

Molecular and morphological characterization of the root-lesion nematode, *Pratylenchus neglectus*, on corn from Henan Province of China

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Summary

Root-lesion nematodes, *Pratylenchus* spp., are economically important pathogens because of their detrimental and economic impact on a wide range of crops. In August 2018, two samples of both roots and rhizosphere soil were collected from a corn field in Liangyuanqu of Shangqiu city, Henan Province, China. Root-lesion nematodes were recovered from the roots and soil samples using the modified Baermann funnel extraction method. Both the morphological characters and molecular analysis of the internal transcribed spacer (ITS) and D2-D3 expansion region of 28S ribosomal RNA sequences confirmed that the root-lesion nematode population collected from corn in this study was *P. neglectus*. Phylogenetic analyses showed that this isolate formed a highly supported clade with other *P. neglectus* isolates. To the best of our knowledge, this is the first report of *P. neglectus* on corn in Henan Province of China. This study reports the first partial sequences of 28S D2-D3 region of *P. neglectus* on corn in China. Due to the great harmfulness of root-lesion nematodes to corn, care should be taken to prevent the spread of *P. neglectus* to other regions in China. At the same time, further study on the biological characteristics of *P. neglectus* is needed, which will be helpful to develop corresponding management and control strategies.

Keywords: Corn (*Zea mays*); Root-lesion nematodes; Identification; Phylogeny; *Pratylenchus neglectus*

Introduction

Corn (*Zea mays* L.) is a very important cereal crop and serves as food, feed, and industrial material, with 45.0 million hectares planting areas in China (Liu *et al.*, 2016; Qiu *et al.*, 2020). Henan province is the main agricultural producing area in China and occupies an important position in the development of national economy. Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasitic nematodes, which together with root-knot and cyst nematodes are three of the most economically important groups

of plant parasitic nematodes species in the world (Castillo & Vovlas, 2007). Root-lesion nematodes have a wide geographical distribution and can parasitize a wide range of host plants. Within the genus *Pratylenchus*, more than 100 species were recognized worldwide (Janssen *et al.*, 2017). Corn is seriously attacked by several *Pratylenchus* species, including *P. neglectus*, *P. scribneri*, *P. brachyurus*, *P. penetrans* and *P. zeae* in the major production areas, causing significant economic losses (Windham, 1998). Infected roots of corn show dark brown discrete lesions that can serve as secondary infection sites for root-rotting fungi (Jones *et*

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al., 2013). *P. neglectus* Filipjev and Schuurmans Stekhoven, 1941 is widely distributed and has been reported on all continents except Antarctica (Castillo & Vovlas, 2007).

More research suggests that the use of a small set of morphological features increases the risk of misidentification (Handoo *et al.*, 2016; Wang *et al.*, 2016). Thus, molecular approaches and phylogenetic analyses tools will allow more accurate nematode identification, regardless of developmental stage (Handoo *et al.*, 2016). In this study, root-lesion nematodes were extracted from corn samples, and one individual female nematode was selected to propagate on carrot disks at 25 °C by parthenogenesis. The purified root-lesion nematode population was identified based on morphological and molecular analysis of the ITS rRNA gene and D2-D3 expansion region of 28S rRNA gene. Both morphological and molecular data confirmed the presence of *P. neglectus*. *P. neglectus* has only been reported on corn in Shandong province

(Qiu *et al.*, 2016) and Tibet of China (Yu *et al.*, 2017), however, only morphological identification was performed and the relevant molecular data was lacking. To the best of our knowledge, this is the first report of *P. neglectus* on corn in Henan province of China. It is also the first molecular data obtained from *P. neglectus* on corn in China. This study aimed to confirm the species of the root-lesion nematode occurred in Henan province of China.

Methods and Materials

Nematode sampling, extraction, and propagation

In August 2018, two samples of both roots and corresponding rhizosphere soils were collected from a corn field, near Liangyuan district of Shangqiu city, Henan Province, China (N 32°16'16.50", E 115°38'9.48"). Root-lesion nematodes were extracted using the modified Baermann funnel method for 2 days (Hooper *et al.*,

