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Identification and pathogenicity of *Pratylenchus scribneri* on tomato in Sichuan Province of People's Republic of China

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Abstract

Root-lesion nematodes (*Pratylenchus* spp.) are a group of economically important pathogens that have caused serious economic losses in many crops. In 2019, root-lesion nematodes were recovered from tomato (*Solanum lycopersicum*) root samples collected from Sichuan Province, People's Republic of China (PRC). Extracted nematodes were disinfected, and one individual female was cultured on a carrot disc for propagation at 25 °C by parthenogenesis and designated the SC isolate. Afterwards, the isolate was identified on the basis of morphometric and molecular markers. Both morphometric characters and molecular analysis of the internal transcribed spacer region gene (ITS) of ribosomal DNA, the D2-D3 expansion region of the 28S rDNA gene and the mitochondrial cytochrome oxidase I (mtDNA-COI) gene revealed that the species of root-lesion nematode was *Pratylenchus scribneri*. The Bayesian tree inferred from the ITS rDNA, 28S rDNA and mtDNA-COI gene sequences also showed that this isolate formed a highly supported clade with other *P. scribneri* isolates. The pathogenicity of the root-lesion nematode SC isolate on tomato was assessed, showing that tomato was a suitable host for *P. scribneri*. To the best of our knowledge, this is the first report of *P. scribneri* on tomato in Sichuan Province, PRC. These are also the first molecular data obtained from *P. scribneri* on tomato in the PRC, and the pathogenicity of *P. scribneri* to tomato was studied for the first time. This study provides scientific data for the detection, identification and control of tomato root-lesion nematode disease.

Introduction

Tomato (*Solanum lycopersicum*) is the second most common vegetable after potato in global food production (Gondal *et al.*, 2012; Vignesh *et al.*, 2021), and the People's Republic of China (PCR) is the largest area of tomato cultivation in the world (Diao *et al.*, 2019). Sichuan Province, located in southwestern PCR, is a major production area for tomato cultivation. This important crop for the human diet is affected by many pathogens during its growth, which are responsible for high economic losses. Among them, plant-parasitic nematodes are one of the most important pathogens on tomato; for example, *Meloidogyne*, *Pratylenchus*, *Tylenchorhynchus* and *Helicotylenchus* can all infect tomato, causing severe damage and yield loss (Hou, 2001; Talwana *et al.*, 2016). Root-lesion nematodes of the genus *Pratylenchus* are the most economically damaging plant-parasitic nematodes on grains, fruits and vegetables (Jones *et al.*, 2013). To date, according to taxonomic studies, there are more than 100 valid species of root-lesion nematodes (Janssen *et al.*, 2017). *Pratylenchus* species are migratory endoparasites that can cause yield losses of up to 85% (Nicol et al., 2001), and yield losses are even higher when nematodes have synergistic interactions with certain soilborne plant pathogens (Jones & Fosu-Nyarko, 2014). *Pratylenchus scribneri* is one of the most important root-lesion nematodes and is a known economic pathogen that infects a variety of crops, including corn (*Zea mays* L.), soybean (*Glycine max* L.), barley (*Hordeum vulgare* L.), potato (*Solanum tuberosum* L.), sugarcane (*Saccharum officinarum* L.), tobacco (*Nicotiana tabacum* L.), tomato, strawberry (*Fragaria x ananassa Duch.)* and onion (*Allium cepa* L.) (Castillo & Vovlas, 2007; Li *et al.*, 2019).

Among the *Pratylenchus* species found to be associated with vegetables are *P. zeae*, *P. scribneri*, *P. neglectus*, *P. loosi* and *P. brachyurus* (Talwana *et al.*, 2016). *Pratylenchus brachyurus* and *P. delattrei* have also been reported on tomato in Cape Verde (Flis *et al.*, 2018).; Siddiqui *et al.* (1973) listed 22 host plants of *P. scribneri* in California, including tomato. It has also been reported that several species of *Pratylenchus* can infect tomato roots in the PCR, having obvious brown spots and serious rot in roots, causing great losses to tomato production. Based on the morphological characteristics, four species of *Pratylenchus* were identified in the rhizosphere soil of tomato in Henan Province: *P. coffeae*; *P. scribneri*; *P. fallax*; and *P. helophilus* (Li *et al.*, 1985). It has also been reported that *P. scribneri* was isolated from the

