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Incidence and Characterization of Rice Root Nematodes, *Hirschmanniella mucronata*, from Rice Fields in Pathum Thani Province, Thailand

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Abstract

The rice root nematodes, *Hirschmanniella oryzae* and *H. mucronata*, are serious and widespread threats to global rice production, especially in tropical and subtropical zones. Reliable and efficient identification of these nematodes is vital to nematode control and management. In this current study, 36 soil or rice root samples were collected from rice fields located in Pathum Thani province and used for nematode extraction. The results demonstrated that *Hirschmanniella* sp. was found in all paddy fields, especially in Muang District, where RD47 rice cultivars were grown at soil pH of 5.9. Molecular identification based on the analysis of 28S rRNA and 18S-ITS1-1.58S genes revealed 98 – 99 % similarity to *H. mucronata*, and its phylogenetic trees were grouped with *H. mucronata* from Cambodia and Philippines. Likewise, the morphometric characterization revealed remarkable features of *H. mucronata* with long body (1,639 - 2,329 µm), long stylet (24 - 27 µm) and an obvious mucron at the end of terminus. Therefore, the main rice root nematode species found in Pathum Thani's rice fields were *H. mucronata*.

Keywords: Nematode identification, *Hirschmanniella mucronata*, Rice cultivars, Pathum Thani

Introduction

Rice (*Oryza sativa* L.) is a vital economic crop of Thailand; both for exportation and household consumption. In the central plain of Thailand, Pathum Thani province has been recorded as one of the most important places for a rice cultivation, with rice planting area of 55 % of the total area and rice production of up to 213,238 tons per year [1,2]. Currently, the main challenges for rice cultivation include the depletion of soil fertility and plant pests due to the practice of continuous rice cultivation for a long time [3]. Numerous countries reported that one of the most serious pests in rice is plant-parasitic nematodes [4,5]. *Hirschmanniella* spp., rice root nematodes, is one of the most prevalent plant-parasitic nematodes that can be found up to 90 % of paddy rice fields. Moreover, this nematode has a wide host range of more than 30 plant species [6,7]. The estimated rice yield losses caused by *Hirschmanniella* sp. around the world are approximately 25 % [7]. One of the most predominant species of *Hirschmanniella* is *H. oryzae*, which causes stunting and up to 60 % reduction in rice tillering. In most cases, the nematode population densities in soil are low and the below ground symptoms such as discoloration, deterioration, and rotting of rice roots are usually unnoticeable by the farmers. However, a decline in plant growth can happen and be readily observed by the experts [8]. In Asian countries, *Hirschmanniella* sp. is widely distributed and infects rice fields. This nematode was identified by molecular and morphological characters as *H. oryzae* and *H. mucronata* in China and Philippines, respectively [9,10]. Furthermore, *H. mucronata* was reported in Cambodia [11,20]. In recent years, *Hirschmanniella* spp. was reported in Nakhon Nayok's rice fields in Thailand [12]. Nevertheless, Bridge *et al.* [13] reported that *H. oryzae* was found in Thailand. Khun *et al.* [11] summarized that *Hirschmanniella* species may result in intraspecific variation when identification is conducted by a sole morphological analysis and this data should be substantiated by molecular

characterization. Therefore, this study was conducted to examine the extent of infestation of *Hirschmanniella* and to identify the species of this nematode collected from Pathum Thani's rice fields by using molecular and morphological characteristics.

Materials and methods

Soil and rice root sampling

Thirty-six soil or rice root samples were collected from paddy rice fields of 6 districts in Pathum Thani province, including Nong Suea, Thanyaburi, Lam Luk Ka, Khlong Luang, Muang and Sam Khok (2 fields/district). In each field, 6 soil or root samples were randomly collected from the rice rhizosphere between 0 - 20 cm depth from the soil surface. After that, the collected soil or rice root samples were separately mixed and 3 sub-samples were chosen from this mixed soil or root samples to be represented (replications) of each field. These composite subsamples were put into plastic bags, labeled, and transported to the laboratory for nematode extraction. In addition, the field data, including rice cultivars and ages, soil pH (soil pH and moisture tester Takemura, Japan) and GPS position (The IOS 7 Compass app) were recorded.

Nematode extraction

Nematodes were extracted from 150 g soil by the Cobb's Sieving and Decantation and the Modified Baermann's Funnel techniques [14]. In brief, nematodes were extracted from soil by pouring the supernatants of soil suspensions into a series of 250, 105 and 37 µm aperture sieve. Nematodes suspended on the 105 and 37 µm mesh sieves were collected and placed on tissue papers lined on a wire screen that had been suspended on the funnels. Two days later, nematodes at the bottom of the funnel were collected, observed, and counted under a compound microscope (Olympus BX50, USA).

For rice root samples, the collected roots from each field were cut into about 1-inch-long pieces and thoroughly mixed. Subsequently, 10 g of roots were randomly selected and used for nematode extraction. Root samples were washed free of soil with tap water, then cut into around 0.5 - 1 cm length and placed on the tissue papers lined on a plastic sieve in the tray. Later on, water was gently added into the tray until the roots were completely submerged [15]. Two days later, nematodes suspended in the tray were collected by 37 µm mesh sieve, observed, and counted under a compound microscope (Olympus BX50, USA).

