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RESEARCH PAPER

First record of the spiral nematode *Scutellonema brachyurum* (Rhabditida: Hoplolaimidae) in strawberry plants

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ABSTRACT

Strawberries are a popular fruit crop with promising cultivation prospects in Indonesia. However, their production is constrained by various pathogens, including plant-parasitic nematodes. This study aimed to characterize the nematode *Scutellonema brachyurum* infecting strawberry plants in West Java using morphological and molecular approaches. Soil and root samples were selectively collected from both symptomatic and asymptomatic plants. Nematodes were extracted from soil using the flotation–centrifugation technique and from roots using the mist chamber method. Morphological identification was complemented by molecular analysis using universal D2A/D3B primers targeting the D2–D3 region of the 28S rRNA gene, followed by DNA sequencing. Data were processed and analyzed descriptively. The nematode *S. brachyurum* infecting strawberry plants was successfully characterized using both morphological and molecular techniques. PCR amplification produced DNA fragments of approximately 700 bp. Nucleotide sequence analysis revealed that *S. brachyurum* isolates from West Java shared 83.7%–93.8% similarity with isolates from other countries. *Scutellonema brachyurum* was identified in Lebakmuncang (Ciwidey Sub-district) and Alamendah (Rancabali Sub-district), Bandung Regency, as well as in Langensari (Lembang Sub-district), West Bandung Regency. The nematode population detected in the samples ranged from 1 to 6 individuals. These findings indicate that *S. brachyurum* is distributed across several major strawberry-producing areas in West Java.

Keywords: DNA sequencing, *Fragaria x ananassa* Duch, PCR amplification, population, 28S rRNA gene

INTRODUCTION

Strawberries (*Fragaria x ananassa* Duch.) are horticultural crops with numerous nutritional benefits, including vitamin C, vitamins B1 and B2, and provitamin A (Widyastuti et al., 2016). In addition to being consumed fresh, strawberries are widely used as raw materials for processed products such as jam, juice, milk-based beverages, ice cream, and others. Strawberry production in Indonesia has fluctuated over the years. The highest production was recorded at 58 thousand tons in 2014, followed by a sharp decline to 7 thousand tons in 2019. Production then increased to 28 thousand tons in 2020, declined to 8 thousand tons in 2022, and rose again to 27 thousand tons in 2023 (BPS-Statistics Indonesia, 2024). In Indonesia, strawberries are typically cultivated in highland regions; however, climate change has necessitated adjustments in cultivation practices to improve production and fruit quality (Adrian et al., 2025). West Java Province is the largest contributor to national strawberry production, accounting for 97% (24 thousand tons) of Indonesia's total strawberry production in 2023.

Strawberries are among the crops whose pollination is assisted by stingless bees in West Bandung. During observations, environmental conditions, including temperature, humidity, and light intensity, were recorded at 29.4 °C, 60.6%, and 1,047.4 lux, respectively (Atmowidi et al., 2022). One of the major factors contributing to low strawberry production in Indonesia is damage caused by plant pests and diseases, including plant-parasitic nematodes (Bozbuga et al., 2021). Plant-parasitic nematodes are estimated to cause annual strawberry yield losses of up to 12% in Egypt (Abd-Elgawad, 2014). Nematode genera reported to infect strawberry plants in Indonesia include *Aphelenchoides, Criconemella, Helicotylenchus, Hemicycliophora, Meloidogyne, Paralongidorus, Pratylenchus, Rotylenchulus, Rotylenchus, Scutellonema, Tylenchus*, and *Xiphinema* (Kusumawardhani et al., 2025).

Kurniawati et al. (2025) reported that the nematode *A. fragariae* infects strawberry plants, causing aboveground symptoms such as stunted

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growth, reddened foliage, small crimped or curled leaves, and malformed buds and flowers. In Indonesia, *Scutellonema* nematodes were first reported to be associated with strawberry plants in West Java (Kurniawati et al., 2023). However, that report did not describe the specific characteristics of the *Scutellonema* species involved.

