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RESEARCH ARTICLE
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Characterization and Biocontrol Potential of Predatory Nematodes (Mononchida and Dorylaimida) Against *Meloidogyne enterolobii* in Thailand

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ABSTRACT

Predatory nematodes are free-living organisms with significant potential to functional as biological control agents against plant-parasitic nematodes. This study investigated the diversity, molecular characterization, and biocontrol potential of predatory nematode from natural soil ecosystems in Thailand. Comprehensive soil sampling from riverbanks and pond ecosystems yielded three predatory nematode genera from the orders Mononchida and Dorylaimida: *Mylonchulus* sp. (43% occurrence), *Mononchus* sp. (39.5%), and *Paractinolaimus* sp. (17.5%). Molecular identification based on 18S and 28S rRNA gene sequences, coupled with morphological examination using De Man Formulae, confirmed the species as *Mylonchulus hawaiiensis*, *Mononchus tunbridgensis*, and *Paractinolaimus* sp. The most abundant species, *Mylonchulus hawaiiensis*, was selected for targeted bioassays to assess its predation efficacy against *Meloidogyne enterolobii*, a major root-knot nematode affecting chili crops in Thailand. Experimental results revealed that *Mylonchulus hawaiiensis* is an active predator of *Meloidogyne enterolobii* second-stage juveniles (J2s), with an average daily consumption of 16.1 J2s and a cumulative total consumption of 80 J2s over five days. This study provides the first comprehensive documented evidence of *Mylonchulus hawaiiensis* as a potential biocontrol agent against *Meloidogyne enterolobii* in Thailand. Although confined to laboratory conditions, these findings establish a critical foundation for future field-based research. The study underscores the potential use of predatory nematodes in developing sustainable, environmentally friendly pest management strategies for agricultural systems.

Keywords: Biological control, Chili, Nematode identification, Predatory nematodes, Root-knot nematodes.

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INTRODUCTION

Root-knot nematodes (RKNs), *Meloidogyne* spp., is one of the most economically significant plant-parasitic nematodes, causing severe agricultural losses worldwide. Their impact is particularly pronounced in tropical and subtropical regions, where environmental conditions favor rapid population growth and infection cycles (Jones et al., 2013). These microscopic soil-dwelling organisms pose a critical threat to global food security by compromising plant root functionality and agricultural productivity. RKNs infect plant roots and induce the formation of characteristic galls or root-knots, disrupting normal root function and leading to impaired water and nutrient uptake (Karssen et al., 2013). As a result, infected plants exhibit stunted growth, reduced vigor, increased susceptibility to secondary infections, and diminished yields. The ability of RKNs to thrive in various soil types, reproduce rapidly, and persist even in the absence of host plants further complicates their management, necessitating the development of effective and sustainable control strategies. Currently, the genus *Meloidogyne* comprises over 90 recognized species, many of which exhibit high adaptability and pathogenic diversity (Moens et al., 2009). The four most widespread and agriculturally

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important species are *Meloidogyne javanica*, *M. arenaria*, *M. incognita*, and *M. hapla* (Eisenback & Triantaphyllou, 1991). These species are particularly destructive due to their ability to infect a broad range of host plants, including economically valuable crops such as vegetables, fruits, cereals, and legumes (Sikandar et al., 2023). In Thailand, the nematological landscape is particularly complex where researchers have identified the presence of ten *Meloidogyne* species−the four globally prevalent species already mentioned plus six additional variants: *Meloidogyne exigua*, *M. graminicola*, *M. microcephala*, *M. naasi*, *M. thailandica*, and *M. enterolobii* (Jindapunnapat et al., 2023). Among the identified species, *Meloidogyne enterolobii* has emerged as one of the most virulent and economically significant species, posing a serious threat to multiple agricultural sectors. This highly aggressive species is capable of overcoming host resistance mechanisms that are effective against other RKN species, making it a formidable challenge for growers (Philbrick et al., 2020). In Thailand, *M. enterolobii* is particularly problematic in chili (*Capsicum annuum*) and guava (*Psidium guajava*) production, notably in northeastern provinces such as Ubon Ratchathani and Si Sa Ket, which experience severe yield losses (Jindapunnapat et al., 2013; Boonrin et al., 2024). Infected chili crops exhibit extensive root galling, leading to significant reductions in growth and fruit production. Due to its rapid reproduction, high infectivity, and resilience to conventional control methods, losses attributed to *M. enterolobii* can reach 65% of the yield, surpassing the economic impact of other RKN (Salazar-Mesta et al., 2024). Given the increasing prevalence and severity of *M. enterolobii*-induced damage, the urgent need for effective and sustainable management strategies is evident. Traditionally, chemical nematicides have been the primary method for controlling RKN populations in agricultural settings. Synthetic nematicides such as carbamates, organophosphates, and fumigants have been widely used because of their broad-spectrum efficacy (Perry et al., 2009). However, the extensive reliance on chemical control has raised significant concerns regarding environmental safety and human health risks, thus prompting development of effective nematode resistance (Nicol et al., 2011). The persistence of chemical residues in soil and water sources has led to regulatory restrictions and increasing calls for more environmentally sustainable alternatives (Collange et al., 2011). Moreover, the high cost of chemical nematicides makes them economically unfeasible for smallholder farmers, further exacerbating the challenge of effective nematode management in developing agricultural systems. As an alternative to chemical control, biological approaches have gained increasing attention for their potential to provide long-term and environmentally friendly nematode management (Khan & Kim, 2007). Various antagonistic microorganisms, including *Bacillus* spp., *Trichoderma* sp., *Paraboeremia taiwanensis*, and *Samsoniella* sp. have demonstrated promising nematicidal activity against *M. enterolobii* (Jindapunnapat et al., 2013; Liang et al., 2020; Puttawong et al., 2024). These microbes function with diverse mechanisms, such as producing nematotoxic metabolites, inducing systemic resistance in plants, and directly parasitizing nematodes. Despite these promising developments, the application of biological control strategies, such as using predatory nematodes as natural enemies of *M. enterolobii*, continue to be underutilized in Thailand.

