

Prevalence and Characterization of Plant-Parasitic Nematodes Existing in RD41 Rice Fields in Pathum Thani Province, Thailand, with Emphasis on *Hirschmanniella mucronata* and *Meloidogyne graminicola*

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Abstract

Plant parasitic nematodes (PPNs) are one of the most destructive pests affecting rice growth worldwide. In this study, populations of PPNs associated with RD41 rice fields in Pathum Thani Province, Thailand, were surveyed and the dominant PPNs found in the fields were identified using a combination of morphological and molecular traits. A total of 105 soil or root samples were taken from 21 paddy fields in 7 districts of Pathum Thani, and nematode extraction was processed in a laboratory. In all sampled rice fields, 6 nematode genera, namely *Hirschmanniella* sp., *Meloidogyne* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Helicotylenchus* sp., and *Tylenchus* sp., were extracted. Of these 6 nematode genera, *Hirschmanniella* sp. was the most prevalent in both soil and root samples (66.7 - 100 % occurrence observed), followed by *Meloidogyne* sp., which was found in only 4 districts of the province (0.2 to 8.7 % occurrence observed). The species of these predominant PPNs were morphologically and molecularly characterized. The identification results remained consistent across morphological features and molecular techniques, confirming the presence of *Hirschmanniella mucronata* and *Meloidogyne graminicola* in the studied rice fields. The results of this study provide useful baseline data of the occurrence of PPNs in Thailand's rice fields. The incidence of some PPNs found in this study is likely to become a significant factor limiting rice production yields if they are not better managed.

Keywords: Nematode identification, Paddy fields, Plant-parasitic nematodes, RD41 rice variety

Introduction

Rice is one of the major agricultural commodities of Pathum Thani Province, Thailand, which has about 33,500 ha of planted areas, covering about one-fourth of the total area of the province [1]. In Thailand, rice farms can generate 2 to 3 crop cycles per year, especially in areas with convenient access to irrigation water [2]. The rice varieties most commonly cultivated in the central region of Thailand include Rice Department (RD) 47, RD61, RD57, Pathum Thani 1, and RD41 [3]. Of these rice varieties, RD41 is recognized for its ability to thrive in challenging environments and still yield as much as 4.51 tons per hectare [4]. Moreover, this particular rice variety has been documented to resist the brown planthopper (*Nilaparvata lugens*) and blast disease caused by

Pyricularia oryzae Cavara [5,6]. Presently, there is limited information available regarding the outbreaks of plant-parasitic nematodes (PPNs) in RD41 rice cultivations, and comprehensive studies or documentation focusing on this matter are scarce.

PPNs represent a significant biotic factor, causing about 20 % of economic losses in rice production throughout the world [7]. The primary PPNS posing a threat to rice crops globally include *Meloidogyne* spp., *Heterodera* spp., *Hirschmanniella* spp., *Pratylenchus* spp., *Aphelenchoides besseyi*, and *Ditylenchus angustus*, as documented by Kyndt *et al.* [8]. The study conducted by Pascual *et al.* [9] in the Philippines, involved surveys of PPNS that revealed the presence of 5 nematode genera (*Meloidogyne*, *Hirschmanniella*, *Pratylenchus*, *Tylenchorhynchus*, and *Helicotylenchus*) in lowland rice fields and 9 genera in upland rice fields (the 5 genera found in lowland rice fields plus *Rotylenchulus*, *Aphelenchoides*, Criconematidae, and *Rotylenchus*). A different study conducted in Ecuador determined that *Hirschmanniella oryzae* was the predominant species in rainfed lowland rice fields [10]. *Hirschmanniella* sp. is a migratory endoparasitic nematode that feeds intracellularly in the parenchyma cells of the cortex and migrates within the root [11]. The *H. oryzae* nematode has caused 24 to 56 % reduction in rice crop yield [12, 13].

Additional extensive studies have been conducted on *Meloidogyne* spp., as a category of endoparasitic nematodes. In particular, *Meloidogyne graminicola* and *M. incognita* have been shown to cause enormous yield losses in rice production, approaching 70 % in field studies and 100 % in greenhouse conditions [14,15]. *Meloidogyne graminicola* infection causes hook-shaped galls to form on young root tips, leading to various conditions the harm rice growth, such as yellowing, stunting, decreased tillering, delayed maturation, and decreased root proliferation [16].

In Thailand, both *Hirschmanniella* spp. (*H. oryzae* and *H. mucronata*) and *Meloidogyne* spp. (*M. graminicola* and *M. incognita*) have been detected in rice-growing areas across almost all parts of the country [15,32,44]. In 2021, *H. mucronata* was initially identified in RD41 rice fields in Khlong Luang, Pathum Thani [32]. Aside from this report, no additional information is available concerning nematode surveys in RD41 rice fields across Thailand. So far, various effective methods have been used to manage *Hirschmanniella* spp. and *Meloidogyne* spp., such as soil amendments/crop rotation with certain legumes [30,45], botanical plant extracts [46-48], biological control agents [49,50], and synthetic nematicides [51]. Nevertheless, successfully controlling PPNS requires an understanding of the types of nematodes and their biology, as different PPNS necessitate distinct control approaches [26]. Thus, accurately diagnosing nematode species, typically through a combination of morphological and molecular identification, is essential for selecting appropriate management methods in rice fields [26].

Given the crucial information regarding the impacts of PPNS, conducting surveys is valuable for assessing the potential threat posed by these nematodes to rice yields. Indeed, the data collected from such surveys can be utilized by researchers and agricultural growers to determine effective integrated management strategies. For these reasons, this study was conducted specifically to investigate the existence of PPNS in rice fields, focusing on RD41 (one of the most commonly grown varieties) in Pathum Thani Province, Thailand. The second objective was to characterize the predominant PPNS found in these rice fields using a combination of morphological and molecular techniques.

Materials and methods

Nematode surveys

A total of 105 soil and root samples were collected in 2022 from the rhizosphere of rice in paddy fields (3 fields per district) located in 7 districts of Pathum Thani Province: Sam Khok, Lat Lum Kaeo, Thanyaburi, Khlong Luang, Mueang, Nong Suea, and Lum Luk Ka. The soil texture of paddy fields in Pathum Thani is typically heavy clay, with a soil pH ranging from 5.4 to 6.4. This study was conducted

during the ripening stage of the rice, focusing on fields where Rice Department 41 (RD41) rice variety was grown. In each field, soil or root samples were collected at fifteen random points (X shaped) covering 0.16 hectare. The subsamples were homogenized in a bucket and 5 samples (comprising both soil and roots) were randomly collected. The samples were then placed in labelled plastic bags and transported to the Nematology Laboratory at the Faculty of Agriculture, Kasetsart University, Thailand, for nematode processing.

Nematode extraction and fixation

PPNs were extracted from 100 cc soil using Cobb's Sieving and Decantation and Modified Baermann's Funnel techniques [17] and 10 g roots using Baermann's Funnel techniques. After 48 h of incubation, 20 mL of the sample containing nematodes was collected from the funnel, and the nematodes were killed using hot water at 50 °C. Subsequently, the nematode samples were fixed using TAF (triethanolamine formalin), following the method described by Courtney *et al.* [18] and stored at room temperature (25 ± 3 °C) until they underwent morphological identification.

Nematode cultures

Hirschmanniella: Root samples from each isolate (district), remaining from the nematode extraction, were mixed with 500 g sterilized clay soil in a 7 cm diameter pot. Then, one two-week-old RD41 rice seedling was transplanted to the pot. Plants were maintained in a glasshouse at the Department of Plant Pathology, Kasetsart University, for 2 months prior to DNA extraction.

Meloidogyne: A single egg mass, collected from rice roots exhibiting hook-shaped galls, was inoculated onto the root of a two-week-old KDML105 rice plant. KDML105 is a rice variety known to be susceptible to *M. graminicola*, as reported by Beesa *et al.* [19]. The plant was grown in a 50 mL tube, with a hole at the bottom, filled with 50 g of 1:1 (w/w) mixture of sterilized sand and clay soil. Plants were allowed to grow in the glasshouse for 1 month before additional analysis was conducted.