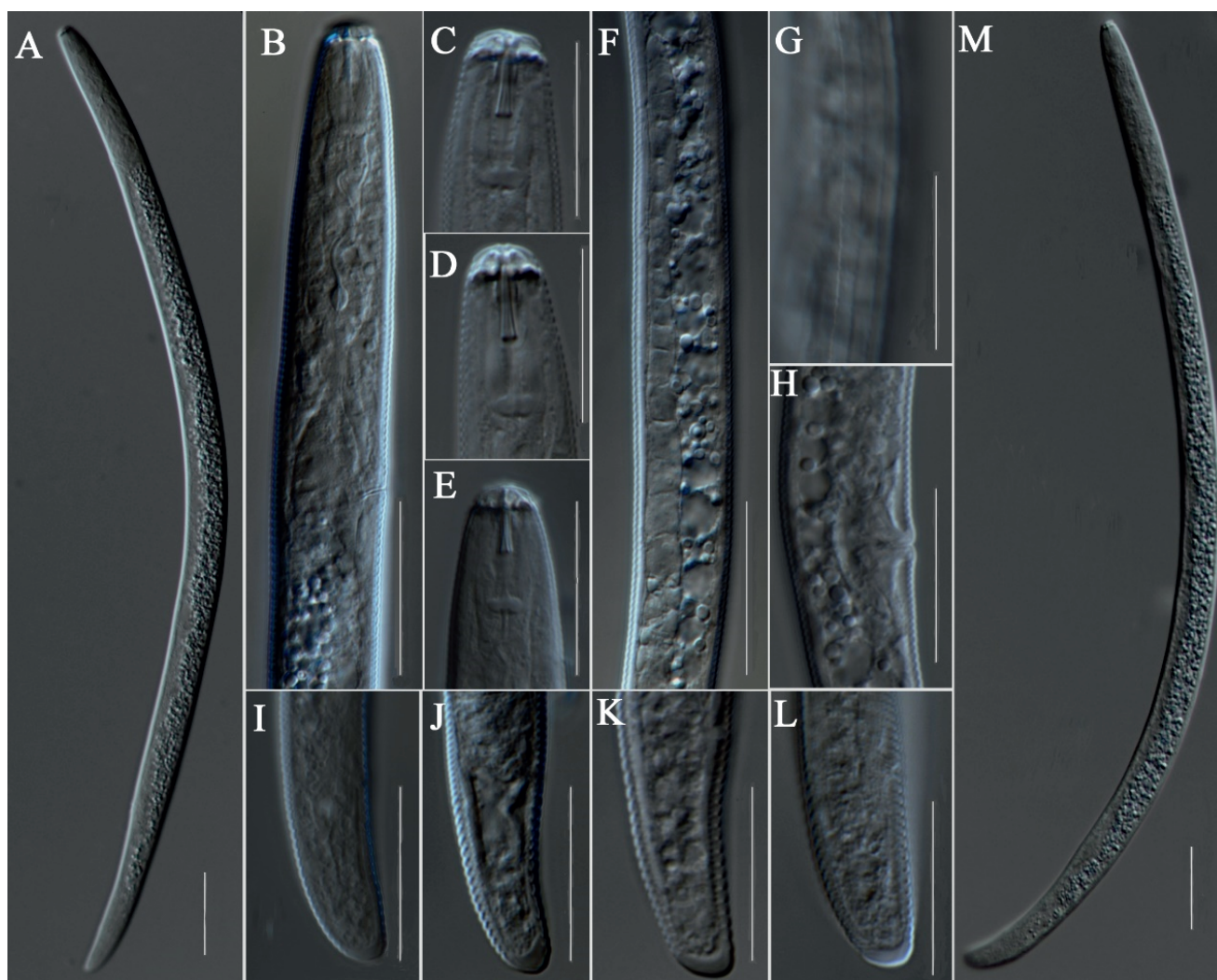


Fig. 1. Light micrographs of *Pratylenchus neglectus* from corn in Henan Province of China. Females (A -M): A and M, Entire body; B: Anterior region; C-E: lip region; F: Anterior end of genital gland; G: Lateral line; H: Post-vulval region and ovary; I-L: tail region. Scale bars: 50µm (A, M) and 20µm (B-L).

Table 1. Measurements of the *Pratylenchus neglectus* population morphological characteristics.