Predatory nematodes offer a unique and potentially transformative solution to RKN management. Unlike plant-parasitic nematodes, predatory nematodes are free-living organisms that naturally inhabit soil ecosystems and actively prey on plant-parasitic nematodes and other invertebrates. As key regulators of nematode populations in soil food webs, they contribute to the natural suppression of plant-parasitic nematodes, making them valuable candidates for biological control. They are classified into four major taxonomic groups: Mononchida, Dorylaimida, Diplogasterida, and Aphelenchida (Ahmad & Jairajpuri, 2010). Each group exhibits distinct feeding mechanisms adapted to their predatory behavior. Mononchids are equipped with a well-developed buccal cavity that contains teeth and denticles, along with strong buccal musculature. These features enable them to employ a combination of cutting, sucking, and engulfing strategies to capture and consume prey (Bilgrami et al., 2005). Dorylaimids, on the other hand, possess a piercing and sucking feeding apparatus, which includes a dagger-shaped odontostyle that allows them to efficiently penetrate and extract the contents of their prey. In contrast, diplogasterids have a relatively smaller buccal cavity with teeth of varying sizes and positions, utilizing a cutting and sucking mechanism. Finally, aphelenchids rely on narrow spears without basal knobs to pierce their prey and extract body contents through suction (Sidhu & Kanwar, 2020). Numerous studies have demonstrated the potential of predatory nematodes to suppress plant-parasitic nematode populations. For instance, *Mylonchulus dentatus* have been observed preying on multiple nematode species, including *Helicotylenchus indicus*, *Helicotylenchus oryzae*, *Hoplolaimus indicus*, *Longidorus* sp., *Meloidogyne incognita*, *Tylenchorhynchus mashhoodi*, and *Tylenchulus semipenetrans* (Jairajpuri & Azmi, 1978; Bilgrami & Kulshreshtha, 1993). Similarly, *Mylonchulus sigmaturus* exhibit a broad predation spectrum, targeting *Meloidogyne javanica, Heterodera mothi, Heterodera oryzae, Anguina tritici, Tylenchorhynchus mashhoodi*, and *Xiphinema americanum* (Bilgrami et al., 2005; Koohkan & Shokoohi, 2014). Laboratory experiments have further validated the predation efficiency of predatory nematodes. For instance, Sidhu & Kanwar (2020) evaluated the predatory efficacy of *Fictor composticola* against second-stage juveniles (J2s) of *Meloidogyne incognita* under controlled conditions. Their findings revealed that predation rates varied depending on prey density, with the highest consumption (74.8%) occurring at 500 J2s per plate and the lowest (38.9%) at 2,000 J2s per plate. These results underscore the significant biocontrol potential of predatory nematodes and highlight the importance of optimizing prey density for effective field application. Despite the proven efficacy of predatory nematodes in other regions, their diversity, ecology, and potential application in Thailand remain largely unexplored. Most studies on predatory

nematodes have focused on temperate environments, with limited research conducted in tropical agricultural systems. Furthermore, the effectiveness of predatory nematodes against *Meloidogyne enterolobii*, in particular, has not been systematically evaluated. Given the increasing incidence of *Meloidogyne enterolobii* infestations in Thai agriculture, there is an urgent need to investigate the role of predatory nematodes in biological control.

To bridge this knowledge gap, this study focuses on the isolation and characterization of predatory nematodes from natural soil environments across Thailand, with an emphasis on species belonging to the orders Mononchida and Dorylaimida. The research aims to determine the diversity of predatory nematodes in different soil types, analyze their morphological and molecular characteristics, and assess their predatory efficacy against *Meloidogyne enterolobii*. Furthermore, this study evaluates the biocontrol potential of *Mylonchulus hawaiiensis*, one of the most abundant predatory nematodes found in the surveyed regions, against *Meloidogyne enterolobii* in chili crops. By exploring the role of predatory nematodes in suppressing *Meloidogyne enterolobii* populations, this research aims to contributes to the development of sustainable and environmentally friendly nematode management strategies for Thai agriculture.

MATERIALS & METHODS

Soil Samplings and Nematode Processing

In total, nine soil samples were collected from natural environments along riverbanks and from ponds across Thailand, seven samples from Bangkok, one from Phra Nakhon Si Ayutthaya and one from Sukhothai (Fig. 1). The selection of these sites was based on their ecological diversity and potential to harbor predatory nematodes. The goals of the soil collection were to assess the relationship between soil characteristics, such as soil type, organic matter content, and pH, and the occurrence of predatory nematodes. Each soil sample consisted of five composite subsamples randomly collected within a 50m^2 area at a depth of 5−30cm. Subsamples were thoroughly mixed in a bucket to ensure homogeneity, and 2kg of soil was selected per sample. The soil samples were placed in sealed plastic bags, labelled with site details (location, GPS coordinates, and collection date), and transported to the Nematode Laboratory, Department of Plant Pathology, Kasetsart University, Thailand for further analysis.

To analyze soil properties, each sample was divided into two parts: 1kg of soil was sent to the Department of Soil Science, Kasetsart University, for the determination of key soil characteristics, including soil type, organic matter content (g/kg soil), and pH. Standard soil analysis protocols were followed to ensure the accuracy and reliability of results. Using the gravity screening Baermann funnel method, nematodes were extracted from the remaining 1kg of soil (Christie & Perry, 1951). Nematodes extraction from the 300g of soil per sample was performed: first, the soil was thoroughly mixed with 500mL of tap water in a plastic bucket and left undisturbed for 20 min to allow particle separation. Then, the soil suspension was stirred manually and passed through a series of sieves with mesh apertures of 250µm, 105µm, and 38µm to retain nematodes of varying sizes. The nematodes trapped on the 105µm and 38µm sieves were rinsed into a funnel using tap water and collected after 48 hours using the Baermann funnel method. The extracted nematodes were observed under a stereo microscope (Olympus SZ60) to identify predatory nematodes, and counted to calculate their percentage of occurrence and absolute frequency (Ahammed et al., 2021).