Statistical analysis

Data were statistically analyzed by the SPSS software (version 26.0; SPSS Inc.; Chicago, IL, USA). Differences in the densities of nematodes among all rice fields were determined by analysis of variance (ANOVA) and the means were compared using Duncan adjustment for multiple comparisons ($p \leq 0.05$).

Molecular identification

Hirschmanniella collected from each district were used for DNA extraction and analysis by the polymerase chain reaction (PCR). Briefly, one adult *Hirschmanniella* was placed in a 0.5 mL PCR tube filled with 25 µL of distilled water. Then, 25 µL of 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 µg/mL proteinase K (Worthington Biochemical, USA)] was added into the tube. The reaction was incubated for 90 min at 65 °C, followed by 5 min at 99 °C in a PCR machine (Biometra Tgradient Thermoblock PCR Thermocycler, UK). Later, the extracted DNA was stored at −20 °C until used as a DNA template [16].

The PCR was conducted by using extracted nematode DNA as the template. 30 µL of PCR reaction included 3 µL of DNA template, 9 µL of sterilized distilled water, 1.5 µL of each 10 µM forward and reverse primers {D2A (5′ ACAAGTACCGTGAGGGAAAGT 3′) and D3B (5′ TGCGAAGGAACCAGCTACTA 3′) [17] and rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') and rDNA1.58s (5'-ACGAGCCCGAGTGATCCACCG-3') [18]}, and 15 µL of 2x PCR master mix with dye solution i-taq (Intron biotechnology, Korea). The PCR condition was programmed as follows: denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, and final extension at 72 °C for 5 min. The PCR products were loaded into 1.5 % agarose buffered gels in 1xTAE buffer. The band size of PCR products was compared with 100+bp DNA marker (Biotechrabbit, Germany). The gel was run for 25 min at 100 V and visualized on an UV box. The PCR products were purified and submitted for sequencing at the SOLGEN Inc., Korea. Then, the DNA

sequences were compared with GenBank in the National Center for Biotechnology Information (NCBI), available online from https://www.ncbi.nlm.nih.gov.

Phylogenetic trees were constructed by the Molecular Evolutionary Genetics Analysis version 7.0 using the DNA sequences at 28S and 18S-ITS1-5.8S gene regions of *Hirschmanniella* obtained from this study. DNA sequences of plant-parasitic nematodes associated with rice were selected from GenBank. Consequently, the alignments between these GenBank nucleotide sequences with those generated from each primer set were done using ClustalW. Phylogenetic trees were performed via the Maximum Likelihood (ML) methods based on Gamma distribution (GTR + G) model and the test of phylogeny done by the rapid bootstrap algorithm (1000 iterations) [19,20].

Morphological identification

A total of 24 *Hirschmanniella* males and females (4 nematodes/district) were killed by hot water at 50 °C and mounted on a drop of distilled water on a glass slide. Then, nematodes were observed and pictured by a digital camera (Canon Power Shot A640), which had been equipped with EOS Utility program and mounted on the compound microscope (Olympus BX50). Sizes of nematodes were measured via Axio Vision SE64 Rel. 4.9.1 program, and finally compared with the polytomous key [11]. The morphometrics were then calculated as follows: L = Total 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×100, Stylet length, Maximum body width, Pharynx length, Anterior end to PIJ, Head to vulva length, Maximum tail width, and Tail length [11].

Results and discussion

Incidence of *Hirschmanniella* in Pathum Thani's rice fields

Hirschmanniella was found in all surveyed rice fields in Pathum Thani province (**Table 1**). In addition, more abundance of *Hirschmanniella* was observed in rice roots than in soil. Highest numbers of nematodes (1,214.6 nematodes/10 g roots) were significantly evident in Muang district no. 1 where RD47 rice cultivar was grown, followed by Sam Khok district no.1 and no.2 (992 and 772.3 nematodes/10 g roots, respectively; RD31 and Pathum Thani 1 cultivars, respectively). However, lowest numbers of nematodes were found in Nong Suea district no.2 and Muang district no. 2 (79.3 and 136 nematodes/10 g roots; RD49 and RD14 cultivars, respectively). The age of rice and soil pH might influence nematode abundance, where greater numbers of nematodes extracted from rice stubbles were observed at soil pH 5.9 - 6.0 than soil pH 5.3 - 5.4. These results were similar to those reports by [11,18,20] who examined *Hirschmanniella* populations in paddy rice fields of Takeo, Battambang and Kampong Thom provinces, Cambodia. In addition, as *Hirschmanniella* are migratory endoparasites by nature, they are more prevalent in rice roots than in soil [21]. Burns [22] mentioned that *Hoplolaimus galeatus* and members of the Tylenchinae-Psilenchinae survived the best at pH 6.0. This information reflects on the results of our experiments, where greater numbers of nematodes were found in rice roots than in soil and the highest density of nematodes was found at pH 5.9 in Muang district's rice field.