Character	SY-X	Pourjam <i>et al.</i> (1999)	Ryss (1988)
n	16	32	10
L	459.0 ± 23.0(431.0 – 498.0)	490(300 – 590)	600(410 – 700)
a	22.9 ± 1.6(20.0 – 26.1)	26.7(22.3 – 32.2)	24(17 – 31)
b	5.7 ± 0.5(5.0 – 6.6)	6.0(4.3 – 7.6)	6.3(4.9 – 7.1)
b'	4.3 ± 0.3(3.9 – 4.7)	4.3(3.4 – 5.2)	—
c	18.7 ± 2.2(15.2 – 24.7)	21.4(14.8 – 28.2)	21(13 – 31)
c'	2.1 ± 0.3(1.5 – 2.6)	2.1(1.5 – 3.0)	1.9(1.5 – 2.5)
V	80.5 ± 2.1(77.5 – 84.6)	82(80 – 85)	83(77 – 85)
Stylet length	15.8 ± 1.1(14.3 – 17.4)	16(14 – 18)	16(15.5 – 17.5)
Stylet shaft	8.1 ± 0.5(7.3 – 9.3)	—	—
Stylet knob width	4.1 ± 0.4(3.2 – 4.8)	—	—
Stylet knob height	2.0 ± 0.3(1.6 – 2.7)	—	—
DGO from stylet base	2.5 ± 0.5(1.4 – 3.3)	—	—
center of metacarpus	45.7 ± 2.7(40.3 – 50.5)	—	—
end of pharyngeal gland lobe	107.0 ± 4.3(98.5 – 113.0)	—	—
Anterior end to excretory pore	74.5 ± 4.5(68.5 – 85.0)	—	—
Pharyngeal overlap	26.7 ± 4.9 (19.6 – 39.4)	—	—
Max body diam.	20.1 ± 1.6 (17.8 – 23.7)	—	—
Vulval body diam.	17.9 ± 1.2(16.0 – 20.00)	—	—
Anal body diam.	12.0 ± 1.1(10.6 – 14.8)	—	—
Anterior genital trace length	147.4 ± 21.9(102.8 – 192.7)	—	—
Tail length	24.8 ± 2.4(20.0 – 29.0)	—	—
No. of tail annuli	20.7 ± 2.3(18 – 24)	—	—
Vulva to annus distance	68.0 ± 11.5(53.5 – 96.5)	—	—
Post- uterine sac length	19.7 ± 4.2(13.0 – 21.7)	—	—
Lateral field width	7.2 ± 1.1(5.8 – 8.8)	—	—
Lip width	8.1 ± 0.4(7.1 – 8.6)	—	—
Lip height	2.4 ± 0.4(1.7 – 3.1)	—	—

Note: All measurements are in μm and in the form of mean \pm SD (range). n: Number of specimens measured; L: Body length; a: Body length/greatest body width; b: Body length/length from the lips to the junction of esophageal gland and intestine; b': Body length/ length from the lips to esophageal gland end; c: Body length/ tail length; c': Tail length/tail diameter at anus; V: Distance of vulva from the lips \times 100/body length; DGO: Distance between dorsal esophageal gland opening and stylet knobs.

2005), by which 100 g of corn soil and roots samples were extracted from each sample. One individual female nematode was picked out and sterilized with 0.3 % streptomycin sulfate, and then transferred to carrot disks at 25 °C for 15 weeks in the dark for propagation (Li *et al.*, 2019; Wang *et al.*, 2021). After that, purified

root-lesion nematodes cultured on carrot disks were extracted and used for morphological and molecular analysis (Xia *et al.*, 2021).

Morphological identification

Root-lesion nematodes were fixed in 4 % formaldehyde and pro-

cessed to glycerin by the formalin glycerin method (Hooper, 1970; Golden, 1990). Photomicrographs and morphometric data of the specimens were obtained using a Nikon Eclipse Ti-S microscope equipped with a Nikon DS-Ri2 camera (Nikon, Tokyo, Japan). Images of key morphological features were processed using Photoshop CS5. Morphometric ratios were performed as defined by De Man. All measurements were expressed in micrometers (μm), unless otherwise stated.

DNA extraction, PCR, and sequencing

DNA was extracted from individual live root-lesion nematode and followed by proteinase K-based lysis (Wang *et al.*, 2011). The rRNA-internal transcribed spacer (ITS) region and D2-D3 expansion region of the 28S rRNA were amplified with primers TW81/AB28 (5'-GTTTCCGTAGGTGAACCTGC-3'/5'-ATATGCTTAAGTTCAGCGGT-3') (Subbotin *et al.*, 2006) and primers D2A-D3B (5'-ACAAGTACCGTGAGGGAAAGTTG-3'/5'-TCGGAAGGAAC-CAGCTACTA-3') (De Ley *et al.*, 1999), respectively. The reaction system was prepared according to the instructions of KOD FX DNA polymerase (TOYOBO, Japan). The thermocycler conditions for amplification comprised initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing for 30 s at 58.2 °C for ITS and 51.7 °C for 28S, extension at 68 °C for 90 s, and a final extension at 72 °C for 10 min. The PCR products were purified using the Biospin Gel Extraction Kit (BioFlux, China) and ligated into pJET1.2/blunt cloning vectors (Thermo Scientific, USA) and transformed to *Escherichia coli* strain DH5 α , and then sequenced by Sangon Biotech Co. Ltd (Shanghai, PR China). The obtained ITS sequences and D2-D3 expansion region of 28S rRNA sequences in this study were submitted to GenBank database.