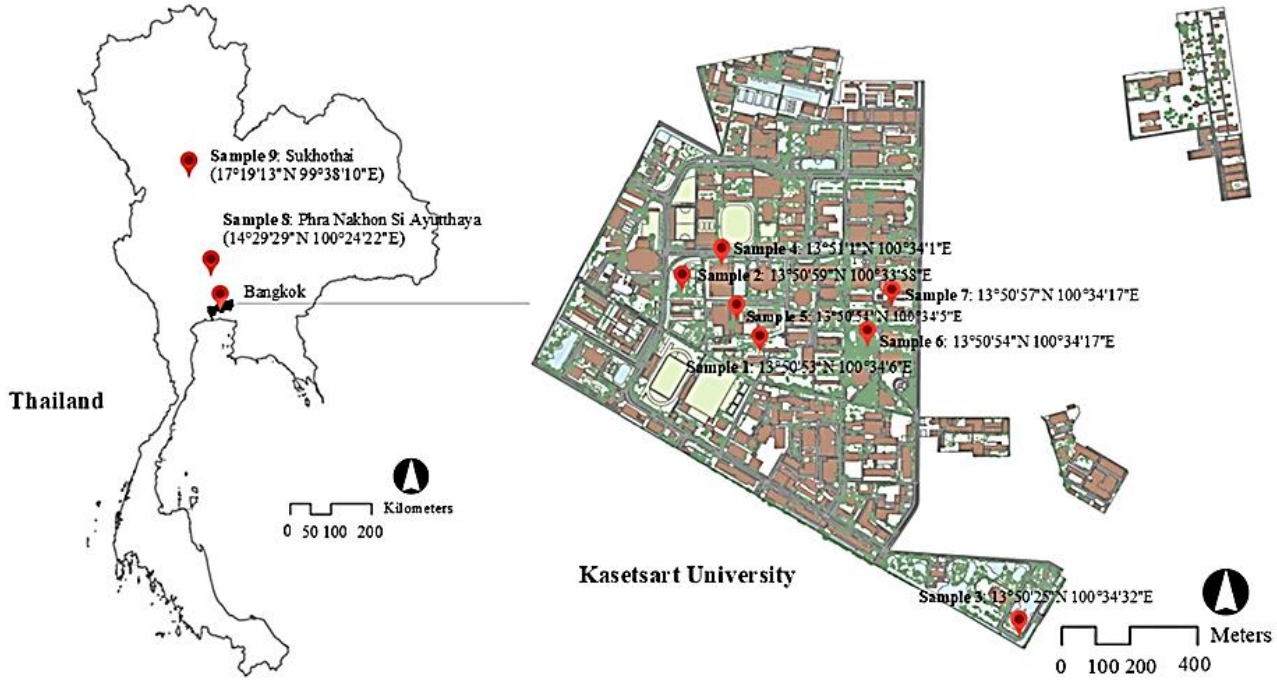

Fig. 1: Map showing location of soil samples collected for this study. Adapted from Davivongs et al. (2024).

Morphological characterization

Detailed morphological analysis was conducted on *Mylonchulus* sp. collected from soil in various districts of diverse Thai provinces−Phak Hai, Phra Nakhon Si Ayutthaya; *Mononchus* sp. from Thung Saliam, Sukhothai; and *Paractinolaimus* sp. from Chatuchak, Bangkok. The nematode specimens were killed by immersion in hot water (~50°C) and subsequently fixed in Triethanolamine Formalin (TAF) solution, following the protocol outlined by Ryss (2017). The fixed specimens were mounted on glass slides with a drop of glycerine, and candle wax was used to support the cover slip to avoid specimen distortion. The mounted nematodes were observed and photographed using a compound microscope (Olympus BX50) equipped with a Canon EOS750 camera and the EOS Utility program. Measurements of the nematode specimens were performed using the Axio Vision SE64 Rel. 4.9.1 program. Morphometric data were calculated per the De Man Formulae (Thorne, 1949), which provided the following parameters were recorded: L = Total body length, a = body length/body width, b = body length/anterior end to pharyngo-intestinal junction (PIJ), c = body length/tail length, c′ = tail length/maximum tail width, V% = head to vulva length/body length × 100. Additional measurements included maximum body width, distance from the anterior end to PIJ, head to vulva length, tail width at the anus, and tail length.

Molecular characterization

To determine their molecular characterization, DNA was extracted from three nematodes representing each genus. A single nematode was placed inside a 0.2mL PCR tube containing 40µL of 1:1 (v/v) lysis buffer, which included 200mM NaCl, 200mM Tris–HCl (pH 8), 1% (v/v) β-mercaptoethanol, and 800µg/mL proteinase K, along with sterilized distilled water. The DNA extraction process was initiated by incubating the tube in a PCR thermal cycler under the following conditions: 65°C for 90min, followed by 99°C for 10min to ensure complete cell lysis. The resulting nematode DNA was then stored at -20°C for future use.

The extracted DNA was amplified using two primer sets targeting different genetic regions: 648/136 (GTATGGTTGCAAAGCTGAAAC/TGATCCTTCTGCAGGTTCAC CTA C) for 18S rRNA, and D2A/D3B (ACAAGTACCGTGAGGGAAAGT/TGCGAAGGAACCAGCTACT A) for the D2D3 region of 28S rRNA (Berg et al., 2009; Phadungkit et al., 2024). A 30µl PCR mixture was prepared, consisting of 15µL of 2x PCR master mix with dye solution (i-taq, Intron Biotechnology, Korea), 10µL of sterilized distilled water, 1µL of both 10µM forward and reverse primer, and 3µL of DNA template. The thermal cycler program was set as follows: initial denaturation at 95°C for 3min, followed by 35 cycles of 95°C for 30 s, 56°C for 30 s (for both primer sets) and then 72°C for 1min, with a final extension at 72°C for 5min. Representative PCR product from each nematode genus were purified and sequenced by Solgen Inc., Korea. The resulting DNA sequences were subjected to quality checks and then compared against available reference sequences in the National Center for Biotechnology Information (NCBI) database to confirm the identity of the nematodes. Subsequent sequence alignment and phylogenetic analyses were conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software, version 7.0. The sequences obtained (18S rRNA and D2-D3 rRNA) were aligned with published sequences from relevant nematode species within the orders Mononchida and Dorylaimida using the ClustalW alignment tool, applying default settings. Phylogenetic trees were constructed using Maximum Likelihood (ML) methods based on the Gamma distribution (GTR+G) model. Phylogenetic support was evaluated by performing 1,000 bootstrap replicates, as outlined by Subbotin et al. (2006), to assess the robustness of the tree topology.