The genus *Scutellonema* comprises approximately 50 species and has a wide host range (Talavera et al., 2019). Members of this genus can cause yield losses in cultivated plants due to their root-feeding activity. *Scutellonema* species known to infect strawberry plants include *S. brachyurus* and *S. clathricaudatum*. *Scutellonema brachyurus* is widely distributed worldwide, including in Egypt, South Africa, Japan, India, Thailand, Greece, Italy, the United States, Australia, and Brazil. This polyphagous nematode infects a broad range of horticultural, ornamental, and wild plants, with primary hosts including banana, cotton, soybean, and tobacco. In the United States, *S. brachyurus* has been reported to cause a 48% reduction in cotton plant growth (Haque & Khan, 2021). Despite its global distribution, data on *S. brachyurum* infection in Indonesia remain limited.

Identification of *S. brachyurum* nematodes in strawberry plants has not been widely reported in Indonesia. Molecular approaches are commonly used to identify species that lack distinct morphological characteristics. Ribosomal DNA regions, particularly the D2–D3 expansion segments of 28S rRNA, have been widely used for the identification of *Scutellonema* species (Trinh et al., 2022). Therefore, this study aims to identify and characterize *S. brachyurum* nematodes infecting strawberry plants based on both morphological and molecular characteristics.

MATERIALS AND METHODS

Research Site. Sampling was conducted in Lebakmuncang Village (7°06'07.3" S, 107°25'37.4" E), Ciwidey District; Alamendah Village (7°07'50.8" S, 107°25'49.5" E), Rancabali District, Bandung Regency; and Langensari Village (6°49'52.2" S, 107°38'37.6" E), Lembang District, West Bandung Regency, West Java. Nematode identification was carried out at the Plant Nematology Laboratory and the Education Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. The study was conducted from September 2023 to March 2024.

Sampling. Soil and plant root samples were collected purposively by distinguishing between asymptomatic and symptomatic plants. Symptoms of nematode infection in strawberry plant crowns included stunted growth, chlorotic and reddened leaves, and small leaves that were curled or wrinkled. Soil samples were collected at a depth of 15–20 cm, while root samples were taken from both primary and fine roots. All samples were placed in plastic clip bags and stored in a cooling box.

Nematode Extraction. Nematodes were extracted from soil samples using the flotation-centrifugation method (Cavaness & Jensen, 1955). A 100 g soil sample was mixed with 900 mL of water, sieved through 20- and 400-mesh sieves, and then transferred into 15 mL Falcon tubes for centrifugation at 1500 rpm for 5 min. The supernatant was discarded, and the sediment was mixed with a 40% sugar solution, followed by centrifugation at 1700 rpm for 1 min. The resulting supernatant was passed through a 400-mesh sieve, rinsed thoroughly with water to remove residual sugar, and collected in a storage bottle maintained at 10 ºC.

Nematodes from root samples were extracted using a modified mist chamber method (Hooper et al., 2005). Roots were cut into 1–2 cm segments, and 5 g of root material was used. The root pieces were placed on a funnel lined with a sieve with a 0.2 cm mesh diameter and positioned inside the mist chamber. After 72 hours of misting, the suspension was sieved through a 400-mesh sieve and collected in a 25 mL container. The samples were stored at 10 ºC and were ready for identification.

Identification Based on Morphological Characters. Nematode identification was performed using standard morphological taxonomic methods, following the reference book **"**Pictorial Key to Genera of Plant-parasitic Nematodes" (Mai & Lyon, 1975), supported by additional relevant literature.

Identification Based on Molecular Characters. Molecular identification followed the DNA extraction method described by Holterman et al. (2006). DNA amplification was performed using a Bio-Rad T100™ Thermal Cycler with universal primers D2A (forward) (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (reverse) (5′-TCG GAA GGA ACC AGC TAC TA-3′), targeting an amplicon of approximately 700 bp (Nunn, 1992). The PCR protocol consisted of 30 cycles of denaturation at 95 ºC for 30 s, annealing at 54 ºC for 30 s, and elongation at 72 ºC for 15 s.

The amplification process was initiated with an initial denaturation at 95 ºC for 3 min and concluded with a final extension at 72 ºC for 10 min.

