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Morphological and Molecular Characterization of *Trichotylenchus dispersus* (Nematoda: Dolichodoridae), a Newly Recorded Plant-Parasitic Nematode in the Rhizosphere Soil of Tomato Plants in Thailand

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Abstract

Trichotylenchus sp. is a migratory ectoparasitic nematode (PPN) that causes hypoplasia disease symptoms in economic plants, such as maize and banana. In this study, soil samples were collected from 6 locations in a tomato field in Khon Kaen Province, Thailand, for the purpose of nematode extraction using the Cobb's sieving and flotation-centrifugation techniques. Morphological and molecular characterization of the collected PPN identified it as *Trichotylenchus* sp., a PPN not previously found in Thai tomato fields. Morphologically, the nematode has a C-shaped body, a rounded lip region, robust stylet and dorsal gland orifice located 2.0 ± 0.5 µm from the base of basal knobs. The tail is conically shaped, and the cuticle exhibits annulations with 3 incisures in the lateral field. The morphological characteristics observed closely matched *Trichotylenchus* sp. To confirm the identity, diagnosis was done using Polymerase Chain Reaction (PCR) molecular technique. Nematode DNA amplification was conducted with different target genes using primer sets AB28/TW81 for ITS1-5.8S-ITS2 and D2A/D3B for D2-D3 region of the 28S rRNA. The PCR amplification showed DNA fragments of approximately 950 and 800 bp, respectively. The sequences obtained were compared with those in the NCBI GenBank, which showed a 98.0 - 99.4 % identity match with *Trichotylenchus dispersus* specimens previously documented in China. In addition, the phylogenetic trees reiterated a nematode grouping with *T. dispersus*, 100 % bootstrap value. To the best of our knowledge, this is the first report of the presence of *T. dispersus* in the soils of tomato field in Thailand. This nematode is likely to become a major factor limiting tomato production yields if not properly managed.

Keywords: Morphometrics, Molecular characterization, Phylogenetic trees, Plant-parasitic nematodes

Introduction

Tomato is globally known as one of the most widely consumed and cultivated vegetable crops. It is highly popular among consumers as both a vegetable and a fruit, serving as a crucial source of essential vitamins and minerals [1]. In Thailand, it is cultivated for fresh consumption and for use as raw material in food processing industry. In 2023, 15,764 acres of planted tomato crops yielding 139,760 tons was recorded [2]. The major cultivated areas are in northern and northeastern regions, especially in Khon Kaen Province, which accounts for more than 18 % of the total area under production [2]. Tomato has been widely used as a plant model to study plant-microorganism interactions due to its susceptibility to various plant pathogens, namely fungi (*Alternaria solani*, *Colletotrichum* sp. and *Septoria lycopersici*), bacteria (*Ralstonia solanacearum* and *Xanthomonas perforans*), viruses (*Tomato yellow leaf curl virus* and *Tobacco mosaic virus*), viroids (*Tomato apical stunt viroid* and *Tomato planta macho viroid*) and different species of nematodes (*Meloidogyne* spp.) [3]. Of these disease-causing agents, plant-parasitic nematodes (PPNs) are considered one of the most significant biotic factors in tomato fields, causing 25 to 100 % yield losses in many countries [4]. The dynamics of reductions caused by PPNs vary, depending on environmental factors, cropping systems and pest management practices [5]. The presence of high populations of nematodes infecting tomato causes plants to suffer different levels of damage and show varied symptoms, depending on the type and species of nematodes. Generally, PPNs cause root necrosis and the development of root galls which results in nutrient deficiency symptoms and secondary infections evidenced by stunted growth and leaf yellowing [6,7]. Currently, several PPNs have been identified and associated with damage to tomato cultivation, including root-knot nematodes (*Meloidogyne chiwoodi*, *M. incognita*, *M. javanica*, *M. enterolobii*, *M. ethiopica*, *M. exigua*, *M. fallax*, *M. floridensis*, *M. graminicola*, *M. luci* and *M. mali*), the false root-knot nematode (*Nacobbus aberrans*), the stem and bulb nematode (*Ditylenchus dipsaci*) and the rice nematode (*Hirschmanniella oryzae*) [8].