Table 1 Incidence and density of *Hirschmanniella* spp. on various rice cultivars and rice fields from Pathum Thani province.

Districts	Location (GPS)	Rice varieties (Age)	Soil pH	Number of nematodes	
				150 g soil	10 g root
Nong Suea 1	14°6'39"N 100°50'45"E	RD47 (110 days)	5.9	8.3±2.6cd[/1]	190.6±21.6e
Nong Suea 2	14°6'46"N 100°50'31"E	RD49 (110 days)	5.8	2.3±0.3cd	79.3±13.3f
Thanyaburi 1	14°2'28"N 100°49'27"E	Phitsanulok 2 (rice stubble)	5.4	5±1.2cd	316.6±18.1d
Thanyaburi 2	14°2'29"N 100°49'28"E	Phitsanulok 2 (rice stubble)	5.3	8±1.7cd	199±16.2e
Lam Luk Ka 1	14°1'42"N 100°49'28"E	Pathum Thani 1 (rice stubble)	5.3	4.6±1.5cd	159±4ef
Lam Luk Ka 2	14°1'41"N 100°49'32"E	Pathum Thani 1 (rice stubble)	5.3	43.3±7.2a	330±13.85d

Districts	Location (GPS)	Rice varieties (Age)	Soil pH	Number of nematodes	
				150 g soil	10 g root
Khlong Luang 1	14°5'35"N 100°38'31"E	RD41 (rice stubble)	5.6	11±2.9c	313±12.7d
Khlong Luang 2	14°5'38"N 100°38'28"E	RD41 (rice stubble)	5.5	44.3±3.7a	349.3±29.7d
Muang district 1	14°2'8"N 100°30'19"E	RD47 (rice stubble)	5.9	34±3.5b	1,214.6±97.8a
Muang district 2	14°2'1"N 100°30'55"E	RD14 (rice stubble)	6.0	2±0.5cd	136±32.3ef
Sam Khok 1	14°6'41"N 100°33'53"E	RD31 (60 days)	6.4	2.33±0.3cd	992±13.85b
Sam Khok 2	14°6'40"N 100°33'38"E	Pathum Thani 1 (rice stubble)	6.7	0.33±0.3d	772.3±17.7c

[1/]mean of the numbers of *Hirschmanniella* ± standard error (n = 3) were compared using Duncan adjustment for multiple comparisons ($p < 0.05$). Similar lower-case letters in each column indicated that means are not significantly different.

Molecular identification

DNA fragments of *Hirschmanniella*, amplified by D2A/D3B and rDNA2/rDNA1.58s, showed the size of 750 and 470 bp, respectively (**Figure 1**). Consequently, a comparison of nucleotide sequences based on 28S rRNA (GenBank accession no. MT597911–MT597916) and 18S-ITS1-5.8S (MT60262 MT602633) gene regions (**Table 2**) showed high homology with *H. mucronata* (more than 98 - 99 % similarity) from Cambodia, Philippines, Taiwan and China. This result coincided with reports by Berg *et al.* [17] who reported the length of amplicon D2-D3 of 28S rRNA and partial 18S rRNA for *Hirschmanniella* spp. of 650 - 800 bp. On the contrary, [20] reported that the size of DNA fragments amplified by rDNA2/rDNA1.58s was 470 bp.