Biocontrol efficacy

Mylonchulus sp., the predominant predatory nematode identified in this study, was selected to assess its biocontrol potential against root-knot nematodes (*Meloidogyne enterolobii*). The *Meloidogyne enterolobii* population used in this experiment was obtained from the Department of Plant Pathology, Kasetsart University. To maintain the nematode culture, *Meloidogyne enterolobii* was inoculated into 2-week-old okra plants and grown in a greenhouse for 8 weeks. To extract second-stage juveniles (J2s) for use in the experiment, the infected okra plants were carefully uprooted, washed with tap water, and cut into 1−2cm segments. The root pieces were then placed in a 50mL Falcon tube containing 30mL of 0.6% sodium hypochlorite solution and gently shaken by hand for 2min to release nematode eggs. The egg suspension was filtered through a series of sieves with 250µm and 25µm apertures, eggs were retained on the 25µm sieve. The collected eggs were rinsed with tap water and placed in a Baermann funnel setup for 5 days to facilitate hatching (Beesa et al., 2022). The hatched J2s were collected and used as prey in subsequent experiments.

The biocontrol efficacy experiment was performed using 24-well plates. Each well contained 1mL of a nematode suspension with approximately 100 J2s of *Meloidogyne enterolobii*. A single *Mylonchulus* sp. individual was introduced into each well as the predator. Control wells contained only *Meloidogyne* J2s−no predatory nematodes were added. The experiment was arranged in a completely randomized design, with three replications per treatment, and repeated once to ensure reliability. The predatory activity of *Mylonchulus* sp. was evaluated by recording the number of *Meloidogyne enterolobii* J2s consumed daily over a 5-day incubation period. Data were statistically analyzed using the SPSS software (version 16.0, SPSS Inc., Chicago, Illinois, USA) and differences among means were determined by Duncan's New Multiple Range Test ($p < 0.05$).

RESULTS & DISCUSSION

Nematode extraction and ecological distribution

A comprehensive survey of the predatory nematodes collected from diverse soil ecosystems in different regions of Thailand revealed a total of 86 individuals from the orders Mononchida and Dorylaimida. Three distinct genera were

identified with unique distribution patterns: *Mylonchulus*, *Mononchus*, and *Paractinolaimus*. Of the identified genera, *Mylonchulus sp.* was the most abundant (37 individuals), with an occurrence of 43% and an absolute frequency of 55.5%. It was predominantly found in Phak Hai, Phra Nakhon Si Ayutthaya (30 individuals) in sandy loam soil which had the highest organic matter content (60.96g/kg). *Mononchus sp.* was the second most abundant (34 individuals); it had an occurrence of 39.5% and an absolute frequency of 77.7%. It was predominantly found in Thung Saliam, Sukhothai (24 individuals); the soil sample was silt loam with high organic matter (53.49g/kg). *Paractinolaimus sp.* was the least abundant (15 individuals), found exclusively in the sample collected in Chatuchak, Bangkok, which was sandy loam soil containing 33.90g/kg of organic matter; it had an occurrence of 17.5% and an absolute frequency of 22.2%. Interestingly, *Mylonchulus* and *Mononchus* were more abundant in soils with high organic matter, whereas no significant correlation was observed between nematode abundance and soil pH (Table 1). Recent studies have highlighted the positive influence soil organic matter has on soil nematode diversity and abundance. Beesa et al. (2025) reported that organic amendments contribute to an increase in predatory nematodes, leading to improved soil food web stability and ecosystem functioning. Shi et al. (2023) determined that organic material amendments enhance the population density of soil nematodes by improving nutrient availability and microbial diversity, which serve as prey for predatory nematodes. These findings align with previous research indicating that nematode communities thrive in environments with rich organic matter, as it indirectly supports their survival by promoting microbial activity. Indeed, predatory nematodes such as mononchids (*Mylonchulus* and *Mononchus*) are widely distributed across various soil types and are often more abundant in undisturbed soils (Ahmad & Jairajpuri, 2010). The presence of *Mylonchulus* sp. has been reported in several Asian countries, Pakistan, Nepal, India, Malaysia, Singapore, Iran, and Indonesia (Shahabi et al., 2016; Gafur & Ajizah, 2022). In Thailand, previous studies recorded *Mylonchulus* sp. in Rayong Province and *Mononchus sp.* in Chiang Mai Province (Jom-in et al., 2019), though these studies lacked detailed morphological or molecular descriptions. A significant finding of this study is the first recorded presence of *Paractinolaimus* sp. in Thailand. Recent research by Mwamula et al. (2024) has provided detailed morphological and molecular characterizations of *Paractinolaimus* species, highlighting their role in soil ecosystems. Their presence in Bangkok's Chatuchak District suggests that urban and semi-urban environments may support diverse nematode communities, particularly in soils with moderate organic matter levels. This discovery underscores the need for further studies to determine the ecological role, genetic diversity, and distribution of *Paractinolaimus* in Thailand, most usefully for agricultural systems, where predatory nematodes could serve as potential biological control agents. Given the ecological significance of predatory nematodes, long-term monitoring of their populations under different land-use conditions would provide valuable insights into their roles in soil health and pest suppression. Additionally, further research should investigate the influence of soil moisture, microbial communities, and organic amendments on predatory nematode abundance. Understanding these interactions will contribute to sustainable soil management and biodiversity conservation in Thailand's diverse ecosystems.