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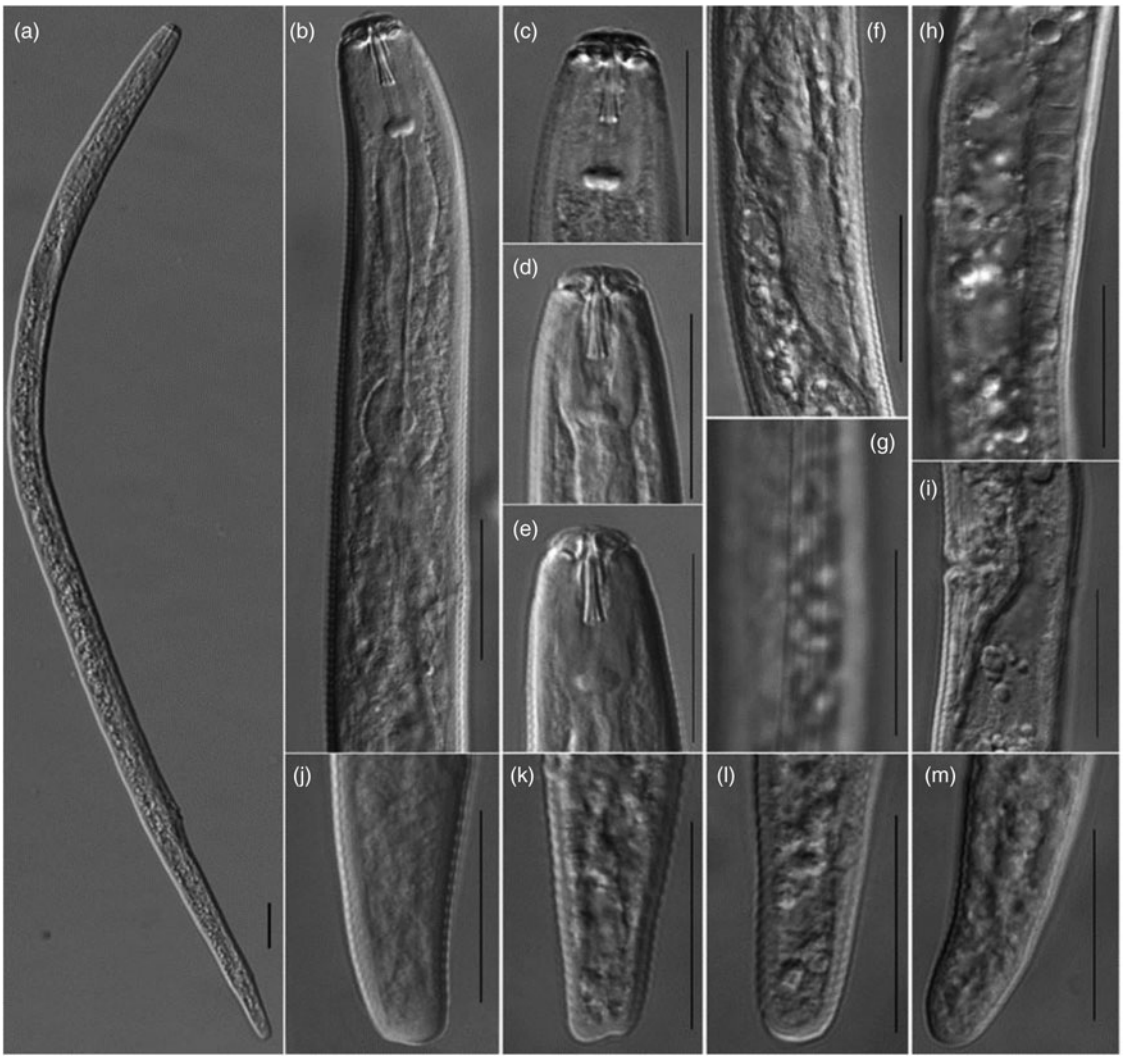

Fig. 1. Light micrographs of *Pratylenchus scribneri* from tomato in Sichuan Province, People's Republic of China. Females (a–m): (a) entire body; (b) anterior region; (c–e) lip region; (f) junction of genital gland and intestine; (g) lateral line; (h) anterior end of genital gland; (i) post-vulval region and ovary; and (j–m) tail region. Scale bars: 50 μm (a, n) and 20 μm (b–m).

rhizosphere of tomato in Shandong Province, PCR (Liu & Liu, 2007). However, the identification of most of the *Pratylenchus* species was only based on morphological characters and lacked molecular data, and further analysis of their pathogenicity to tomato has not been reported. In this study, a purified root-lesion nematode isolate from Sichuan Province was identified on the basis of morphological and molecular markers or characters, and the pathogenicity of this isolate on tomato was assessed in pot experiments. Both morphometric characters and molecular markers revealed that the species of root-lesion nematode from Sichuan Province was *P. scribneri,* and it has strong pathogenicity to tomato. To the best of our knowledge, this is the first report of *P. scribneri* on tomato in Sichuan Province, PCR, using morphological and molecular characters. These are also the first molecular data obtained from *P. scribneri* on tomato in the PCR, and the pathogenicity of *P. scribneri* to tomato was studied for the first time. The purpose of this study was to understand the pathogen species of root-lesion nematodes on tomato in the PCR, which provides a scientific basis for the detection and control of tomato root-lesion nematode diseases.

Materials and methods

Nematode isolate sampling and culturing

Five soil samples were collected from the rhizosphere of tomatoes (cv. Maohong 801), which had weak growth in a field near Shizishu village in Jingtang County of Chengdu city, Sichuan Province, PCR. Root-lesion nematodes were extracted using the modified Baermann funnel method (Hooper *et al.*, 2005). To obtain purified isolates, individual females were isolated, sterilized with 0.3% streptomycin sulphate and transferred to carrot discs, prepared according to the methods described by Reise *et al.* (1987) and Kaplan & Davis (1990) and maintained at 25 °C in the dark (Li *et al.*, 2019; Wang *et al.*, 2021). The selected purified root-lesion nematode SC isolate was used for subsequent morphological and molecular identification.