Sequencing analysis and phylogenetic relationships

The obtained ITS sequences and D2-D3 expansion region of 28S rRNA sequences were compared with other nematode species sequences available in the GenBank database using the BLAST homology search tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple alignments of the nematode rRNA-ITS and rRNA 28S D2-D3 sequence were performed using Clustal W in MEGA 7 (Tamura *et al.*, 2011). Phylogenetic analysis of the sequence dataset was conducted by Bayesian inference (BI) using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001). The best-fit model of nucleotide evolution was determined with MrModeltest 2.3 (Nylander, 2004) to be GTR+I+G, according to Akaike Information Criteria (AIC). Bayesian analysis was run with random starting trees, four Markov chains for 1×10^6 generations, with Markov chains sampled every 100 generations. Two runs were performed for each analysis. Outgroups were selected based on the previous reports (Subbotin *et al.*, 2008; Wang *et al.*, 2015). After discarding burn-in samples, the remaining samples were used to generate a 50 % majority rule consensus tree. Posterior probabilities (pp) were given on appropriate clades.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors.

Results

Morphology of the root-lesion nematodes

The morphology of the root-lesion nematodes SY-X population isolated from corn in this study was photographed (Fig. 1). The morphometric measurements of the SY-X population of *P. neglectus* (Table 1) were consistent with *P. neglectus* as described previously (Ryss, 1988; Pourjam *et al.*, 1999; Castillo & Vovlas, 2007). Female: The body vermiform, straight or slightly ventrally curved after heat-killing (Fig. 1A, M); Labial region bluntly rounded, with two annuli, anterior one distinctly narrower than second, apical one comprising the lips (Fig. 1C, D, E); Stylet is robust, with rounded knobs that vary little in shape and typically indented on anterior surfaces, stylet length 14.3 – 17.4 μm (Fig. 1C, D, E); Lateral field composed of four lines at midbody (Fig. 1G); Pharyngeal gland overlapping intestine ventrally or ventrolaterally (Fig. 1B); Excretory pore 69 – 85 μm from anterior end. Hemizonid just anterior to the excretory pore, extending over 2 – 3 body annules (Fig. 1B); Female monodelphic, prodelphic, ovary outstretched with oocytes in tandem (Fig. 1F); Post-vulval uterine sac equal to or less than body diameter, 13 – 21.7 μm long and normally undifferentiated (Fig. 1H); Tail variable in shape, usually conoid with little curvature