Morphological identification

The predominant PPNs found in each paddy field were further studied to identify the specific nematode. Semi-permanent slides were prepared from individual 20 fixed nematodes of each isolate. The fixed nematode specimens were placed in a droplet of glycerin on a glass slide, covered with a cover slip, and sealed using nail polish. Subsequently, nematode specimens were observed and photographed using a digital camera (Canon Power Shot A640) equipped with EOS utility program and mounted on a compound microscope (Olympus BX50). Nematode measurements were carried out via Axio Vision SE64 Rel. 4.9.1 program and the morphometrics were calculated as follows: L = mean body length, a = body length/body width, b = body length/anterior end to pharyngo-intestinal junction (PIJ), b' = body length/pharynx length, c = body length/tail length, c' = tail length/maximum tail width, V% = head to vulva length/body length \times 100, stylet length, maximum body width, dorsal pharyngeal gland orifice (DGO), anterior end to PIJ, pharynx length, head to vulva length, tail width at anus, and tail length [20].

Morphometric values of the nematodes were statistically analyzed using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). Differences among values were determined by analyzing the variance (ANOVA), and means were compared using the Duncan's Multiple Range Test ($p \leq 0.05$).

Molecular characterization

Twenty-one *Hirschmanniella* or 9 *Meloidogyne* adult females were used for DNA extraction which followed a method described by Holterman *et al.* [21]. The 40- μ l mixture, containing a 1:1 (v/v) ratio of 1

nematode in distilled water and lysis buffer (200 mM NaCl (A&D Technology, Japan), 200 mM Tris-HCl pH 8.0 (A&D Technology, Japan), 1 % (v/v) β -mercaptoethanol (Sigma, Japan), and 800 μ g/mL proteinase K (Worthington Biochemical, USA)) was prepared in a 0.2 mL PCR tube. Subsequently, the mixture was incubated in a PCR machine (Biometra Tgradient Thermoblock) at 65 °C for 90 min, followed by 99 °C for 10 min. After incubation, the mixture was maintained at -20 °C until it was used as DNA template.

The species of the studied nematodes were validated using the PCR-based method with 3 different target gene regions: For *Hirschmanniella* sp. (18S rRNA, D2-D3 of 28S rRNA, and ITS1-5.8S-ITS2) and for *Meloidogyne* sp. (18S rRNA, D2-D3, and COII-16s rRNA). The primer sets included SSU18A/SSU26R for 18S rRNA [22], D2A/D3B for D2-D3 [23], Vrain2F/Vrain2R for ITS1-5.8S-ITS2 [24], and C2F3/1108 for COII-16s rRNA [25].

A final volume of 15 μ L of PCR mixture contained 4 μ L of ddH₂O, 7 μ L of 2x PCR master mix solution (i-Taq) (Intron biotechnology, Korea), 0.5 μ L of each forward and reverse primers, and 3 μ L of DNA template. The PCR profile was as follows: 94 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 56 °C (18A/26R and D2A/D3B) or 52 °C (Vrain2F/Vrain2R) or 48 °C (C2F3/1108) for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR products were screened in 1.5 % agarose gel and electrophoresed at 100 volts for 30 min in 0.5xTAE solution. The DNA purification and sequencing of 3 representative samples derived from each primer set and the nematode isolate were delineated by Solgen Inc., Korea. The obtained sequences were aligned using Contig Assembly Program in the BioEdit version 7.0. Then, all nucleotide sequences were compared to the nematode sequences already available and deposited in the GenBank NCBI database.

The multiple sequence alignments among the studied specimens and the sequences selected from the NCBI GenBank were performed using CLUSTALW. Maximum Likelihood (ML) phylogenetic analyses for each gene sequence were conducted using Molecular Evolutionary Genetics Analysis (MEGA) 7.0 under the General Time Reversible model with parameters for invariable sites and gamma-distributed rate heterogeneity. The test of phylogeny was carried out using the rapid bootstrap algorithm (1,000 iterations) [26].

Results and discussion

Prevalence of plant-parasitic nematodes in RD41 rice-growing fields

Following several surveys, 6 PPN genera were recovered from rice fields of the major rice-producing areas in Pathum Thani Province (**Figure 1**). Five nematode genera, including *Hirschmanniella* sp., *Meloidogyne* sp., *Tylenchorhynchus* sp., *Helicotylenchus* sp., and *Tylenchus* sp., were observed in the soil, but only *Hirschmanniella* sp., *Meloidogyne* sp. and *Pratylenchus* sp. were observed in the roots. Of nematodes found in these surveys, the most abundant PPNs in both the soil and rice roots were *Hirschmanniella* sp. (2,910.9 nematodes; occurrence frequency 66.7 to 100 %) and *Meloidogyne* sp. (58.5 nematodes; occurrence frequency 0.2 to 8.7 %) (**Table 1**). These 2 species were the most prevalent nematodes occurring in all sampled paddy fields, except for Thanyaburi, Khlong Luang and Lum Luk Ka districts where no *Meloidogyne* sp. was observed.

The presence of these nematodes signals an additional menace to rice production in the surveyed areas, indicating potential inoculum build-up in the field, thereby posing a risk of causing reductions in crop yield. In a similar study conducted by Pascual *et al.* [9] in the Philippines, similar genera of PPNs were observed, particularly *Hirschmanniella* sp. and *Meloidogyne* sp., which were found in both upland and lowland rice fields. Likewise, in Vietnam and Cambodia, these 2 genera are considered the predominant PPNs, found widely in lowland rice fields [27,28].

Nematode growth and survival are influenced by various factors, including temperature, soil moisture levels, soil composition and structure, soil pH, and the availability of suitable host plants [26]. For *Hirschmanniella* spp., optimal growth conditions include heavy clay soil with a pH level between 4 and 9, temperatures ranging from 20 to 34 °C, and a tendency to thrive in flooded environments [41]. This explains why *Hirschmanniella* spp. are commonly found in rice fields across Asia, particularly in lowland areas of the Philippines, Vietnam, Cambodia, and Thailand, where conditions are ideal for nematode growth [9,27,28]. Conversely, the study observed lower populations of *Meloidogyne* spp. compared to *Hirschmanniella* sp. This is likely due to *M. graminicola* tending to reproduce more successfully in rice grown in loamy sand and sandy loam soils rather than in clay loam [42]. However, both are regarded as the primary PPNs that infest rice crops in various regions worldwide [8].

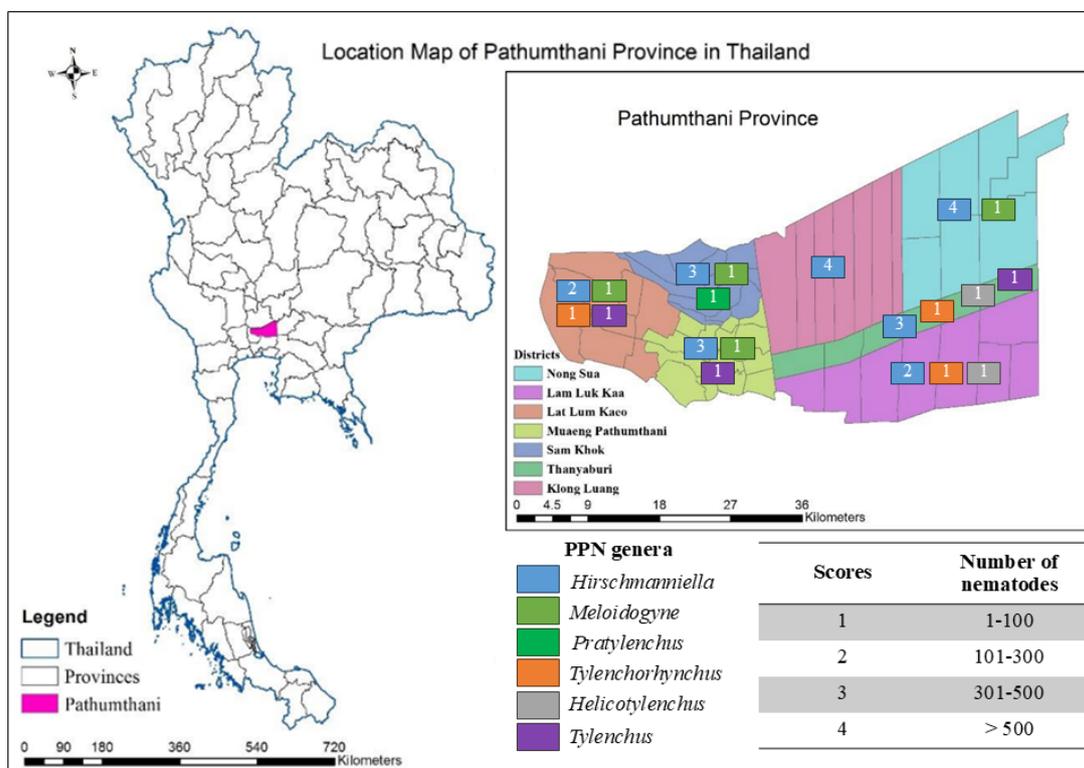


Figure 1 Distribution of plant-parasitic nematodes in RD41 rice fields from Pathum Thani province, Thailand. The map adapted from Pathak [40].