Morphometric identification

Nematodes were heat-killed and fixed in FG solution (formalin:glycerin:water = 10:1:89) (Xie, 2005). The fixed specimens were

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Table 1. Morphometrics of females of *Pratylenchus scribneri*.

Character	SC isolate (female)	Roman & Hirschmann (1969)
n	16	50
L	501.5 ± 16.0(465.6–530.3)	504.4 ± 23.0(436.8–553.2)
a	24.7 ± 2.4(21.3–29.0)	26.3 ± 1.6(21.4–29.0)
b	5.5 ± 0.5(5.0–6.2)	6.3 ± 0.3(5.7–7.0)
b’	4.0 ± 0.3(3.7–4.7)	
c	17.7 ± 1.1(16.0–19.5)	18.4 ± 0.8(16.9–20.0)
c’	2.4 ± 0.1(2.2–2.6)	
V	78.2 ± 0.9(76.8–80.0)	77.4 ± 1.2(75.0–82.0)
stylet length	15.6 ± 0.2(15.1–16.0)	15.7 ± 0.4(14.4–16.8)
stylet shaft	7.3 ± 0.2(7.0–7.7)	—
stylet knob width	4.2 ± 0.4(3.8–4.9)	4.5 ± 0.3(4.2–4.8)
stylet knob height	2.5 ± 0.3(1.9–3.1)	2.4 ± 0.1(2.4–3.0)
DGO from stylet base	2.7 ± 0.2(2.5–3.3)	2.2 ± 0.3(1.8–2.4)
anterior end to centre of metacorpus	53.8 ± 2.3(49.7–58.2)	—
end of pharyngeal gland lobe	125.0 ± 8.8(109.6–138.3)	—
anterior end to excretory pore	82.7 ± 1.9(79.2–86.2)	80.9 ± 3.1(74.0–86.0)
pharyngeal overlap	32.9 ± 6.1(23.2–43.9)	—
maximum body diameter	20.5 ± 2.1 (16.5–23.7)	19.3 ± 1.5(16.8–23.4)
vulval body diameter	17.6 ± 1.1(16.0–19.5)	—
anal body diameter	11.8 ± 0.9(10.4–13.2)	—
Tail length	28.4 ± 1.7(26.4–32.4)	27.3 ± 1.6(24.0–30.6)
number of tail annuli	23.5 ± 1.4(21–25)	—
Vulva to annus distance	80.9 ± 5.7(72.1–91.3)	84.2 ± 7.6(77.6–100.4)
post-uterine sac length	21.1 ± 2.2(18.3–26.2)	—
Lateral field width	6.2 ± 0.7(5.1–7.3)	—
Lip width	8.1 ± 0.3(7.4–8.7)	7.8 ± 0.1(7.2–7.8)
Lip height	2.5 ± 0.3(2.2–3.2)	2.3 ± 0.2(1.8–2.4)

Notes: all measurements are in μm and in the form of mean ± standard deviation (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 oesophageal gland and intestine; *b*’: body length/length from the lips to oesophageal gland end; *c*: body length/tail length; *c*’: tail length/tail diameter at anus; *V*: distance of vulva from the lips × 100/body length; and DGO: distance between dorsal oesophageal gland opening and stylet knobs.

transferred to anhydrous glycerine according to the methods described by Seinhorst (1959) and mounted on permanent slides. Measurements and light photomicrographs of nematodes were performed using a Nikon Eclipse Ti-S inverted microscope (Japan). Images of key morphological features were processed by Photoshop CS5 software. The De Man formula was used to calculate the measurements. All measurements are in micrometres unless otherwise stated.

Molecular characterization and phylogenetic relationships

The DNA of one individual nematode was extracted using liquid nitrogen, followed by proteinase K (Wang *et al.*, 2011). The internal transcribed spacer region (ITS) rDNA gene was amplified using primers TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Subbotin et al., 2006). The D2-D3 region of the 28S rDNA gene was amplified using primers D2A (5′-ACAAGTACCGTGAGGG AAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (De Ley *et al.*, 1999). The mitochondrial cytochrome oxidase I (mtDNA-COI) gene was amplified using primers JB3 (5’-TTTTTTGGGCATCCTGAGGTTTAT-3’) and JB5 (5’-AGC ACCTAAACTTAAAACATAATGAAAATG-3’) (Liu *et al.*, 2018). Polymerase chain reaction (PCR) amplifications were carried out in 25 μL of reaction mixture with the following components: 1 × KOD FX PCR buffer’ 0.4 mM of each dNTP; 0.3 μM of each primer; 3 μL of DNA template; and 0.5 units of KOD FX (Toyobo, Japan). The reaction protocol was as follows: predenaturation at 94 °C for 2 min, followed by 35 cycles (denaturation at 98 °C for 10 s, annealing at 58.2 °C (ITS rDNA) or 51.7 °C (28S rDNA) or 52 °C (mtDNA-COI) for 30 s, extension at 68 °C for 90 s) and final extension at 72 °C for 10 min (Wang *et al.*, 2016). The PCR products were purified using the Biospin Gel Extraction Kit (BioFlux, PCR), cloned into pJET 1.2/blunt cloning vectors (Thermo Scientific, USA) and then sequenced by Sangon Biotech Co., Ltd. (Shanghai, PCR). The newly obtained sequences were deposited in the GenBank database (NCBI).

