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Morphological and Molecular Characterization of Predominant Plant-Parasitic Nematodes Associated with Pattawia Pineapple-Cultivated Areas in Prachuap Khiri Khan, Thailand

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Abstract

The predominant plant-parasitic nematodes associated with Pattawia pineapple-cultivated areas were investigated using combined molecular and morphological techniques. In total, 134 soil samples were collected for nematode extraction from the main pineapple crops in 3 districts (Sam Roi Yot, Mueang and Hua Hin) of Prachuap Khiri Khan, Thailand. Based on the morphology, 6 plant-parasitic nematode genera were identified: *Rotylenchulus* sp., *Meloidogyne* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Mesocriconema* sp. and *Pratylenchus* sp. The main plant-parasitic nematodes that occurred in Sam Roi Yot and Mueang districts were *Rotylenchulus* sp. (70.27 and 97.01 %, respectively), while in Hua Hin district, *Helicotylenchus* sp. (37.73 %) was dominant. Based on molecular identification, nucleotide sequence comparisons and the phylogenetic tree analyses based on the 18S and 28S rRNA genes revealed the species of the nematodes were *R. reniformis* and *H. dihystera*, showing 99 % identity with the nematode populations from Japan and China, respectively. Notably, the morphometric and morphological characters also identified these 2 species correctly; however, this technique required more time and may be less accurate. While *R. reniformis* has been identified as a common species in pineapple fields in the USA, India, Kenya, the Philippines and Nigeria, the current study was the 1st to report *H. dihystera* isolated from soil in Pattawia pineapple crops in Thailand.

Keywords: Plant-parasitic nematodes, Pineapple, *Rotylenchulus reniformis*, *Helicotylenchus dihystera*

Introduction

The Pattawia or Smooth Cayenne pineapple (*Ananas comosus*) is a tropical plant with an edible fruit and is one of the most important crops processed into canned food of Thailand, with planting areas around 72,371 ha [1]. The 5 major provinces of pineapple production in Thailand are Prachuap Khiri Khan (27,058 ha), Ratchaburi (6,346 ha), Phetchaburi (4,470 ha), Phitsanulok (4,162 ha) and Rayong (3,594 ha) [1]. Currently, several diseases cause problems in pineapple production such as wilt disease vectored by mealybugs, citrus pink disease, bacterial heart rot, anthracnose, fungal heart rot, root rot, black rot, fruitlet core rot, butt rot and yellow spot virus [2]. In addition, plant-parasitic nematodes (PPNs) are a pathogen that causes the loss of approximately 1 - 40 % in pineapple production [3-4]. There have been reports on

the widespread occurrence of PPNs such as *Rotylenchulus reniformis*, *Helicotylenchus* spp., *Meloidogyne* spp., *Hoplolaimus* sp., *Tylenchorhynchus* spp., *Pratylenchus brachyurus*, *Criconemoides* spp. and *Paratylenchus* spp. in pineapple fields in the USA, India, Kenya, the Philippines and Nigeria [5-11]. Damage by PPNs to pineapple roots results in reduced root function, deterioration of the plants and eventual death, and reduced yield (> 60 %), as well as diminished fruit quality (smaller fruit size when compared with healthy plants) [12]. In Thailand, there have been few published reports on surveys of PPNs in pineapple fields. For example, Saipetara [13] studied pineapple fields in Chonburi province using morphological analysis and identified 7 PPNs, *Meloidogyne incognita*, *Helicotylenchus erythrinae*, *Hoplolaimus seinhorsti*, *Criconemoides curvatum*, *Pratylenchus brachyurus*, *Aphelenchus eremitus* and *Tylenchus filiformis*. The correct diagnosis is very important for successful nematode management, due to the different species of PPNs as well as using different methods for identification [14]. For example, tomato plants containing the *Mi* gene are resistant to *Meloidogyne incognita*, but not to *M. enterolobii* due to its high virulence and aggressiveness [15]. Currently, polymerase chain reaction (PCR) techniques have been widely applied for nematode identification because of their accuracy and the speed of use as a morphological technique [14]. Berg *et al*. [16], Douda *et al.* [17], Nyaku *et al.* [18], Riascos-Ortiz *et al.* [19] and Beesa *et al.* [20] successfully amplified the DNA target of 18S and 28S rRNA genes for *Hirschmanniella* spp., *Globodera* spp., *Rotylenchulus* sp. and *Helicotylenchus* spp. However, the combination of morphological and molecular techniques can be used to obtain more precise and reliable information. Therefore, the objectives of the current study were to identify the predominant PPN species in pineapple fields of Prachuap Khiri Khan province, the most planting area in Thailand using molecular and morphological techniques.

Materials and methods

Soil sampling

In total, 134 soil samples were collected from randomly selected Pattawia pineapple-cultivated areas (1 sample per field) in Prachuap Khiri Khan province, Thailand, during September 2016: 42 samples from Sam Roi Yot district, 40 samples from Mueang district and 52 samples from Hua Hin district. Each soil sample consisted of 4 composite subsamples that were randomly collected in the rhizosphere of heathy plants at a depth of 5 - 20 cm from the soil surface in each field. For each composite soil sample, 500 g were selected, packed into plastic bags and identified based on a sample number. Furthermore, soil temperature (Hanna HI98509), soil moisture and pH (Takemura DM-15, Japan) were also noted.