Overall, this study indicates that *Hirschmanniella* sp. and *Meloidogyne* sp. are the most prevalent PPN genera in RD41 rice fields in Pathum Thani Province, Thailand. Consequently, these 2 genera were subjected to further investigation for nematode identification as discussed and detailed in the following sections.

Table 1 Average population density and frequency of occurrence of plant-parasitic nematodes associated with RD41 rice fields in Sam Khok (SK), Lat Lum Kaeo (LAT), Thanyaburi (TH), Khlong Luang (KL), Mueang (MU), Nong Suea (NS), and Lum Luk Ka (LAM) districts in Pathum Thani Province, Thailand.

District	Number of plant-parasitic nematodes ^b							
	10 g rice roots			100 cc soil				
	Hir	Mel	Pra	Hir	Mel	Tyr	Heli	Tys
SK	380.9 ± 37.7 ^a (93.2)	27.6 ± 15.4 (6.7)	0.4 ± 0.4 (0.1)	38.6 ± 10.6 (97.8)	-	0.9 ± 0.9 (2.2)	-	-
LAT	243.1 ± 26.4 (91.3)	23.1 ± 11.6 (8.7)	-	32.1 ± 10.1 (85.8)	0.4 ± 0.4 (1.2)	4.4 ± 2.2 (11.8)	-	0.4 ± 0.4 (1.2)
TH	337.7 ± 33.0 (100.0)	-	-	8.0 ± 2.4 (66.7)	-	2.2 ± 1.5 (18.5)	0.9 ± 0.9 (7.4)	0.9 ± 0.6 (7.3)
KL	1,135.5 ± 174.3 (100.0)	-	-	58.2 ± 20.3 (99.2)	-	-	-	0.4 ± 0.4 (0.8)
MU	169.3 ± 15.3 (99.6)	0.7 ± 0.5 (0.4)	-	262.2 ± 63.6 (90.6)	0.4 ± 0.4 (0.2)	-	-	0.4 ± 0.4 (0.2)
NS	523.5 ± 51.0 (98.7)	7.1 ± 3.9 (1.3)	-	11.5 ± 3.6 (96.3)	-	-	0.4 ± 0.4 (3.7)	-
LAM	120.9 ± 13.7 (100.0)	-	-	143.1 ± 32.3 (99.1)	-	0.4 ± 0.4 (0.3)	0.9 ± 0.9 (0.6)	-
Total	2,910.9	58.5	0.4	553.7	0.8	7.9	2.2	2.1

^aValues are mean of nematodes ± SE (n = 15). The percent frequency of occurrence is shown in the parenthesis.

^bHir: *Hirschmanniella* sp.; Mel: *Meloidogyne* sp.; Pra: *Pratylenchus* sp.; Tyr: *Tylenchorhynchus* sp.; Heli: *Helicotylenchus*; Tys: *Tylenchus* sp.

Morphological characterization of *Hirschmanniella* sp.

Female: The morphological characterization of nematodes corresponded to those described for *H. mucronata* in previous studies conducted in the Philippines by Pascual *et al.* [9], and in Cambodia by Khun *et al.* [29] and Beesa *et al.* [30]. The features discerned had the following characteristics: Slender and slightly C-shaped bodies ranging 1,452.0 to 2,558.7 µm in length; maximum body diameters were 24.7 to 38.0 µm; hemispherical lip region, distinctly offset; robust stylet ranging 24.0 to 30.5 µm in length with round knob basal; pharyngeal glands were elongated with ventrally overlapping intestines; anterior end to pharyngo-intestinal junction (PIJ) was 102.5 to 137.8 µm in length; pharynx lengths were 209.0 to 428.8 µm; vulva position occupied 46.9 to 61.4 % of corresponding body length with didelphic-amphidelphic ovaries; the lateral field has 4 incisures; tail lengths ranged 61.2 to 103.4 µm; and tail widths ranged 17.0 to 28.8 µm, with a terminal mucron evident at the end, which was occasionally absent in some nematode specimens (**Figures 2(a) - 2(d)**). The morphometric values of the females were (in range); a = 46.3 to 82.3, b = 12.6 to 21.7, c = 16.4 to 34.1, and c' = 2.6 to 4.8 (**Table 2** and **Figure 2**).

Significant morphometric differences were observed among the 7 studied nematode isolates, except for certain values of c, c', v, and stylet length (**Table 2**). The value (mean ± STD) of body length was longer in isolate NS (2,060.9 ± 261.8 µm) than in isolates TH (1,831.2 ± 156.2 µm) and KL (1,972.5 ± 293.8 µm). The maximum body width of isolate LAT (33.9 ± 2.4 µm) and NS (33.6 ± 2.8 µm) was greater than that of isolate KL (29.8 ±

2.7 μm). Isolate MU ($121.4 \pm 6.6 \mu\text{m}$) exhibited a longer value of PIJ than isolate TH ($116.3 \pm 6.8 \mu\text{m}$). Pharynx length was longer in isolate LAM ($352.6 \pm 41.4 \mu\text{m}$) than in isolates KL ($328.4 \pm 36.8 \mu\text{m}$) and NS ($328.0 \pm 39.6 \mu\text{m}$). Tail length of isolate MU ($81.8 \pm 7.8 \mu\text{m}$) was longer than that of isolate LAM ($75.9 \pm 9.0 \mu\text{m}$). Isolates NS ($22.7 \pm 2.6 \mu\text{m}$), MU ($23.2 \pm 2.0 \mu\text{m}$), and TH ($22.7 \pm 1.6 \mu\text{m}$) exhibited a tail width at the anus larger than that of isolates KL ($21.2 \pm 2.0 \mu\text{m}$) and LAM ($20.9 \pm 2.0 \mu\text{m}$). The a value of isolate KL (62.4 ± 6.4) and NS (61.4 ± 6.6) was higher than that of isolate LAT (54.6 ± 6.7) and TH (57.0 ± 4.3). Isolate NS (17.3 ± 1.9) displayed a higher value of b than isolates LAT (15.7 ± 2.0), TH (15.7 ± 1.0), KL (15.5 ± 1.3), and LAM (15.7 ± 1.3). The b' value was higher in isolate NS (6.3 ± 0.6) than in the other isolates (between 5.4 ± 0.7 to 5.7 ± 0.9).

Male: Similar to female in general morphological traits (**Figures 2(e) - 2(h)**), except for a slight decrease in size (body length and width) compared to females. The bodies ranged from 1,358.0 to 2,249.0 μm in length and 24.0 to 38.0 μm in width, stylet lengths were 15.0 to 31.0 μm , PIJ were 95.0 to 131.0 μm , pharynx lengths were 270.0 to 415.0 μm , tail lengths ranged 50.0 to 115.0 μm , and tail widths ranged 15.0 to 23.0 μm with a distinct bursa, and spicules 27 to 37 μm in length. The morphometric characteristics of the nematode specimens were; a = 42.4 to 72.4, b = 11.7 to 19.2, b' = 4.0 to 6.9, c = 16.6 to 28.7, and c' = 2.8 to 6.1 (**Table 3** and **Figure 2**).