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The *P. scribneri* sequences were aligned with other *Pratylenchus* species published in GenBank using the nucleotide BLAST program in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). *Nacobbus aberrans*, *Tylenchorhynchus dubius* and *Meloidogyne incognita* were chosen as the outgroup taxa according to the results published by Nguyen *et al.* (2017) and Handoo *et al.* (2020). Multiple alignments of the sequences were performed using Clustal W in MEGA 7 (Tamura *et al.*, 2011). Phylogenetic relationships were established with Bayesian inference (BI) using MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001). The best-fit model (GTR + I + G) was selected by the Akaike information criterion using MrModeltest 2.3 (Nylander, 2004). BI analysis was initiated with a random starting tree and was run with four Markov chains for 1,000,000 generations. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples, the remaining samples were retained to generate a 50% majority rule consensus tree. Posterior probabilities were given on appropriate clades.

Pathogenicity assays

Seeds of tomato (cv. Maohong 801) were purchased from Qiule, Zhengzhou, PRC, placed in 0.75% sodium hypochlorite solution for disinfection for 30 min, washed with sterile water and sown in pots with sterilized sand soil. Thirty days after sowing, five seedlings with the same growth status (one/pot) were selected for the pathogenicity assays. These studies were performed with the *P. scribneri* SC isolate extracted from carrot discs and adjusted to a suspension with 500 nematodes/ml. A 2 mL suspension containing 1000 nematodes was then pipetted into three small holes 5 cm deep. For the blank controls, 2 mL of sterile water was pipetted into holes. To permit undisturbed root invasion by *P. scribneri*, the seedlings were not watered during the first three days after inoculation (Hahn *et al.*, 2010). Seventy-five days after inoculation, plant growth parameters, the number of nematodes, and the reproduction factor (Rf) were assessed. The symptoms of root-lesion nematode infection on tomato roots were also photographed. Tomato plants were removed from the pots and nematodes were extracted from soil and roots using the modified Baermann funnel method. The total number of nematodes (Pf) was the sum of nematodes isolated from soil and roots, and then the Rf was calculated. There were five replications for the inoculation period, and the experiment was performed twice. According to the method described by Byrd *et al.* (1983), the nematodes in the roots were stained and then detected under a microscope.

Results

Morphometric identification

The morphological characters analysed in females were as follows: entire body; anterior region; lip region; junction of genital gland and intestine; lateral line; anterior end of genital gland; post-vulval region and ovary, and tail region (fig. 1). The morphometric measurements of the SC isolate of *P. scribneri* (table 1) were consistent with *P. scribneri* as described previously (Roman & Hirschmann, 1969).

Female: body stout, straight or slightly bent ventrally after heat relaxation; labial region slightly offset from body, composed of two annuli of approximately the same height, anterior one distinctly narrower than second and inner part of lateral lips narrower than outer part; stylet robust, with rounded knobs that vary little in shape, stylet length 15.1–16.0 µm; orifice of dorsal pharyngeal gland approximately 2.5–3.3 µm posterior to stylet base; lateral fields with four longitudinal lines, occasionally a fifth line may be present at mid-body; excretory pore immediately posterior to hemizonid; pharyngeal gland overlapping intestine ventrally or ventrolaterally; anterior genital branch consisting of ovary with oocytes in single file except for a short zone near anterior end; short oviduct; empty spermatheca; post-vulval uterine sac generally 18.3–26.3 µm body diameter long; vulva–anus distance generally 2.2–3.5 times tail length; and tail tapering slightly, terminus mostly broadly rounded, varying from somewhat narrower to almost truncate, usually with 21–25 annuli.

Male: not found.

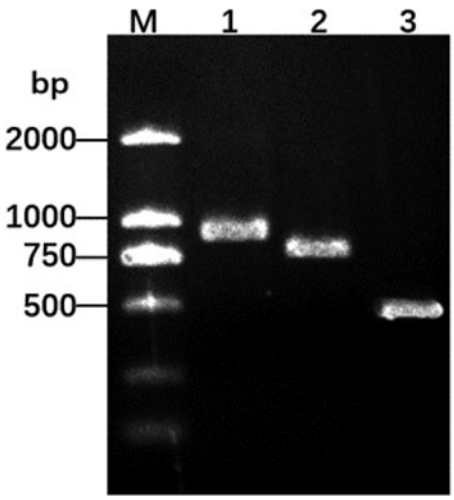

Fig. 2. Polymerase chain reaction amplification of the internal transcribed spacer region (ITS) rDNA gene, the D2-D3 region of the 28S rDNA gene and the mtDNA-COI gene of *Pratylenchus scribneri*. M: DL2000 marker; 1: ITS rDNA; 2: D2-D3 region of 28S rDNA; and 3: mtDNA-COI.

