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First Report of Stubby-Root Nematode, *Paratrichodorus minor*, on Onion in Georgia, U.S.A

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Abstract

Onions (*Allium cepa* L.) are the leading vegetable crop in Georgia accounting for 13.7% of total state vegetable production (Wolfe and Stubbs, 2017). In November 2017, two samples each of onion (var. Candy Ann) seedlings and soil were received from the University of Georgia Cooperative Extension office in Tattnall County, GA. The samples were collected from a nursery fumigated with metam sodium and used for sweet onion transplant production. Symptoms of the damaged plants included stunted growth both in the root system and foliage, tip die-back of the leaves (Fig. 1A,B), and slight swelling at the tip of roots. Vermiform life stages from the soil samples were extracted using centrifugal-flotation technique (Jenkins, 1964). On an average, 67 stubby-root nematodes per 100 cm^3 of soil were obtained. Additional two soil samples were collected from the nursery in December 2017 to confirm the presence of the nematode. On an average, 1 and 75 nematodes per 100 cm^3 of soil were recovered from areas with healthy and infested plants, respectively. Because the male individuals were not found in the soil samples, females were used for species identification. Morphological and molecular analyses of females (Fig. 2A-C) identified the species as *Paratrichodorus minor* (Colbran) Siddiqi; (Decraemer, 1995). Nematode body shape was "cigar-shaped" with dorsally curved "onchiostyle" stylet Females had an oval-shaped vagina, vulva a transverse slit, and lateral body pores were absent. The measurements of females (n = 20) included: body length 671.1 (570.1–785.3) µm; body width 32.5 (27.8–37.0) µm; onchiostyle 32.5 (31.1–34.8) µm; anterior end to esophagus-intestinal valve 117.6 (101.2–128.5) µm; a 21.5 (15.3-28.1) µm; b 5.2 (4.9–6.3) µm; V 52.9% (48.1–55.4%) µm; and vagina length 8.7 (7.8–10.7) µm. To confirm the identity of *P. minor,* DNA was extracted from single females (n = 3) using Extract-N-Amp™ Tissue PCR Kit (Sigma-Alredich Inc., St. Louis, MO). The partial 18S rRNA, the D2-D3 expansion segments of 28S rRNA, and ITS1 rDNA were amplified using primer pairs 360F (5′ CTACCACATCCAAGGAAGGC 3′)/932R (5′ TATCTGATCGCTGTCGAACC 3′), D2A (5′ ACAAG TACCGTGAGGGAAAGTTG 3′)/D3B (5′ TCGGAAGGAACCAGCTAC TA 3′), and BL18 (5′ CCCGTCGCTACTACCGATT 3′)/5818 (5′ ACGARCCGAGTGATCCAC 3′), respectively (Riga et al., 2007; Duarte et al., 2010; Ye et al., 2015; Shaver et al., 2016). The obtained PCR fragments were purified using QIAquick Gel Extraction Kit (Qiagen Inc., Santa Clara, CA, USA), sequenced and deposited in the GenBank databases (18S rRNA: MG856931; 28S rRNA: MG856933; ITS1 rDNA: MH464152). The 18S rRNA, 28S D2-D3, and ITS1 rDNA sequences shared 99% similarity (100% coverage) with GenBank accessions of *P. minor* from California, Arkansas, and China (18S rRNA: JN123365;

28S D2-D3: JN123395; ITS1 rDNA: GU645811). In a pathogenicity test, five sweet onion seeds var. Pirate were planted (one per pot) in 11.5-cm-diameter polyethylene pots containing 1,000 cm^3 of equal parts of pasteurized field soil and sand, and then inoculated with 1,000 fresh *P. minor*. Plants were grown for 9 wk in a greenhouse at 25 ± 2°C prior to extraction of nematodes from soil. Plant roots were abbreviated and final population density of *P. minor* was 2,856 ± 104 per pot (285 nematodes/100 cm^3 of soil) confirming the nematode parasitism on onion. To our knowledge, this is the first report of *P. minor* parasitizing onion in Georgia. Stubby-root nematode (*Paratrichodorus* sp.) has already been reported on corn, St. Augustine grass, and switchgrass in Georgia (Heald and Perry, 1969; Davis and Timper, 2000; Mekete et al., 2011). In the U.S.A, *P. minor* is known to occur on diverse crops in most of the states (Decraemer, 1995; CABI/EPPO, 2002). A survey of vegetable-producing areas in Georgia is currently under investigation to determine the distribution of this economically important nematode species.

Key words
Detection, Georgia, *P. minor*, Sweet onion.

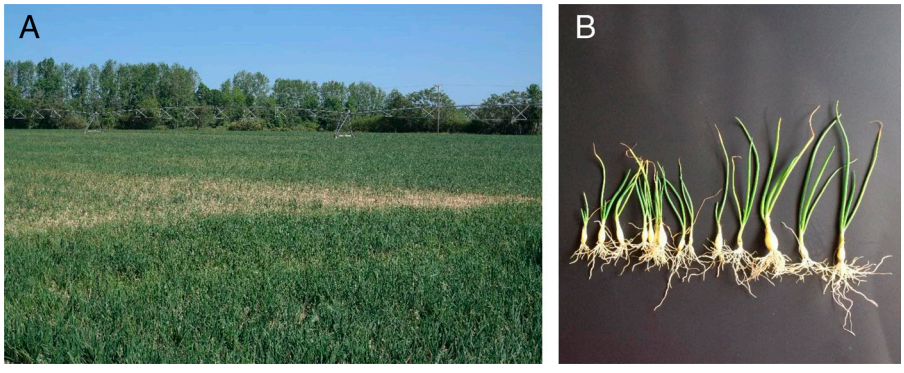

Figure 1: Damage symptoms caused by stubby-root nematode *P. minor* on sweet onion in Georgia. A large area of stunted and chlorotic plant foliage (A); Infested seedlings with abbreviated roots and necrotic leaf tips (B).

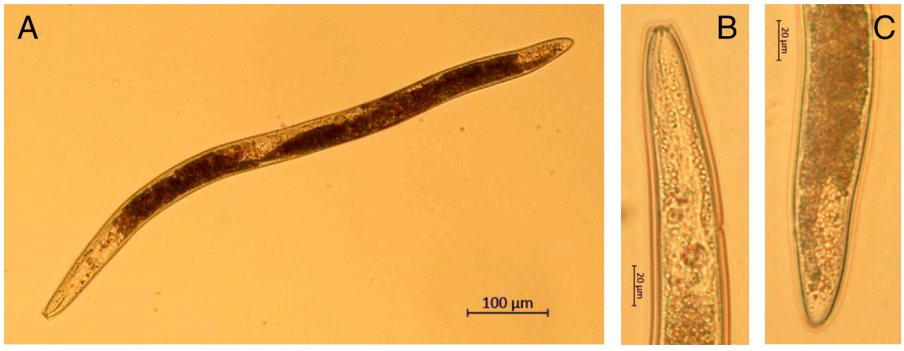

Figure 2: Light microscopy micrographs showing morphological characters of stubby-root nematode, *P. minor*. Entire body (A), anterior end (B), and posterior region (C) of female nematode.

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