Morphological characterization

In this study, no adult males from any nematode genera were found in the soil samples. Consequently, morphometric analysis was conducted exclusively on female specimens.

The morphological features of *Mylonchulus* sp. include body lengths ranging from 633.70 to 1436.65µm and body widths 27.80 to 63.26µm. The buccal cavity shape varies from goblet to funnel; a dorsal tooth is prominent, large, and claw-like, with a pointed tip positioned in the anterior half of the dorsal side. The mean distance from the anterior end to the esophageal-intestinal valve measured 202.72 to 542.03µm. The reproductive system is located at the mid-body region, accounting for 46.96 to 77.59% of the body length, and features two ovaries (didelphic-amphidelphic). The tail is short to slightly elongated, measuring 25.60 to 52.74µm in length and 34.17 to 17.18µm in width, with a terminal or subterminal spinneret (Fig. 2). The morphometric values are presented in Table 2. These morphological observations are consistent with reports of *Mylonchulus hawaiiensis* found in Iran, Indonesia, and South Africa (Shokoohi et al., 2013; Pradana & Yoshiga, 2023; Shokoohi, 2024). Notably, the Thai specimens share morphometric similarities with those found in Iran and Indonesia but are generally smaller than the South African specimens, particularly in the "a" and "c" values.

Table 1: Number of predatory nematodes isolated from soil samples collected from various locations

Sample No.	District, Province	Soil type	Soil pH	Soil organic matter (g/kg soil)	No. of nematodes		
					Mylonchulus	*Mononchus*	*Paractinolaimus*
1	Chatuchak, Bangkok	Loamy Fine Sand	7.0	31.78	2	1	-
2	Chatuchak, Bangkok	Silty Clay	7.6	24.15	1	4	-
3	Chatuchak, Bangkok	Clay Loam	7.3	16.40	-	1	1
4	Chatuchak, Bangkok	Sandy Clay Loam	7.3	3.54	3	1	-
5	Chatuchak, Bangkok	Sandy Clay Loam	7.3	14.12	1	1	
6	Chatuchak, Bangkok	Sandy Loam	6.9	14.85	-	2	-
7	Chatuchak, Bangkok	Sandy Loam	7.0	33.90	-	-	14
8	Phak Hai, Phra Nakhon Si Ayutthaya	Sandy Loam	6.3	60.96	30	-	-
9	Thung Saliam, Sukhothai	Silt Loam	7.0	53.49	-	24	-
Total					37	34	15
Occurrence (%)					43	39.5	17.5
Absolute frequency (%)					55.5	77.7	22.2

Table 2: Morphometric measurements of predatory nematodes isolated from soil samples in this study. All measurements are in µm and are presented as mean±SD (range)

Character	*Mylonchulus*	*Mononchus*	*Paractinolaimus*
n	22	6	5
L	1136.21±199.08 (633.70–1436.65)	1262.61±173.58 (1113.22–1520.39)	1839.73±265.78 (1419.50–2094.86)
V%	58.87±5.66 (46.96–77.59)	53.44±2.72 (48.84–56.42)	53.89±4.77 (48.35–61.38)
a	23.43±2.85	31.32±3.19	37.46±2.69
(L/MB)	(18.95–30.25)	(27.98–35.93)	(34.26–41.56)
b	3.34±0.32	4.31±0.25	3.91±0.52
(L/ES)	(2.07–3.68)	(3.94–4.68)	(3.49–4.79)
c	26.97±4.72	10.88±0.48	15.10±2.94
(L/TL)	(18.12–34.28)	(9.64–10.88)	(11.98–19.86)
c′	1.42±0.16	4.7±0.40	4.38±0.51
(TL/MT)	(1.17–1.72)	(4.34–5.22)	(3.88–5.14)
Maximum body width	48.57±7.13	40.22±1.78	48.95±4.69
(MB)	(27.80–63.26)	(38.14–42.32)	(41.17–53.43)
Head to esophageal-intestinal valve	343.28±72.33	292.6±31.07	472.55±61.07
(ES)	(202.72–542.03)	(260.05–346.83)	(404.63–534.63)
Head to vulva length	670.88±136.48	675.66±108.39	983.92±106.73
(VL)	(303.24–871.54)	(594.52–857.84)	(482.75–1185.58)
Tail length	42.57±6.42	123.12±13.8	122.88±11.66
(TL)	(25.60–52.74)	(106.94–140.36)	(105.46–135.83)
Maximum tail width	29.98±3.53	25.99±1.20	28.28±3.45
(MT)	(17.18-34.17)	(23.64–26.91)	(22.90–31.86)

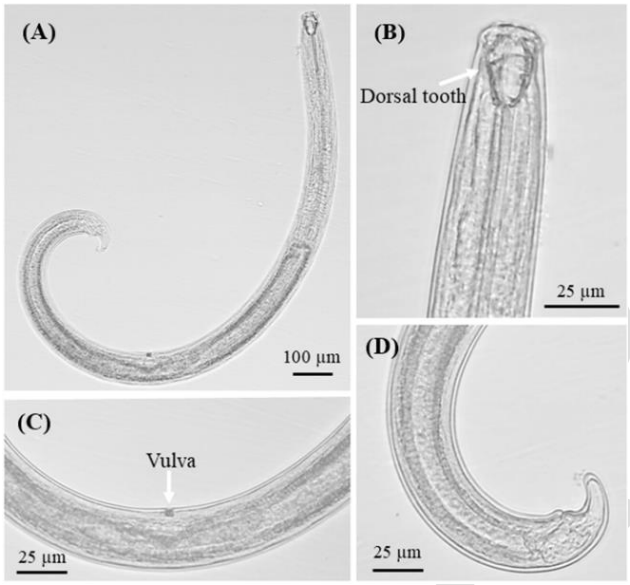

Fig. 2: Photomicrographs of *Mylonchulus hawaiiensis*; (A) Female entire body, (B) Head region showing anterior structures, (C) Vulva position of the female, (D) Tail region of the female.