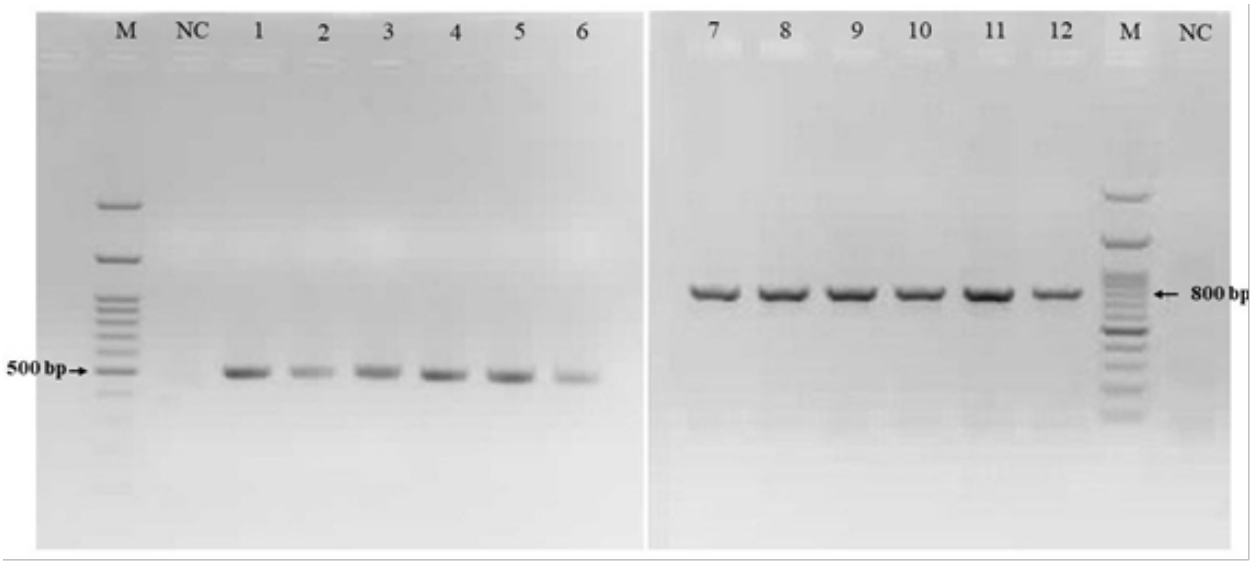

Figure 1 Agarose gel electrophoresis of PCR-amplified DNA of *Hirschmanniella mucronata* by using primers rDNA2/rDNA1.58s (Lane 1 - 6) and D2A/D3B (Lane 7 - 12). M: 100+bp DNA leader, NC: negative control, Lane 1,7: Nong Suea's sample, Lane 2,8: Thanyaburi's sample, Lane 3,9: Lam Luk Ka's sample, Lane 4,10: Khlong Luang's sample, Lane 5,11: Muang district's sample, Lane 6,12: Sam Khok's sample.

Table 2 List of new GenBank accession numbers.

Nematode species	isolate	Location	Sequenced region	GenBank accession number
Hirschmanniella mucronata	PN	Nong Suea	28S	MT597911
H. mucronata	PT	Thanyaburi	28S	MT597912
H. mucronata	PL	Lam Luk Ka	28S	MT597913
H. mucronata	PK	Khlong Luang	28S	MT597914
H. mucronata	PM	Muang district	28S	MT597915
H. mucronata	PS	Sam Khok	28S	MT597916
H. mucronata	PN	Nong Suea	18S-ITS1-5.8S	MT602628
H. mucronata	PT	Thanyaburi	18S-ITS1-5.8S	MT602629
H. mucronata	PL	Lam Luk Ka	18S-ITS1-5.8S	MT602630
H. mucronata	PK	Khlong Luang	18S-ITS1-5.8S	MT602631
H. mucronata	PM	Muang district	18S-ITS1-5.8S	MT602632
H. mucronata	PS	Sam Khok	18S-ITS1-5.8S	MT602633

The phylogenetic tree showed similarity between 28S rRNA and 18S-ITS1-5.8S gene regions (**Figure 2** and **3**), where the nematodes were identified as *H. mucronata* at bootstrap values > 98 and related to *H. kwazuna* and *H. loofi* sisters, while *H. oryzae* were grouped in a different clade with low support. This result was similar to [11,20], who reported that *H. mucronata* was relative *to H. kwazuna* and *H. loofi* sisters, with the single nucleotide polymorphisms (SNPs) at 28S region differed from *H. loofi* and *H. kwazuna* by 7.7 - 8.3 and 8.1 - 8.7 %, respectively, while at ITS1-5.8S-ITS2 by 22.8 - 23.4 and 22.7 – 23 %, respectively.