In Thailand, several PPNs have been recorded in economic plants: Some of the species are *Hirschmanniella mucronata*, *Helicotylenchus* sp., *Tylenchorhynchus* sp., *M. graminicola* in rice fields [9,10]; *Helicotylenchus crenacauda*, *H. dihystera*, *H. retusus*, *Hoplolaimus seinhorsti*, *M. incognita*, *M. javanica*, *Paratrichodorus christiei*, *Pratylenchus brachyurus*, *P. penetrans*, *Tylenchorhynchus clavicaudatus*, *T. crassicaudatus* and *T. martini* in soybean [11]; *Rotylenchulus reniformis* and *Helicotylenchus dihystera* [12], *M. graminicola* in shallot fields [13]; and *M. enterolobii* in chili, rain tree and mulberry [14-16]. Several of the aforementioned nematode genera have been listed among the top 10 most damaging PPNs, according to Jones *et al.* [17]. In Thai tomato fields, previous studies have mainly focused on managing root-knot nematodes, such as *M. incognita* and *M. javanica*, using biological control agents [18,19]. However, there is no recently published information regarding nematode surveys or the identification of nematodes (besides root-knot nematodes) conducted in the region. Information derived from nematode surveys is crucial for assessing the potential risk of yield losses due to these PPNs. It also provides essential data for researchers and the agronomic community to design effective integrated management programs [20].

From the present surveys conducted in tomato field in Thailand, several PPNs were recorded. Notably, a new PPN genus, *Trichotylenchus* sp., has been documented from this study. According to the

literature, this nematode can cause severe damage to economically important plants, such as stunting and thinning in maize [21] and hypoplasia in banana [22]. Nevertheless, information regarding the characteristics of these nematodes had not been documented previously in Thailand. With this view, this study was conducted to characterize *Trichotylenchus* species based on a combination of morphological and morphometric features along with molecular characterization, using nucleotide sequence comparisons and phylogenetic relationships (based on ITS1-5.8S-ITS2 and D2D3 genes) with other related taxa.

Materials and methods

Soil sampling and nematode processing

Fresh soil samples were collected in 2023 from 6 rhizospheric regions of tomato plants from Mueang District, Khon Kaen Province, Thailand. Each sample (300 g) was collected at the depth of 5 - 20 cm below the soil surface. The collected soil was placed into a plastic bag, and transported to laboratory for nematode extraction. Nematodes were extracted from 100 g of sub-soil samples using the modified Cobb's sieving [23,57] and flotation-centrifugation methods [24]. The nematode populations obtained from each sample were observed and counted under an inverted microscope (Olympus CKX53, Japan).

Morphological identification

For morphological examination, the collected *Trichotylenchus* specimens were killed using hot water (50 °C) and fixed by FA 4:1 fixative solution (40 % formaldehyde, 1 % glacial acetic acid and 89 % distilled water). After 48 h of nematode fixation, the specimens were further processed in anhydrous glycerol and glycerol-ethanol, following the method outlined by Seinhorst [25]. The fixed nematode specimens were permanently mounted in a droplet of anhydrous glycerol on the glass microscope slide [21,26], photographed under compound light microscope (Olympus CX23, Japan) using EP view program version 510, and subsequently merged with Helicon Focus program version 8.2.2. The nematode measurements (morphometric studies) were conducted using the AxioVision program version LE Rel 4.1. Morphometric values were then calculated using the De Man formula [27] as follows: a = body length/greatest body diameter, b = body length/distance from anterior to esophago-intestinal valve, c = body length/tail length, c' = tail length/tail diameter at anus and other parameters such as distance from anterior to esophago-intestinal valve, tail diameter at anus, body width, stylet length, stylet shaft length, stylet knob width, stylet knob height, median bulb width, median bulb length, dorsal pharyngeal gland orifice (DGO), lip width, lip height and tail length [27].

