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Morphological, Molecular, and Morphometric Analysis of *Aphelenchoides besseyi* on Organic Rice (*Oryza sativa*) Seeds in the Beringin District, Deli Serdang Regency, Indonesia

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Abstract

Aphelenchoides besseyi, a nematode, can infect rice resulting in the white shoot disease, which decreases the germination rate and even causes seedling death. *A. besseyi* has recently been reported to mainly attack the organic rice varieties especially Pandanwangi and Ciherang in the Pasar Kawat, Beringin District, Deli Serdang, Indonesia. This research investigated *A. besseyi* via morphological, molecular, and morphometric approaches. The nematodes were observed in an area of 2000 m^2, divided into five sample plots each with an area of 400 m^2 on which Pandan Wangi and Ciherang rice were cultivated. Nematode morphology was observed using a stereo microscope at a 10–100x magnification. Molecular identification employed PCR, followed by sequencing and phylogenetic analysis. This nematode possessed typical morphological characteristics namely a longer female body (457.07–738.86 µm) than males (385.23–505.29 µm), and cured spicules, 17 to 21 µm long. The tail was ribbon-shaped with a tapered tip that had a mucrone with 2–4 points. Sequencing and phylogenetic analysis revealed that *A. besseyi* isolated from Indonesia (Deli Serdang/North Sumatra) was closely related to those from China, India, Russia, Taiwan, and Portugal with a 98% homology level.

Keywords: Aphelenchoides besseyi, molecular detection, Oryza sativa, PCR

Introduction

Rice production in North Sumatra declined in 2021 with a total production of 2,004,143 tons compared to 2020 with a total production of 2,040,500 tons [1]. One group of pathogens that can reduce rice production is parasitic nematodes [2], especially *Aphelencoides besseyi* (Rice white tip nematode [RWTN]). It causes white shoot disease and is a serious threat to rice cultivation which can reduce production by 17–54% in susceptible and 0–24% in resistant plants [3]. It can be transmitted via seeds and is widespread in the world's rice-growing centers [3–5], causing a loss of US$16 billion per year [4–7].

RWTN has also spread to various rice cultivation locations in Indonesia. The existence of *A. besseyi* in the Bogor Region, West Java, and North Sumatra has been reported [7–9]. Various rice varieties, such as SL8SHS, HIPA14, IPB3S, IR-64, Inpari 31, Inpari 32, Pertiwi, Deli Serdang, Pandan Wangi Bogor, Mekongga, Inpari Sidenuk, Ciherang, Situ Bagendit, Batu Tegi, Gondosari, and Ciherang have been infected [8, 10]. Attacks have been identified in the five rice-growing districts in North Sumatra: Serdang Bedagai, Simalungun, Batubara, and Langkat. *A. besseyi* can survive on seeds under anhydrobiotic conditions during storage in warehouses [8, 11] and on seeds stored under dry conditions for 2–3 years. Infection with seed-borne pathogens can suppress seed germination, change the chemical components of seeds, cause the death of seedlings, enhance the risk of disease development in the field, and reduce crop yields [12].

Information regarding the identification of *A. besseyi* which infects various rice varieties in North Sumatra is still very limited. Severe symptoms of infection onset were reported by an organic farmer group in Pasar Kawat. This study aims to identify the parasitic nematodes that cause white shoot disease in the rice plantations of the organic farmer group in Pasar Kawat, Karanganyar Village, Beringin District, Deli Serdang, North Sumatra.

Methods

Sampling of rice seeds, roots of infected plants, and soil samples in the rice plantations of the farmer group. A total of 500 g seeds of the Pandan Wangi (Tani Mandiri, West Java ID) and Ciherang (Jaya Mandiri, East Java ID) varieties obtained from the Pasar Kawat Farmers Group were stored for 6 months in plastic bags at room temperature. Both varieties are unique and have typical properties such as smell, taste, and texture. Furthermore, Ciherang is a new variety that contains 23% amylose with a preferable texture along with a production potential of 10 tons/Ha. Infested rice plants were taken from an area of 2000 m^2, each was divided into five sample plots with an area of 400 m^2. Rice plants attacked by nematodes were sampled using the diagonal method where rice plants were collected by crossing the field diagonally. Clumps of rice plants with white tip symptoms were uprooted with the soil still attached to the roots. They were then put into a plastic bag which was labeled, tied with plastic rope, and put into a container for further examination at the nematode laboratory of the IPB University.

