platform. A total of 754,356 reads (9.6 Gb) were assembled with Canu v1.3 (10), and the contigs were polished with Quiver (SMRT Analysis suite v2.3, Pacific Biosciences) (11). The assembled genome contains 2,224 contigs (all of the contigs are greater than 500 bp) with an  $N_{50}$  contig length of 204,551 bp and a total length of 284.05 Mb. The assembly was estimated to cover 94.76% of the coding space according to Core Eukaryotic Genes Mapping Approach (CEGMA) analysis (12) and is more contiguous than the previously published *M. arenaria* genome (7), with 14-fold fewer contigs and a 12.9-fold increased  $N_{50}$  contig length. This long-read-based high-quality assembly of *M. arenaria* should promote identification of virulence-related genes that often exist in repeat-rich or highly variable regions in the genome (13).

Accession number(s). The sequences obtained by this whole-genome shotgun project have been deposited in DDBJ/ENA/GenBank under the accession number QEUI00000000.

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## REFERENCES

- 1. Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-López R, Palomares-Rius JE, Wesemael WM, Perry RN. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961. https://doi.org/10.1111/mpp.12057.
- Trudgill DL, Blok VC. 2001. Apomictic, polyphagous, root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annu Rev Phytopathol 39:53–77. https://doi.org/10.1146/ annurev.phyto.39.1.53.
- Castagnone-Sereno P, Danchin EG. 2014. Parasitic success without sex the nematode experience. J Evol Biol 27:1323–1333. https://doi.org/10 .1111/jeb.12337.
- 4. Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EG, Deleury E, Perfus-Barbeoch L, Anthouard V, Artiguenave F, Blok VC, Caillaud MC, Coutinho PM, Dasilva C, De Luca F, Deau F, Esquibet M, Flutre T, Goldstone JV, Hamamouch N, Hewezi T, Jaillon O, Jubin C, Leonetti P, Magliano M, Maier TR, Markov GV, McVeigh P, Pesole G, Poulain J, Robinson-Rechavi M, Sallet E, Ségurens B, Steinbach D, Tytgat T, Ugarte E, van Ghelder C, Veronico P, Baum TJ, Blaxter M, Bleve-Zacheo T, Davis EL, Ewbank JJ, Favery B, Grenier E, Henrissat B, Jones JT, Laudet V, Maule AG, Quesneville H, Rosso MN, et al. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nat Biotechnol 26:909–915. https://doi.org/10.1038/nbt.1482.
- Opperman CH, Bird DM, Williamson VM, Rokhsar DS, Burke M, Cohn J, Cromer J, Diener S, Gajan J, Graham S, Houfek TD, Liu Q, Mitros T, Schaff J, Schaffer R, Scholl E, Sosinski BR, Thomas VP, Windham E. 2008. Sequence and genetic map of *Meloidogyne hapla*: a compact nematode genome for plant parasitism. Proc Natl Acad Sci U S A 105:14802–14807. https://doi.org/10.1073/pnas.0805946105.
- Lunt DH, Kumar S, Koutsovoulos G, Blaxter ML. 2014. The complex hybrid origins of the root knot nematodes revealed through comparative genomics. PeerJ 2:e356. https://doi.org/10.7717/peerj.356.

- Blanc-Mathieu R, Perfus-Barbeoch L, Aury JM, Da Rocha M, Gouzy J, Sallet E, Martin-Jimenez C, Bailly-Bechet M, Castagnone-Sereno P, Flot JF, Kozlowski DK, Cazareth J, Couloux A, Da Silva C, Guy J, Kim-Jo YJ, Rancurel C, Schiex T, Abad P, Wincker P, Danchin EGJ. 2017. Hybridization and polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes. PLoS Genet 13:e1006777. https://doi.org/ 10.1371/journal.pgen.1006777.
- Szitenberg A, Salazar-Jaramillo L, Blok VC, Laetsch DR, Joseph S, Williamson VM, Blaxter ML, Lunt DH. 2017. Comparative genomics of apomictic root-knot nematodes: hybridization, ploidy, and dynamic genome change. Genome Biol Evol 9:2844–2861. https://doi.org/10.1093/gbe/ evx201.
- Uehara T, Tateishi Y, Kadota Y, Iwahori H. 2017. Differences in parasitism of *Meloidogyne incognita* and two genotypes of *M. arenaria* on *Solanum torvum* in Japan. J Phytopathol 165:575–579. https://doi.org/10.1111/jph .12594.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi .org/10.1101/gr.215087.116.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/ nmeth.2474.
- Parra G, Bradnam K, Ning Z, Keane T, Korf I. 2009. Assessing the gene space in draft genomes. Nucleic Acids Res 37:289–297. https://doi.org/ 10.1093/nar/gkn916.
- Dong S, Raffaele S, Kamoun S. 2015. The two-speed genomes of filamentous pathogens: waltz with plants. Curr Opin Genet Dev 35:57–65. https://doi.org/10.1016/j.gde.2015.09.001.