Molecular identification

DNA extraction was performed using the method outlined by Holterman *et al*. [28]; Beesa *et al*. [29]. In brief, 20 µL of worm lysis buffer (1 mL of the mixture included 176 µL of 1M NaCl [A&D Technology, Japan], 176 µL of 1M Tris-HCl (pH 8) [A&D Technology, Japan], 508 µL of ddH_2O [Invitrogen, USA], 100 µL of DTT [Merck, Canada], and 40 µL of 20 mg/mL Proteinase K [Worthington Biochemical, USA]) which was added to a 0.2 mL PCR tube containing 20 µL of distilled water with 1 nematode. Then, the

mixture was incubated, first at 65 °C for 2 h and then at 99 °C for 7 min in a PCR thermocycler (MiniAmp plus Thermal cycler, USA). The extracted DNA was stored at −20 °C until used as a DNA template.

Polymerase chain reaction (PCR) analysis was conducted using 2 universal primer sets: TW81/AB28 (5'GTTTCCGTAGGTGAACCTGC-3'/5' - ATATGCTTAAGTTCAGCGGGT-3') targeting the internal transcribed spacer 1 (ITS1)-5.8S-ITS2 gene [30] and D2A/D3B (5'-ACAAGTACCGTGAGGGAAAGTTG-3'/5'-TCGGAAGGAACCAGCTACTA-3') targeting the D2-D3 expansion of 28S rRNA [31]. The 15 µL PCR reaction included 2 µL of DNA template, 4 µL of sterilized distilled water, 0.75 mL each of forward and reverse primers and 7.5 µL of 2X PCR master mix (Wizbio solutions, Korea).

The PCR condition was processed as follows: Initial denaturation at 95 °C for 5 min; followed by 40 cycles of denaturation at 95 °C for 1 min; annealed at 55 °C (TW81/AB28) or 56 °C (D2A/D3B) for 1 min; with an extension at 72 °C for 1 min and final extension at 72 °C for 7 min. Subsequently, PCR products were screened on a 1.5 % agarose gel in 1X TAE buffer with fluorescent staining (Prime juice; Biohelix, Taiwan). The DNA size was compared with a 100 bp DNA ladder (Biohelix, Taiwan). The gel was screened in the electric field of 100 V for 25 min and visualized using a Blue light transilluminator (Blue pad Dual LED Blue, Taiwan). The PCR products were purified and sent for sequencing by Solgen Inc., South Korea. The 5 nucleotide sequences of each sample derived from ITS1-5.8S-ITS2 refer to regions in the ribosomal RNA (rRNA) gene cluster and D2-D3 expansion segments of 28S rRNA amplicon which were assembled using the CAP contig assembly program in the BioEdit version 7.0.5.3. Then, DNA sequences were compared with other PPN species available at the GenBank sequence database of the National Center for Biotechnology Information (NCBI), available online at https://www.ncbi.nlm.nih.gov/. The new DNA sequences were submitted to the GenBank, PP193858-PP193862 for ITS1-5.8S-ITS2 genes and PP193328-PP193332 for the D2-D3 gene.

Phylogenetic analyses

The multiple gene sequence alignments and phylogenetic analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0. In brief, the recently acquired ITS1-5.8S-ITS2 or D2-D3 sequences were aligned using ClastalW, with default parameters of published ITS1-5.8S-ITS2 or 28S rRNA sequences for relevant nematode groups in the family Telotylenchidae [54,55]. *Meloidogyne enterolobii* served as an outgroup taxon. Maximum Likelihood (ML) methods based on Gamma distribution (GTR + G) model was used to construct phylogenetic trees. The test of phylogeny was conducted using 1,000 bootstrap replicates [29,30].

Results and discussion

Results

Nematode surveys

Following nematode extraction, 6 nematode genera were found: *Trichotylenchus* sp., *Meloidogyne* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp. and *Helicotylenchus* sp. Among these, the most abundant nematode was *Tylenchorhynchus* sp. (51.0 %), followed by *Hoplolaimus* sp. (20.9 %),

Helicotylenchus sp. (13.1 %), *Meloidogyne* sp. (11.8 %), *Trichotylenchus* sp. (8.5 %) and *Pratylenchus* sp. (6.5 %) (**Table 1**). Each of the PPNs recorded in the tomato field had been previously documented, except for *Trichotylenchus* sp. For this reason, *Trichotylenchus* sp. was selected for further characterization using combined morphological and molecular diagnostic techniques.

Table 1 Number of plant-parasitic nematodes found in 100 g soil collected from a tomato field in Khon Kaen Province, Thailand.

