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First Report of the Ring Nematode *Mesocriconema nebraskense* from a Corn Field in North Dakota

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In May 2016, two soil samples were collected from a corn field in Sargent County, ND from which nematodes were extracted from soil using sieving and decanting followed by a sugar centrifugal flotation method (Jenkins, 1964). Plant-parasitic nematodes were identified to genus based on morphological features and their population densities were quantified. Both samples contained ring nematodes at 85 to 87 per kg of soil. In September 2017, 12 soil samples were collected from the same field, 4 of the samples had ring nematodes ranging from 100 to 900 per kg of soil (Fig. 1). One large, composite sample with 75 ring nematodes per kg of soil obtained after mixing positive and negative soil samples was used to inoculate three corn cultivars DK 43-48-RIB, DKC 43-46, and DKC 44-13 each in four replicates. After 14 wk of growth at 22°C in a greenhouse room, the average population of ring nematodes increased. The final population density from DK 43-48-RIB, DKC 43-46, and DKC 44-13 was 158 ± 151, 283 ± 154, and 156 ± 140 per kg of soil, respectively. The Reproductive Factor was 2.1, 3.8, and 2.1 for DK 43-48-RIB, DKC 43-46, and DKC 44-13, respectively, indicating that this ring nematode was able to infect and reproduce on these three corn cultivars. Individual ring nematodes were hand-picked from nematode suspensions isolated from field and greenhouse samples and examined morphologically and molecularly for species identification. Morphometric measurements of adult females (n = 10) included body length (mean ± standard deviation = 546.6 ± 64.0 µm, range = 425.0-627.0 µm), stylet (56.2 ± 1.6, 54.0–60.0), tail length (27.4 ± 3.2, 22.0–32.0), maximum body width (50.9 ± 2.5, 49.0–55.0), anterior end to basal bulb (124.3 ± 8.8, 110.0–140.0), a (total body length divided by maximum body diameter: 10.7 ± 1.5, 8.5–13.6), b (total body length divided by pharyngeal length: 4.4 ± 0.7, 3.9-4.8), c (total body length divided by tail length: 20.0 ± 2.5, 16.1–25.0), V (percentage of length from anterior end to vulva position in total body length: 91.7 ± 0.6%, 90.7–92.8), R (total body annules:105.0 ± 6.0, 98.0–118.0), Rex (annules from anterior end to excretory pore: 28.3 ± 1.3, 26.0–32.0), RV (total annules from vulva to tail terminus: 9.0 ± 0.7, 8.0–10.0), Rvan (total annules from anus to tail terminus: 5.1 ± 0.3, 5.0-6.0), body diameter at vulva (39.4 ± 3.0, 33.0–40.0), and body diameter at anus (29.5 ± 2.8, 25.0–33.0). Lip region having two annules that are smaller and narrower than other body annules but are not set off. Body annules are retrorse with smooth margins, labial disc elevated surrounding the oral opening, stylet robust with well-developed knobs, submedian lobes present, vulva on 8-10th annule and anus on 5-6th annule from posterior end of body, and post-vulval body portion conical to more or less rounded, with single to multiple lobed terminus. The nematode species was identified as *Mesocriconema nebraskense* Olson et al., 2017 based on morphological and morphometric characteristics

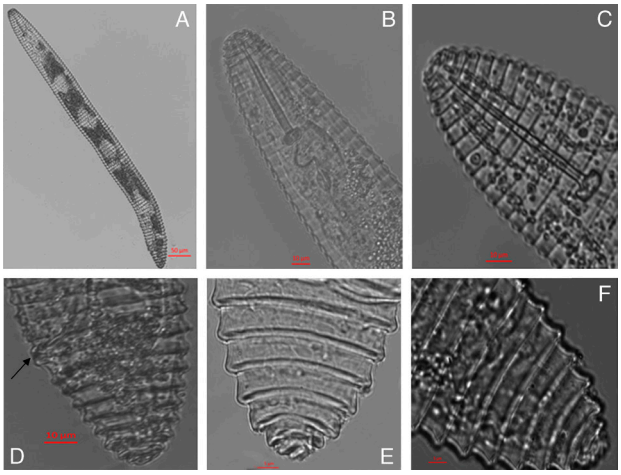

Figure 1: *Mesocriconema nebraskense* female. A: entire body; B, C: anterior end; D, E, F: posterior end, with the arrow pointing at the vulva.