PCR products were separated by electrophoresis at 100 V DC for 30 min using an Advanced Mupid Exu Electrophoresis system, visualized under a UV transilluminator (Bio-Rad® UView™ Mini Transilluminator) and documented using a digital camera. DNA sequencing was conducted by a commercial sequencing service provider. Sequence data were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website. Nucleotide sequence alignment and homology analyses were performed using ClustalW in BioEdit Sequence Alignment Editor version 7.2.5.0. Phylogenetic relationships were inferred using Molecular Evolutionary Genetics Analysis software (MEGA version 12) with the maximum likelihood method and 1000 bootstrap replications (Khan et al., 2008).

RESULTS AND DISCUSSION

Disease Symptoms in Strawberry Plants. Disease symptoms in strawberry plants were observed primarily on the upper part of the plants in the form of chlorotic leaves (Figure 1A). Infected roots exhibited lesions and reduced root mass (Figure 1B). In contrast, healthy roots appeared brighter and showed a greater root mass (Figure 1C). Affected plants showed stunted growth and, in advanced stages, failed to produce fruit (Figure 1D). These symptoms are consistent with those reported by Machado et al. (2018) in soybean plants infected by *Scutellonema*, which included stunted growth, chlorotic foliage, and root lesions. *Scutellonema* nematodes cause mechanical damage to root cells, leading to the formation of necrotic lesions. These lesions may also affect cells that are not directly penetrated by nematodes, indicating damage resulting from both mechanical injury and the chemical activity of cell wall–degrading enzymes. Feeding activity is intracellular and is limited to epidermal and cortical cells (Schuerger & McClure, 1983). Nematode feeding on roots leads to the development of necrotic lesions that interfere with the plant's ability to absorb water and nutrients, resulting in stunted growth and reduced fruit yield (Trinh et al., 2022). The pathogenicity of these nematodes is further enhanced by their ability to secrete enzymes that degrade plant cell walls, facilitating feeding activity and increasing the severity of root damage (Shokoohi, 2021; Trinh et al., 2022).

Identification of *Scutellonema* Based on Morphological Characters. *Scutellonema* nematodes are cylindrical (vermiform) and possess four lateral lines. The body is curved into a 'C' shape to spiral during the resting (dormant) phase (Figure 2A). The lip region is slightly set off, and the stylet is well developed with distinct, rounded, and flattened knobs (Figure 2B). The median bulb is round to oval, and the esophageal gland overlaps the intestine. The female gonad is amphidelphic, and the vulva is located at the mid-body region (Figure 2D). An enlarged phasmid, known as the scutellum, is located opposite the anus (Figure 2C). The female tail is short with a rounded terminus and lacks a mucro (Figure 2C). Male nematodes were not observed, suggesting parthenogenetic reproduction. *Scutellonema* reproduces primarily by parthenogenesis,

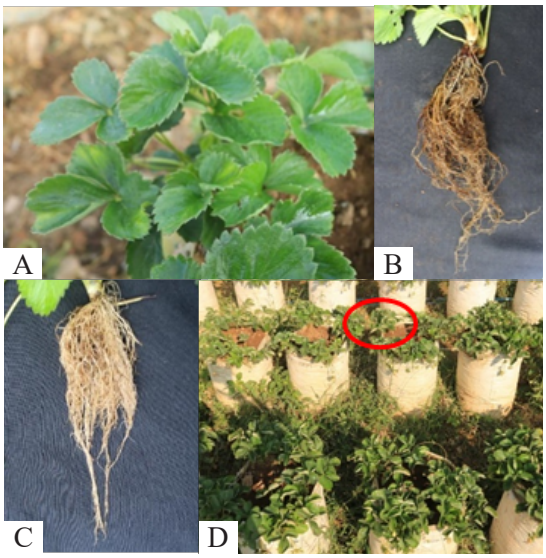

Figure 1. Variation of symptoms in strawberry plants. A. Chlorosis; B. Root lesions and reduced root number; C. Healthy plant roots; D. Stunted plants.

with males comprising only about 9% of the total population when present (Grosmaire et al., 2019).