Molecular characterization and phylogenetic relationships

Primers TW81/AB28, D2A/D3B and JB3/JB5 were used to amplify the rDNA-ITS gene, the D2-D3 region of the 28S rDNA gene and the mtDNA-COI gene of root-lesion nematode SC isolates, respectively (fig. 2). The obtained ITS rDNA, D2-D3 region of 28S rDNA and mtDNA-COI sequences were submitted to GenBank for a BLAST search. The obtained ITS rDNA sequences (GenBank Accession Numbers MZ203866, OK021634, OK021635 and OK021636) showed 99%–100% identity with *P. scribneri* sequences available in GenBank; the D2-D3 region of the 28S rDNA sequences (MZ215788, OK021637, OK030711 and OK030712) shared more than 99% identity with several *P. scribneri* sequences available in GenBank; and the mtDNA-COI sequences (MZ203870, OK021638, OK021639 and OK030210) shared more than 99% identity with several *P. scribneri* sequences available in GenBank.

The phylogenetic tree based on the ITS rDNA gene (fig. 3), which contained 58 ingroups and one outgroup taxon, revealed that the newly obtained sequence of the SC isolate is clearly different from other *Pratylenchus* species and forms a 100% supported clade with *P. scribneri*. The phylogenetic tree based on the D2-D3 region of the 28S rDNA gene contained 51 ingroups and one outgroup taxon, showing that the newly obtained

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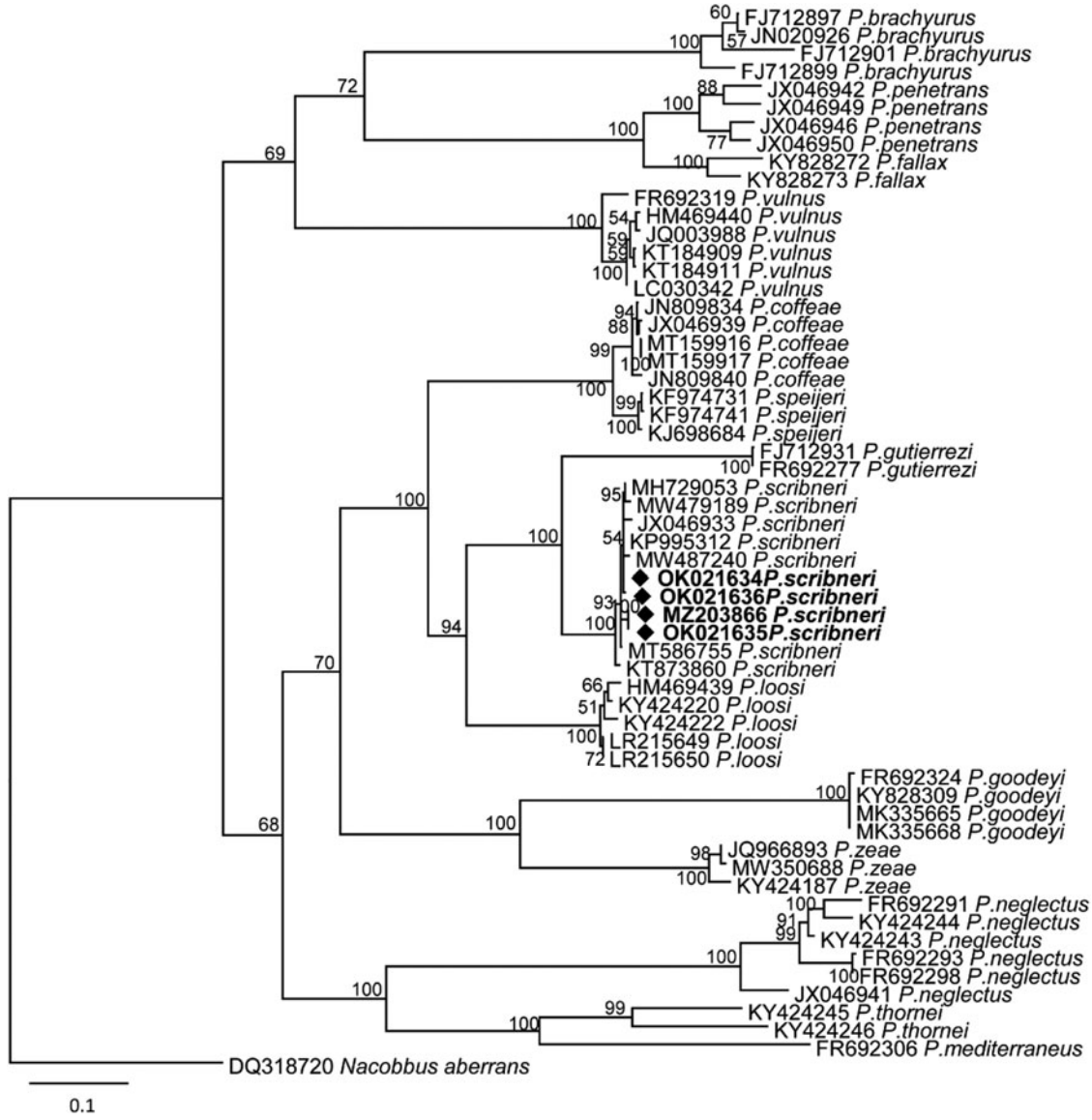