Nematode extraction

PPNs were extracted from a 300 g soil sample using a combination of Cobb's Sieving and Baermann's Funnel techniques [21,43]. In brief, PPNs were extracted from each soil sample by pouring the supernatants of the soil suspensions into a series of 250, 105 and 38 µm aperture sieves. The PPNs suspended on 105 and 38 µm mesh screens were collected and poured onto 2 pieces of tissue papers lining a wire screen that had been suspended on the funnels**.** The PPNs at the bottom of the funnel were collected after 48 h, observed, and counted under a stereoscopic microscope (Olympus SZ60).

Molecular analysis

The highest abundance of observed PPNs from each district was identified to the species level using PCR based on the 18S and 28S rRNA genes. The genomic DNA samples were extracted from 1 adult PPNs using the Holterman *et al.* [22] method. PCR was carried out using extracted nematode DNA as the

template. In total, the 30 µL of PCR reaction consisted of 3 µL of DNA template, 9 µL of sterilized distilled water, 1.5 µL of each 10 µM forward and reverse primer (648/136 primer for the 18S rRNA gene [23] and D2A/D3B primer for the D2 - D3 expansion segments of 28S rRNA [16] and 15 µL of 2×PCR master mix with dye solution i-taq (Intron Biotechnology, Korea). The PCR conditions were denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min with a final extension at 72 °C for 5 min. The PCR products were loaded into 1.5 % agarose buffered gels in 1×TAE buffer. The band sizes of the PCR products were compared with the 100 bp DNA ladder (Biotechrabbit, Germany). Each gel was run for 25 min at 100 V and visualized in an UV chamber. The PCR products were purified and sequenced by Solgen Inc., Korea. Then, the obtained DNA sequences were compared and deposited in the National Center for Biotechnology Information (NCBI), available online at https://www.ncbi.nlm.nih.gov/.

Phylogenetic trees were studied using the Molecular Evolutionary Genetics Analysis software version 7.0 with the DNA sequences at the 18S and 28S rRNA gene regions of the PPNs obtained from the current study. In addition, DNA sequences of other PPNs were chosen from GenBank as the out group. Subsequently, the alignments of these nucleotide sequences of each primer set were carried out using the ClustalW software. Phylogenetic trees were built using maximum likelihood (ML) methods based on the gamma distribution (GTR + G) model and the phylogeny was tested using 500 iterations of the rapid bootstrap algorithm [24].

Morphometric and morphological analyses

The predominant PPNs were identified according to the procedures of Suwanngam [23]. The nematodes were killed using immersion in hot water at 50 °C before mounting on a drop of distilled water on a glass slide that was supported and sealed with colorless enamel. Then, the PPNs were observed and photographed using a digital camera (Canon EOS 750D, Japan) equipped with the EOS Utility program and mounted on a compound microscope (Olympus BX50). Nematodes were measured using the Axio Vision SE64 program, and finally compared with the Key to Genera of Plant-Parasitic Nematodes [25-27]. Then, the morphometric values were calculated according to the De Man Formula [28] based on the parameters; L = total body length; a = body length/body width; b' = body length/distance from anterior end of body to posterior end of pharyngeal glands; c = body length/tail length; c' = tail length/body diameter at the anal/cloacal aperture; V % = head to vulva length/body length×100; stylet length; maximum body width; pharynx length; anterior end to vulva length; maximum tail width and tail length. For *R. reniformis* from Sam Roy Yot and Mueang districts, differences among nematode males or females were determined based on the Student's paired-plot design test at the 0.05 significance level.

Results and discussion

Occurrence of plant-parasitic nematodes

The soil properties in the pineapple fields in the 3 districts of Prachuap Khiri Khan were Sam Roi Yot (pH 3 - 7, soil moisture content 5.02 - 20.90 % and soil temperature 29.3 - 37.2 °C); Mueang (pH 3 - 6.8, soil moisture content 4.82 - 19.13 % and soil temperature 29.5 - 35.9 °C) and Hua Hin (pH 3 - 7, soil moisture content 1.87 - 14.95 %). In total, 1,992 PPNs were isolated from the 134 soil samples that were identified into 6 genera based on their morphological features, consisting of *Rotylenchulus* sp., *Meloidogyne* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Mesocriconema* sp. and *Pratylenchus* sp. (**Figure 1**). In Sam Roi Yot and Mueang districts, the dominant PPNs were *Rotylenchulus* sp. (70.27 and 97.01 %, respectively), followed by *Helicotylenchus* sp. (12.04 and 2.08 %, respectively) and *Hoplolaimus* sp. (7.37 and 0.78 %, respectively) (**Table 1**). In contrast, *Rotylenchulus* sp. was not very common in the sampled pineapple fields

in Hua Hin district, with *Helicotylenchus* sp. (37.73 %) and *Mesocriconema* sp. (26.42 %) being the most abundant, respectively. Thus, the results indicated that *Rotylenchulus* sp. were very common in pineapple fields in Sam Roi Yot and Mueang districts. In Thailand, *Rotylenchulus* sp. were first observed in pineapple fields (unidentified isolated source) approximately 50 years ago by Chunram [29]. To date, there has been no further report on the occurrence of *Rotylenchulus* sp. damaging pineapple in Thailand.