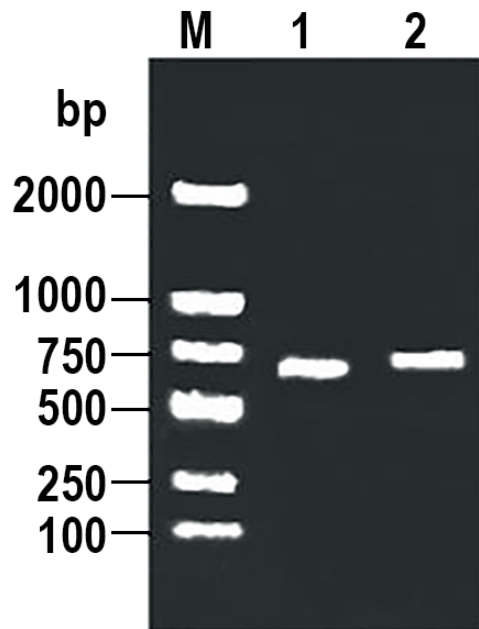
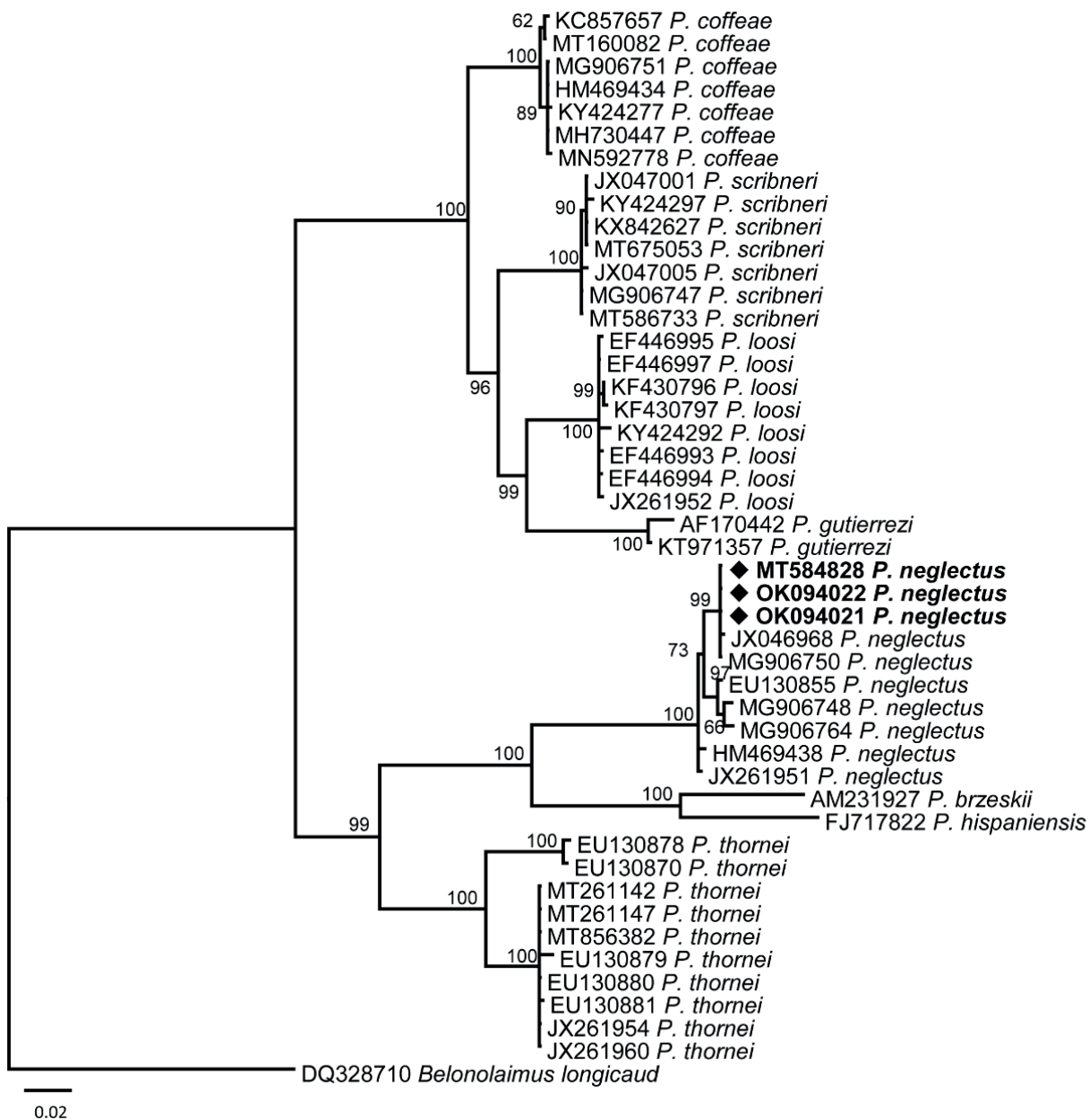


Fig. 2. PCR amplification of the rDNA-ITS and rDNA 28S D2-D3 expansion region of *Pratylenchus neglectus*. M: DL2000 Marker; 1: rDNA-ITS; 2: D2-D3 expansion region of the 28S rDNA.



0.02

Fig. 4. Bayesian tree of *Pratylenchus* as inferred from rDNA 28S D2-D3 sequences under GTR+I+G model. Posterior probabilities more than 50% are given for appropriate clades. Newly obtained sequence is indicated in bold font.

The phylogenetic tree generated from rRNA ITS is presented in Figure 3, which contained 44 ingroups and one outgroup taxon. This tree showed that the newly obtained sequence of *P. neglectus* in this study (MT584827, OK090917 and OK090916) is clearly different from other *Pratylenchus* species ITS sequences, and formed a 100 % supported clade with *P. neglectus*. The phylogenetic tree generated from 28S D2-D3 region in Figure 4, contained 46 ingroups and one outgroup taxon. This tree indicated that the newly obtained sequence of *P. neglectus* in this study (MT584828,

OK094022 and OK094021) is clearly different from other *Pratylenchus* species 28S of D2-D3 region sequences in the GenBank, and formed a highly supported clade with *P. neglectus* (100 %). These results further confirmed that the root-lesion nematodes obtained in this study were *P. neglectus*.

Discussion

Despite the agricultural importance of some *Pratylenchus* spe-

cies, information about these organisms of description is relatively scarce. In addition, identification of root-lesion nematodes must take into account countries and hosts, due to the presence of the intraspecific variation (Dobosz *et al.*, 2013). Therefore, some descriptions of *Pratylenchus* species from new host are also available information.