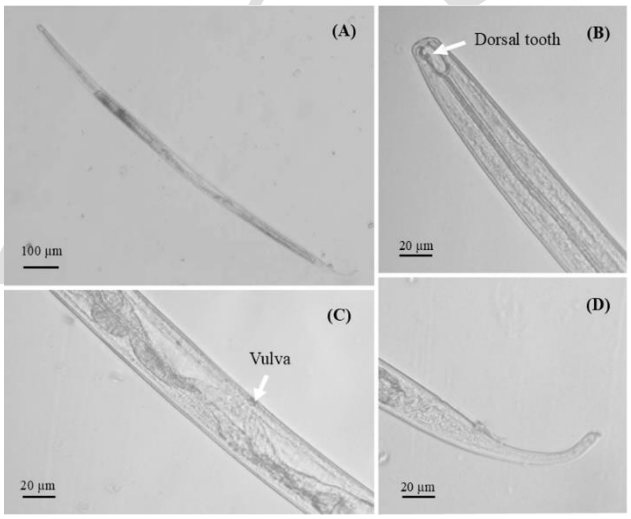

Fig. 3: Photomicrographs of *Mononchus tunbridgensis*; (A) Female entire body, (B) Head region showing anterior structures, (C) Vulva region of the female, (D) Tail region of the female.

The body of *Mononchus* sp. is nearly cylindrical, measuring 1,113.22 to 1,520.39µm in length and 38.14 to 42.32µm in width. The buccal cavity is elongated, oblong, and barrel shaped. The dorsal tooth is large, with a pointed tip positioned towards the anterior half of the buccal cavity, approximately 60% of its length from the base. The mean distance from the anterior end to esophageal-intestinal valve measures 260.05 to 346.83µm. The reproduction system is situated at approximately 48.84 to 56.42% of the body length. The tail is ventrally curved, tapering gradually to a cylindrical posterior end, and measures 106.94 to 140.36µm in length and 23.64 to 26.91µm in width (Fig. 3). The morphometric values are detailed in Table 2. These morphological traits align with descriptions of *Mononchus tunbridgensis* from studies conducted in the UK and Japan (Bastian, 1865; Nakazawa, 1999; Andrássy, 2011).

The body of *Paractinolaimus* sp. is ventrally curved into a C-shape, measuring 1,419.50 to 2,094.86µm in length and 41.17 to 53.43µm in width. The lip region is offset by a depression. The cheilostome is robust, with four onchia and multiple sharp denticles. The odontostyle is simple and rod-like, supported by a straightforward odontophore. The mean distance from the anterior end to the esophageal-intestinal valve measured 404.63 to 534.63µm. The reproduction system is located at the mid-body region, comprising two ovaries (didelphic-amphidelphic) and accounting for 48.35 to 61.38% of the body length. The tail is generally long and either ventrally curved or straight, measuring 105.46 to 135.83µm in length and 22.90 to 31.86µm in width (Fig. 4). Morphometric values are provided in Table 2. These characteristics are consistent with *Paractinolaimus* spp. as described by Vinciguerra et al. (2013) and Mwamula et al. (2024). Molecular analysis confirmed close similarity to *Paractinolaimus* sp., leading to its identification at the genus level.

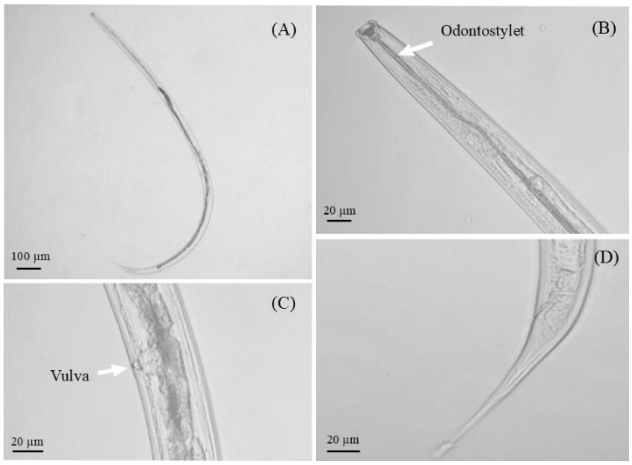

Fig. 4: Photomicrographs of *Paractinolaimus* sp.; (A) Female entire body, (B) Head region showing anterior structures, (C) Vulva region of the female, (D) Tail region of the female.

Based on both molecular and morphological characterization, the predatory nematodes identified in the current study were *Mylonchulus hawaiiensis*, *Mononchus tunbridgensis*, and *Paractinolaimus* sp.

Molecular characterization

Nematode identification was achieved through DNA sequencing analysis of the 18S and D2-D3 regions of the 28S rRNA genes. The amplification of nematode DNA using primers 648/136 (18S region) and D2A/D3B (28S region) resulted in amplicons of approximately 850bp and 700bp, respectively, for all nematode specimens. The nucleotide sequences obtained were compared with those available in the NCBI GenBank using BLAST, which revealed a high degree of similarity to previously identified species. Specifically, the 18S rRNA sequences showed 98% similarity to *Mylonchulus hawaiiensis* (GenBank accession number: PQ867116), 99% similarity to *Mononchus tunbridgensis* (PQ867117), and 100% identity to a *Paractinolaimus* sp. population (PQ867118). Similarly, the 28S rRNA sequences yielded 99% similarity to *Mylonchulus hawaiiensis* (PQ869812), 100% similarity to *Mononchus tunbridgensis* (PQ869813), and 100% identity to *Paractinolaimus* sp. (PQ872093). The phylogenetic analysis using these sequences alongside reference sequences from GenBank, revealed that the specimens clustered in distinct branches corresponding to *Mylonchulus hawaiiensis*, *Mononchus tunbridgensis*, and *Paractinolaimus* sp. populations, with strong bootstrap support (Fig. 5 and 6). This confirmed the molecular identity of the nematodes as *Mylonchulus hawaiiensis*, *Mononchus tunbridgensis*, and *Paractinolaimus* sp. These molecular findings align with previous studies in the field of nematode identification. The high sequence identity (98-100%) between these sequences and those already in GenBank further confirms the accuracy of this molecular identification. The use of the 18S and 28S rRNA genes as molecular markers for nematode species identification has been well-established by recent literature. Shokoohi & Moyo (2022) investigated molecular diversity of predatory nematodes in Southern African ecosystems, highlighting the importance of multi-gene approaches in nematode taxonomy. Altash et al. (2024) demonstrated the

