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[Previous Article](#) | [Next Article](#)

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Page 2536

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DISEASE NOTES

First Report of the Lance Nematode *Hoplolaimus stephanus* From a Soybean Field in North Dakota

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ABSTRACT

Lance nematodes (*Hoplolaimus* spp.) are an important group of ecto-endo parasitic nematodes that feed on plant roots and cause damage to crop production (Holguin et al. 2016). In August 2015, a soil sample was collected from a soybean field near Cogswell, Sargent County, ND, to investigate the occurrence of lance nematodes. Nematodes were extracted from soil using the sugar centrifugal flotation method, revealing that this sample contained 210 lance nematodes per 100 cm³ of soil. In October 2015, four soil samples were collected from the same field and had lance nematodes ranging from 30 to 100. One soil sample with 60 lance nematodes per 100 cm³ of soil was used to inoculate a soybean cultivar Lamoure ($n = 3$). After 12 weeks of growth in a greenhouse room maintained at 22°C, the average of lance nematode populations slightly increased (68 ± 50 per 100 cm³ soil), and stunted and shortened lateral roots branching from the main root were observed. Individual lance nematodes were hand-picked and characterized morphologically and molecularly for species identification. Morphological measurements of adult females ($n = 8$) included body length (range = 1,048 to 1,410 μm , mean = 1,272.0 μm), stylet (43 to 48, 45.0), body width (45 to 60, 51.8), anterior end to basal bulb (140 to 190, 169.3), tail length (26 to 35, 31.0), a (22 to 27, 24.5), b (6 to 9, 7.4), c (32 to 52, 40.8), and V (52 to 58%, 53.6%). Morphological measurements of adult males ($n = 4$) were body length (935 to 1,045, 1,004.0), stylet (40 to 42, 40.7), body width (44 to 48, 46.0), anterior end to basal bulb (145 to 160, 152.5), tail length (30 to 32, 31.2), a (21 to 23, 21.7), b (6 to 7, 6.5), c (31 to 34, 32.0), spicules (36 to 40, 39.0), and capitulum (18 to 22, 20.5). The nematode species was identified as *Hoplolaimus stephanus* Sher, 1963 according to morphological and

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morphometric characteristics (Handoo and Golden 1992). DNA was extracted from single nematodes ($n = 10$) isolated from soil in 20 μ l of extraction buffer. D1/D3 region of 28S rRNA and ITS1 of rDNA were amplified (Bae et al. 2008). PCR products from three nematodes were cloned using pGEM-T easy vector and sequenced. The consensus sequence from each genomic region was deposited into GenBank. The D1/D3 sequence (GenBank accession no. KX347887, 1,076 bp) was 99% identical to a population of *H. stephanus* (HQ678717) from Pennsylvania (Ma et al. 2011) and was 96% or less identical to other *Hoplolaimus* spp. The ITS1 rDNA sequence (KX347888, 612 bp) was 98% identical with 13 *H. stephanus* isolates among which *H. stephanus* (KP303643) from South Carolina ranked first in the list. This sequence had no significant similarity with other *Hoplolaimus* spp. including *H. galeatus*. Species-specific primers were used to amplify DNA of seven nematodes and produced single bands specific for *H. stephanus* (Ma et al. 2011). The molecular tests above confirmed the species identity as *H. stephanus*. This species mainly has been reported in the southeastern United States and was found to be the most predominant lance nematode species on soybean in North Carolina (Holguin et al. 2016). *H. galeatus* was mentioned as common in the northern Great Plains (Thorne and Malek 1968), but *H. stephanus* has never been reported in the northern United States. To our knowledge, this is the first report of *H. stephanus* in North Dakota.

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