Similar morphological characteristics were reported by Trinh et al. (2022), who described adult females as vermiform with four lateral lines, a set-off lip region, a stylet with rounded knobs, an oval median bulb, a vulva located at mid-body, an enlarged phasmid positioned posterior to the vulva and opposite the anus, and a rounded female tail tip. Males were also absent in their observations.

Identification of *Scutellonema* Based on Molecular Characters. DNA amplification demonstrated that the *Scutellonema* population from West Java was successfully amplified, producing a fragment of approximately 700 bp (Figure 3). The D2–D3 expansion region of the 28S rRNA gene was sequenced for nucleotide analysis.

The *Scutellonema* sequence obtained from Indonesia has been deposited in the GenBank database under accession number LC801363.1. Alignment analysis was conducted using isolates from other countries for comparison. BLAST analysis revealed that the *Scutellonema* sample from West Java exhibited the highest sequence similarity (95.18%) with an *S. brachyurus* isolate from the United States (KX959259.1) (Table 1).

Homology analysis of six ingroup isolates showed that nucleotide similarity among *S. brachyurus* isolates ranged from 87.8% to 93.8% (Table 2). The Indonesian isolate (LC801363.1) showed the highest homology (93.8%) with the isolate from the United States (KX959259.1) and the lowest homology (87.8%) with isolates from South Africa (JX472056.1). The outgroup isolate from Taipei (FJ799117.1), identified as *Pratylenchus penetrans*, exhibited a homology of 39.7%.

Phylogenetic analysis of the 28S rRNA D2–

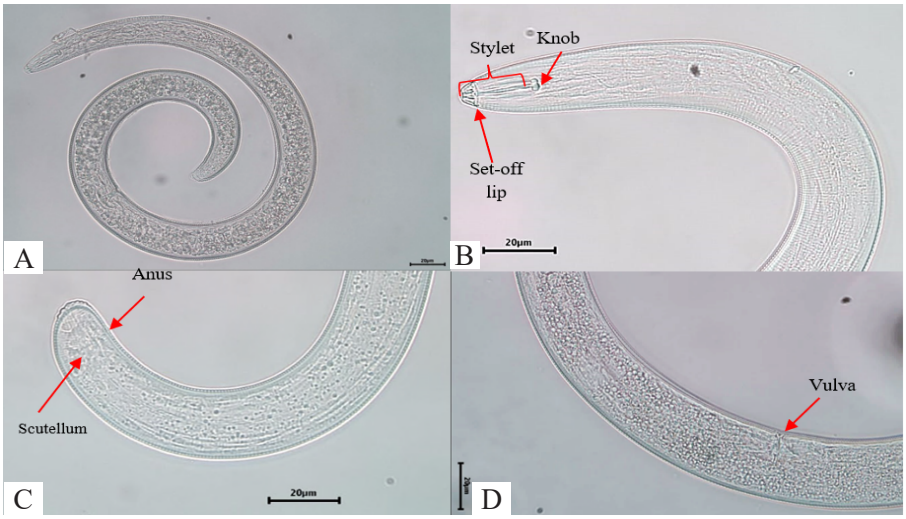

Figure 2. Morphology of *Scutellonema* nematodes. A. Female nematode; B. Anterior region; C. Posterior region; D. Vulva.