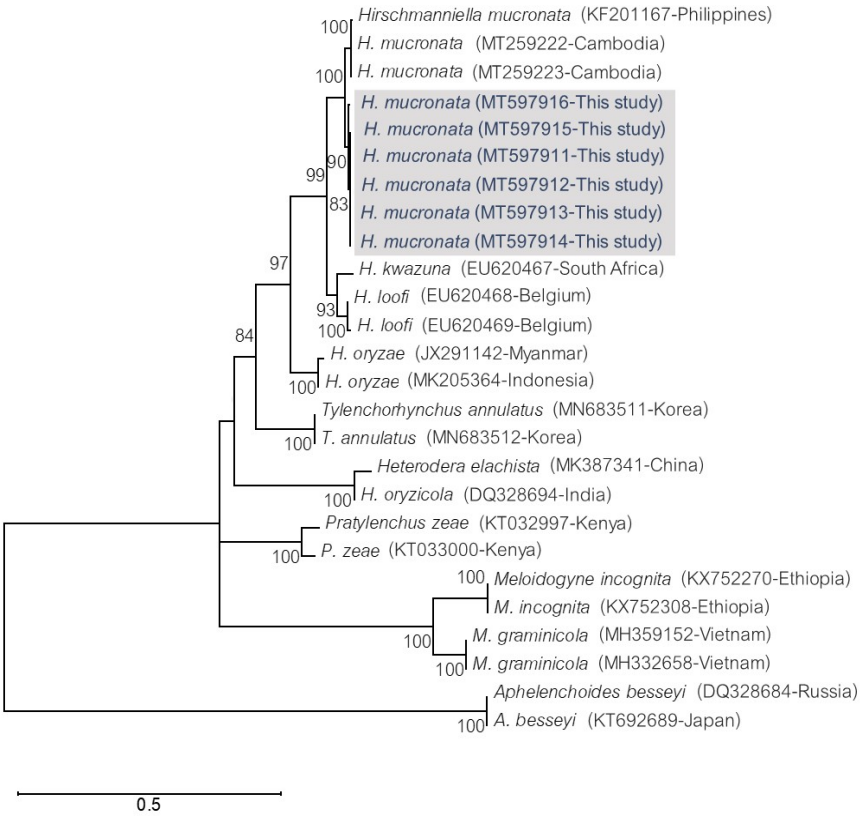

Figure 2 The phylogenetic tree of *Hirschmanniella mucronata* collected from Pathum Thani's rice fields and some other plant-parasitic nematodes in rice based on 28S rRNA gene. Maximum likelihood method was used to create a bootstrap consensus tree inferred from 1,000 replicates. Numbers beside branches represent ML bootstrap support values ≥ 70%. Scale bar represents substitutions per nucleotide position. NCBI accession numbers are listed behind the species names.

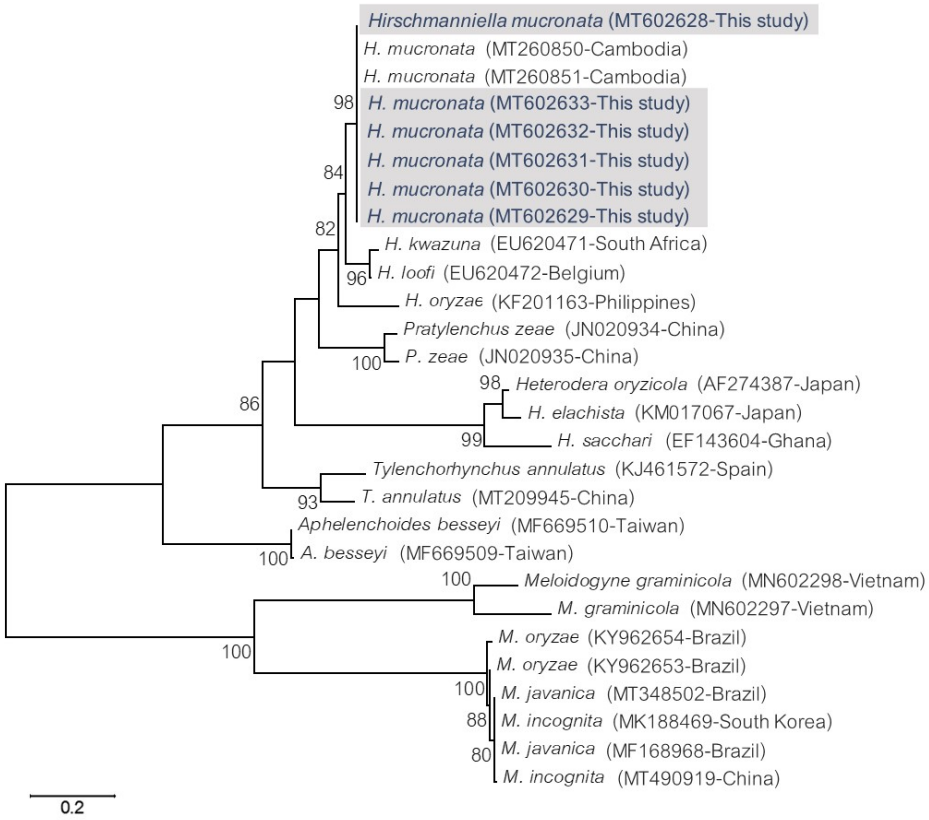

Figure 3 The phylogenetic tree of *Hirschmanniella mucronata* collected from Pathum Thani's rice fields and some other plant-parasitic nematodes in rice based on 18S-ITS1-5.8S genes. Maximum likelihood method was used to create a bootstrap consensus tree inferred from 1,000 replicates. Numbers beside branches represent ML bootstrap support values ≥ 70 %. Scale bar represents substitutions per nucleotide position. NCBI accession numbers are listed behind the species names.

Morphological identification

Morphological characteristics of *Hirschmanniella* were similar between males and females (**Figure 4** and **5**); Body length ranged from 1,639 - 2,329 µm, stylet length 24 - 27 µm, basal knob round, distinctly offset, values of anterior end to pharyngo-intestinal junction (PIJ) 105 - 147 µm, long overlapping of esophagus over intestine, pharyngeal glands elongated, pharynx lengths 217 - 431 µm, tail lengths 65 - 108 µm, maximum tail widths 15 - 25 µm and the end of tails terminal an obvious mucron. For females, the position of the vulva was located approximately 49.2 - 57.3 % of body length with 2 ovaries, while *Hirschmanniella* male tail was possessed with a distinct bursa and spicules of 29 - 36 µm long. Morphometric characteristics were as follows; L= 1,639 - 2,329 µm, V (%) = 49.2 - 57.3, a = 52.4 - 72.8, b = 12.4 - 19, b' = 4.2 - 8.7, c = 17.5 - 30 and c' = 3.1 - 6 (**Table 3**). These characteristics were matched with *H. mucronata*, which [11,20] reported in Battambang and Takeo provinces, Cambodia. Although this study found *H. mucronata*, Bridge *et al.* [13] reported the occurrence of *H. oryzae* in Thailand. The difference between *H. oryzae* and *H. mucronata* includes shorter body length (1,090 - 1,330 µm) and stylet length (15 - 17 µm) of *H. oryzae* than those of *H. mucronata* [9].