The morphometric comparisons among male populations showed significantly divergent variations in the values (mean \pm SD), except for a, c', stylet lengths, PIJ, and pharynx lengths. Isolate MU ($1,828.0 \pm 145.6 \mu\text{m}$) and NS ($1,887.1 \pm 91.8 \mu\text{m}$) had bodies longer than those of other isolates ($1,659.2 \pm 147.0 \mu\text{m}$ to $1,729.4 \pm 140.1 \mu\text{m}$). Bodies were wider in isolate NS ($32.3 \pm 2.5 \mu\text{m}$) than in isolates SK ($29.6 \pm 2.0 \mu\text{m}$), TH ($29.6 \pm 3.0 \mu\text{m}$), and KL ($28.7 \pm 2.8 \mu\text{m}$). Tail lengths were longer in isolate NS ($82.6 \pm 12.6 \mu\text{m}$) as compared to isolate SK ($75.2 \pm 10.6 \mu\text{m}$), LAT ($75.4 \pm 8.1 \mu\text{m}$), and TH ($73.0 \pm 8.6 \mu\text{m}$), while smallest tail widths were found in isolate TH ($17.4 \pm 1.0 \mu\text{m}$). Spicule lengths were longer in isolate SK ($31.6 \pm 2.4 \mu\text{m}$) than in isolate NS ($33.4 \pm 2.8 \mu\text{m}$). The values of b and b' were greater in isolate NS (16.1 ± 0.9 and 5.7 ± 0.4 , respectively) than in the rest of the populations, while isolate KL showed the lowest value for c (21.1 ± 2.1).

Table 2 Morphometrics of *Hirschmanniella* females isolated from rice var. RD41 in Sam Khok (SK), Lat Lum Kaeo (LAT), Thanyaburi (TH), Khlong Luang (KL), Mueang (MU), Nong Suea (NS), and Lum Luk Ka (LAM) in Pathum Thani Province, Thailand and their comparison with specimens from Cambodia [29]. All measurements are in μm and in the form: mean \pm SD (range).

Character	Nematode isolate							Cambodia [29]
	SK	LAT	TH	KL	MU	NS	LAM	
N	20	20	20	20	20	20	20	30
L	1919.8 \pm 159.1 ^{ab} (1,614.0 - 2,198.0)	1849.6 \pm 245.0 ^b (1,477.6 - 2,428.0)	1831.2 \pm 156.2 ^b (1,452.0 - 2,111.0)	1,850.1 \pm 178.0 ^b (1,515.9 - 2,149.4)	1,972.5 \pm 293.8 ^{ab} (1,488.4 - 2,558.7)	2,060.9 \pm 261.8 ^a (1,540.9 - 2,561.0)	1,887.3 \pm 199.9 ^b (1,501.4 - 2,268.4)	1,775 \pm 188.0 (1,260 - 2,160)
a	58.6 \pm 4.5 ^{ab} (46.3 - 65.8)	54.6 \pm 6.7 ^c (47.7 - 75.9)	57.0 \pm 4.3 ^{bc} (46.9 - 62.1)	62.4 \pm 6.4 ^a (52.7 - 74.1)	59.4 \pm 7.4 ^{ab} (49.2 - 82.3)	61.4 \pm 6.6 ^a (48.3 - 73.9)	60.1 \pm 4.9 ^{ab} (49.0 - 67.3)	58.0 \pm 5.2 (46 - 67)
b	16.2 \pm 1.2 ^{ab} (13.9 - 18.8)	15.7 \pm 2.0 ^b (13.0 - 21.7)	15.7 \pm 1.0 ^b (13.8 - 17.2)	15.5 \pm 1.3 ^b (13.1 - 18.2)	16.2 \pm 2.3 ^{ab} (13.3 - 21.2)	17.3 \pm 1.9 ^a (13.2 - 20.6)	15.7 \pm 1.3 ^b (12.6 - 17.3)	14.0 \pm 1.1 (12 - 16)
b'	5.7 \pm 0.5 ^b (4.9 - 6.8)	5.6 \pm 0.8 ^b (4.2 - 7.2)	5.4 \pm 0.4 ^b (4.4 - 6.1)	5.7 \pm 0.9 ^b (4.7 - 8.2)	5.7 \pm 0.5 ^b (4.8 - 6.8)	6.3 \pm 0.6 ^a (4.7 - 7.1)	5.4 \pm 0.7 ^b (4.0 - 7.4)	5.9 \pm 0.7 (4.4 - 7.4)
c	24.5 \pm 2.8 ^a (19.9 - 30.9)	24.2 \pm 2.5 ^a (20.7 - 29.9)	23.7 \pm 2.3 ^a (19.8 - 28.7)	24.3 \pm 2.7 ^a (16.4 - 29.6)	24.0 \pm 2.0 ^a (20.3 - 27.9)	25.6 \pm 3.6 ^a (20.1 - 34.1)	25.0 \pm 2.7 ^a (21.0 - 31.3)	22.0 \pm 2.7 (16 - 28)
c'	3.6 \pm 0.5 ^a (2.9 - 4.8)	3.5 \pm 0.4 ^a (2.7 - 4.1)	3.4 \pm 0.5 ^a (2.8 - 4.5)	3.6 \pm 0.5 ^a (2.9 - 4.7)	3.6 \pm 0.5 ^a (2.6 - 4.6)	3.6 \pm 0.5 ^a (2.9 - 4.7)	3.6 \pm 0.3 ^a (3.0 - 4.2)	3.7 \pm 0.4 (2.8 - 5.0)

Character	Nematode isolate							Cambodia [29]
	SK	LAT	TH	KL	MU	NS	LAM	
N	20	20	20	20	20	20	20	30
V (%)	54.3 ± 2.1 ^a (48.4 - 57.6)	53.9 ± 2.1 ^a (49.6 - 58.3)	54.6 ± 3.2 ^a (48.1 - 61.4)	53.8 ± 2.8 ^a (46.9 - 58.9)	53.4 ± 2.5 ^a (48.5 - 57.9)	53.1 ± 2.1 ^a (49.3 - 57.4)	53.7 ± 2.2 ^a (48.6 - 58.6)	52.0 ± 2.3 (49.0 - 59.0)
Body width	32.9 ± 2.6 ^{ab} (29.0 - 38.0)	33.9 ± 2.4 ^a (28.7 - 37.1)	32.2 ± 2.3 ^{ab} (27.0 - 37.0)	29.8 ± 2.7 ^b (24.7 - 35.1)	33.2 ± 2.4 ^{ab} (28.8 - 37.8)	33.6 ± 2.8 ^a (27.8 - 38.0)	31.4 ± 2.8 ^{ab} (26.1 - 37.5)	30.5 ± 2.3 (25.0 - 35.0)
Stylet length	27.8 ± 1.3 ^a (25.0 - 30.0)	27.7 ± 1.5 ^a (24.4 - 29.8)	27.6 ± 1.2 ^a (24.0 - 29.0)	27.6 ± 1.0 ^a (26.0 - 29.0)	27.4 ± 1.2 ^a (25.2 - 29.7)	28.2 ± 1.9 ^a (24.1 - 30.5)	27.5 ± 1.3 ^a (25.0 - 30.3)	22.2 ± 0.6 (21.0 - 23.0)
PIJ	118.3 ± 6.0 ^{ab} (107.0 - 129.0)	118.1 ± 7.1 ^{ab} (102.5 - 130.4)	116.3 ± 6.8 ^{ab} (105.0 - 129.0)	119.5 ± 7.6 ^{ab} (104.5 - 130.5)	121.4 ± 6.6 ^a (107.1 - 129.9)	119.1 ± 6.5 ^{ab} (107.1 - 131.1)	120.4 ± 6.6 ^{ab} (105.6 - 137.8)	124.0 ± 12.0 (84.0 - 147.0)
Pharynx length	339.2 ± 25.3 ^{ab} (290.0 - 378.0)	333.4 ± 28.6 ^{ab} (279.0 - 405.8)	336.6 ± 22.9 ^{ab} (298.0 - 380.0)	328.4 ± 36.8 ^b (209.0 - 367.7)	345.1 ± 29.5 ^{ab} (274.0 - 401.3)	328.0 ± 39.6 ^b (229.2 - 405.9)	352.6 ± 41.4 ^a (271.4 - 428.8)	300.0 ± 40.0 (229.0 - 399.0)
Head to vulva length	1,041.2 ± 90.3 ^{ab} (849.0 - 1235.0)	997.7 ± 147.5 ^b (829.9 - 1,416.4)	996.7 ± 77.7 ^b (853.0 - 1,103.0)	996.6 ± 114.6 ^b (805.6 - 1,267.0)	1,050.4 ± 135.2 ^{ab} (802.4 - 1,330.2)	1,092.1 ± 127.3 ^a (841.4 - 1,329.0)	1012.1 ± 108.5 ^b (839.0 - 1,208.8)	936.0 ± 104.0 (630.0 - 1,160)
Tail length	78.8 ± 6.9 ^{ab} (64.0 - 93.0)	76.4 ± 7.5 ^{ab} (66.8 - 92.0)	77.7 ± 8.8 ^{ab} (65.0 - 99.0)	76.7 ± 8.8 ^{ab} (63.5 - 103.4)	81.8 ± 7.8 ^a (69.2 - 99.2)	81.1 ± 8.9 ^{ab} (68.0 - 99.0)	75.9 ± 9.0 ^b (61.2 - 97.4)	81.0 ± 8.2 (60.0 - 99.0)
Tail width at anus	22.1 ± 2.0 ^{ab} (17.0 - 25.0)	22.1 ± 1.8 ^{ab} (19.0 - 25.2)	22.7 ± 1.6 ^a (19.0 - 26.0)	21.2 ± 2.0 ^b (17.2 - 24.2)	23.2 ± 2.0 ^a (20.8 - 28.8)	22.7 ± 2.6 ^a (18.2 - 27.0)	20.9 ± 2.0 ^b (17.1 - 25.0)	22.0 ± 2.3 (18.0 - 27.0)