Sample	Number of plant-parasitic nematodes					
	Meloidogyne	***Helicotylenchus***	***Hoplolaimus***	***Tylenchorhynchus***	***Pratylenchus***	***Trichotylenchus***
1	13.3 ± 5.8	13.3 ± 5.8	6.7 ± 5.8	40.0 ± 10.0	13.3 ± 5.8	3.3 ± 1.5
2	3.3 ± 5.8	10.0 ± 10.0	3.3 ± 5.8	10.0 ± 10.0	0	3.3 ± 2.5
3	3.3 ± 5.8	0	6.7 ± 5.8	26.7 ± 11.6	3.3 ± 5.8	5.0 ± 1.7
4	0	3.3 ± 5.8	13.3 ± 11.6	16.7 ± 5.8	0	3.3 ± 2.3
5	3.3 ± 5.8	3.3 ± 5.8	16.7 ± 5.8	6.7 ± 5.8	0	3.3 ± 1.5
6	6.7 ± 5.8	3.3 ± 5.8	6.7 ± 5.8	30.0 ± 10.0	0	3.3 ± 2.1
Nematode occurrence (%)	11.8	13.1	20.9	51.0	6.5	8.5

Note: Values (number of nematodes) are mean ± SD (n = 3).

Morphological identification

The juveniles (immature stages) of *Trichotylenchus* sp., collected from soil in tomato field, were evaluated for their morphometric characteristics. It can be noted that after nematode fixation, the body becomes straight and slightly curved downward (**Figure 1(A)**). The cuticle showed annulations, with 3 incisures in the lateral line (**Figures 1(E) - 1(F)**). The cephalic region exhibited continuity, featuring a subtle concavity at the intersection of the lip and body. Their bodies have an average length of 518.4 ± 40.5 µm (mean ± SD). The lip region has a broad, rounded shape, characterized by a limited number of indistinct lip annuli, measuring 4.1 ± 0.4 µm in height and 6.5 ± 0.4 µm in width (**Figure 1(C)**). The stylet has a robust morphology, measuring between 17.1 and 18.0 µm in length. It is characterized by rounded basal knobs with posteriorly sloping borders. DGO is located 2.0 ± 0.5 µm posterior to basal knobs. The median bulb exhibits a rounded shape and is highly developed (**Figure 1(C)**). The excretory pore is near the isthmus with a pharyngeal gland junction. The basal pharyngeal bulb is located adjacent to the intestine (**Figure 1(B)**). The tail conoid is characterized by prominent annulations and exhibited a gradual tapering towards a termination that resembles a heart or V shape, and the tail has an average length of 44.0 ± 1.8 µm (**Figure 1(D)**). The phasmid is located anteriorly to the midpoint of the tail.