Testing of seeds suspected of being infected with *A. Besseyi*. Plant testing was carried out in two rice varieties that showed symptoms of *A. besseyi* attack. Rice seeds numbering 50 were soaked in 5.25% NaOCl (Merck, US) for 30 s and were rinsed thrice in sterile distilled water. The seeds were air-dried and sowed in seeding tanks filled with wet rice field soil sterilized by steaming at 100 ℃. The seedling tub was covered with a plastic sheet for 3 days. On the fourth day, the plastic sheet was removed and the tub was placed under sunlight. The appearance of the early symptoms of an attack by *A. besseyi* was observed. Then, the proportion(%) and intensity of attacks for three weeks were calculated using the following formula [13].

$$Is = \frac{\sum(ni \times vi)}{Z \times N} \times 100\%$$

Is: Insect intensity; ni: Plant Number; vi: Severity Scale; N: Total Plant Number; and Z: The Highest Severity Scale

Nematode extraction from the Pandanwangi and Ciherang seeds. Extraction of the nematodes from the seeds and soil was carried out in the Nematode laboratory of IPB University. A total of 200 rice seeds each of (Tani Mandiri, West Java ID) and Ciherang (Jaya Mandiri, East Java ID) were collected and their hila were cut off. The seeds were soaked in dd H_2O for 24 h in the dark. The nematode suspension was filtered through a 45 µm sieve (VWR Scientific, US) and observed using a stereo compound microscope (Olympus SZ40) at 10–100x magnification.

Nematode extraction from the soil sample. Extraction of nematodes from the soil samples utilized the centrifugation flotation method in which, the centrifugation time was modified and a speed of 1000 g. Soil samples were separated from the rocks, gravel, and other impurities. Then 100 g of the soil was mixed with 800 ml of water in a bucket, stirred well, and then left aside for 30 s. The mixture was then filtered through a multi-level filter consisting of 50, 400, and 500 mesh at an inclined position of 30°. The soil substrate collected from the 500-mesh sieve was added to a centrifuge tube, centrifuged for 5 min at 1700 rpm, and the supernatant was then discarded. The precipitate was added to a 40% sugar solution and stirred until evenly distributed. Next, the suspension was centrifuged for 1 min at 1500 rpm. The supernatant was filtered through a 500-mesh sieve and rinsed with running water to obtain a nematode suspension. It was then placed in a collection bottle for observation and identification per a previous protocol [14].

Identification of nematode morphology and Morphometry. Morphological identification, morphometry, and molecular analysis of the parasitic nematodes were carried out in the Nematology laboratory at IPB University. Identification was carried out by matching the morphological and morphometric characters of the specimens with the nematode identification book, Plant Parasitic Nematodes: A Pictorial Key and Genera [15] (May *et al*. 1996) and Nematology [16] (Eisenback 2003). Morphometric measurements were based on the formula of De Man (1876) which was modified by Cobb (1914) and then Thorne [17] (1949).

DNA extraction. For this, 1–3 nematodes were placed into a 0.2 ml PCR collection tube (Biologix, US) containing 25 µl of nuclease-free water (Thermoscientific, US). An extraction buffer containing 200 mM NaCl (Merck, US) was added with 200 mM Tris-HCL (Merck, US) (pH 8.2), 1% 2-mercaptoethanol (Merck, US), and 25 µl of 800 µg/ml Proteinase K (Geneaid, TW). The mixture was vortexed for 1 min and incubated at 65 ℃ for 1.5 h and 99 ℃ for 5 min in a PCR machine (T100 Thermal Cycler, Biorad US). The mixture was stored at −20 ℃ [18].

ITS rDNA amplification of *A. Besseyi*. DNA amplification used ITS-specific primer pairs: sequence forward (5'-ACA ATC GAG TTG GGA GTG-3') and sequence reverse (5'-GGT CAG TGT CAT CAA TCG-3'). The amplification reaction consisted of: pre-denaturation at 94 ℃ for 4 min; a cycle of denaturation at 94 ℃ for 1 min, annealing at 55 ℃ for 1 min, and elongation at 72 ℃ for 2 min, for 30 times; and the final post-elongation at 72 ℃ for 10 min.

Visualization of RT-PCR Amplicons. The DNA amplicons were then separated using 1% agarose gel electrophoresis, 0.5x Tris-borate EDTA electrophoresis buffer (40 mM Tris (Merck, US), 20 mM sodium acetate (Merck, US), and 1 mM EDTA (Merck, US) [pH 7.0]), and 10000x Gelred nucleic acid stain (Biotium, CA, USA); at 50 V for 50 min. DNA bands were visualized on a UV transilluminator (Labnet, US). The DNA bands were documented using a digital camera (Nikon D700, US).

