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LETTER

First report of cereal cyst nematode (*Heterodera filipjevi*) on winter wheat in Shandong Province, China

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The cereal cyst nematodes (*Heterodera avenae*, *Heterodera filipjevi*, *Heterodera latipons*) are considered to be one of the most important plant parasitic nematodes attacking most cereals and can cause significant crop losses (Sikora 1988). In China, *H. filipjevi* (Madzhidov 1981) Stelter, 1984, was first reported from Henan Province (Peng *et al.* 2010) and a few years later in Anhui Province and Xinjiang Uygur Autonomous Region (Peng *et al.* 2016, 2018). In December 2017, a survey for cereal cyst nematodes on winter wheat was conducted in Shandong Province, China. A total of 79 samples that including roots and rhizosphere soil were collected. Cysts and second-stage juveniles (J2s) were isolated from each soil sample using the sieving-decanting method. Wheat roots were stained with acid fusion to observe the development of cereal cyst nematodes. One sample collected from Yangzhuang Village in Huanggang Town, Shan County of Heze City (GPS 34°38'23.10"N, 116°05'42.95"E), Shandong Province, was found that the wheat roots were heavily parasitized by cyst nematodes, and most of the nematodes in roots had developed to fourth-stage (J4) in mid-December of 2017. The morphological and molecular studies of cyst and J2s were carried out to confirm the identification of *H. filipjevi* in one winter wheat field soil and root sample from Shan County. The cysts were lemon shaped with prominent vulval cone, brown to black in colour. Cuticle with irregular zig-zag pattern. Neck prominent, vulval cone bifenestrated with horseshoe-shaped fenestra, bullae and underbridge strongly developed. The main morphometrics of cysts ($n=8$) were length (including neck) (688 to 948 μm , mean=794 μm , standard deviation=87 μm), width (465 to 620 μm , mean=529 μm , standard deviation=63 μm), neck length (71.5 to 126.3 μm , mean=86.5 μm , standard deviation=9.2 μm), fenestra length (43.8 to 71.3 μm , mean=58.0 μm , standard deviation=15.1 μm), fenestra width (19.8 to 32.0 μm , mean=25.0 μm , standard deviation=3.9 μm), length of vulval slit (8.1 to 9.7 μm , mean=9.1 μm , standard deviation=0.5 μm) and length of underbridge (64.5 to 101.3 μm , mean=82.6 μm , standard deviation=12.8 μm). Measurements of J2s ($n=10$); body length (556.7 to 617.0 μm , mean=584.3 μm , standard deviation=23.2 μm); stylet

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(22.8 to 24.1 μm , mean=23.3 μm , standard deviation=0.4 μm), tail (59.6 to 68.6 μm , mean=65.8 μm , standard deviation=3.5 μm) and hyaline tail terminus (35.9 to 41.1 μm , mean=38.6 μm , standard deviation=2.1 μm). Genomic DNA was isolated from single cysts ($n=6$), and the internal transcribed spacer regions were amplified with primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Joyce et al. 1994) and 28S rDNA-D2/D3 regions were amplified with primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Subbotin et al. 2006). The obtained internal transcribed spacer regions (ITSs) sequences (GenBank accession MG859977) is 99% identical to those of *H. filipjevi* from Turkey (KR704292.1 and KR704304.1), the United States (KP878490.1 and GU079654.1) and China (KY448473.1 and KY448473.1). The obtained 28S rDNA-D2/D3 sequences (GenBank accession MG859980) also to be 99 to 100% identical to those of *H. filipjevi* from China (GU083597.1, KT314235.1, GU083592.1). The species-specific primers of *H. filipjevi* (HfF1, 5'-CAGGACGAACTCATTCAACCAA-3'; HfR1, 5'-AGGGCGAACAGGAGAAGATTAGA-3') were also used to identify this population (Peng et al. 2013), the specific band was obtained species-specific primers of *H. filipjevi*. Based on the morphological and molecular data, the species of the cyst-forming nematode was identified as *H. filipjevi*.

As far as we know, this is the first report of *H. filipjevi* in Shandong Province, China. The population density of *H. filipjevi* were found much higher than those of other CCN, it can serious infect winter wheat at seedling stage which often cause economically damaging to wheat, so the spread of *H. filipjevi* would be a risk for the cereal production of Shandong Province.

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