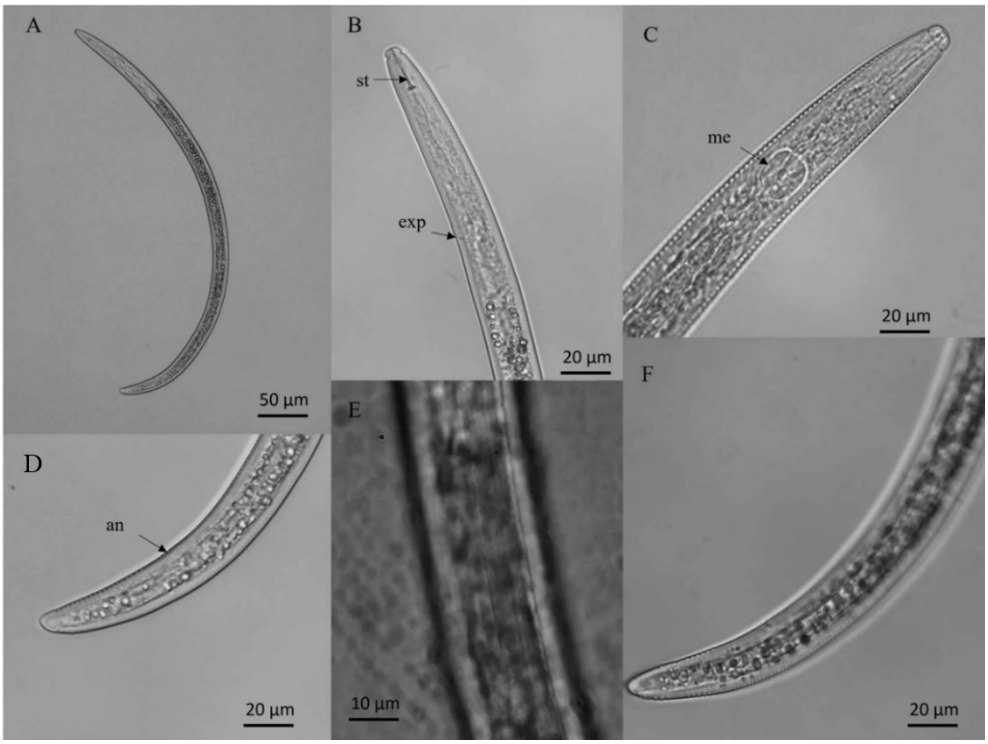

Figure 1 Photomicrographs of *Trichotylenchus dispersus* isolated from Thai tomato field: (A) entire body of a juvenile, (B,C) anterior region, (D) tail region, (E) lateral field with 3 incisures and (F) v-shaped tail, (an) anus, (exp) excretory pore, (st) stylet and (me) median bulb.

The morphometric characteristics of the juveniles are as follows (mean ± SD): a value = 32.8 ± 4.0, b value = 4.4 ± 0.3, c value = 11.8 ± 0.9 and c' value = 3.3 ± 0.3 (**Table 2**). These features closely matched the *T. dispersus* species as previously identified in banana-cultivated areas in China [22].

Table 2 Morphometrics of juveniles of *Trichotylenchus* sp. from tomato field in the current study and its comparisons with *T. dispersus* reported in banana-cultivated areas, China. The values are mean ± SD (min - max) and all measurements are in µm.

Characters	Isolation source	
	Rhizosphere of banana roots Guangdong province, China [22]	**Rhizosphere of tomato roots Khon Kaen province, Thailand (Current study)**
n	8	6
L	527.0 ± 55.3 (466.0 - 612.0)	518.4 ± 40.5 (458.9 - 563.3)
a	31.2 ± 1.2 (28.7 - 32.6)	32.8 ± 4.0 (27.9 - 37.7)
b	4.7 ± 0.4 (4.4 - 5.2)	4.4 ± 0.3 (3.7 - 4.7)
c	12.0 ± 0.7 (11.2 - 13.1)	11.8 ± 0.9 (10.4 - 12.7)

Characters	Isolation source	
	Rhizosphere of banana roots Guangdong province, China [22]	**Rhizosphere of tomato roots Khon Kaen province, Thailand (Current study)**
c’	3.2 ± 0.3 (2.8 - 3.6)	3.3 ± 0.3 (2.9 - 3.7)
Distance from anterior to esophago-intestinal valve	-	120.3 ± 8.7 (103.7 ± 126.7)
Tail diameter at anus	-	13.3 ± 1.1 (11.6 ± 14.6)
Body width	-	15.9 ± 1.0 (14.2 - 17.2)
Stylet length	17.7 ± 0.7 (17.0 - 19.1)	17.5 ± 0.3 (17.1 - 18.0)
Stylet shaft length	-	9.2 ± 0.3 (8.9 - 9.6)
Stylet knob width	3.0 ± 0.3 (2.5 - 3.6)	3.0 ± 0.6 (2.3 - 3.8)
Stylet knob height	1.6 ± 0.3 (1.3 - 2.1)	2.1 ± 0.1 (2.0 - 2.2)
Median bulb width	8.6 ± 0.5 (7.6 - 9.2)	9.3 ± 0.4 (8.9 - 10.0)
Median bulb length	12 ± 0.7 (11.0 - 13.3)	13.2 ± 0.8 (12.3 - 14.1)
DGO	1.6 ± 0.3 (1.2 - 2.0)	2.0 ± 0.5 (1.3 - 2.3)
Lip width	5.8 ± 0.3 (5.2 - 6.3)	6.5 ± 0.4 (6.0 - 7.0)
Lip height	3.1 ± 0.3 (2.6 - 3.6)	4.1 ± 0.4 (3.6 - 4.6)
Tail length	44.2 ± 4.5 (38.4 - 50.4)	44.0 ± 1.8 (42.2 - 46.7)

Note: n = number of measured juvenile specimens, L = body length, a = body length/diameter of the body, b = body length/distance from anterior to esophago-intestinal valve, c = body length/tail length and c’ = tail length/tail diameter at the anus.

