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SHORT COMMUNICATION

Description of the morphology, morphometric, and molecular of *Aphelenchoides fragariae* (Aphelenchida: Aphelenchoididae) causing crimp disease of strawberry in Indonesia

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ABSTRACT

Aphelenchoides fragariae, commonly known as strawberry crimp nematodes, primarily target the aerial parts of plants, affecting both internal and external structures. In Indonesia, where strawberries are predominantly cultivated in highland regions, the presence of strawberry crimp disease has been confirmed. Infected plants exhibit symptoms such as stunted growth, reddened foliage, crimped or curled leaves, and malformed buds and blooms. Aboveground damage caused by the nematodes includes contorted shoots, undersized leaves, and reddish petioles, often accompanied by discolored patches on the foliage. These symptoms significantly impair the growth and productivity of strawberry plants, highlighting the nematode's potential as a serious pest in these regions. The identification of *A. fragariae* was achieved through a combination of morphological and molecular characterization methods. Species confirmation relied on PCR amplification of the nematode's cytochrome oxidase subunit I (COI) gene, using primers (COI F and COI R) designed in the laboratory. The amplification yielded a specific fragment of approximately 550 base pairs, which was sequenced for further analysis. Sequence alignment revealed identity levels ranging from 82.8% to 99.7%, confirming the presence of *A. fragariae*. The resulting sequences were deposited in GenBank under the accession numbers LC804455 (*A. fragariae* isolate RB) and LC804456 (*A. fragariae* isolate LB), providing a valuable resource for future studies on this nematode species.

Key words: foliar nematode, *Fragaria x ananassa* Duch, nematode identification

INTRODUCTION

The recent discovery of *Aphelenchoides fragariae*, commonly known as strawberry crimp nematodes, infesting strawberry plants in Indonesia marks a significant milestone in the country's agricultural landscape. Until now, there have been no documented reports of *A. fragariae* affecting strawberry crops in Indonesia, making this finding a groundbreaking revelation. This newly identified occurrence unveils a previously unreported threat to strawberry cultivation, a crop that holds economic and cultural significance in various regions. Strawberry crimp nematodes are notorious for their ability to attack both internal and external plant structures, resulting in symptoms such as stunted growth, reddened foliage, crimped or curled leaves, and malformed buds and blooms growth, reddened foliage, crimped or curled leaves, and malformed buds and blooms (Cobon et al., 2011). These symptoms not only compromise the health of the plants but also reduce the marketable yield and quality of strawberries, potentially leading to substantial economic losses for farmers.

The identification of *A. fragariae* in Indonesian strawberry fields highlights the urgent need for vigilance and the implementation of proactive management strategies. Early detection through regular field monitoring and diagnostic testing is crucial to minimize the spread and impact of this nematode. Integrated pest management (IPM) approaches, including cultural practices, resistant varieties, and biological control agents, may offer sustainable solutions for affected growers.

Additionally, this discovery underscores the necessity of further research into the biology, ecology, and host range of *A. fragariae* to better understand its behavior in local conditions. The study by Nabilah et al. (2021) on the diversity and abundance of nematodes in guava (Psidium guajava L.) cultivation in Lampung provides insights into nematode diversity in Indonesia,

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emphasizing the need for similar investigations in strawberry cultivation. Collaborative efforts among researchers, extension workers, and farmers will be instrumental in developing effective strategies to mitigate the nematode's impact. This pioneering report not only raises awareness of an emerging threat but also sets the stage for informed interventions to protect and sustain strawberry cultivation in Indonesia.

MATERIALS AND METHODS

Research Site. The research commenced with sample collection from strawberry plantations located in Alamendah Village, Rancabali District, Bandung Regency (7°07'17.2"S,107°25'27.3"E), and Langensari Village, Lembang District, West Bandung Regency (6°49'52.2"S,107°38'37.6"E) from September to December 2023. The identification of *A. fragariae* nematodes was conducted in the Plant Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University, from January to March 2024.