The taxonomy of the genus *Pratylenchus* is always difficult due to intraspecific variability in the diagnostic characters and the overlap of many measurements and characters among different species (Ryss, 2002). Loof (1978) pointed out that a few characteristics were reliable and useful for differentiating *Pratylenchus* species, such as stylet length, structure of lateral fields, position of the vulva, head shape and shape of tail. In this study, the morphological identification of root-lesion nematodes collected from the corn rhizosphere soils and roots in Henan province of China was carried out. The main morphological characteristics of SY-X population of *P. neglectus* were compared with data of Pourjam *et al.* (1999) and Ryss (1988). We found the c values of female nematodes was relatively the less than data of Pourjam *et al.* (1999) and Ryss (1988); L and b values of female nematodes were less than data of Ryss (1988); a value of female nematodes were slightly less than data of Pourjam *et al.* (1999). The reason for differences among different geographical isolates may be related to the small activity range and weak active dispersal ability of *P. neglectus*.

Nematode species difficult to distinguish morphologically from other species can be successfully identified using molecular identification. Hence, it is important to complement the specific identification with analysis of different molecular markers to reinforce the diagnosis (Lax *et al.*, 2021). And molecular identification is also useful in resolving the possible existence of species complexes. In our study, molecular identification found the ITS and 28S rRNA region sequences were the highest identity with one population of *P. neglectus* and our phylogenetic analysis showed that these obtained sequences clustered within the strongly supported *P. neglectus* clade.

Plant parasitic nematodes are important pathogens of corn, and it has been reported that many plant parasitic nematodes can infect corn in several provinces of China (Qiu *et al.*, 2017; Xu *et al.*, 2012). *P. neglectus* is one of the most important root-lesion nematodes. It can infect *Triticum aestivum*, *Zea mays*, *Glycine max*, *Helianthus annuus*, *Solanum tuberosum*, *Brassica oleracea*, *Lactuca sativa*, *Juglans regia*, *Cucumis sativus*, *Nicotiana tabacum*, *Gossypium sp.*, *Brassica napus*, *Arachis hypogaea*, *Fragaria ananassa* and other food and commercial crop in China (Liu & Liu, 2007; Yu *et al.*, 2017; Li *et al.*, 2019). Additionally, *P. neglectus* is widely distributed in the world and has been reported to infect corn both in China and abroad (Castillo & Vovlas, 2007). It has been recorded to infect corn in Iowa, USA (Williams, 1982) and several European countries (Tacconi *et al.*, 1988; Urek *et al.*, 2013). *P. neglectus* has only been reported on corn in Shandong province (Qiu *et al.*, 2016) and Tibet of China (Yu *et al.*, 2017), however, only morphological identification was performed in these two reports

and the relevant molecular data were lacking. Both morphological and molecular data confirmed the identity of *P. neglectus* on corn in Henan province of China in this study. As far as we know, this is the first report of *P. neglectus* on corn in Henan Province of China. It is also the first molecular data of 28S D2-D3 regions obtained from *P. neglectus* on corn in China. Detection of *P. neglectus* on corn is of great value for further study of their potential harm and control measures. Because the root-lesion nematode can cause great harm to corn, attention should be paid to prevent the spread of *P. neglectus* to other corn producing areas in China.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgments

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References

- CASTILLO, P., VOVLAS, N. (2007): *Pratylenchus* (Nematoda: Pratylenchus): *Diagnosis, biology, pathogenicity and management. Nematology monographs and perspectives, vol. 6.* Leiden, The Netherlands: Brill. DOI: 10.1163/ej.9789004155640.i-523
- DOBOSZ, R., WINISZEWSKA, G., MALEWSKI, T., RYBARCZYK-MYDŁOWSKA, K., TEREBA, A., KOWALEWSKA, K., GAWLAK, M., BOGDANOWICZ, W. (2013): Morphological and molecular features of *Punctodeira stonei* Brzeski, 1998 (Nematoda: Heteroderidae) – species associated with roots of grasses. *Ann Zoo*, 63: 157–162. DOI: 10.3161/000345413X669487
- DE, L.P., FELIX, M.A., FRISSE, L.M., STEVEN, A.N. (1999): Molecular and morphological characterization of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology*, 1: 591 – 612. DOI: 10.1163/156854199508559
- GOLDEN, A.M. (1990): Preparation and mounting nematodes for microscopic observation. In ZUCKERMAN B.M., MAI W.F. AND KRUSBERG L.R. (Eds) *Plant nematology laboratory manual University of Massachusetts Agricultural Experiment Station*, Amherst, MA, pp. 197 – 205
- HANDOO, Z.A., CARTA, L.K., SKANTAR, A.M., SUBBOTIN, S.A., FRAEDRICH, S.W. (2016): Molecular and morphological characterization of *Xiphinema chambersi* population from live Oak in Jekyll Island, Georgia, with comments on morphometric variation. *J. Nematol*, 48: 20 – 27. DOI: 10.21307/jofnem-2017-005
- HOOPER, D.J., HALLMANN, J., SUBBOTIN, S.A. (2005): Methods for ex-