Fig. 3. Bayesian tree of *Pratylenchus* as inferred from internal transcribed spacer region gene rDNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 50% are given for appropriate clades. Newly obtained sequence is indicated in boldface type.

sequence of this isolate is clearly different from other *Pratylenchus* species and formed a highly supported clade with *P. scribneri* (100%) (fig. 4). The Bayesian phylogenetic tree generated from the mtDNA-COI region dataset contained 46 ingroups and one outgroup taxon, showing that the newly obtained sequence of the SC isolate is clearly different from other *Pratylenchus* species and formed a highly supported clade with *P. scribneri* (fig. 5). These results confirmed that the sequence of this root-lesion nematode isolate formed a highly supported clade with all the reported *P. scribneri* sequences.

Pathogenicity assays

Seventy-five days after nematode inoculation, obvious disease symptoms were observed, including reduced plant growth and height and chlorotic leaves, compared with the uninoculated control plants. The plant height, fresh shoot weight and fresh root weight were 43.50 cm, 28.27 g and 4.44 g, respectively, which were significantly lower than those in uninoculated control plants ($P < 0.05$) (table 2). Due to the infection and damage of *P. scribneri*, the roots of tomato plants decreased significantly and showed distinct brown lesions (fig. 6a). At the initial stage of nematode infection, tomato roots showed small and light brown spots (fig. 6b) that became larger and brown or dark brown (fig. 6c, d), eventually leading to the whole roots appearing in a large area of necrosis and rot (fig. 6e). A large number of root-lesion nematodes were extracted from the rhizosphere soil and roots of the tomato plants. The average number of *P. scribneri* nematodes extracted from each inoculated plant was 4293. Also, the Rf of *P. scribneri* in tomato soil and roots reached 4.29 (table 2). According to the measuring standards of Goo & Sipes (1997), tomato is a suitable host plant for the *P. scribneri* SC isolate. Staining results showed a large number of *P. scribneri* nematodes and eggs in the tomato root tissues, which indicated that *P. scribneri* can propagate and complete its life cycle in tomato roots (fig. 6f–i).

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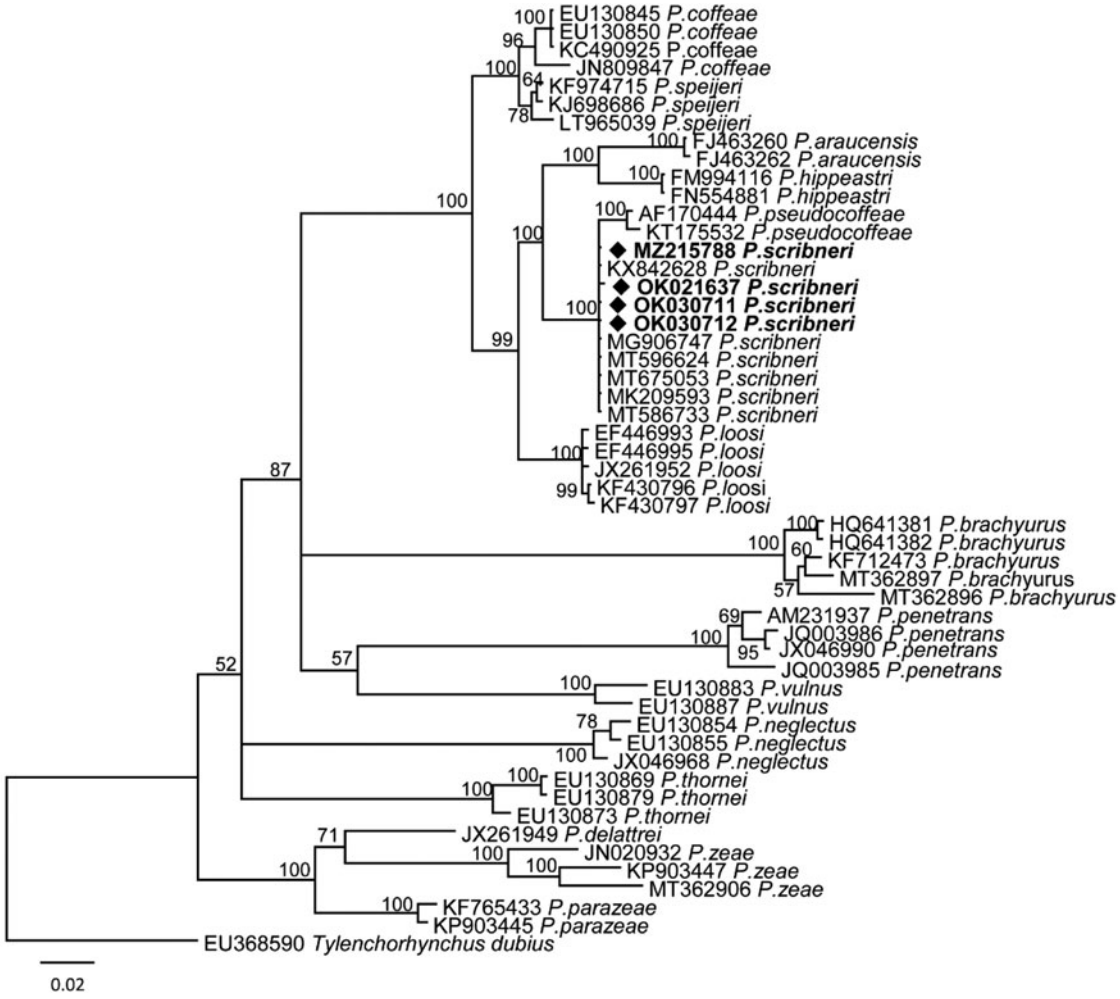