Molecular characterization

Nematode DNA amplification with 2 universal primer sets yielded approximate DNA fragments: TW81/AB28 (950 bp) and D2A/D3B (800 bp) (**Figure 2**). Subsequently, the nucleotide sequences obtained were compared with those sequences already deposited in NCBI GenBank. The BLAST analysis of ITS1-5.8S-ITS2 (PP193858-PP193862) and D2-D3 amplicon (PP193858-PP193862) revealed identities closely matching the *Trichotylenchus dispersus* specimens from China: ON622716 (98.0 %) and ON622717 (99.4 %).

Phylogenetic tree analyses

The phylogenies based on both genes were constructed using 5 newly obtained sequences of the nematodes in this study, along with reference nucleotides from nematodes classified in the family Telotylenchidae available

in the GenBank database. The results showed similarities in the D2D3 and ITS1-5.8S-ITS2 regions, where the nematodes occupied the same clade with *T. dispersus* (bootstrap values of 100), which were previously reported in rhizosphere regions of banana roots from China (ON622716 and ON622717), and are distinguishable from *Trichotylenchus changlingensis* clades (OM294653, MH545694, KM204134 and OM276857). Moreover, the studied nematodes were clearly clustered in a different clade from other nematode members in the family Telotylenchidae (**Figure 3**). Based on these analytics, this present study has revealed for the first time the presence of *T. dispersus*, associated with tomato-cultivated areas in Khon Kaen Province, Thailand.

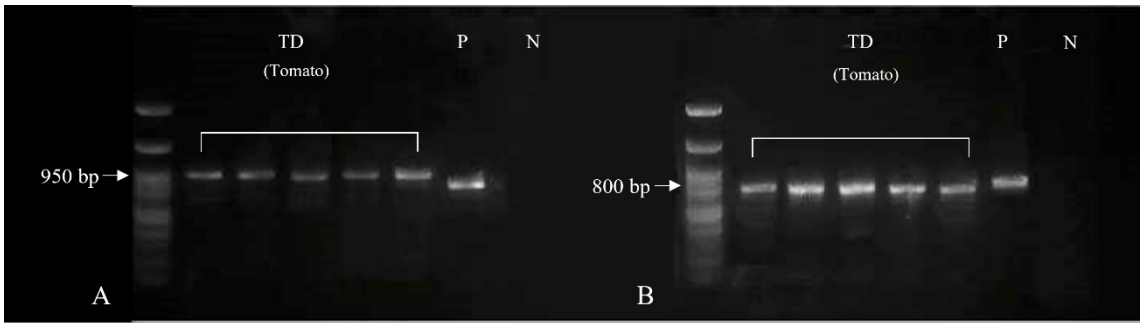

Figure 2 Agarose gel electrophoresis of the DNA of *Trichotylenchus dispersus* isolated from tomato field amplified by PCR with primers (A) TW81/ AB28 and (B) D2A/D3B: (M) 100 bp DNA ladder, (TD) DNA samples from *T. dispersus* in this study, (N) negative control (without DNA template) and (P) DNA sample from *Pratylenchus* spp.