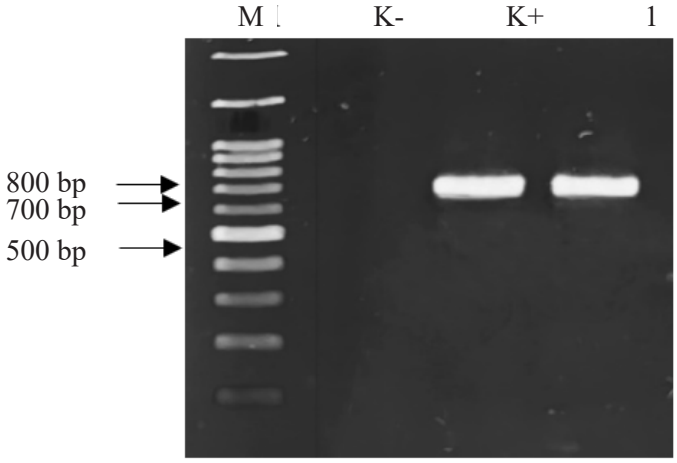

Figure 3. PCR amplification results of the 28S rRNA region (~ 700 bp). M = 100 bp DNA ladder marker (Thermo Scientific, USA); K− = Negative control; K+ = Positive control; 1 = *Scutellonema* isolate from West Java.

D3 region using six *S. cutellonema* isolates and *P. penetrans* as the outgroup revealed that Indonesian *S. brachyurum* isolates were closely related to isolates from other countries but formed distinct clades (Figure 4). The branch length between the Indonesian isolate and the South African isolate was longer than that between the Indonesian and U.S. isolates (clades 3), indicating a closer evolutionary relationship with the U.S. isolate. Longer branches on the cladogram represent greater nucleotide divergence resulting from longer evolutionary processes, whereas shorter branches indicate fewer nucleotide changes and closer taxonomic relationships (Suvorov & Schrider 2024).

Population of *Scutellonema*. *Scutellonema brachyurum* was identified at all three sampling locations (Table 3). Population of *S. brachyurum* were higher in symptomatic plants than in asymptomatic plants, and nematodes were detected in both soil and root samples. This distribution is consistent with the

Table 1. BLAST results of *Scutellonema brachyurum* isolates and the outgroup based on 28S rRNA gene sequences

Nematode species	Isolate	Accession number	Country	Host	Similarity (%)
Scutellonema brachyurum	RB	LC801363.1	Indonesia	*Fragaria* spp.	-
S. brachyurus	X50	KU059494.1	Greece	-	95.15
S. brachyurus	CD1510	KX959259.1	USA	*Ficus nitida*	95.18
S. brachyurus	-	DQ328753.1	Italy	-	93.82
S. brachyurus	CD549	JX472056.1	South Africa	-	90.24
S. brachyurus	CD1380	KX959263.1	Costa Rica	-	94.32
Pratylenchus penetrans	-	FJ799117.1	Taipei	*Fragaria* spp.	47.00

Table 2. Percentage homology of Indonesian *Scutellonema brachyurum* isolates from West Java compared with isolates from several countries based on 28S rRNA nucleotide sequences analyzed using BioEdit version 7.7.1

Accession number	Isolate	Homology (%)						
		1	2	3	4	5	6	7
LC801363.1	*Scutellonema brachyurum* Indonesia	ID	93.4	93.8	92.9	87.8	93.1	39.7
KU059494.1	*S. brachyurus* Greece	93.4	ID	99.2	98.7	92.4	98.9	40.9
KX959259.1	*S. brachyurus* USA	93.8	99.2	ID	99.0	92.9	99.2	40.7
DQ328753.1	*S. brachyurus* Italy	92.9	98.7	99.0	ID	92.4	98.7	40.7
JX472056.1	*S. brachyurus* South Africa	87.8	92.4	92.9	92.4	ID	92.6	40.2
KX959263.1	*S. brachyurus* Costa Rica	93.1	98.9	99.2	98.7	92.6	ID	40.6
FJ799117.1	*Pratylenchus penetrans* Taipei	39.7	40.9	40.7	40.7	40.2	40.6	ID

