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Five life stage-specific transcriptome assemblies for the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira

Kurt C. Showmaker,[1,2,3] William S. Sanders,[2,3,4] Sebastian Eves-van den Akker,[5] Brigitte E. Martin,[6] Franklin E. Callahan,[7] Daniel G. Peterson[3,8] and Martin J. Wubben[2,7]*

[1]Department of Crop Science, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

[2]Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, MS, USA.

[3]Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, MS, USA.

[4]The Jackson Laboratory, Farmington, CT, USA.

[5]Department of Plant Sciences, University of Cambridge, Cambridge, UK.

[6]Department of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

[7]USDA-ARS, Crop Science Research Laboratory, Mississippi State, MS, USA.

[8]Department of Plant and Soil Sciences, Mississippi State University, MS, USA

*E-mail: Martin.Wubben@ars.usda.gov.

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Abstract

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) is a semi-endoparasitic nematode that is a pathogen of numerous major crops such as cotton and soybean. Here, the authors present transcriptome assemblies of the egg, second-stage juvenile (J2), J3, vermiform adult, and sedentary female life stages of this important plant pathogen.

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) is a sedentary semi-endoparasitic nematode that causes significant economic damage to many important crops such as cotton, soybean, sweet potato, and pineapple (Robinson et al., 1997). Evolutionary studies indicated that *R. reniformis* is most closely related to the cyst nematode genera and share common ancestry with *Radopholus* spp. (Holterman et al., 2009). While both *R. reniformis* and the cyst nematodes are sedentary in nature and form similar feeding sites within the host root, the life cycles of these genera differ significantly. While the cyst nematodes initiate host root infection immediately upon egg hatching as second-stage juveniles (J2), *R. reniformis* J2 become immobile soon after hatching (Robinson et al., 1997). Syncytia formation is induced by cyst nematode J2 and the nutrients provided by this feeding site allow the cyst nematode to develop through the J3 and J4 life stages until adulthood. In contrast, *R. reniformis* proceeds through all juvenile stages in the soil, without feeding, until reaching the adult male and female vermiform life stage (Robinson et al., 1997). It is the *R. reniformis* vermiform female that infects the host root and initiates syncytium formation (Robinson et al., 1997).

The *R. reniformis* life stages of egg, J2, J3, vermiform adult (VA), and sedentary female (SF) were isolated as previously described (Ganji et al., 2013). Total RNA was extracted from individual life stages using Trizol reagent (Life Technologies, Grand Island, NY, USA) (Wubben et al., 2010). RNA quantity and integrity were determined with the Qubit RNA HS assay kit (Life Technologies) and with an Agilent Bioanalyzer 2100 via the Agilent RNA 6000 Nano kit (Agilent Technologies, Palo Alto, CA, USA), respectively. For each life stage, three technically replicated Illumina Truseq V2 RNA (Illumina, San Diego, CA) libraries were prepared by Global Biologics (Columbia, MO). Libraries were pooled and paired-end sequenced (2×100bp) on five lanes of the Illumina Hiseq 2000 using TruSeq v3 chemistry (Illumina) at the USDA-ARS Bovine Functional

Genomics Laboratory (Beltsville, MD). The resulting reads were deposited in the SRA archive under the BioProject PRJNA286314.

Sequences were trimmed and filtered for adapters and low-quality base calls with Trimmomatic (v0.32; Bolger et al., 2014), and trimmed reads for each life stage were aligned to the reniform nematode reference genome (RREN1.0, GCA_001026735.1) with the STAR aligner (v2.6.0c; Dobin et al., 2013), and subsequently assembled with Trinity (v2.6.5; Grabherr et al., 2011). The resulting assemblies for each life stage were deposited in the Transcriptome Shotgun Assembly (TSA) database. Completeness assessment by the programs CEGMA (v2.5; Parra et al., 2007) and BUSCO (v3.0.1; Simão et al., 2015) ranged from 81.45 to 83.06 (92.34-93.95 including partial alignments) and 60.0 to 67.2% complete, respectively. Coding regions were predicted with TransDecoder (v5.1.0; http://transdecoder.github.io) guided by Diamond (v0.9.16; Buchfink et al., 2015), Blastp alignments to Swiss-Prot (release 2018_07), and hmmscan (v3.2.1; Eddy, 2011) alignments to Pfam-A (vPfam31.0; Finn et al., 2016). Clustering of all the predicted proteins with CD-HIT (v4.6.6; Li and Godzik, 2006) resulted in 71,321 protein clusters at the 90% identity level.

GenBank accession numbers: the raw sequence data and transcriptome assemblies were deposited at GenBank under BioProject no. PRJNA286314. The Transcriptome Shotgun Archive (TSA) projects has been deposited at DDBJ/ENA/GenBank and the versions described in this paper are the first versions; GGVV01000000 (egg), GGVO01000000 (J2), GGVP01000000 (J3), GGVQ01000000 (VA), and GGVR01000000 (SF). Disclaimer: Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

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