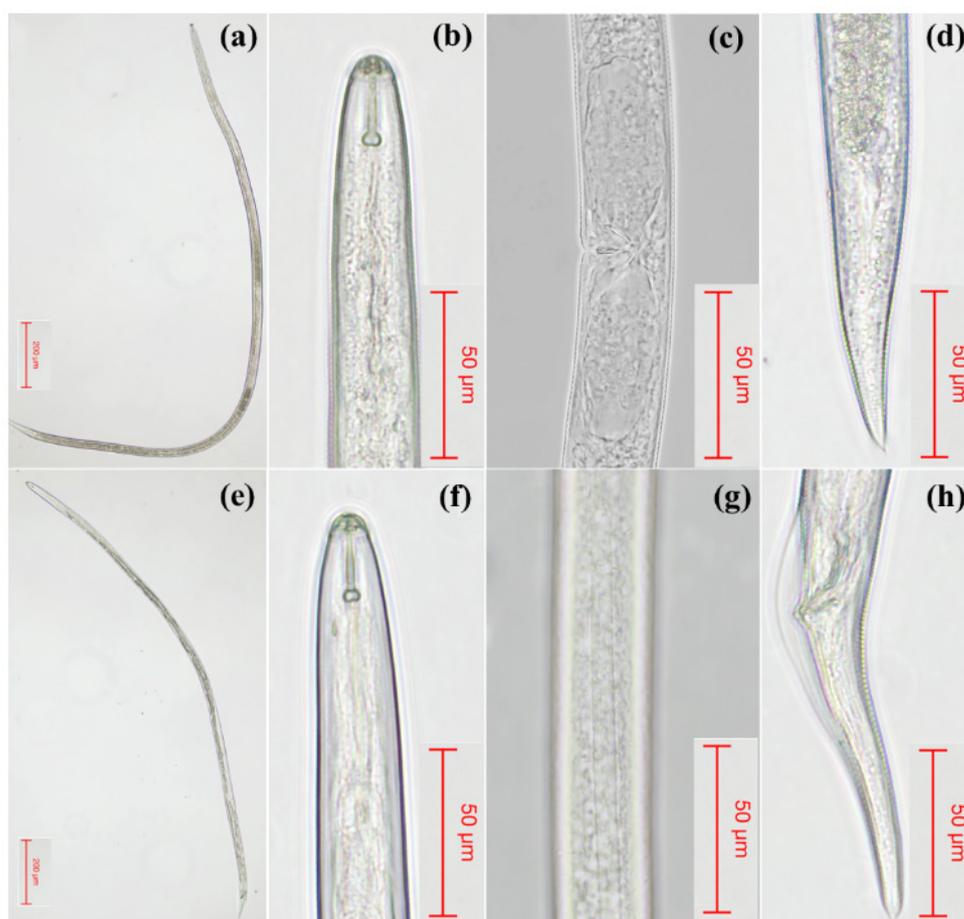


Figure 2 Photomicrographs of *Hirschmanniella mucronata* isolated from RD41 rice fields in Pathum Thani Province, Thailand. (a) Whole body of female; (b) Head region of female; (c) Vulva position of female; (d) Tail region of female; (e) Whole body of male; (f) Head region of male; (g) Lateral field with 4 incisures; (h) Tail region of male.

Table 3 Morphometrics of *Hirschmanniella* males isolated from rice var. RD41 in Sam Khok (SK), Lat Lum Kao (LAT), Thanyaburi (TH), Khlong Luang (KL), Mueang (MU), Nong Suea (NS), and Lum Luk Ka (LAM) in Pathum Thani Province, Thailand and their comparison with specimens from Cambodia [29]. All measurements are in μm and in the form: mean \pm SD (range).