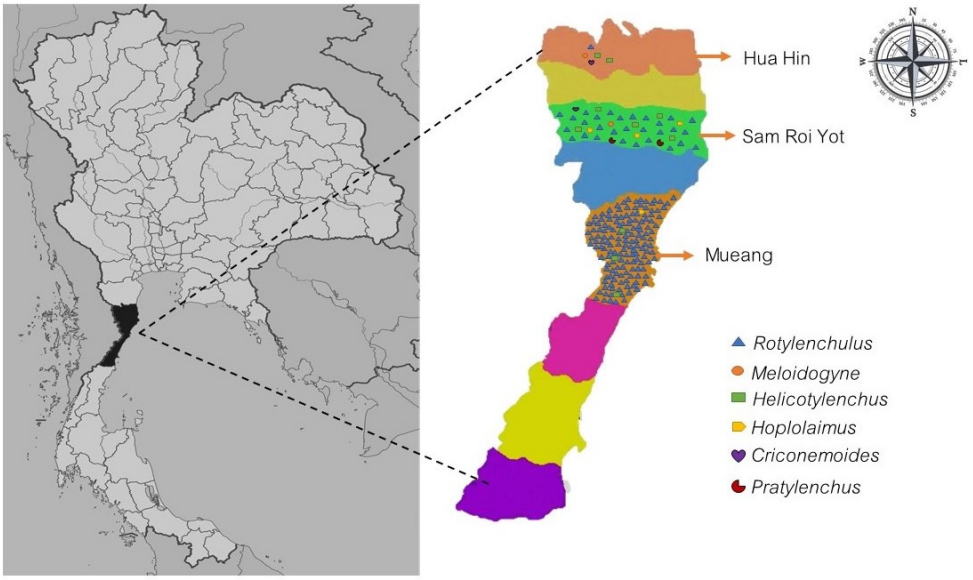

Figure 1 Occurrence of plant-parasitic nematodes associated with Pattawia pineapple cultivated-areas in Prachuap Khiri Khan, Thailand, where symbols refer to 10 individual nematodes.

Table 1 Plant-parasitic nematode genera extracted from soil in Pattawia pineapple fields of Prachuap Khiri Khan, Thailand.

District	Total number of soil samples or fields	Plant-parasitic nematode genera	Total number of presented nematodes	Number of soil samples presenting nematode genera	Nematode occurrence (%)
Sam Roi Yot	42	*Rotylenchulus*	286	22	70.27
		Helicotylenchus	49	13	12.04
		Hoplolaimus	30	6	7.37
		Pratylenchus	17	9	4.18
		Mesocriconema	13	5	3.19
		Meloidogyne	8	5	1.97
Mueang	40	*Rotylenchulus*	1,492	24	97.01
		Helicotylenchus	32	6	2.08
		Hoplolaimus	12	3	0.78
Hua Hin	52	*Helicotylenchus*	20	9	37.73
		Mesocriconema	14	5	26.42
		Meloidogyne	13	4	24.53
		Rotylenchulus	6	3	11.32

The 6 PPNs identified in pineapple fields in the current study were similar to those reported by Rohrbach and Apt [5], Chinnasri *et al.* [6], Devi [7], Kiriga [8], Daramola and Afolami [9], Benzonan *et al.* [10] and Kiriga *et al.* [11], who found several kinds of PPN, including *R. reniformis*, *Helicotylenchus* spp., *Meloidogyne* spp., *Hoplolaimus* sp., *Tylenchorhynchus* spp., *Pratylenchus brachyurus*, *Criconemoides* spp. and *Paratylenchus* spp., in pineapple fields in the USA, India, Kenya, the Philippines and Nigeria. Population densities of preplant PPNs are mostly related to crop yield, with the economic threshold of the reniform nematode (*R. reniformis*) being 310 nematodes/250 cm^3 soil and root-knot nematodes (*Meloidogyne* spp.) being 1 egg/100 cm^3 soil [30,31]. Therefore, nematode management should be considered when high populations of these nematodes are observed in crop fields.

Environmental factors affecting nematode growth include soil pH, soil moisture, soil temperature, soil structure and the kinds of plant [32,33]. The soil type requirement for the growth of pineapple is a well-drained sandy loam at pH 4.5 - 5.5, which ideally promotes the growth of several PPNs [10]. For example, the optimal pH levels for *Rotylenchulus* spp. and *M. javanica* are within wide ranges of approximately 4.8 - 5.2 and 4.0 - 8.5, respectively, in which pineapple is usually grown [34]. Peacock [32] and Wallace [35] reported that the optimal temperature of *M. javanica* for egg hatching, movement and invasion is approximately 20 - 30 °C. Burns [36] mentioned that *P. alleni*, *Hoplolaimus galeatus* and members of the Tylenchinae-Psilenchinae survived best at soil pH 6.0. These reports supported our results where the numerous PPNs were observed in the Pattawia pineapple fields of Prachuap Khiri Khan, Thailand.