Fig. 4. Bayesian tree of *Pratylenchus* as inferred from the D2-D3 region of 28S rDNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 50% are given for appropriate clades. Newly obtained sequence is indicated in boldface type.

Discussion

Most vegetable crops may be attacked by one or more species of *Pratylenchus*, and root-lesion nematodes of the genus *Pratylenchus* have been reported to parasitize tomato plants at home and abroad. The accurate identification of *Pratylenchus* species is crucial for applying appropriate control measures. The taxonomy of the genus *Pratylenchus* is always difficult because of the relatively large and significant intraspecific variability in the diagnostic characters and the similarity of the diagnostic characters among different species (Ryss, 2002). Loof (1978) pointed out that a few characteristics were reliable and useful for the identification of species of *Pratylenchus*, such as structure of lateral fields, stylet length, lip annuli, shape of labial region, position of vulva, presence and shape of spermatheca, length of overlapping gland lobe, shape of female tail and terminus and presence or absence of males, and length of the post vulval uterine sac. Although morphology is still used for species identification of this genus, new technologies based on biochemical and molecular analyses are becoming increasingly important in nematode taxonomy and practical identification (Andrés *et al.*, 2000; De Luca *et al.*, 2004). However, these new molecular approaches should still be integrated with morphological data. In this study, a combination of morphometric characters and molecular markers was used to identify the species of the purified root-lesion nematode SC isolate. The results revealed that the species of this root-lesion nematode extracted from the rhizosphere of tomato in Chengdu City of Sichuan Province was *P. scribneri*. The Bayesian tree showed that the phylogenetic analysis of the ITS rDNA gene, D2-D3 region of the 28S rDNA gene and mtDNA-COI gene were consistent, which clearly separated *P. scribneri* from other *Pratylenchus* species. This is the first report of *P. scribneri* on tomato in Sichuan Province, PCR, using morphological and molecular data. It is also the first molecular data obtained from *P. scribneri* on tomato in the PCR.

Pathogenicity is usually used in plant pathology to indicate the capacity of an organism to induce disease or the amount of physiological damage caused to the host plant by the presence of a pathogen (Shaner *et al.*, 1992). The most obvious aboveground symptoms of root-lesion nematode disease on plants are stunting and chlorotic (yellowish) colouring, which give the field a ragged appearance. The symptoms of root-lesion nematode damage to plant roots are similar to those of other soil-borne diseases, nutrient deficiencies, insect damage and environmentally induced stress. Therefore, the damage caused by *Pratylenchus* species in agriculture is frequently neglected. In this study, we