Nematode Extraction. Extraction was carried out using the soaking method. Strawberry leaves were cut into 1 cm pieces, placed in Petri dishes (ø 9 cm), and submerged in distilled water until fully covered. The samples were incubated at a cold temperature in a refrigerator (4 ºC) for two days. Nematode suspensions collected in the Petri dishes were filtered using 100-mesh and 400-mesh sieves, then stored in collection bottles. The obtained nematodes were observed under a stereoscopic microscope (Olympus SZ61, US).

Identification Based on Morphological Characters. The extracted nematodes were identified following standard morphological taxonomy methods for the genus *Aphelenchoides* Fischer (Aphelenchoididae) as described in several references, including: 1). Pictorial Key to Genera of Plant-parasitic Nematodes (Mai & Lyon, 1975); 2). Taxonomy and Identification of Principal Foliar Nematode Species (*Aphelenchoides* and *Litylenchus*) (Handoo et al., 2020); 3). C.I.H. Description of Plant-parasitic Nematodes (Siddiqi, 1975).

Identification Based on Morphometric Characters. Morphometric identification was conducted alongside morphological identification. Nematode suspensions were analyzed based on key morphometric characters described by De Man (1880). Morphological characters used for the identification of *A. fragariae* species included body shape, lip region shape, metacorpus shape, stylet shape, stylet length, vulva position, body length and width, and mucro in female nematodes. For male nematodes, specific morphological characters included body shape and tail-end shape. The presence of males in a population is an important factor supporting nematode species determination (Siddiqi, 1975).

Nematode body measurements were performed using a calibrated Olympus 16 BX51 binocular compound microscope (De Man, 1880). The collected data were processed and analyzed using Microsoft Excel 2013.

Identification Based on Molecular Characters. DNA extraction of nematodes was performed following the method described by Holterman et al. (2006). One to three nematodes were placed into a 0.2 mL PCR collection tube containing 25 µL of nuclease-free water. An extraction buffer solution containing 200 mM NaCl, 200 mM Tris-HCL (pH 8), 1% 2-mercaptoethanol, and 800 µg/mL Proteinase K (25 µL) was then added. The mixture was vortexed for 1 min, incubated at 65 ºC for 90 min, and subsequently heated at 99 ºC for 5 min in a water bath. The prepared extract was either used immediately for PCR or stored at -20 ºC.

COI DNA amplification was conducted using PCR with primer pairs COI-F1 (5'-CCTACTATGATTGGTGGTTTTGGTAATTG-3') and COI-R2 (5'-GTAGCAGCAGTAAAATAAGCAC G-3'). The PCR program included the following steps: pre-denaturation at 94 ºC for 4 min, 30 cycles of denaturation at 94 ºC for 1 min, primer annealing at 55 ºC for 1 min, and DNA extension at 72 °C for 2 min. A final extension step was performed at 72 °C for 10 min, followed by stored at 4 ºC. The PCR product were then electrophoresed and visualized under a UV transilluminator to observe the DNA bands, which were documented using a digital camera (Nikon D7000, Japan).

Nucleotide sequencing was performed by sending the amplified DNA fragments to a commercial sequencing service provider. The sequencing results were analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGETYPE=BLASTHome). The obtained nucleotide sequences were further analyzed using multiple sequence alignments in the ClustalW algorithm via the Bioedit sequence alignment editor (version 7.0.3.5). Phylogenetic relationships among

isolates were constructed using the Molecular Evolutionary Genetic Analysis (MEGA XI) software (Tamura et al., 2021) with the neighbor-joining method and 1000 bootstrap replicates.

RESULTS AND DISCUSSION

The Symptoms of Crimp Disease in Strawberries. *A. fragariae*, also known as strawberry crimp nematodes, are both endo- and ectoparasites that primarily target the aerial parts of plants. Their host range is extensive, encompassing over 250 plant species from 47 botanical families (Siddiqi, 1975). In Indonesia, strawberries are predominantly cultivated in highland regions such as Lembang (West Bandung Regency) and Rancabali (Bandung Regency) in West Java Province (Figure 1). The presence of strawberry crimp disease, caused by *A. fragariae,* has been confirmed in these areas through field surveys.