Molecular identification

The predominant PPNs, *Rotylenchulus* sp. (from Sam Roy Yot and Mueang districts) and *Helicotylenchus* sp. (from Hua Hin district), were identified using a PCR technique based on amplification of the 18S and 28S rRNA genes. The DNA products showed a fragment size of approximately 900 and 790 bp in length, respectively. These results coincided with Berg *et al.* [16], Douda *et al.* [17], Nyaku *et al.* [18], Riascos-Ortiz *et al.* [19], Beesa *et al.* [20] and Singh *et al.* [37] who reported the length of the amplicon D2 - D3 of 28S rRNA for several PPNs as 650 - 800 bp and the 18S rRNA of *R. reniformis* and *Rotylenchus* sp. in the family Hoplolaimidae was 653 - 903 bp. The DNA products were sequenced and compared with the NCBI database, with a 98 - 99 % sequence similarity with those of *R. reniformis* and *H. dihystera* in both DNA regions. Consequently, all nucleotide sequences were deposited in GenBank under the accession numbers in **Table 2**. In addition, these 18S and D2 - D3 rRNA sequences were used to construct a phylogenetic tree using the MEGA7 software. The observed PPNs of the current study were grouped with *R. reniformis* samples from Japan and China, and *H. dihystera* samples from China and the USA for both the amplified gene regions (**Figures 2** and **3**).

Table 2 List of new accession numbers of *Rotylenchulus reniformis* and *Helicotylenchus dihystera* found in Pattawia pineapple fields from Prachuap Khiri Khan, Thailand.

Nematode species	Isolate	Location	Accession number	
			18S rRNA	28S rRNA
Rotylenchulus reniformis	SRY1	Sam Roi Yot	OL614940	OK275496
R. reniformis	SRY2	Sam Roi Yot	OL614941	OL415760
R. reniformis	SRY3	Sam Roi Yot	OL614942	OL415761
R. reniformis	AM1	Mueang	OL614943	OL415762
R. reniformis	AM2	Mueang	OL614944	OL415763
R. reniformis	AM3	Mueang	OL614945	OL415764

Nematode species	Isolate	Location	Accession number	
			18S rRNA	28S rRNA
Helicotylenchus dihystera	HH1	Hua Hin	OL614946	OL415765
H. dihystera	HH2	Hua Hin	OL614947	OL415766
H. dihystera	HH3	Hua Hin	OL614948	OK275497