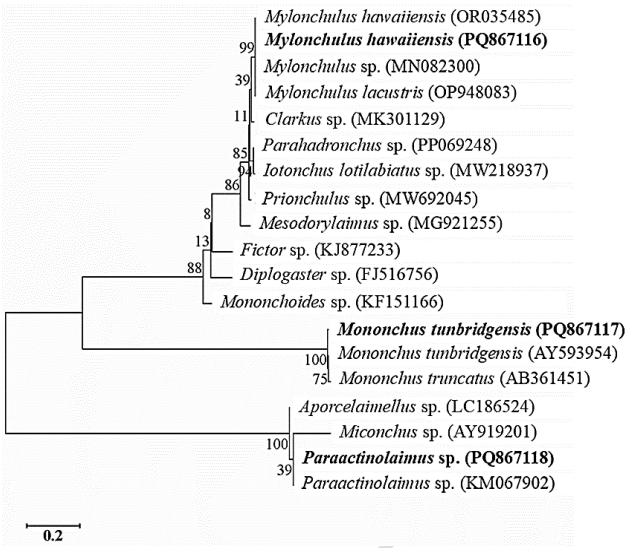

Fig. 5: Phylogenetic analysis of predatory nematodes based on 18s rRNA sequences. Bootstrap support values from Maximum Likelihood (ML) analysis are shown next to the branches. The scale bar represents the number of substitutions per nucleotide position. Sequences generated in this study are highlighted in bold, with NCBI accession numbers provided alongside the species names.

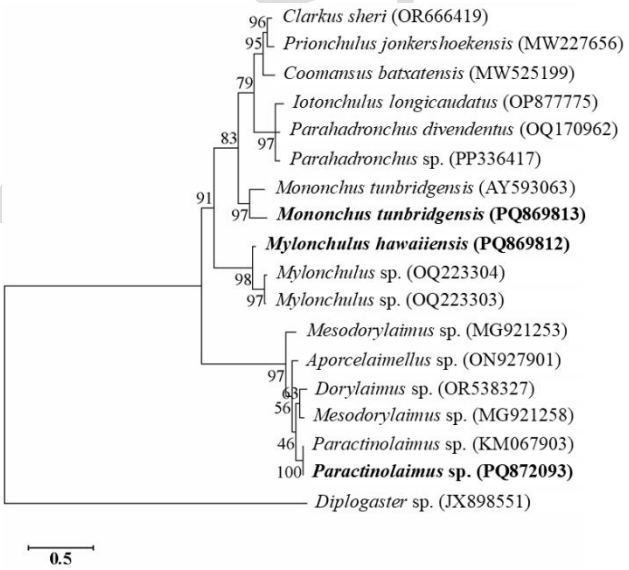

Fig. 6: Phylogenetic analysis of predatory nematodes based on the D2-D3 region of 28s rRNA sequences. Bootstrap support values from Maximum Likelihood (ML) analysis are shown next to the branches. The scale bar represents the number of substitutions per nucleotide position. Sequences generated in this study are highlighted in bold, with NCBI accession numbers provided alongside the species names.

utility of 18S and 28S rRNA regions in resolving cryptic species complexes within predatory nematode genera. The high degree of sequence similarity and the strong phylogenetic support obtained from this study confirm the reliability of these markers for accurate nematode species identification. The rRNA gene regions, particularly the 18S and 28S regions, have become essential in molecular taxonomy because of their universality and relatively high variability at the species level. Recent advancements in sequencing technologies have further enhanced the precision of these markers for nematode identification. For example, Shokoohi (2024) highlighted the usefulness of these markers in resolving species-level distinctions in

morphologically similar nematodes. The phylogenetic analysis, which clustered the specimens in this study with those of previously identified populations, highlights the importance of molecular characterization in expanding an understanding of nematode biodiversity. These findings not only support the identification of these species but also demonstrate the potential for molecular techniques to track the distribution and ecological roles of nematodes in different environments.

Biocontrol efficacy

The predatory potential of *Mylonchulus hawaiiensis* against *Meloidogyne enterolobii* was systematically evaluated under controlled laboratory conditions. The results demonstrated a progressive increase in the accumulative attack rate of *Mylonchulus hawaiiensis* on *Meloidogyne enterolobii* second-stage juveniles (J2s) over the course of the experiment (Fig. 7). The total predation accumulated during the 5-day observation period was 80.5 J2s, with an average daily consumption rate of 16.1 J2s.

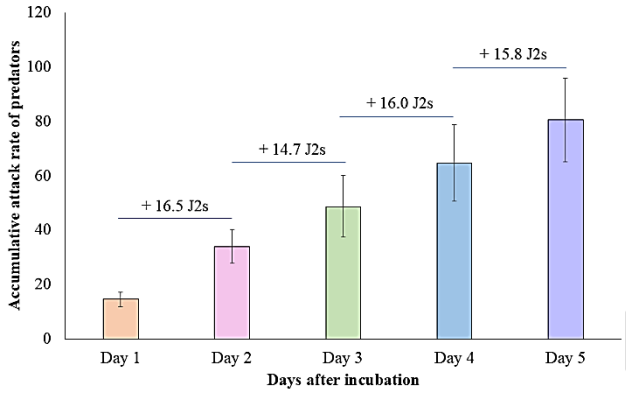

Fig. 7: Daily predation rate of second-stage juveniles (J2s) of *Meloidogyne enterolobii* by *Mylonchulus hawaiiensis*. The number of J2s consumed per day is shown across 5−day period.