Aboveground symptoms include stunted growth, reddened foliage, diminutive crimped or curled leaves, and malformed buds and blooms (Figure 2). These symptoms align closely with those described by Subbotin (2024), indicating that the

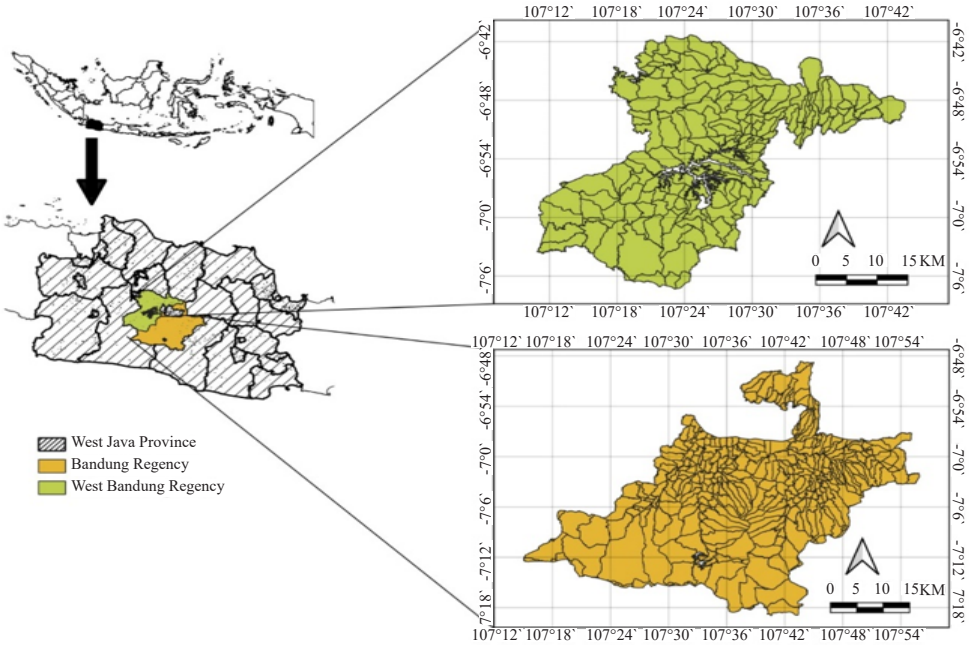

Figure 1. Map of sample locations in Lembang (West Bandung Regency) and Rancabali (Bandung Regency), West Java Province, Indonesia.

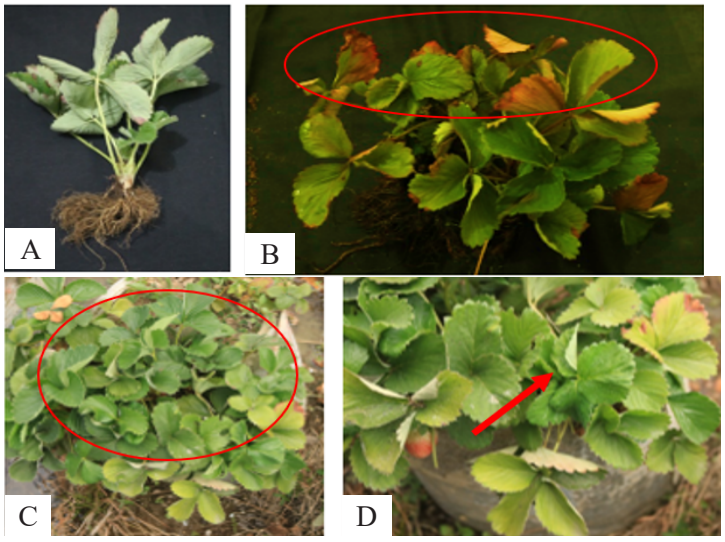

Figure 2. Above-ground symptoms on strawberry plants infested with the nematode *Aphelenchoides fragariae*. A. Stunted growth; B. Reddish leaves; C. Tiny, crimped leaves; D. Malformed buds and blooms.

nematode predominantly affects the plant's aerial structures, causing anomalies such as contorted shoots and undersized leaves with wrinkled margins. Additionally, reddish petioles and discolored patches with rough, hardened surfaces may also be observed. According to Anindita et al. (2021), symptom caused by *A. fragariae* have also reported on shallot bulbs in several regions in Indonesia, including curled leaves, reduced bulb size, and wilting.