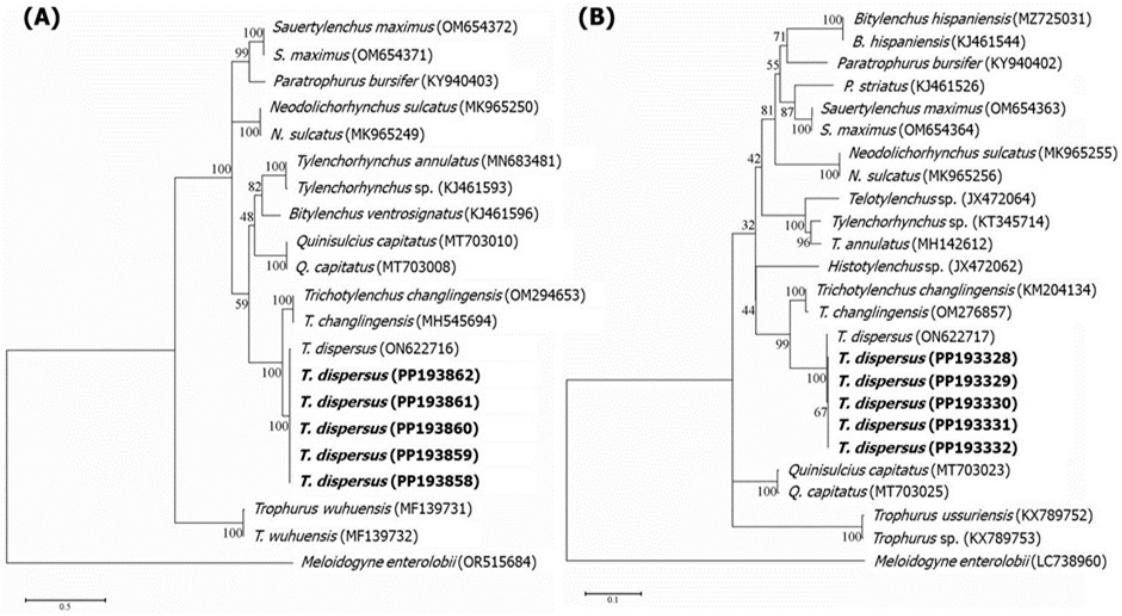

Figure 3 Phylogenetic reconstruction based on: (A) ITS1-5.8S-ITS2, and (B) D2D3 regions of *Trichotylenchus* sp. and some other plant-parasitic nematodes in family Telotylenchidae. Numbers beside branches represent ML bootstrap support values. The scale bar represents substitutions per nucleotide position. NCBI accession numbers are listed behind the species names. Specimens obtained in this study are in bold.

Discussion

Plant-parasitic nematodes (PPNs) are one of vital biotic constraints affecting yield losses from different crop, including tomato and chili [15,32]. In the current study, 6 nematode genera were found in a tomato field in Khon Kaen Province, Thailand. Similar results were previously observed and reported by Osei *et al*. [33] who discovered and identified PPNs in Ghana tomato fields, specifically *M. incognita*, *Pratylenchus* sp., *Rotylenchulus* sp., *Xiphinema* sp., *Tylenchulus* sp. and *Helicotylenchus* sp. Similarly, Shokoohi and Masoko [32] conducted a survey of PPNs in tomato fields in South Africa and found species like *Criconema mutable*, *Rotylenchus brevicaudatus*, *Tylenchorhynchus* sp., *M. incognita*, *Pratylenchus* sp., *Helicotylenchus pseudorobustus* and *Xiphinema* sp. Of these nematodes, *Meloidogyne* sp. is the most commonly recognized root-knot nematode in many parts of the world and is regarded as one of the most devastating nematode species [34]. Several researchers have studied its distribution, identification, biology and management [17,35,56]. In Thailand, many root-knot species found on tomato plants have been identified, most notably *M. incognita* and *M. javanica* [18,19]. However, for the *Trichotylenchus* sp., there appears to be no information regarding its presence and identification in Thailand. For these important reasons, the morphological and molecular characterization of *Trichotylenchus* sp. is provided in this study. Morphological features of the studied nematode specimens closely matched those described for *T. dispersus*, as reported in China. Indeed, most of the morphometrics of the current studied specimens were similar to the specimens reported by Chen *et al*. [22], except for the following slight variations (Thai study vs. China study): Body length (518.4 vs. 527.0 µm), stylet knob height (2.1 vs. 1.6 µm), median bulb width (9.3 vs. 8.6 µm), lip width (6.5 vs. 5.8 µm), lip height (4.1 vs. 3.1 µm) and DGO (2.0 vs. 1.6 µm). If based only on these morphological observations, the studied species could be misidentified because these general characteristics are similar for many PPNs, such as *Telotylenchus* sp. and *Belonolaimus* sp. However, the *Trichotylenchus* sp has distinctive characteristics that distinguishes it from other nematodes: *Trichotylenchus* sp. (18.6 µm) has a longer stylet than *Telotylenchus* sp. (17.5 µm), but is shorter than *Belonolaimus* sp. (24.0 µm) [22,36,37]; basal knobs are more rounded in *Telotylenchus* sp. than in *Trichotylenchus* sp. [38]; and the median blub of *Belonolaimus* sp. is smaller than *Trichotylenchus* (30.8 vs. 49.1 µm) [22,39]. To confirm the species identity, the nematode specimens in this study were further processed using molecular characterization, which is more accurate and reliable [15].