Notably, the predation rate remained relatively stable across the experimental days, with slight daily variations ranging from 14.7 to 16.5 J2s. This consistent predation behavior emphasizes the robustness and adaptability of *Mylonchulus hawaiiensis* as a promising biocontrol agent against *Meloidogyne enterolobii*, a major plant-parasitic nematode that threatens agricultural productivity, especially in tropical and subtropical regions. The observation that the highest predation rate occurred on Day 5 suggests that *Mylonchulus hawaiiensis* may maintain effective predation over extended periods, suggesting its realistic potential for effective sustained biocontrol. These findings are consistent with previous studies related to the predatory abilities of *Mylonchulus* species. For instance, Koohkan & Shokoohi (2014) demonstrated that *Mylonchulus sigmaturus* significantly reduced populations of *Meloidogyne javanica* in laboratory trials. In the current study, similar predation behavior was observed, with *Mylonchulus hawaiiensis* effectively preying on the juvenile stages of *Meloidogyne enterolobii*. The predation mechanism of *Mylonchulus* species is facilitated by its unique morphological features. As described by Bilgrami (2008), these nematodes possess a specialized mural tooth and serrated, rasp-like denticles that enable them to penetrate and destabilize the cuticle of their prey, facilitating efficient feeding. This morphological strategy aligns with earlier behavioral studies by Jairajpuri & Azmi (1978), who observed selective predation by *Mylonchulus dentatus* on juvenile stages of various nematode species, including *Meloidogyne* spp. Furthermore, Cohn & Mordechai (1974) reported a negative correlation between *Mylonchulus sigmaturus* populations and the density of *Tylenchulus semipenetrans*, noting the generalist predation behavior of *Mylonchulus* species across a broad spectrum of plant-parasitic nematodes. Kanwar et al. (2021) expanded on the biocontrol potential of *Mylonchulus* species, reporting their effectiveness against several plant-parasitic nematodes, including *Meloidogyne incognita*, *Meloidogyne javanica*, *Tylenchulus semipenetrans*, *Xiphinema americanum*, *Rotylenchulus reniformis*, *Heterodera schachtii* (eggs), *Radopholus similis*, and *Subanguina radicicola*. This broad prey range highlights the versatility of *Mylonchulus* species as biocontrol agents, capable of suppressing multiple nematode pests that significantly affect agricultural production. The present study further supports these findings, confirming that *Mylonchulus hawaiiensis* is an effective predator of *Meloidogyne enterolobii*, a particularly damaging nematode in tropical and subtropical regions. While the current results establish the predatory potential of *Mylonchulus hawaiiensis* under laboratory conditions, further research is needed to evaluate its biocontrol efficacy in field settings. Field trials are essential for assessing the effectiveness of *Mylonchulus hawaiiensis* in controlling *Meloidogyne* infestations under natural conditions, where environmental factors such as soil type, temperature, and prey availability may influence the nematode's predatory behavior. Moreover, optimizing mass production techniques for *Mylonchulus hawaiiensis* will be critical for any practical application in integrated pest management (IPM) programs. Scalable production methods would facilitate the large-scale release of *Mylonchulus hawaiiensis* into agricultural systems. Recent advancements in nematode biocontrol have emphasized the need for effective and scalable production systems for biocontrol agents. Studies by Abd-Elgawad (2016) have underscored the importance of producing nematode-based biocontrol agents efficiently for enhanced field applications. As agricultural systems increasingly move toward sustainable practices, the use of *Mylonchulus* species as biocontrol agents will be crucial in managing nematode populations in an eco-friendly manner.

Conclusion

This study successfully isolated and identified predatory nematodes collected from diverse Thai soil ecosystems. *Mylonchulus* was the predominant genus, followed by *Mononchus* and *Paractinolaimus*. Molecular techniques, including nucleotide sequence comparisons and phylogenetic analysis of 18S and 28S rRNA, alongside morphological characterization, conclusively identified *Mylonchulus hawaiiensis*, *Mononchus tunbridgensis*, and *Paractinolaimus* sp. Of most significant, the biocontrol potential of *Mylonchulus hawaiiensis* against *Meloidogyne enterolobii* was demonstrated, with the predatory nematode

consistently consuming an average of 16.1 root-knot nematode juveniles daily under controlled laboratory conditions. These findings underscore the substantial predatory capabilities of *Mylonchulus hawaiiensis* and present a promising, eco-friendly alternative to conventional chemical nematode management strategies, positioning this species as a potential key player in sustainable agricultural pest control. The observed biocontrol efficacy suggests that *Mylonchulus hawaiiensis* could be incorporated into sustainable integrated pest management (IPM) strategies, reducing the reliance on chemical pesticides and minimizing the ecological footprint of nematode management. However, further research is necessary to evaluate its effectiveness in field conditions and to optimize mass production for large-scale applications. Long-term studies, including field trials, are essential to assess its impact in natural environments and its integration into existing pest management frameworks.

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Conflict of Interests: None

Data Availability: All the data is available in the article.

Ethics Statement: This study did not involve any human participants or vertebrate animals. All procedures involving nematode collection and experimentation compiled with institutional guidelines. The field sampling was conducted in publicly accessible areas and did not require specific permits. No endangered or protected species were involved in this study.

Author's Contributions: Conceptualization and Data curation: MP and ANS; Formal analysis: MP, NB and ARS; Funding acquisition: MP and ANS; Investigation: MP, NB, KJ and ARS; Methodology: MP; Project administration: ANS; Resources: BC, SC and ANS; Software: MP and KJ; Supervision: BC and ANS; Validation: MP, NB, ARS and ANS; Visualization: MP, NB and ARS; Writing - original draft: NB and ARS; Writing - review and editing: KJ, BC, SC and ANS. All authors read and approved the final manuscript.

Generative AI statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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