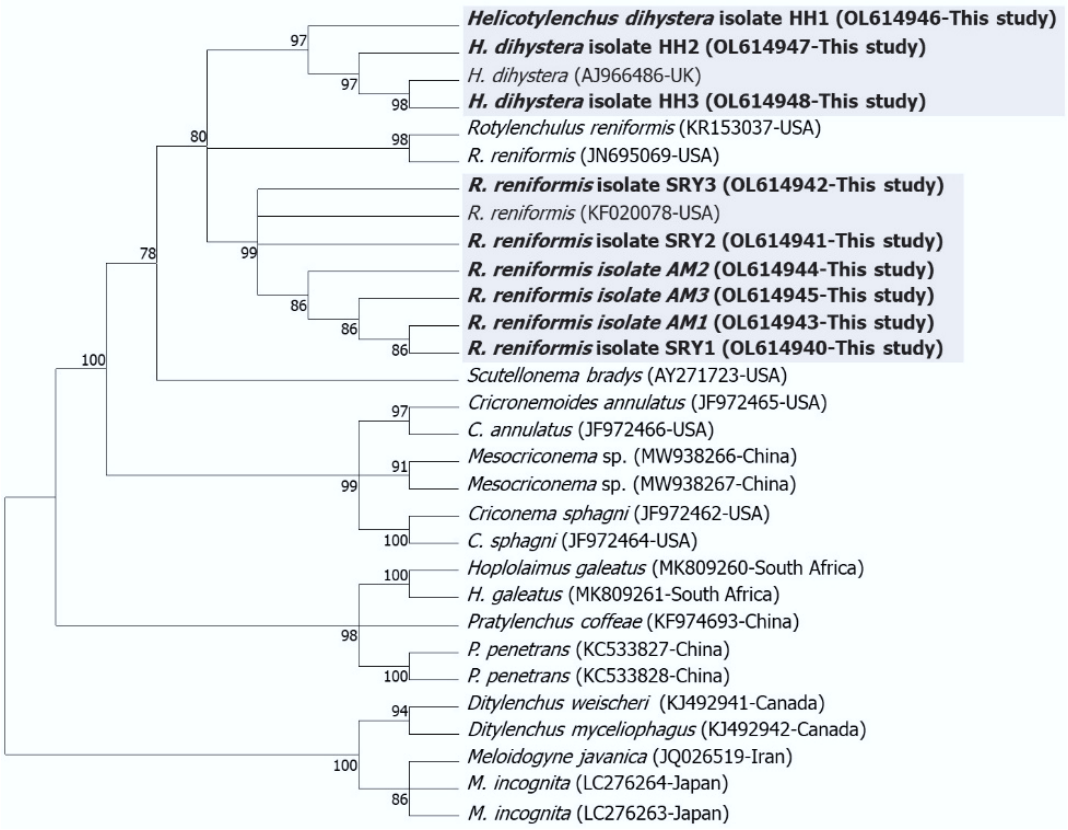

Figure 2 Phylogenetic tree of predominant plant-parasitic nematodes extracted from Pattawia pineapple fields of Prachuap Khiri Khan, Thailand and some other plant-parasitic nematodes based on the 18S rRNA gene. ML method used to create a bootstrap consensus tree inferred from 500 replicates. Numbers beside branches represent ML bootstrap support values 70 % NCBI accession numbers are shown after species names.

Morphological and morphometric identification

The most abundant PPNs were identified based on their morphological features.

Rotylenchulus reniformis males: The results illustrated that there were no significant differences in the morphometric values between Sam Roy Yot and Mueang specimens with ranges for body length (n = 20) of 366.0 - 466.0 µm (**Figures 4(B) - 4(D)**), stylet length from 14.0 - 18.4 µm with rounded basal knobs, pharynx length from 94.6 - 134.2 µm, maximum body width from 13.3 - 19.3 µm, tail length from 23.7 - 43.0 µm and anal body width (maximum tail width) from 9.0 - 13.5 µm. The spicule was elongate-slender with 16.0 - 21.0 µm long and ventrally curved. De Man ratios were: a = 22.2 - 31.4, b' = 2.9 - 4.6, c = 9.40 - 17.8 and c' = 1.8 - 3.9 (**Table 3**).

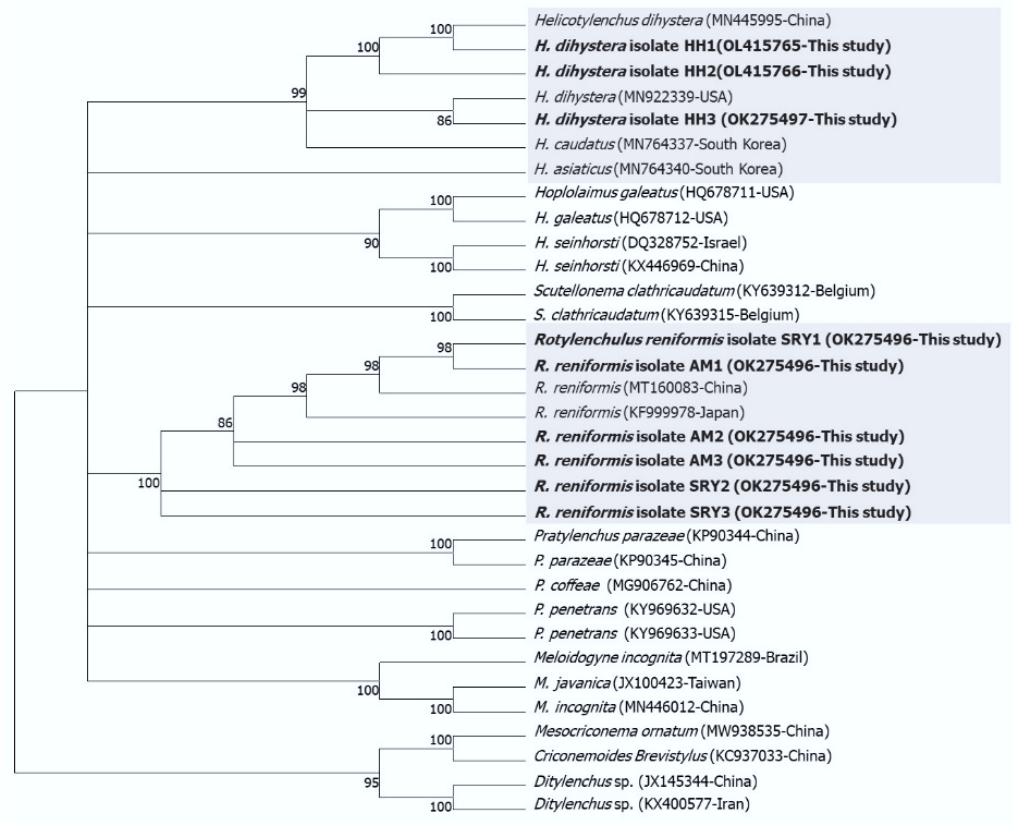

Figure 3 Phylogenetic tree of predominant plant-parasitic nematodes extracted from Pattawia pineapple fields of Prachuap Khiri Khan, Thailand and some other plant-parasitic nematodes based on the 28S rRNA gene. ML method used to create a bootstrap consensus tree inferred from 500 replicates. Numbers beside branches represent ML bootstrap support values 70 % NCBI accession numbers are shown after species names.