- traction, processing and detection of plant and soil nematodes. 53 – 86. In LUC, M., SIKORA, R.A., BRIDGE J. (Eds) *Plant parasitic nematodes in subtropical and tropical agriculture. 2nd edn.* UK, CABI Publishing. DOI: 10.1079/9780851997278.0053
- HUELSENBECK, J.P., RONQUIST, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754 – 755
- HOOPER, D.J. (1970): Handling, fixing, staining, and mounting nematodes. In SOUTHEY, J.F. (Ed) *Laboratory methods for work with plant and soil nematodes 5th ed.*, Her Majesty's Stationery Office, London, pp. 39 – 54
- JANSSEN, T., KARSSSEN, G., ORLANDO, V., SUBBOTIN, S.A. BERT, W. (2017): Molecular characterization and species delimiting of plant parasitic nematodes of the genus *Pratylenchus* from the penetrans group (Nematoda: *Pratylenchidae*). *Mol. Phylogenet. Evol.*, 117: 30 – 48. DOI: 10.1016/j.ympev.2017.07.027
- JONES, J.T., HAEGEMAN, A., DANCHIN, E.G.J., GAUR, H.S., HELDER, J., JONES, M.G.K., KIKUCHI, T., MANZANILLA-LÓPEZ, R., PALOMARES, J.E., WESEMAEL, W.M.L., PERRY, R.N. (2013): Top ten plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathology*, 14: 946 – 961
- LAX, P., RONDAN, J.C., ANDRADE, A.J., DOUCET, M.E. (2021): *Punctodera achalensis* n. sp. (Nematoda: *Punctoderidae*), a new cyst nematode from Argentina. *Eur J Plant Pathol*, 161: 397 – 409. DOI:10.1007/s10658-021-02331-7
- LI, L., HE, J., LIU, L.L., GU, J.F., ZHANG, J.G. (2018): Identification of *Pratylenchus neglectus* from sugarbeet in Xinjiang. *Plant Quar*, 32: 20–23. DOI: 10.19662/j.cnki.issn1005-2755.2018.02.007
- LI, Y., WANG, S., DU, J., LU, Q.S., WANG, Z.Y., WANG, K., LI, H.L. (2018): First report of the root-lesion nematode (*Pratylenchus neglectus*) on wheat in Anhui province, China. *Plant Dis*, 102: 5663 – 5663
- LI, Y., WANG, S., LIU, Y.K., LU, Q.S., WANG, K., LI, H.L. (2019): Occurrence of soybean root rot caused by *Pratylenchus coffeae* in Henan province, China. *Plant Dis*, 103: 1435 – 1435.
- LIU, X.Y., LIU, W.Z. (2007): Species of nematodes of *Pratylenchidae* in Shandong province. *Journal of Qingdao Agricultural University*, 24(3): 175 – 176. DOI: 10.3969/j.issn.1674-148X.2007.03.006
- LIU, Q.S., HALLERMAN, E., PENG, Y.F., LI, Y.H., MASSIMO, M., FRANCESCO, B. (2016): Development of Bt rice and Bt maize in China and their efficacy in target pest control. *Int. J. Mol. Sci.*, 17: 1561 – 1576. DOI: 10.3390/ijms17101561
- LOOF, P.A.A. (1978) The genus *Pratylenchus* Filipjev, 1936 (Nematoda: *Pratylenchidae*): a review of its anatomy, morphology, distribution, systematics and identification. *Sveriges Landbruksuniversitet Växtskyddsrapporter*, 5, 1 – 12
- NYLANDER, J.A.A. (2004): *MrModeltest 2.3*. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden
- QIU, H.G., LI, X.H., YU, J.L. (2020): China maize industry: development trends and policy suggestions. *Issues in Agricultural Economy*, 7: 4 – 16. DOI: 10.13246/j.cnki.iae.2021.07.002
- QIU, Z.Q., MO, A.S., HE, Q., WU, H.Y., ZHOU, X.B. (2016): Root-lesion nematodes on maize in Shandong, China. *J. Gen. Plant. Pathology*, 82: 224 – 227. DOI: 10.1007/s10327-016-0660-0
- QIU, Z.Q., WU, H.Y., LONG, Y.R. (2017): Research progress in the occurrence and biology of *Heterodera zeae*. *Plant Protec*, 43: 16 – 20. DOI: 10.3969/j.issn.0529-1542.2017.04.003