Character	Nematode isolate							Cambodia [29]
	SK	LAT	TH	KL	MU	NS	LAM	
N	20	20	20	20	20	20	20	20
L	1,703.2 \pm 132.5 ^b (1,448.0 - 1,953.0)	1,729.4 \pm 140.1 ^b (1,375.0 - 1,949.0)	1,663.7 \pm 117.0 ^b (1,424.0 - 1,922.0)	1,659.2 \pm 147.0 ^b (1,397.0 - 1,859.0)	1,828.0 \pm 145.6 ^a (1,522.0 - 2,142.0)	1,887.1 \pm 91.8 ^a (1,740.0 - 2,025.0)	1,703.2 \pm 176.8 ^b (1,358.0 - 2,249.0)	1,607.0 \pm 179.0 (1,260 - 1,876)
a	57.8 \pm 4.4 ^d (51.6 - 66.0)	56.7 \pm 3.5 ^d (50.9 - 65.7)	56.6 \pm 5.4 ^a (45.9 - 65.3)	58.2 \pm 6.2 ^a (50.7 - 72.4)	58.8 \pm 4.5 ^a (47.1 - 67.3)	58.9 \pm 5.5 ^a (48.6 - 69.8)	55.6 \pm 4.9 ^a (42.4 - 63.0)	57.0 \pm 6.4 (47.0 - 75.0)
b	14.9 \pm 1.4 ^{bc} (11.8 - 17.4)	15.3 \pm 1.3 ^{abc} (12.8 - 18.6)	14.6 \pm 1.0 ^c (12.4 - 17.6)	14.8 \pm 1.8 ^{bc} (11.7 - 19.2)	15.7 \pm 1.3 ^{ab} (13.6 - 18.6)	16.1 \pm 0.9 ^a (14.2 - 18.6)	14.9 \pm 0.9 ^{bc} (13.6 - 16.5)	12.8 \pm 1.6 (9.0 - 16.2)
b'	5.1 \pm 0.5 ^{bc} (4.4 - 6.5)	5.3 \pm 0.6 ^b (4.3 - 6.9)	5.0 \pm 0.5 ^c (4.0 - 5.7)	5.1 \pm 0.5 ^{bc} (4.3 - 6.1)	5.4 \pm 0.5 ^b (4.6 - 6.5)	5.7 \pm 0.4 ^a (4.8 - 6.6)	5.3 \pm 0.4 ^b (4.6 - 6.2)	5.3 \pm 0.5 (4.6 - 6.1)
c	23.0 \pm 3.3 ^a (18.0 - 33.5)	23.1 \pm 2.0 ^a (20.0 - 28.5)	23.0 \pm 2.5 ^a (18.3 - 27.7)	21.0 \pm 2.1 ^b (18.8 - 28.0)	23.0 \pm 2.4 ^a (17.3 - 27.7)	23.3 \pm 3.3 ^a (16.6 - 28.7)	22.0 \pm 2.7 ^{ab} (17.0 - 26.8)	22.0 \pm 2.0 (18.0 - 25.3)
c'	4.1 \pm 0.7 ^a (2.8 - 5.3)	4.0 \pm 0.5 ^a (3.1 - 4.8)	4.2 \pm 0.5 ^a (3.4 - 5.3)	4.1 \pm 0.3 ^a (3.5 - 4.6)	4.2 \pm 0.5 ^a (3.2 - 5.5)	4.4 \pm 0.8 ^a (3.1 - 6.1)	5.0 \pm 0.5 ^a (3.2 - 5.0)	4.4 \pm 0.5 (3.2 - 5.5)
Body width	29.6 \pm 2.0 ^{bc} (27.0 - 33.0)	30.6 \pm 2.1 ^{ab} (26.0 - 34.0)	29.6 \pm 3.0 ^{bc} (25.0 - 35.0)	28.7 \pm 2.8 ^c (24.0 - 33.0)	31.2 \pm 2.3 ^{ab} (28.0 - 36.0)	32.3 \pm 2.5 ^a (28.0 - 36.0)	30.7 \pm 2.9 ^{ab} (26.0 - 38.0)	28.0 \pm 3.8 (19.0 - 33.0)
Stylet length	27.3 \pm 1.1 ^a (25.0 - 29.0)	27.3 \pm 1.1 ^a (25.0 - 29.0)	27.4 \pm 1.3 ^a (25.0 - 30.0)	27.5 \pm 0.9 ^a (26.0 - 29.0)	27.8 \pm 1.3 ^a (25.0 - 30.0)	27.2 \pm 1.3 ^a (25.0 - 30.0)	27.3 \pm 1.5 ^a (25.0 - 31.0)	22.0 \pm 1.0 (20.0 - 25.0)
PIJ	114.9 \pm 6.9 ^a (95.0 - 123.0)	113.2 \pm 6.7 ^a (98.0 - 125.0)	114.4 \pm 6.2 ^a (104.0 - 128.0)	112.6 \pm 9.3 ^a (97.0 - 124.0)	116.9 \pm 6.8 ^a (101.0 - 131.0)	117.6 \pm 6.7 ^a (106.0 - 129.0)	114.2 \pm 9.0 ^a (100.0 - 114.0)	125.0 \pm 13.3 (91.0 - 150.0)
Pharynx length	334.4 \pm 27.4 ^a (277.0 - 379.0)	327.8 \pm 22.9 ^a (276.0 - 357.0)	337.3 \pm 32.3 ^a (270.0 \pm 394.0)	325.8 \pm 37.1 ^a (275.0 - 389.0)	342.7 \pm 31.1 ^a (292.0 - 415.0)	334.0 \pm 22.7 ^a (304.0 - 378.0)	321.7 \pm 28.3 ^a (271.0 - 385.0)	302.0 \pm 29.0 (245.0 - 362.0)
Tail length	75.2 \pm 10.6 ^{bc} (50.0 - 95.0)	75.4 \pm 8.1 ^{bc} (59.0 - 88.0)	73.0 \pm 8.6 ^c (60.0 - 92.0)	79.5 \pm 7.7 ^{abc} (63.0 - 92.0)	80.4 \pm 10.9 ^{ab} (63.0 - 115.0)	82.6 \pm 12.6 ^a (66.0 - 115.0)	78.0 \pm 7.5 ^{abc} (67.0 - 93.0)	78.1 \pm 9.1 (54.0 - 100.0)
Tail width at anus	18.5 \pm 1.5 ^a (16.0 - 23.0)	18.7 \pm 1.6 ^a (16.0 - 22.0)	17.4 \pm 1.0 ^b (15.0 - 19.0)	19.4 \pm 1.8 ^a (17.0 - 22.0)	19.2 \pm 1.2 ^a (17.0 - 21.0)	19.0 \pm 1.1 ^a (17.0 - 21.0)	18.9 \pm 1.6 ^a (16.0 - 22.0)	17.4 \pm 1.0 (16.0 - 19.0)
Spicule length	31.6 \pm 2.4 ^b (28.0 - 36.0)	31.8 \pm 1.8 ^{ab} (29.0 - 35.0)	31.9 \pm 2.0 ^{ab} (27.0 - 35.0)	31.9 \pm 2.5 ^{ab} (27.0 - 37.0)	31.9 \pm 2.2 ^{ab} (28.0 - 36.0)	33.4 \pm 2.8 ^a (29.0 - 37.0)	32.1 \pm 2.3 ^{ab} (27.0 - 36.0)	31.0 \pm 1.2 (28.0 - 32.0)

Although variations were found among the nematode populations, they were identified as *H. mucronata* based on their morphological and morphometric features, consistent with the characteristics previously detailed by Khun *et al.* [29] for the *Hirschmanniella* dichotomous key. Comparing the specimens in this study to those documented in Taiwan [31] and Cambodia [29] indicated that the body lengths, maximum body widths, tail lengths, and % of the specimens studied closely resembled those observed in the Cambodian specimens. Compared to the Taiwanese study specimens, both the male and female body lengths and tail lengths were smaller in this study's samples. Interestingly, the stylet in these Thai specimens were longer (> 2 - 4 μm) compared to specimens from other populations. However, So *et al.* [43] and Phadungkit *et al.* [26] observed that nematode size is influenced by factors such as food availability, sex, and temperature.

In Thai rice fields, only the *H. mucronata* and *H. oryzae* [32] have been documented as prevalent rice root nematode species. Comparing *H. oryzae* to *H. mucronata*, *H. oryzae* have shorter body lengths (1,090 to 1,330 μm) and stylet lengths (15 to 17 μm) [31,32]. Noting these physical distinctions, the nematodes observed in this study were conclusively identified as *H. mucronata*. Subsequently, the nematodes underwent molecular characterization for additional confirmation.

Morphological characterization of *Meloidogyne* sp.

The perineal pattern characteristics of adult females obtained from 3 isolates were similar; generally, the pattern was oval-shaped with rounded dorsal arches, and with smooth striae covering most of the region but with no detectible lateral fields (**Figure 3**).

Adult males exhibited cylindrical bodies ranging 942.3 to 1,575.3 μm in length and 24.2 to 35.0 μm in width. The lip region was either continuous with the body or slightly offset. They possessed a robust stylet ranging 16.6 to 21.8 μm in length, with a round basal knob. The orifice of the dorsal esophageal gland was located 2.8 to 4.0 μm from the base of the stylet. The spicules displayed a slight ventral bend near the middle, measuring 19.8 to 31.4 μm in length. The tail was mostly straight and short, measuring 8.5 to 15.4 μm in length, and 17.5 to 26.5 μm in width at the anus. The morphometric characteristics of the nematode specimens were; $a = 38.9$ to 47.0 , $b = 9.1$ to 16.4 , $c = 91.2$ to 142.8 , and $c' = 0.5$ to 0.7 (**Figure 3** and **Table 4**). No morphometric differences were observed among the nematode populations, except for maximum body width (mean \pm SD) that was greater in isolate SK ($35.0 \pm 0.9 \mu\text{m}$) than in isolate LAT ($31.0 \pm 4.1 \mu\text{m}$).