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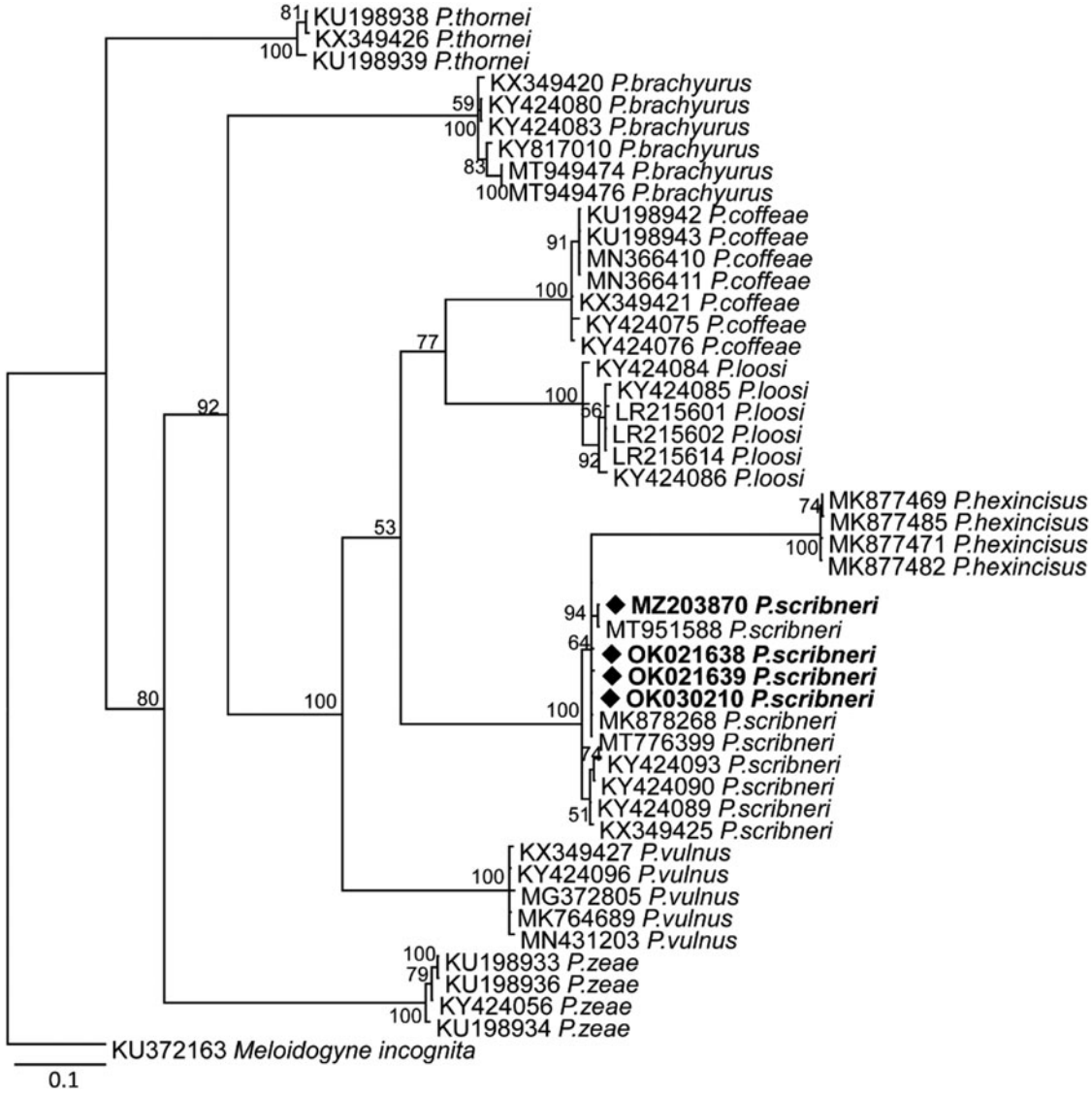

Fig. 5. Bayesian tree of *Pratylenchus* as inferred from mtDNA-COI gene sequences under the GTR + I + G model. Posterior probabilities greater than 50% are given for appropriate clades. Newly obtained sequence is indicated in boldface type.

Table 2. Effects of *Pratylenchus scribneri* SC-1 isolate on tomato growth, final population density (Pf) 75 days after inoculation and reproduction factor (Rf).

Treatment	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Pf	Rf
inoculated	43.50 ± 1.07*	28.27 ± 1.52*	4.44 ± 0.47*	4293 ± 334.31	4.29 ± 0.33
Noninoculated (control)	53.50 ± 1.15	34.03 ± 0.63	6.75 ± 0.41	0.0	0.0

Notes: values represent the mean of five replicates ± standard error; * indicates significant differences between two treatments based on *t*-test ($P < 0.01$). Reproduction factor (Rf) = final population density (Pf)/initial population density (Pi)

demonstrated that the slow decline of tomato caused by *P. scribneri* is a disease that fulfils Koch's postulate. The pathogenicity of *P. scribneri* to tomato was confirmed via pot experiments in the greenhouse, and the microscopic detection of tomato roots inoculated with *P. scribneri* confirmed the presence of these nematodes. Rebois (1986) reported the ectoparasitic feeding behaviour of *P. scribneri* on root hairs of corn, tomato and soybean. However, the endoparasitic feeding of *P. scribneri* on tomato roots was also demonstrated, and the entire body of the nematode and eggs were observed in the inoculated roots, which indicated that *P. scribneri* could propagate and complete its life cycle in root cells. The present study also showed that the *P. scribneri* SC isolate collected from Sichuan Province has a strong pathogenicity to tomato. These results provide an effective theoretical basis for the diagnosis and control of tomato root-lesion nematode disease. In addition, root-lesion nematodes are poikilothermic organisms, and consequently, temperature influences the rates of physiological processes, such as movement, growth and reproduction,

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Fig. 6. Symptoms of tomato roots infected by *Pratylenchus scribneri*. (a) CK: healthy plant with a good root system; 1: tomato roots infected by *P. scribneri* 75 days after inoculation' (b) early symptoms of small and light brown spots infected by *P. scribneri*; (c) expanding lesions on tomato roots; (d) deep brown spots on roots seriously infected by *P. scribneri*; (e) severely rotted root system caused by *P. scribneri*; (f–i) photomicrograph of *P. scribneri* within tomato roots; (f) healthy roots; (g, h), a large number of *P. scribneri* and eggs in the infected tomato roots; and (i) *P. scribneri* in tomato root cells. Scale bars: 50 μm (A, B, C) and 25 μm (D). n = nematode; e = egg; and s = stylet.

expression of nematode damage to plants and sex determination (Freckman & Caswell, 1985). Therefore, whether growing *Pratylenchus*-infected tomato plants under different temperature conditions will affect the pathogenicity of *P. scribneri* to tomato requires further investigation.

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Conflict of interest. None.

Ethical standards. All procedures contributing to this study comply with the ethical standards of the relevant national and institutional guides on the care and use of animals.

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