Table 3 Morphometrics of *Hirschmanniella mucronata* isolated from Pathum Thani province and their comparison with those of the report from Takeo, Cambodia.

Character	***Hirschmanniella mucronata*** **(This study)**		**Takeo, Cambodia** ***H. mucronata*** **[11]**	
	Female	**Male**	**Female**	**Male**
n	24	24	30	21
L	2,023±140 (2,329 – 1,779)	1,864±131 (2,221 – 1,639)	1,775±188 (2,160 – 1,260)	1,734±186 (2,109 – 1,421)
V (%)	53±1.7 (57.3 – 49.2)	–	52±2.3 (59 – 49)	–
a	63.7±4.2 (72.8 – 55.8)	63.8±6 (75.5 – 52.4)	58±5.2 (67 – 47)	59±7.6 (81 – 45)
b	16±1.2 (19 – 14)	14.5±1.4 (18.5 – 12.4)	14±1.1 (16 – 12)	13.9±1.7 (16.7 – 11)
b’	6.1±1.1 (8.7 – 4.8)	5.5±0.6 (7 – 4.2)	5.9±0.7 (7.4 – 4.4)	5.8±0.7 (7 – 4.5)
c	23.6±3 (30 – 18)	22.3±2.3 (26.7 – 17.5)	22±2.7 (28 – 16)	21±2.2 (25 – 17)
c’	4±0.4 (4.6 – 3.1)	4.5±0.6 (6 – 3.6)	3.7±0.4 (5 – 2.8)	4.4±0.4 (5.1 – 3.5)
Stylet length	25.8±1[/1] (27 – 24)	25.7±1.1 (27 – 24)	22.2±0.6 (23 – 21)	23±1.2 (26 – 21)
Maximum body width	31.8±2.2 (35 – 27)	29.4±2.5 (34 – 23)	30.5±2.3 (35 – 25)	30±3.6 (35 – 22)
Pharynx length	338.4±51.2 (417 – 217)	341.4±39.1 (431 – 263)	300±40 (399 – 229)	299±38 (371 – 215)
Anterior end to PIJ	127.7±5.5 (140 – 117)	128.9±10.5 (147 – 105)	124±12 (147 – 84)	125±10.9 (144 – 108)
Anterior to vulva length	1,077.3±65.5 (1,188 – 958)	–	936±104 (1,160 – 630)	–
Spicule length	-	31.9±1.9 (36 – 29)	–	34±1.6 (37 – 31)
Maximum tail width	21.8±1.8 (25 – 19)	18.9±2.1 (22 – 15)	22±2.3 (27 – 18)	18.5±1 (20 – 16)
Tail length	86.5±7.9 (102 – 74)	84.3±9 (108 – 65)	81±8.2 (99 – 60)	82.6±9.9 (98 – 63)

[/1]All measurements are in µm and in the form: Mean ± S.D. (Max-Min).