Furthermore, research on the mass rearing of *A. fragariae* had been conducted by Kurniawati et al. (2024) using fungal cultures for future research purposes. Their study found that the fungus *Alternaria porri,* incubated at a temperature of 28 ºC, is suitable for the mass rearing of *A. fragariae*.

Identification of *A. fragariae* Nematodes through Morphological Characters. To identify the nematode *A. fragariae* responsible for this disease, both morphological and molecular characterization methods were employed. Female and male *A. fragariae* have slender, worm-like bodies that closely conform to the contours of the host (Figure 3a and 3e). They possess a distinct protruding lip region and a slender stylet, with a visible, fully rounded, and well-developed median bulb (Figure 3b). The vulva is visibly divided and slightly protruding. The tail of females is elongated, conoid, and ends in blunt, spine-like points (Figure 3c).

In contrast, male *A. fragariae* exhibit spicules resembling roses thorns on a comparatively thinner body than females. The males' elongated, conoid tail features a single mucro at the tip and curves from 45º to 90º when relaxed (Figure 3d). These morphological characteristics align with the observations of Khan et al. (2007), who describe female nematodes as having slender bodies that are nearly straight or gently curved ventrally when relaxed. The body tapers towards a pointed end with a peg-like structure, and the lip region blends seamlessly with the body. The stylet is thin, with a well-developed, rounded or oval median bulb. The post-uterine sac extends more than halfway between the vulva and anus. The tail is elongated and cone-shaped, and terminates with a small mucro-like structure.

Males are prevalent as females and exhibit a relaxed tail that curves, ending in a simple blunt terminal spine. Their spicules resemble rose thorns, accompanied by caudal papillae. Notably, male lack a bursa and gubernaculum.

Identification of *A. fragariae* Nematodes through Morphometric Characters. Morphometric measurements of adult female and male *A. fragariae* nematodes (n = 10), collected from strawberry plants, were as follows:

Female: Body length ranged from 440.1–642.5 µm (548.7 ± 56.2 µm), mid-body width from 11.6–15.7 µm (13.05 ± 1.4 µm), stylet length from 10.4–13.2 µm (11.5 ± 0.8 µm), and tail length from 28.05–39.3 µm (34.1 ± 3.9 µm). The distance from the anterior and to the vulva (V) was 61.8–70.9% (67.01 ± 2.7%). The following ratio values were recorded for females:

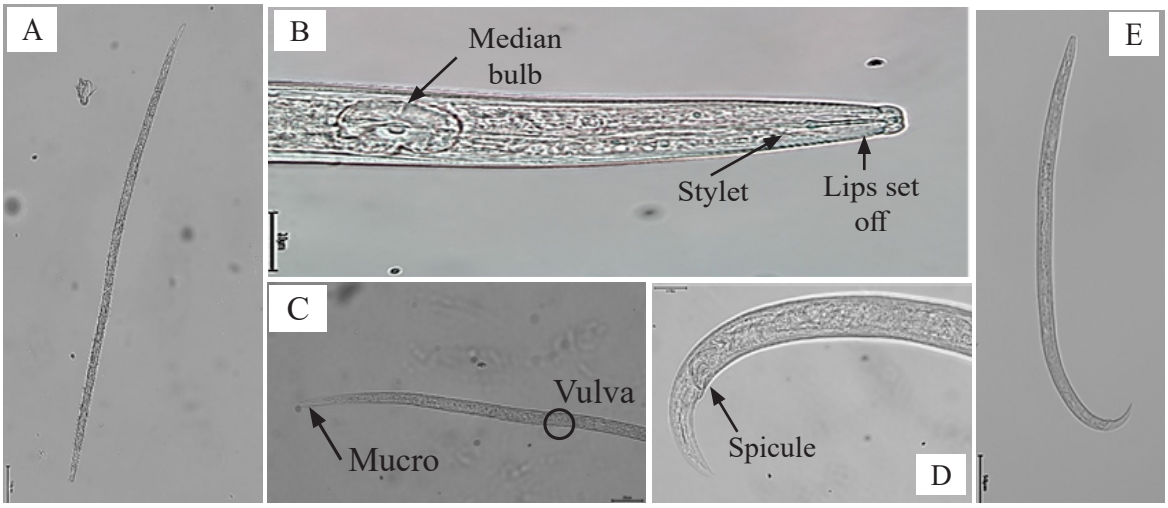

Figure 3. The characteristics of *Aphelenchoides fragariae*. In females (A-C), features include. A. slender body of female; B. lips off set, a delicate stylet, and large median bulb; C. Vulva and one mucro in the tip of tail. In males (D-E), consist of; D. Spicule like rose thorns, and a mucro positioned at the tail's tip; E. Slender body with a conoid tail. Specimens a, c, and e were examined using a compound microscope at a 20 × 10 magnification, while specimens b, d were observed at a 40× 10 magnification.

a = 33.8–49.5 (42.3 ± 4.6)
b = 4.5–7.0 (5.4 ± 0.7)
b‘ = 4.1–6.04 (4.8 ± 0.6)
c = 13.4–19.6 (16.2 ± 2.2)
c‘ = 52.4–74.7 (66.5 ± 8.3).

Male: Body length ranged from 449.4–603.7 µm (523.4 ± 44.2 µm), stylet length from 9.4–13.8 µm (11.3 ± 1.4 µm), tail length (T) from 26.7–35 µm (32.4 ± 2.6 µm), spicule length from 13.2–21.03 µm (17.8 ± 2.6 µm), and testis length from 36.4–65.7 µm (50.5 ± 9.4 µm). The following ratio values were recorded for males:

a = 33.3–49.4 (43.0 ± 4.9)
b = 3.6–6.8 (5.1 ± 0.8)
b‘ = 3.6–6.0 (4.6 ± 0.6)
c = 13.8–18.0 (16.3 ± 2.1)
c‘ = 44.6–62.9 (53.8 ± 7.4).

These morphometric features align with *A. fragariae* as described by Chizhov et al. (2006), which parasitizes fern plants in Moscow, and Khan et al. (2007) parasitizes *Helianthus tuberosus* and *Weigela subsessilis* in Korea.

Identification of *A. fragariae* Nematode Through Molecular Characters. Species identification was confirmed through PCR analysis. The partial gene sequence of cytochrome oxidase subunit I (*COI*) was amplified using primers designed in our laboratory: COI F (5’-GTTGCTGCCTGTTTCGTTATT-3’), COI R (5’-ACACAACCAATAAGCCCAATTC-3’). This amplification resulted in a specific fragment of approximately 550 bp (Figure 4).

Nucleotide Sequencing Analysis and Phylogenetic Tree Construction. The sequences of the amplicons showed 99.7% similarity with *A. fragariae* (GenBank Accession No. MF669524), 94.2% similarity with *A. fragariae* (GenBank Accession No. KX356857), and 82.8% similarity with *A. fragariae* (GenBank Accession No. AB067761). This homology data was further analyzed using Sequence Demarcation Tools (SDT) (Figure 5). All sequences were submitted to GenBank as LC804455 (*A. fragariae* isolate RB) and LC804456 (*A. fragariae* isolate LB) for the partial gene of cytochrome oxidase subunit I (*COI*).

The phylogenetic tree was constructed based on nucleotide sequences and revealed three main branches. *A. fragariae* from Lembang (LC804456) and Rancabali (LC804455) showed close genetic similarity to *A. fragariae* isolate SP (MF669524), followed by *A. fragariae* voucher A.frag ND2 (KX356857) and *A. fragariae* (AB067761). In contrast, other sequence from GenBank, including *Aphelenchoides* sp. (KX356899, GU367859, KX3356885, KX356889, and KX356883), *Laimaphelenchus liaoningensis* (MT808402), and *Xiphinema index* (CV127983), were grouped on a separate branch as outgroups (Figure 6).