(Olson et al. 2017). DNA was extracted from single nematodes (n = 10) from both greenhouse and field samples using the Proteinase K method (Kumari and Subbotin, 2012). Two ribosomal DNA regions and one mitochondrial DNA region were amplified and sequenced for species identification. The ITS region of rDNA was amplified with primers TW81/AB28 (Joyce et al., 1994), D2-D3 region of 28S rRNA gene with primers D2A/D3B (Subbotin et al., 2008), and cytochrome oxidase subunit I (*cox1*) gene with primers COI-F5/COI-R9 (Powers et al., 2014). PCR products were purified and submitted for sequencing directly. The sequences were deposited into the GenBank database and assigned with accession number MH013430 (762bp) for the D2-D3 of 28S rRNA gene, MH013431 (798bp) for the ITS rDNA, and MH023322 (721bp) for the mitochondrial *cox1* gene. The comparative sequence analysis with BLASTn search suggests that the closest species was *Mesocriconema nebraskense*, showing 99% identity in *cox1* gene with KJ787973 and 14 other isolates of *M. nebraskense*, and 90% or less sequence similarity with other *Mesocriconema* spp. The BLASTn search also revealed that the ring nematode from North Dakota had 99% similarity in ITS rDNA with seven isolates of *M. nebraskense*, five isolates of *Mesocriconema* sp., and four isolates of *M. curvatum*. The sequence of D2-D3 of *M. nebraskense* was not available in GenBank, and therefore was deposited in the GenBank database by us for the first time. This nematode population had less than or equal to 98% sequence similarity in D2-D3 with other ring nematode species. The molecular identification in the *cox1* gene supported that the North Dakota specimens is *M. nebraskense*. *M. nebraskense* is a recently described species from the central grasslands (Olson et al., 2017), and was found on golf courses in New Mexico and Arizona (Steve Thomas, unpublished data). However, the impact of this nematode on plant growth and yield has not been documented. To our knowledge, this is the first report of the ring nematode *M. nebraskense* from a corn field in North Dakota.

References

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Joyce, S. A., Reid, A. P., Driver, F., and Curran, J. 1994. Application of polymerase chain reaction (PCR) methods to the identification of entomopathogenic nematodes. In Burnell, A. M., Ehlers, R. U., and Masson, J. P. (Eds), Genetics of entomopathogenic nematode-bacterium complexes. proceedings of symposium and workshop, St. Patrick's College, Maynooth, Co, Kildare, Ireland. European Commission, DGXII, Luxembourg: 178–87.

Kumari, S., and Subbotin, S. A. 2012. Molecular characterization and diagnostics of stubby root and virus vector nematodes of the family Trichodoridae (Nematoda: Triplonchida) using ribosomal RNA genes. Plant Pathology 61:1021–31.

Olson, M., Harris, T., Higgins, R., Mullin, P., Powers, K., Olson, S., and Powers, T. O. 2017. Species delimitation and description of *Mesocriconema nebraskense* n. sp. (Nematoda: Criconematidae), a morphologically cryptic, parthenogenetic species from North American grasslands. Journal of Nematology 49:42–66.

Powers, T. O., Bernard, E. C., Harris, T., Higgins, R., Olson, M., Lodema, M., Mullin, P., Sutton, L., and Powers, K. S. 2014. COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations. Zootaxa 3827:101–46.

Subbotin, S. A., Ragsdale, E. J., Mullens, T., Roberts, P. A., Mundo-Ocampo, M., and Baldwin, J. G. 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters. Molecular Phylogenetics and Evolution 48:491–505.