Characteristics of J2s: Their body is vermiform and slender, and tapers at both ends, but more so towards their posterior extremity (**Figure 3**). The body lengths ranged 407.2 to 470.7 μm and body widths were 14.0 to 19.3 μm . Their lip region was flat anteriorly and continuous with the body. The stylet lengths varied from 13.7 to 15.4 μm , with rounded knobs. The orifice of the dorsal oesophageal gland was located 2.2 to 4.4 μm from the base of the stylet. Tail lengths were 57.0 to 77.6 μm and widths were 8.8 to 13.9 μm . The tail terminus was rounded, often slightly clavate. The hyaline tail length ranged from 17.1 to 23.6 μm . The morphometric characteristics of the nematode specimens were; $a = 22.4$ to 33.0 , $b = 5.2$ to 8.1 , $c = 5.9$ to 7.6 , $c' = 4.7$ to 7.8 , and $h\% = 24.7$ to 39.1 . No variations in morphometric measurements were observed among the nematode populations, except for DGO and maximum body width. Specifically, DGO was longer in isolate LAT ($3.1 \pm 0.6 \mu\text{m}$) compared to isolate NS ($2.8 \pm 0.4 \mu\text{m}$), while maximum body width was greater in isolate LAT ($16.5 \pm 1.5 \mu\text{m}$) than in isolates SK ($15.5 \pm 1.1 \mu\text{m}$) and NS ($15.6 \pm 0.9 \mu\text{m}$).

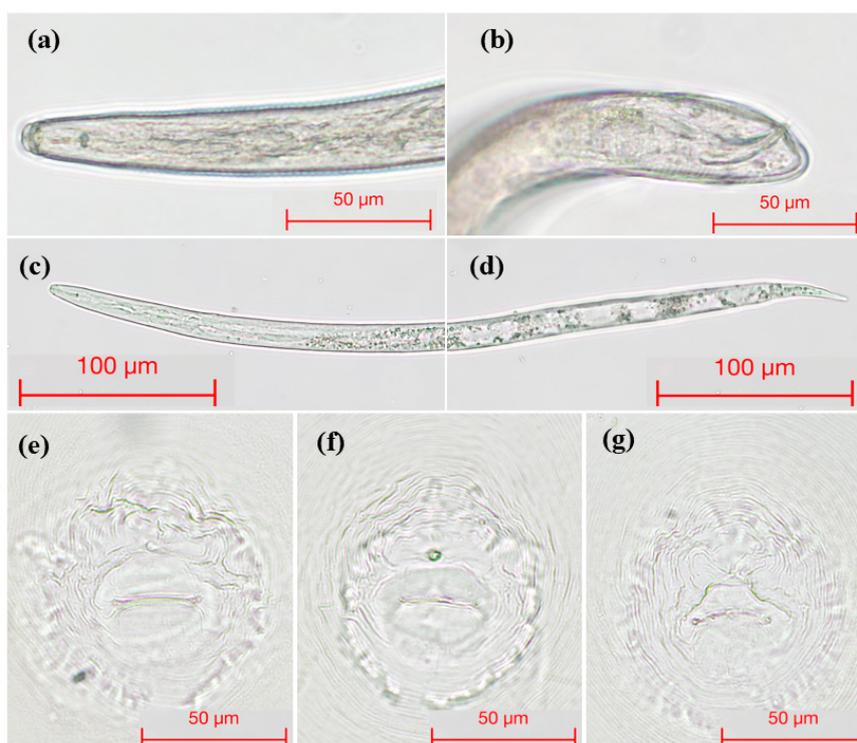


Figure 3 Photomicrographs of *Meloidogyne graminicola* isolated from RD41 rice fields in Pathum Thani Province, Thailand. (a) Head region of male; (b) Tail region of male; (c) Head region of second stage juvenile; (d) Tail region of second stage juvenile; (e) - (g) perineal patterns of adult female specimens from Sam Khok, Lat Lum Kaeo, and Nong Suea districts, respectively.

Table 4 Morphometrics of *Meloidogyne* second-stage juveniles and males isolated from rice var. RD41 in Sam Khok (SK), Lat Lum Kaeo (LAT), and Nong Suea (NS) in Pathum Thani Province, Thailand. All measurements are in μm and in the form: mean \pm SD (min-max).

Character	Nematode isolate							
	Second-stage juveniles				Males			
	SK	LAT	NS	Golden and Birchfield [36]	SK	LAT	NS	Golden and Birchfield [36]
N	20	20	20	20	5	5	5	20
L	439.7 \pm 16.2 ^a (407.2 - 470.7)	447.7 \pm 12.2 ^a (426.8 - 468.6)	446.3 \pm 12.0 ^a (416.6 - 464.0)	441.0 (415.0 - 484.0)	1,494.4 \pm 54.5 ^a (1,433.9 - 1,575.3)	1,312.6 \pm 231.9 ^a (942.3 - 1491.5)	1,350.9 \pm 108.7 ^a (1,181.2 - 1,470.4)	1,222.0 (1,020 - 1,408)
a	28.4 \pm 1.8 ^a (24.4 - 31.1)	27.4 \pm 2.9 ^a (22.4 - 33.0)	28.6 \pm 2.0 ^a (24.7 - 31.5)	24.8 (22.3 - 27.3)	42.7 \pm 1.2 ^a (41.0 - 44.0)	42.2 \pm 3.1 ^a (38.9 - 47.0)	42.4 \pm 2.8 ^a (39.7 - 47.0)	-
b	6.5 \pm 0.6 ^a (5.2 - 8.1)	6.4 \pm 0.3 ^a (5.7 - 6.9)	6.6 \pm 0.5 ^a (5.8 - 7.6)	-	15.1 \pm 1.5 ^a (12.5 - 16.4)	13.1 \pm 2.5 ^a (9.1 - 15.3)	14.0 \pm 1.7 ^a (11.7 - 15.6)	-
c	6.9 \pm 0.4 ^a (5.9 - 7.4)	6.7 \pm 0.4 ^a (6.0 - 7.5)	6.9 \pm 0.5 ^a (6.0 - 7.6)	6.2 (5.5 - 6.7)	108.4 \pm 7.5 ^a (101.6 \pm 120.2)	107.5 \pm 10.8 ^a (99.1 - 125.1)	121.6 \pm 18.8 ^a (91.2 - 142.8)	-
c'	6.0 \pm 0.5 ^a (4.7 - 6.7)	6.0 \pm 0.7 ^a (4.8 - 7.6)	6.1 \pm 0.6 ^a (5.3 - 7.8)	-	0.6 \pm 0.1 ^a (0.5 - 0.7)	0.6 \pm 0.1 ^a (0.5 - 0.6)	0.6 \pm 0.1 ^a (0.5 - 0.7)	-
h	20.3 \pm 1.7 ^a (17.3 - 23.6)	20.3 \pm 1.8 ^a (17.1 - 23.6)	20.6 \pm 1.9 ^a (17.6 - 23.6)	17.9 (14.0 - 21.2)	-	-	-	-
h%	31.7 \pm 2.2 ^a (26.9 - 35.5)	30.2 \pm 3.1 ^a (24.7 - 35.7)	31.6 \pm 3.4 ^a (25.8 - 39.1)	-	-	-	-	-
Max. body width	15.5 \pm 1.1 ^b (14.0 - 18.3)	16.5 \pm 1.5 ^a (14.2 - 19.3)	15.6 \pm 0.9 ^b (14.3 - 17.8)	-	35.0 \pm 0.9 ^a (34.2 - 36.6)	31.0 \pm 4.1 ^b (24.2 - 35.0)	31.8 \pm 1.8 ^{ab} (29.0 - 33.3)	29.8 (24.0 - 34.7)
Stylet length	14.5 \pm 0.4 ^a (13.9 - 15.2)	14.6 \pm 0.5 ^a (13.7 - 15.4)	14.6 \pm 0.4 ^a (13.8 - 15.1)	11.4 (11.2 - 12.3)	19.9 \pm 1.3 ^a (18.4 - 21.8)	19.1 \pm 1.7 ^a (16.6 - 20.6)	20.0 \pm 1.0 ^a (18.7 - 20.8)	16.8 (16.2 - 17.3)
DGO	3.0 \pm 0.2 ^{ab} (2.4 - 3.4)	3.1 \pm 0.6 ^a (2.2 - 4.4)	2.8 \pm 0.4 ^b (2.2 - 3.7)	2.8 (2.8 - 3.4)	3.4 \pm 0.4 ^a (2.8 - 4.0)	3.5 \pm 0.3 ^a (3.0 - 3.8)	3.3 \pm 0.4 ^a (2.9 - 4.0)	3.3 (2.8 - 3.9)
PIJ	68.3 \pm 6.7 ^a (55.1 - 86.4)	70.2 \pm 4.1 ^a (62.8 - 77.3)	67.6 \pm 5.0 ^a (60.0 - 76.2)	-	99.9 \pm 12.4 ^a (88.5 - 120.4)	100.8 \pm 2.5 ^a (96.6 - 103.3)	97.7 \pm 14.9 ^a (84.7 - 120.2)	-
Tail length	64.3 \pm 4.7 ^a (57.0 - 77.1)	67.2 \pm 4.4 ^a (61.6 - 75.3)	65.4 \pm 5.1 ^a (58.5 - 77.6)	70.9 (67.0 - 76.0)	13.8 \pm 1.2 ^a (12.5 - 15.4)	12.3 \pm 2.4 ^a (8.5 - 14.8)	11.3 \pm 2.0 ^a (9.3 - 14.5)	11.1 (6.1 - 15.1)
Tail width at anus	10.9 \pm 1.2 ^a (8.8 - 13.0)	11.3 \pm 1.0 ^a (9.7 - 13.9)	10.7 \pm 0.8 ^a (9.5 - 12.5)	-	22.5 \pm 2.6 ^a (20.0 \pm 26.5)	21.6 \pm 2.4 ^a (17.5 - 23.6)	19.8 \pm 0.9 ^a (18.9 - 20.8)	-
Spicule length	-	-	-	-	25.0 \pm 4.7 ^a (19.8 - 31.4)	22.7 \pm 1.3 ^a (20.7 - 23.8)	25.1 \pm 2.1 ^a (23.0 - 27.7)	28.1 (27.4 - 29.1)