Rotylenchulus reniformis immature females: We found similar morphological traits in the comparison of specimens from Sam Roy Yot and Mueang districts (**Figures 4(A) - 4(C)**). The body length of immature females (n = 7) ranged from 376.0 - 429.0 µm, stylet length from 14.0 - 18.0 µm, pharynx length from 91.0 - 145.0 µm, maximum body width from 15.0 - 20.0 µm, tail length from 26.0 - 37.0 µm and anal body width (maximum tail width) from 10.0 - 14.0 µm. De Man ratios were: V = 68.8 - 76.7, a = 21.1 -27.6, b' = 2.9 - 4.3, c = 10.4 - 15.1 and c' = 2.1 - 3.4 (**Table 3**).

These morphological and morphometric features matched with *R. reniformis*, found in cotton from the USA [38], tomato from China [39] and bananas from Brazil and Colombia [19]. In the current study, *R. reniformis* immature females slightly appeared, due probably to the soil samples being collected during a period of nematode attack and developing into mature females in the kidney shape on the pineapple roots.

Helicotylenchus dihystera females: Their morphological features illustrated the body of the nematodes as completely spiraled (**Figure 5**), some of them were slightly C-shaped. The body length was in the range 498.0 - 849.3 µm, lip region rounded, stylet robust with length in the range 21.8 - 29.8 µm, esophageal glands overlapping the intestine ventrally, pharynx length in the range 73.4 - 169.8 µm, the reproductive system (vulva) was located at 45.9 - 67.7 % of the body length, maximum body width was in the range 21.7 - 31.6 µm, tail length in the range 9.2 - 32.9 µm and maximum tail width in the range 9.6 - 19.5 µm. The morphometric values were; a = 17.6 - 28.2, b' = 3.8 - 9.6, c = 20.3 - 67.5 and c' = 0.7 - 2.2 (**Table 4**). These

characteristics corresponded well with *H. dihystera* populations from maize in Argentina [40] and *Musa* sp. in Columbia [41]. However, the nematode sizes in the current study were slightly bigger than in these other reports **[**40,41**]**. So *et al.* [42] reported that nematode size was dependent on sex and growth temperature. In the current study, *H. dihystera* male was not observed in the sampled pineapple fields, as was also reported by Brucher *et al.* [40] in maize fields.

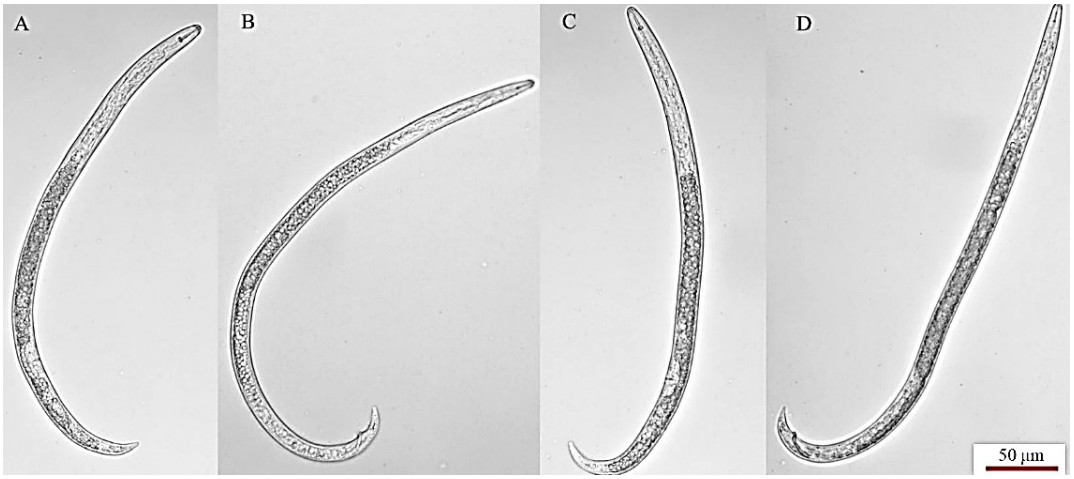

Figure 4 Photomicrographs of *Rotylenchulus reniformis* immature females **(**(A**)** and **(**C**))** and adult males **(**(B**)** and **(**D**))** extracted from Pattawia pineapple fields of Sam Roy Yot **(**(A**)** and **(**B**))** and Mueang ((C) and D**))** districts, Prachuap Khiri Khan province, Thailand**.**