CONCLUSION

Aphelenchoides fragariae, commonly known as Strawberry crimp nematodes, were identified as the causative agent of crimp disease in strawberries, primarily affecting the aerial parts of the plant. The disease has been confirmed in major strawberry cultivation areas in Indonesia, particularly in highland regions such as Lembang and Rancabali. Observed symptoms, including stunted growth, reddened foliage, and malformed buds and blooms, closely align with those reported in previous studies. Morphological and molecular characterization methods were employed

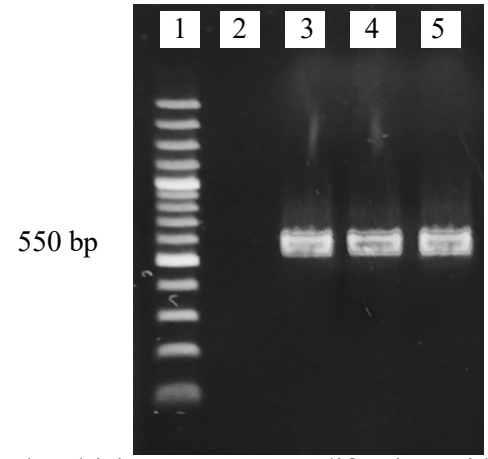

Figure 4. Visualization of mitochondrial DNA COI amplification with specific primers for *Aphelenchoides fragariae*: Line 1: DNA ladder 100 bp (Thermo Scientific, US), line 2: Negative control, line 3: Positive control, line 4: Sample from Rancabali, line 5: Sample from Lembang.