Universal primers D2A/D3B and TW81/AB28 have been extensively employed in nematode identification, including *Paratylenchus aculentus* [40], *Heterodera filipjevi* [41], *Heterodera ripae* [42], *Globodera rostochiensis* [43], *Rotylenchulus reniformis* [12] and *Meloidogyne* spp. [16,44], *Pratylenchus parazeae* [45], *Hirschmanniella mucronata* [29] and *Quinisulcius curvus* [46]. Moreover, these primers are effective for identifying *Trichotylenchus* spp. such as *T. gorganiensis* [47], *T. changlingensis* [21] and *T. dispersus* [22]. In this study, 950- and 800-bp DNA fragments were obtained from PCR amplification - nucleotide sequence comparisons of both genes showed 98 to 99.4 % identity with *T. dispersus* specimens from China (ON622716) and 98 % identity to *T. dispersus* specimens from China (ON622717). Likewise, the phylogenetic studies confirmed that the nematodes identification as *T. dispersus*, and stand in a different clade from other nematode genera in Family Telotylenchidae, as observed and documented by Saeedi *et al*. [48].

Trichotylenchus spp., a migratory ectoparasitic nematode, belongs to the same family (Hoplolaimidae) as *Hoplolaimus* sp., *Rotylenchus* sp., *Helicotylenchus* sp. and *Scutellonema* sp. [49,50]. These nematodes were first observed by Whitehead [51] in the rhizosphere of *Hyparrhenia* sp. in southern Tanganyika, where it was identified as *Trichotylenchus falciformis*. In 2011, Geraert [52] combined the genera *Uliginotylenchus*, *Triversus, Divittus* and *Morasinema* into a single genus called *Trichotylenchus*, recognizing 32 species as valid within this newly unified genus. So far, this nematode has been found in association with rhizosphere of many plants, such as grass (*Hyparrhenia* sp.) in East Africa [51], maize in Shaanxi, China [21], camel thorn (*Alhagi* sp.) in Golestan province, Iran [47] and banana in Leizhou City, China [22]. The important nematode species are *T. astriatus*, *T. bifasciatus*, *T. cicerus*, *T. changlingensis*, *T. chonai*, *T. conicaudatus*, *T. dispersus*, *T. divittatus*, *T. falciformis*, *T. gorganiensis*, *T. impar*, *T. kangwonensis*, *T. liginosus*, *T. obscurisulcatus*, *T. palustris*, *T. papyrus*, *T. pruni*, *T. rectangularis*, *T. rhopalocercus*, *T. sabournesis*, *T. sculptus*, *T. triglyphus*, *T. trilineatus* and *T. yuhanensis* [21,22,52,53]. Because this nematode is a migratory ectoparasite, the damage symptoms that appear on plants are not clear and specific. For instance, Chen *et al*. [21]; Chen *et al*. [22] noted that the symptoms demonstrated by maize and banana plants include stunting, thinning and hypoplasia. Although no damage symptoms were observed on the tomato plants in the current study, the presence of this nematode in the rhizosphere soil suggests an emerging threat to tomato production in the surveyed areas. The potential for inoculum build-up in the field poses a grave risk of reduced crop yields. Therefore, this study highlights the need for additional research, focusing particularly on the impacts, biological characteristics and host preferences of this specific nematode, which should be further investigated.

Conclusions

This study has discovered the presence of *T. dispersus* in a tomato field in Khon Kaen Province, Thailand. This is the first report on the morphological and molecular characteristics of *T. dispersus* found in Thailand. The incidence of this nematode is likely to become a significant factor limiting tomato production yields if they are not well managed. Therefore, immediate control measures are likely necessary to restrict their spread to other tomato-growing areas and to prevent potential yield loss related to this nematode.

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