- POURJAM, E., KHEIRI, A., GERAERT, E., ALIZADEH, A. (1999): Variations in Iranian population of *Pratylenchus neglectus* and *P. thornei* (Nematoda: *Pratylenchidae*). *Iran. J. Plant. Pathology*, 35: 67
- RYSS, A.Y. (1988): World fauna of the root parasitic nematodes of the family *Pratylenchidae* (*Tylenchida*). Leningrad, USSR, 367 pp
- RYSS, A.Y. (2002): Genus *Pratylenchus* Filipjev: multientry and monentry keys and diagnostic relationships (Nematoda: *Tylenchida: Pratylenchidae*). *Zoosyst Ross*, 10: 241 – 255
- SUBBOTIN, S.A., RAGSDALE, E.J., MULLENS, T., ROBERTS, P.A., MUNDO, M., 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. *Mol. Phylogenet. Evol.*, 48: 491 – 505. DOI: 10.1016/j.ympev.2008.04.028
- SUBBOTIN, S.A., STURHAN, D., CHIZHOV, V.N., VOVLAS, N., BALDWIN, J.G. (2006): Phylogenetic analysis of *Tylenchida* Thome, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology*, 8(3), 455 – 474. DOI: 10.1163/156854106778493420
- TACCONI, R., SANTI, R., POLA, R. (1988): *Pratylenchus neglectus* sugranturco (*Zea mays*) in Emilia Romagna. *Infor Fitopatologico*, 38: 59 – 61
- TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M., KUMAR, S. (2011): MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28(10): 2731 – 2739
- UREK, G., ŠIRCA, S., KARSSSEN, G. (2003): A review of plant-parasitic and soil nematodes in Slovenia. *Nematology*, 5: 391 – 403. DOI: 10.1163/156854103769224386
- WANG, H., ZHUO, K., LIAO, J.L. (2016): Morphological and molecular characterization of *Pratylenchus hippeastri*, a new record of root-lesion nematode associated with apple in China. *Pak. J. Zool*, 48: 665 – 671
- WANG, H.H., ZHUO, K., YE, W.M., LIAO, J.L. (2015): Morphological and molecular characterisation of *Pratylenchus parazeae* n. sp. (Nematoda: *Pratylenchidae*) parasitizing sugarcane in China. *Eur. J. Plant. Pathol*, 143: 173 – 191. DOI: 10.1007/s10658-015-0674-z
- WANG, J.L., ZHANG, J.C., GU, J.F. (2011): DNA extracted method on single nematode. *Plant Quar*, 25: 32 – 35. DOI: 10.19662/j.cnki.issn1005-2755.2011.02.009
- WANG, K., HAO, P.H., LIU, Y.K., XIA, Y.H., SUN, B.J., LI, H.L., LI, Y. (2021): Occurrence of *Pratylenchus coffeae* causing root rot of soybean in Shandong province of China. *Plant Dis*, 2020. DOI: 10.1094/PDIS-08-20-1740-PDN
- WILLIAMS, D.D. (1982): The known *Pratylenchidae* (Nematoda) of Iowa. *Iowa. J. Res*, 56: 419 – 424
- WINDHAM, G.L. (1998): Corn. In: BARKER, K.R., PEDERSON, G.A. &

- WINDHAM, G.L. (Eds) *Plant and nematode interactions*. Madison, WI, USA, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc, pp. 335 – 357
- XIA, Y.H., LIU, Y.K., HAO, P.H., ZHAO, L.Y., WANG, K., YUAN, H.X., LI, H.L., LI Y. (2021): Identification of pathogenic species of three root-lesion nematodes samples from corn fields in Henan Province. *Acta Phytopathol Sin*, 51: 536 – 548. DOI: 10.13926 /j. cnki. Apps.000541
- XU, Z.P., LI, H.X., LIU, Y.J., LI, W.H., ZHANG, S.L. (2020): First report of cyst nematode (*Heterodera elachista*) on zea mays in gansu province, China. *Plant Dis*, 105: 511 – 511
- YU, J., JIN, X.X., QIN, M., WU, W.J., XU, C.L., XIE, H. (2017): Identification and description of three species of genus *Pratylenchus* from crops in Tibet. *J Huazhong. Agri. Uni*, 36: 20 – 24. DOI: 10.13300/j. cnki.hnlkxb.2017.05.004