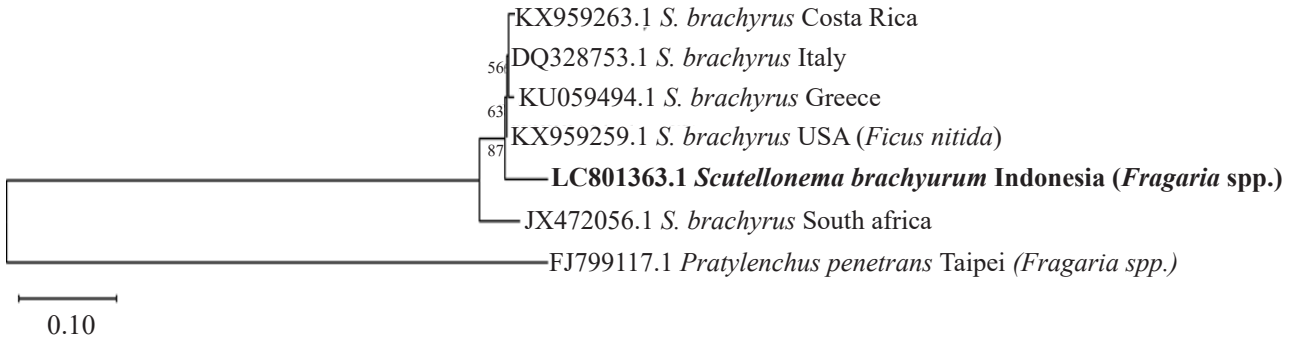

Figure 4. Phylogenetic tree of *Scutellonema brachyurum* isolates from Indonesia, from West Java (bold) against isolates from several countries based on 28S rRNA nucleotide sequencing. The nematode *Pratylenchus penetrans* was used as the outgroup.

Table 3. The population of nematode *Scutellonema brachyurum* in soil and strawberry plant roots in Ciwidey, Lembang, and Rancabali Districts

Sample	Locations	Fields	Population of nematodes *S. brachyurum* (nematode)*	
			SP	AP
Root (5 g)	Ciwidey	1	-	-
		2	-	-
	Lembang	1	-	-
		2	3	-
	Rancabali	1	2	-
		2	-	-
Soil (100 g)	Ciwidey	1	1	-
		2	-	-
	Lembang	1	1	-
		2	-	-
	Rancabali	1	-	1
		2	6	2

*SP: Symptomatic plants, AP: Asymptomatic plants.

feeding behavior of *S. brachyurum*, which which is classified as an ectoparasite or semi-endoparasite, with only the anterior portion of the body penetrating the root cortex (van den Berg et al., 2025).

The higher abundance of *S. brachyurum* in soil samples reflects the fact that part or all of its life cycle occurs in the soil. Males are rarely observed; therefore, reproduction primarily occurs through parthenogenesis. The life cycle of *S. brachyurum* includes egg, juvenile, and adult stages. Eggs are laid in the soil or within cortical or ectodermal root tissues (Haque & Khan, 2021). Nematodes typically overwinter as eggs in the soil, with juveniles emerging in the spring and migrating to host roots, where they feed and develop into adults. Adult nematodes continue feeding on root tissues, causing additional damage and reproducing to complete the life cycle (Shokoohi, 2021).

Scutellonema brachyurus can form large populations on suitable hosts. Population growth is optimal at temperatures around 28 °C, whereas reproduction decreases significantly at 18 °C (Claudius-Cole & Fawole, 2016). The temperatures at the three sampling locations ranged from 23 to 25 °C, which falls within the range that supports nematode activity and reproduction, although not at optimal levels.

CONCLUSION

Based on morphologically identification and confirmation using molecular characterization of the 28S rRNA gene, the nematodes associated with strawberry plants were successfully identified as *Scutellonema brachyurum*. *S. brachyurum* isolates from West Java showed 93.8% sequence similarity with isolates from the United States. Phylogenetic analysis indicated that the West Java isolate is closely related to *S. brachyurus* isolates from the USA. This nematode species was detected in Lebakmuncang Village, Ciwidey District, and Alamendah Village, Rancabali District, Bandung Regency, as well as in Langensari Village, Lembang District, West Bandung Regency.

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AUTHORS' CONTRIBUTIONS

FK and SPN conceptualized and designed the experiments. DSQ conducted the molecular analyses. FK, SPN, and DSQ prepared the manuscript. FK

and SPN analyzed and interpreted plant damage observations. All authors reviewed and approved the final manuscript.

COMPETING INTEREST

The authors declare no competing interests. The research, analysis, and conclusions presented in this study were conducted independently and are free from any financial, personal, or professional conflicts that could have influenced the results.

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