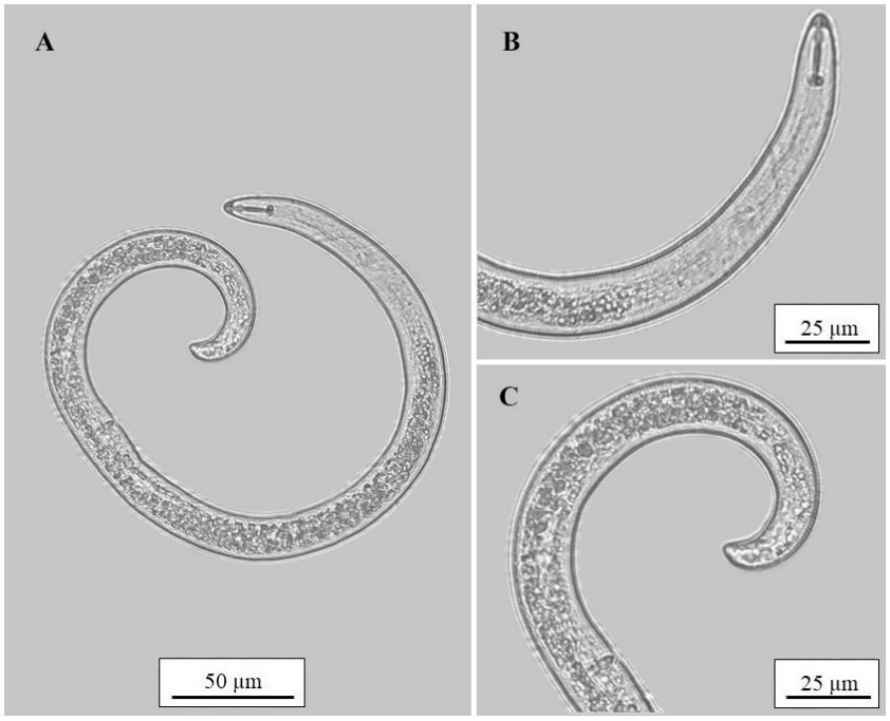

Figure 5 Photomicrographs of *Helicotylenchus dihystera* female collected from Pattawia pineapple fields of Hua Hin district, Prachuap Khiri Khan province, Thailand. (A) Whole body of female, (B) Anterior region and (C) Posterior region.

Table 3 Morphometrics of *Rotylenchulus* sp**.** isolated from Pattawia pineapple fields of Prachuap Khiri Khan, Thailand and their comparison with *Rotylenchulus reniformis* as reported by Agudelo *et al.* **[**38**].** The values are mean ± standard deviation (minimum**-**maximum).

Morphometric character	**This study**				***Rotylenchulus reniformis* [38]**	
	Immature female		**Adult male**		**Immature female**	**Adult male**
Location	**Sam Roy Yot**	**Mueang**	**Sam Roy Yot**	**Mueang**	**USA**	**USA**
N	7	7	20	20	20	20
L	406.4 ± 18.5 (379.0 ± 429.0)	395.9 ± 12.4 (376.0 - 414.0)	417.2 ± 25.9 (366.0 - 466.0)	401.5 ± 24.0 (351.0 - 442.0)	403.8 (365.0 - 430.0)	410.0 (375.0 - 480.0)
V	72.9 ± 2.9 (68.8 - 76.7)	72.7 ± 2.1 (69.4 - 74.9)	-	-	70.4 (66.0 - 74.0)	-
a	24.1 ± 2.3 (21.1 - 27.5)	24.6 ± 2.0 (22.1 - 27.6)	27.9 ± 1.9 (24.2 - 31.4)	25.5 ± 2.1 (22.2 - 29.5)	25.1 (21.5 - 28.)	27.6 (23.4 - 31.9)
b'	3.3 ± 0.6 (2.9 - 4.3)	3.6 ± 0.5 (3.1 - 4.1)	3.7 ± 0.4 (2.9 - 4.6)	3.7 ± 0.3 (3.0 - 4.5)	-	-
c	12.9 ± 1.2 (11.5 - 14.3)	13.2 ± 1.6 (10.4 - 15.1)	14.0 ± 2.1 (10.7 - 17.8)	12.8 ± 1.8 (9.4 - 16.4)	14.1 (10.0 - 16.3)	14.2 (12.2 - 18.3)
c'	2.9 ± 0.4 (2.1 - 3.4)	2.7 ± 0.4 (2.2 - 3.2)	2.8 ± 0.5 (1.8 - 3.6)	2.9 ± 0.4 (2.3 - 3.9)	2.9 (2.3 - 3.7)	2.9 (2.3 - 4.5)
Stylet length	16.7 ± 1.3 (15.0 - 18.0)	16.4 ± 1.6 (14.0 - 18.0)	15.7 ± 1.0 (14.0 - 17.8)	16.3 ± 0.8 (15.0 - 18.4)	19.4 (17.0 - 21.0)	14.9 (13.0 - 16.0)
Max. body width	17.0 ± 1.7 (15.0 - 20.0)	16.1 ± 1.1 (15.0 - 18.0)	15.0 ± 1.4 (13.3 - 18.0)	15.8 ± 1.3 (14.2 - 19.3)	16.2 (15.0 - 18.0)	15.0 (13.0 - 18.0)
Pharynx length	124.1 ± 18.0 (100.0 - 145.0)	111.6 ± 15.0 (91.0 - 129.0)	112.1 ± 10.2 (94.6 - 133.2)	110.6 ± 10.2 (98.4 - 134.2)	128.5 (115.0 - 165.0)	112.8 (100.0 - 125.0)
Anterior end to vulva length	296.1 ± 12.7 (278.0 - 311.0)	288.0 ± 13.0 (272.0 - 302.0)	-	-	284.3 (255.0 - 310.0)	-
Tail length	31.7 ± 2.9 (29.0 - 36.0)	30.4 ± 3.8 (26.0 - 37.0)	30.3 ± 4.1 (23.7 - 37.3)	31.9 ± 4.5 (26.2 - 43.0)	28.9 (25.0 - 38.0)	29.1 (23.0 - 36.0)
Max. tail width	11.0 - 1.4 (10.0 - 14.0)	11.4 ± 1.1 (10.0 - 13.0)	11.0 ± 1.2 (9.0 - 13.5)	10.9 ± 1.2 (9.1 - 13.5)	10.0 (8.0 - 12.0)	10.2 (8.0 - 11.0)
Spicule length	-	-	18.2 ± 1.0 (16.0 - 20.0)	18.2 ± 1.1 (17.0 - 21.0)	-	20.9 (18.0 - 23.0)