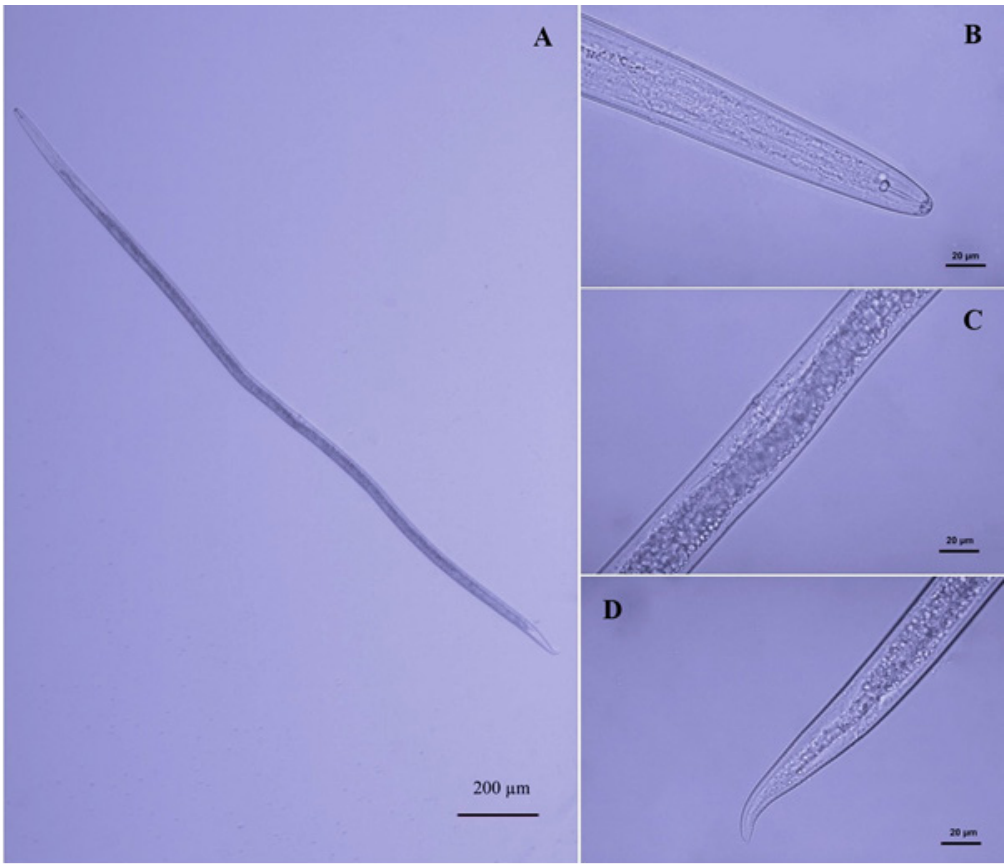

Figure 4 Photographs of *Hirschmanniella mucronata* female collected from paddy rice fields of Pathum Thani province, Thailand. A) Whole body (100×), B) Anterior region (1000×), C) Middle region (1000×), D) Female tail (1000×).

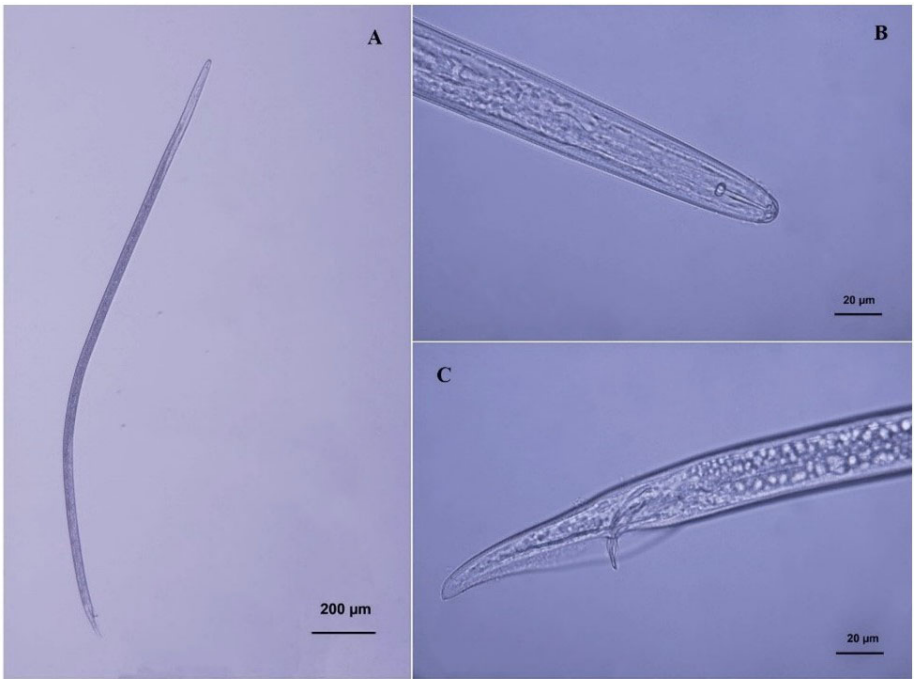

Figure 5 Photographs of *Hirschmanniella mucronata* male collected from paddy rice fields of Pathum Thani province, Thailand. A) Whole body (100×), B) Anterior region (1000×), C) Male tail (1000×).

Conclusions

This study demonstrated that *Hirschmanniella* infested all surveyed rice cultivars and all rice fields located in 6 districts of Pathum Thani province. The highest numbers of nematodes were found in RD47, followed by RD31 and Pathum Thani 1 cultivars, respectively. In addition, soil pH affected the number of nematodes, where higher nematode densities were evident at soil pH 5.9 - 6.0 than pH 5.3 - 5.4. The molecular characteristics based on 28S and 18S-ITS1-5.8S genes revealed 98 - 99 % similarity to *H. mucronata* and its phylogenetic trees were grouped with *H. mucronata* from Cambodia, Philippines, and China. Based on nematode morphology, long body (1,639 - 2,329 µm), long stylet (24 - 27 µm) and obvious mucron at the end of terminus were matched with those of *H. mucronata*. Therefore, the main rice root nematode species found in Pathum Thani's rice fields was *H. mucronata*. Further studies are needed, especially to determine the susceptibility of Thai local cultivars to this nematode.

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References

[1] Rice Department, Available at: http://brrd.ricethailand.go.th/ricemap/riceCD52/index.php-url=detail.php&region_id=1&province_id=13.htm, accessed July 2020.

[2] Office of Agricultural Economics, Available at: http://www.oae.go.th/assets/portals/1/fileups /prcaidata/files/second%20rice%2062%20province%2030-1-63-2(2).pdf, accessed January 2020.

[3] IJ Shelley, M Takahashi-Nosaka, M Kano-Nakata, MS Haque and Y Inukai. Rice cultivation in Bangladesh: present scenario, problems, and prospects. *J. Int. Cooperat. Agr. Dev.* 2016; **14**, 20-9.