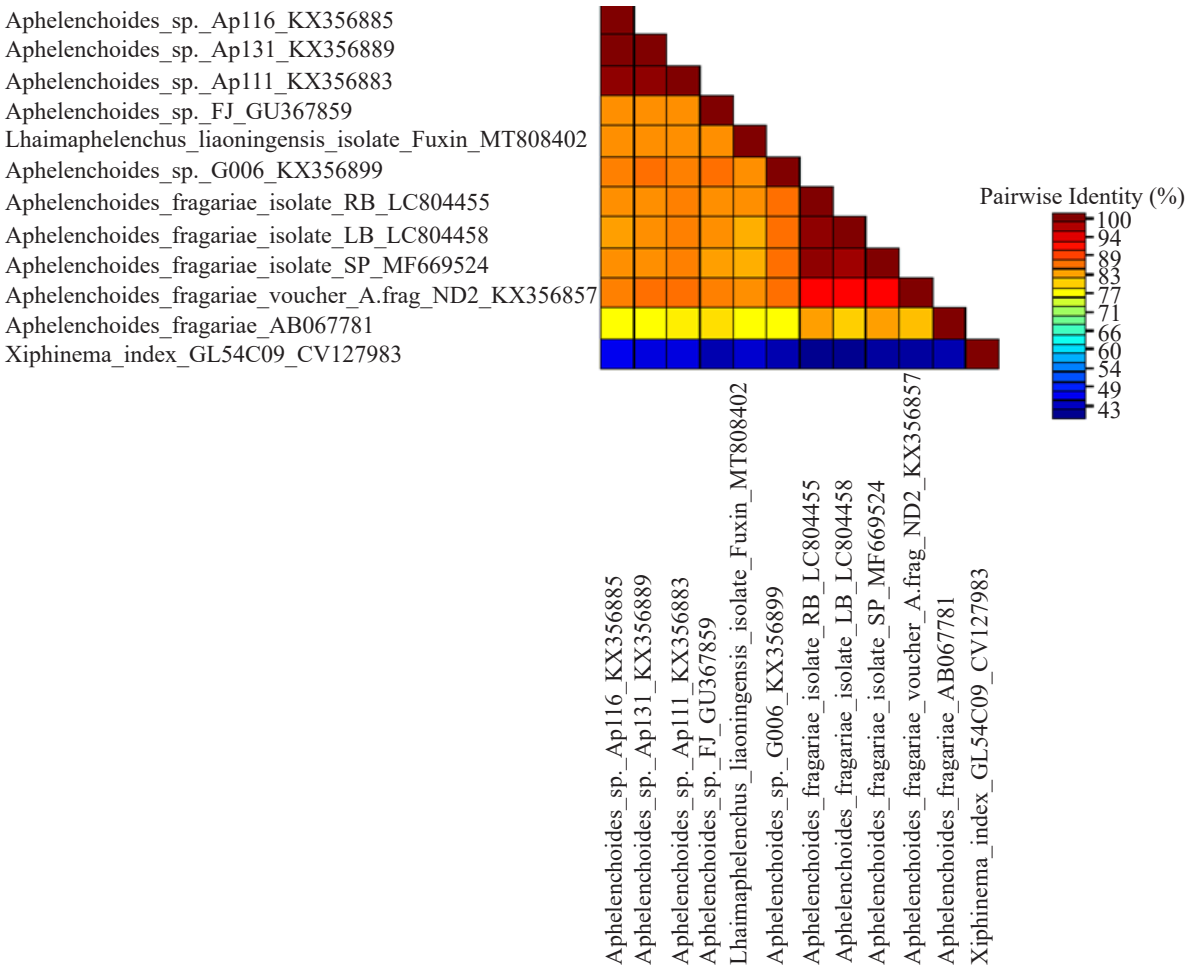

Figure 5. The SDT interface displays a color-coded pairwise identity matrix comparing *Aphelenchoides fragariae* with other nematodes. Each cell in the matrix is color-coded to represent the percentage identity score between the two sequences, with one sequence shown horizontally on the left and the other vertically below. A color key is provided to show the relationship between pairwise identities and the colors displayed in the matrix.

to accurately identify *A. fragariae* nematodes. Morphological features matched previously documented descriptions, and PCR analysis confirming species identification. Nucleotide sequencing revealed high similarity with known *A. fragariae* sequences, further validating the taxonomic classification. Phylogenetic tree construction highlighted the close genetic relationship between *A. fragariae* isolates from different locations.

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AUTHORS' CONTRIBUTIONS

I and SPN conceptualized and planned the experiments. ESH and HSF performed molecular work and analysis. I, SPN, and ETT prepared the manuscript. I and SPN analyzed and interpreted plant damage observations.

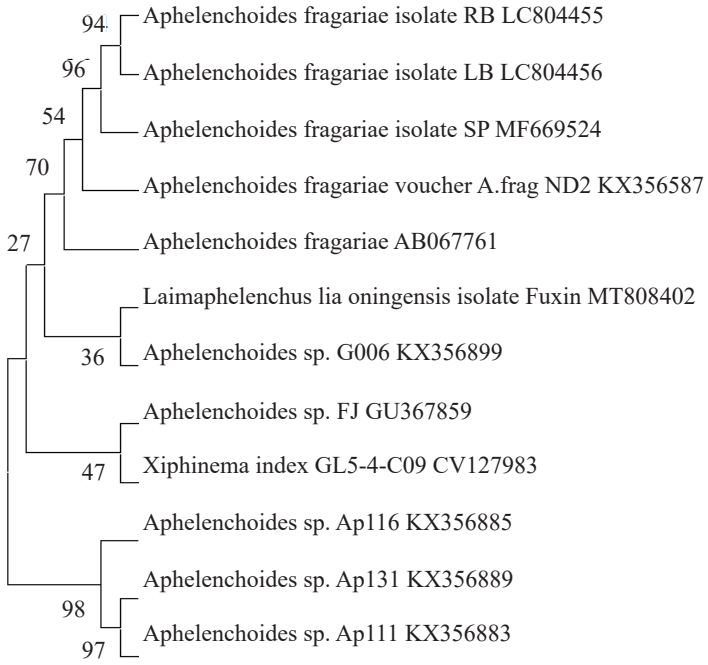

Figure 6. Neighbor joining phylogenetic tree of the partial cytochrome oxidase subunit I (COI) sequences of *Aphelenchoides fragariae* and outgroups.

COMPETING INTEREST

The authors declare no competing interests related to the study of Description of the morphology, morphometric, and molecular of Aphelenchoides fragariae (Aphelenchida: Aphelenchoididae) causing crimp disease of strawberry in Indonesia. The research, analysis, and conclusions presented in this publication were conducted independently and are free from any financial, personal, or professional conflicts that could influence the findings.

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