The morphometrics and morphological features of *Meloidogyne* females, males, and J2s are consistent with those of *M. graminicola* previously reported in rice fields in India [33], China [34], and Vietnam [35], as well as those observed in shallot fields in Thailand [16]. By comparing the characteristics data of this study with previously published data from the USA [36], China [34], and Vietnam [35], this study noted that the average body length of J2s was akin to that reported for the USA population (441 μm), but shorter than that of the Chinese (456 μm) and Vietnamese (472 μm) populations.

Moreover, the stylet length of the samples of this study was approximately 2 μm greater than in other populations. Hyaline length and DGO aligned closely with measurements found in Chinese and Vietnamese populations. For males, the body and stylet lengths resembled the measurements from the Chinese population, albeit with slightly shorter DGO. Nguyen *et al.* [35] noted that the virulent *M. graminicola* isolates from Cambodia possess a significantly longer stylet and hyaline tail terminus length compared to the avirulent *M. graminicola* isolates from Vietnam. However, variations in the morphology and morphometrics of *M. graminicola* are noticeable, particularly when the nematodes are collected from different agroecosystems [33].

This study suggests that variations in nematode body sizes observed across different locations can lead to misidentification. Consequently, the molecular characterization analysis was carried out to validate these observations.

Molecular characterization of *Hirschmanniella* sp.

The amplification of the 18S, 28S, and ITS1-5.8S-ITS2 gene regions yielded specific amplicons of 900, 750 and 950 bp, respectively. All the amplified sequences were identical and displayed a 96 - 100 % similarity for 18S and 28S, and 93 - 97 % for ITS1-5.8S-ITS2, with *H. mucronata* sequences deposited in the GenBank. The newly obtained nucleotide sequences of the nematodes in the present study were deposited in the NCBI GenBank under accession numbers shown in **Table 5**. In this study, the percentage similarity of the ITS1-5.8S-ITS2 amplicon was relatively low compared to other gene segments, primarily due to the limited availability of nucleotide sequences in GenBank (only DQ309589 and KJ923642). However, the identification of *H. mucronata* in this study can be validated using the 18S and 28S rRNA genes.

The phylogenetic analysis of the 18S rRNA (**Figure 4(a)**), 28S rRNA (**Figure 4(b)**), and ITS1-5.8S-ITS2 (**Figure 4(c)**) trees aligned closely with the previously classified arrangement of *H. mucronata* based on their morphological and molecular features [29,30]. The nematodes occupied the same group of *H. mucronata* populations found in rice fields in Thailand and Cambodia, and shared a sister clade with *H. loofi* and *H. kwazuna*. Although some of these nematode isolates were located in different clades, this result is similar to the findings of Indarti *et al.* [37] and Mwamula *et al.* [38], who studied the phylogenetic trees of *Hirschmanniella* spp. and classified *H. mucronata* into several clades. Khun *et al.* [29] observed that the single nucleotide polymorphisms (SNPs) in the D2-D3 region showed variances of 7.7 to 8.7 %, whereas as ITS1-5.8S-ITS2 of *H. mucronata* showed variances of 22.7 to 23.4 %, compared to *H. loofi* and *H. kwazuna*. Indeed, *H. mucronata* is situated in a distinct clade from *H. oryzae* [9,29,30]. This study identified *H. mucronata* as the rice root nematodes found extensively in RD41 rice fields in Pathum Thani Province.

Table 5 Lists of new GenBank accession number of plant-parasitic nematodes isolated from rice var. RD41 in Sam Khok (SK), Lat Lum Kaeo (LAT), Thanyaburi (TH), Khlong Luang (KL), Mueang (MU), Nong Suea (NS), and Lum Luk Ka (LAM) in Pathum Thani Province, Thailand.

Nematode species	Isolate	GenBank accession number			
		18S	28S	ITS1-5.8S-ITS2	COII-16S
<i>H. mucronata</i>	SK	OR365103-05	OR632907-09	OR364751-53	-
	LAT	OR365106-08	OR632910-12	OR364754-56	-
	TH	OR365109-11	OR632913-15	OR364757-59	-
	KL	OR365112-14	OR632916-18	OR364760-62	-
	MU	OR365115-17	OR632919-21	OR364763-65	-
	NS	OR365118-20	OR632922-24	OR364766-68	-
	LAM	OR365121-23	OR632925-27	OR364769-71	-
<i>M. graminicola</i>	SK	PP593436-38	OR625165-67	-	PP593239-41
	LAT	PP593439-41	OR625168-70	-	PP593242-44
	NS	PP593442-44	OR625171-73	-	PP593245-47

significantly supported (bootstrap values of 99 - 100). Moreover, they were distinctly distinguished from the *M. incognita* clade and other PPNs. These results were consistent with previous identifications of *M. graminicola* using phylogenetic analyses [16,39]. As evidenced by Bridge *et al.* [14] and Ruanpanun and Khun-In [15], only two root-knot species (*M. graminicola* and *M. incognita*) are known to damage rice in Thailand. The results of this study recorded prominent and distinctive features of *M. graminicola*, which clearly distinguish it from *M. incognita*, including both perineal pattern characteristics and the percentage similarity of nucleotide sequences. Hence, the morphological and molecular characterization were in agreement, indicating that across the 3 root-knot nematode isolates identified in this study were *M. graminicola*.

Conclusions

This study provides additional information on PPNs found in RD41 rice fields across all districts of Pathum Thani Province, Thailand. Six genera of PPNs were identified based on their morphology; the predominant genera found in this present study were *Hirschmanniella* sp. and *Meloidogyne* sp. These nematodes were identified using a combination of morphological and molecular characterizations. Although there were some variations in morphology among the nematode populations studied, they were conclusively identified as *H. mucronata* and *M. graminicola* using molecular characterization. Unfortunately, this study found that these nematodes have already spread and heavily infested several intensive rice-cultivating areas in Pathum Thani Province. The high population densities of these 2 nematodes in the rice fields of Pathum Thani can cause various issues in plant growth, such as stunting, leaf discoloration, and root damage, ultimately leading to yield losses. Hence, imminent control measures are likely necessary to restrict their spread to other rice growing areas and to prevent future crop yield losses due to these plant parasites. Various control measures, such as crop rotation with certain legumes, the use of biological control agents, botanical plants, and nematicides, can be effectively utilized to manage *Hirschmanniella* sp. and *Meloidogyne* sp. However, integrating these strategies can lead to more efficient nematode control.

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