[4] JT Jones, A Haegeman, EGJ Danchin, HS Gaur, J Helder, MGK Jones, T Kikuchi, R Manzanilla-Lopez, JE Palomares-Rius, WML Wesemael and RN Perry. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol.* 2013; **14**, 946-61.

[5] GC Bernard, M Egnin and C Bonsi. *The impact of plant-parasitic nematodes on agriculture and methods of control*. *In*: MM Shah and M Mohamood (Eds.). Nematology - concepts, diagnosis and control. InTech, London, 2017, p. 121-51.

[6] ZTZ Maung, PP Kyi, YY Myint, T Lwin and D Waele. Occurrence of the rice root nematode *Hirschmanniella oryzae* on monsoon rice in Myanmar. *Trop. Plant Pathol.* 2010; **35**, 3-10.

[7] MMA Youssef and MFM Eissa. The rice root nematode, *Hirschmanniella oryzae*, its identification, economic importance and control measures in Egypt: A review. *Arch. Phytopathol. Pflanzenschutz* 2014; **47**, 2340-51.

[8] T Kyndt, D Fernandez and G Gheysen. Plant-parasitic nematode infections in rice: molecular and cellular insights. *Annu. Rev. Phytopathol.* 2014; **52**, 135-53.

[9] DY Chen, HF Ni, JH Yen, RS Chen and TT Tsay. Distribution of rice root nematode *Hirschmanniella oryzae* and a new recorded *H. mucronata* (Nematoda: Pratylenchidae) in Taiwan. *Plant Pathol. Bull.* 2006; **15**, 197-210.

[10] MLD Pascual, W Decraemer, I Tandingan De Ley, A Vierstraete, H Steel and W Bert. Prevalence and characterization of plant-parasitic nematodes in lowland and upland rice agro-ecosystems in Luzon, Philippines. *Nematropica* 2014; **44**, 166-80.

[11] K Khun, W Decraemer, M Couvreur, G Karssen, H Steel and W Bert. Deceptive morphological variation in *Hirschmanniella mucronata* (Nematoda: Pratylenchidae) and a polytomous key to the genus. *Nematology* 2015; **17**, 377-400.

[12] K Srimuang and P Ruanpanun. Surveillance of rice nematodes in rice field in Nakhon Nayok province (*in Thai*). *In*: Proceedings of 36th Academic Conference on Rice and Cereals. Rice Department, Bangkok, Thailand. 2019, p. 138-45.

[13] J Bridge, RA Plowright and D Peng. *Nematode parasites of rice. In*: M Luc, RA Sikora and J Bridge (Eds.). Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, 2005, p. 87-130.
[14] JR Christie and VG Perry. Removing nematodes from soil. *Proc. Helminthol. Soc. Wash.* 1951; **18**, 106-8.
[15] DL Coyne, JM Nicol and B Claudius-Cole. *Practical plant nematology: A field and laboratory guide*. 2nd ed. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin, 2014, p. 31-48.
[16] M Holterman, A Wurff, S Elsen, H Megan, T Bongers, O Holovachov, J Bakker and J Helder. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol. Biol. Evol.* 2006; **23**, 1792-800.
[17] EVD Berg, SA Subbotin, ZA Handoo and LR Tiedt. *Hirschmanniella kwazuna* sp. n. from South Africa with notes on a new record of *H. spinicaudata* (Schuurmans Stekhoven, 1944) Luc and Goodey, 1964 (Nematoda: Pratylenchidae) and on the molecular phylogeny of *Hirschmanniella* Luc and Goodey, 1964. *Nematology* 2009; **11**, 523-40.
[18] M Suong, E Chapuis, V Leng, F Tivet, D Waele, HN Thi and S Bellafiore. Impact of a conservation agriculture system on soil characteristics, rice yield, and root-parasitic nematodes in a Cambodian lowland rice field. *J. Nematol.* 2019; **51**, 1-15.
[19] G Besnard, N Thi-Phan, H Ho-Bich, A Dereeper, HT Nguyen, P Queneherve, J Aribi and S Bellafiore. On the close relatedness of two rice-parasitic root-knot nematode species and the recent expansion of *Meloidogyne graminicola* in Southeast Asia. *Genes* 2019; **10**, 175.
[20] N Beesa, A Sasnarukkit, K Jindapunnapat, F Tivet, S Bellafiore and B Chinnasri. Species characterization and population dynamics of *Hirschmanniella mucronata* in lowland rice fields managed under conservation agriculture in Cambodia. *J. Saudi Soc. Agr. Sci.* 2021; **20**, 137-45.
[21] D Peng, HS Gaur and J Bridge. *Nematode parasites of rice*. *In*: RA Sikora, D Coyne, J Hallmann and P Timper (Eds.). Plant parasitic nematodes in subtropical and tropical agriculture. 3rd ed. CAB Publishing, Oxfordshire, 2018, p. 120-62.
[22] NC Burns. Soil pH effects on nematode populations associated with soybeans. *J. Nematol.* 1971; **3**, 238-45.