L**:** Total body length, a**:** Body length/body width, b'**:** Body length/distance from anterior end of body to posterior end of pharyngeal glands, c**:** Body length/tail length, c'**:** Tail length/body diameter at the anal/cloacal aperture and V**:** Head to vulva length/body length×100**.** Differences between *R. reniformis* males or females from Sam Roy Yot and Mueang specimens were determined based on Student's paired-plot design test at 0.05 significance level**.**

Table 4 Morphometrics of *Helicotylenchus* sp**.** isolated from Pattawia pineapple fields of Prachuap Khiri Khan, Thailand and their comparisons with *Helicotylenchus dihystera* as reported by Brucher *et al.* **[**40**]** and Riascos-Ortiz *et al.* **[**41**].** The values are mean ± standard deviation (minimum**-**maximum).

Morphometric character	**This study**	***H. dihystera* [40]**	***H. dihystera* [41]**
Location	Hua Hin (Thailand)	Argentina	Columbia
N	16	25	10
L	691.4 ± 105.7 (498.0 - 849.3)	686.0 ± 62.5 (527.0 - 796.0)	620.0 ± 80.0 (530.0 - 770.0)
V	61.5 ± 4.8 (45.9 - 67.7)	63.9 ± 2.0 (61.2 - 69.2)	64.6 ± 1.2 (62.2 - 66.4)
a	24.2 ± 2.7 (17.6 - 28.2)	26.0 ± 3.1 (22.5 - 32.6)	25.5 ± 1.9 (22.9 - 29.7)
b'	5.0 ± 1.3 (3.8 - 9.6)	4.9 ± 0.5 (4.0 - 5.9)	-
c	36.3 ± 13.8 (20.3 - 67.5)	41.2 ± 5.2 (34.2 - 49.2)	43.8 ± 8.4 (25.1 - 55.5)
c'	1.4 ± 0.4 (0.7 - 2.2)	1.2 ± 0.1 (0.8 - 1.4)	1.0 ± 0.2 (0.8 - 1.3)
Stylet length	25.1 ± 2.6 (21.8 - 29.8)	25.6 ± 1.6 (23.7 - 28.0)	24.4 ± 1.0 (23.0 - 26.0)
Max. body width	28.5 ± 2.7 (21.7 - 31.6)	25.6 ± 3.2 (19.6 - 31.6)	-
Pharynx length	142.3 ± 22.4 (73.4 - 169.8)	146.8 ± 11.4 (122.5 - 168.3)	-
Anterior end to vulva length	426.7 ± 77.9 (294.5 - 521.5)	436.4 ± 38.5 (341.5 - 501.2)	-
Tail length	21.4 ± 7.6 (9.2 - 32.9)	17.0 ± 2.2 (11.4 - 21.3)	14.5 ± 3.1 (11.0 - 21.0)
Max. tail width	15.0 ± 3.2 (9.6 - 19.5)	14.2 ± 1.3 (10.8 - 16.2)	-

L**:** Total body length, a**:** Body length/body width, b'**:** Body length/distance from anterior end of body to posterior end of pharyngeal glands, c**:** Body length/tail length, c'**:** Tail length/body diameter at the anal/cloacal aperture and V**:** Head to vulva length/body length×100**.**

Conclusions

These findings indicated that the predominant PPNs distributed in Pattawia pineapple crop soil in Prachuap Khiri Khan province, Thailand were *Rotylenchulus* sp. and *Helicotylenchus* sp., especially in Sam Roi Yot and Mueang districts. The molecular, morphometric and morphological characterization revealed the nematode species *R. reniformis* and *H. dihystera*. This report provided the first description based on the morphological and molecular characterization of *R. reniformis* and *H. dihystera* found in Pattawia

pineapple crops, Thailand. Notably, *R. reniformis* is one of the important PPNs causing crop yield losses in many countries. Therefore, nematode management should be considered and conducted before crops become damaged. Further study is needed, especially on the impacts of *R. reniformis* on pineapple yields in Thailand.

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