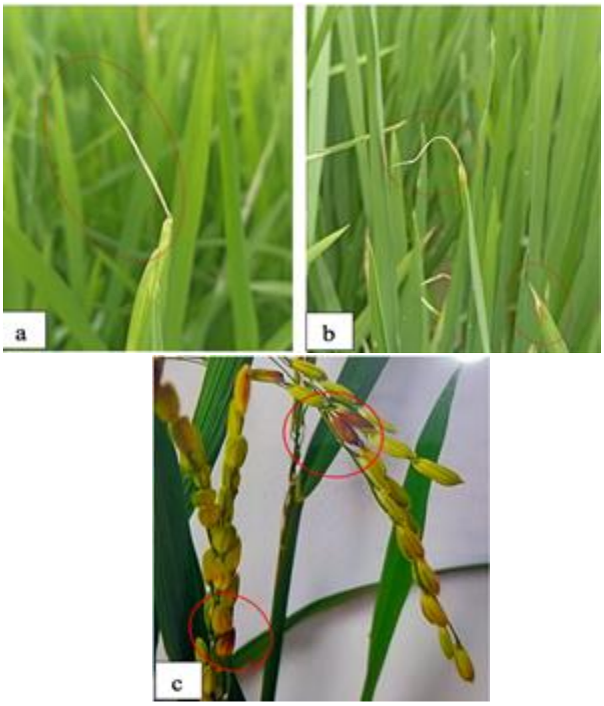
Phylogenetic sequencing and analysis. PCR product sequencing was carried out at PT Genetika, Science Tangerang, Indonesia; 50 µl of amplicons were used. The electropherogram obtained was analyzed using the sequence scanner program v 1.0 (Applied Biosystems, MA, USA). The nucleotide sequences obtained were then confirmed by searching the GenBank with the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The confirmed sequences were then used for multi-alignment employing the Bioedit Sequence Alignment Editor v 7.0.5.3 program (Hall, 1999). Phylogenetic trees were constructed using the Molecular Evolutionary Genetic Analysis Software (MEGA) v. 11 software with the UPGMA method and a boostrap of 1000 repetitions. The reference isolate nucleotide sequence of *Hirschmaniella oryzae* from other countries was obtained from the GenBank.

Results and Discussion

Symptoms of *A. besseyi* attacks on rice plants in the field. *A. besseyi* attacks were found on the rice crops belonging to the Pandanwangi and Ciherang varieties cultivated by the Pasar Kawat farmer group, Beringin District, Deli Serdang. The most typical symptom was a white tip, leaf tips were necrotic and twisted (Figure 1a), the panicle size was shorter, the number of spikelets decreased, and the number of empty spikelets increased. Infected grains appeared black on the surface (Figure 1 b, c).

At the beginning of the survey, it was observed that nematodes attacked rice plants 2 weeks after planting with a greater proportion of attacks on Ciherang than Pandanwangi. Symptoms of the attack would progress to the generative phase of the plant when panicles are produced. The nematodes move to the panicles, reproduce, and return to the anhydrobiosis phase at harvest. Infected panicles were shorter, with a decreased number of grains, and an increased number of empty grains. Infected seeds show a black colour on the surface (Figure 1).

Growth of rice seedlings during the test in plant. In rice seedlings aged 6–10 days after sowing, symptoms of nematode attack were observed in the form of chlorotic leaves with twisted tips (Figure 1 a, b, c). The first leaf dried at 7 days after infection. The symptoms then develop on the second leaf and finally on the plant when it enters the generative phase and grains are formed. Attack symptoms appeared 6 days after planting (DAP) in the Ciherang variety and at 10 DAP in Pandanwangi. The proportion of attacks was high in the Ciherang variety, at 40%–50% but with a moderate attack intensity (25%–50%); the proportion of attacks on Pandanwangi was 20% with light attack intensity (12.5%) (Figure 1 c, d). The intensity of nematode attacks was inversely proportional to the age of the rice seedlings. The Pandanwangi variety is more resistant than others [19].



Source: a, b Khofifah Documentation; c Photo Documen-tation by Suswati

Figure 1. Symptoms of *Aphelenchoides besseyi* Attacks in Pandanwangi Rice Plants; Remarks: a), b). The Tip of the Leaf was Twisted and White (White Tip); c). Seeds Infected by *A. besseyi*

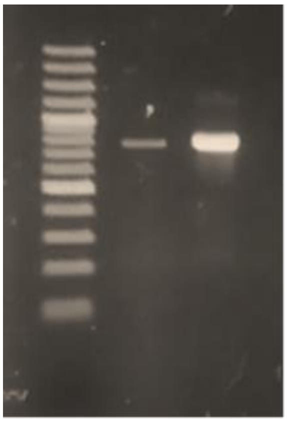


Figure 2. Visualization of the *Aphelenchoides besseyi* ITS rDNA PCR Producing DNA Fragments ~750 bp Long; 100 bp DNA Ladder

A. besseyi can survive on Ciherang and Pandanwangi seeds stored by farmer groups for up to 6 months. This nematode can last even longer, up to 2–3 years under optimal conditions. It can survive for 2–3 years on seeds under dry conditions but dies within 4 months on grains left in the field. Its mobility between plant parts depends on the presence of a water layer on the plant tissue surface [20] (Luc *et al*. 1995). It cannot survive for a long time in the soil [21].

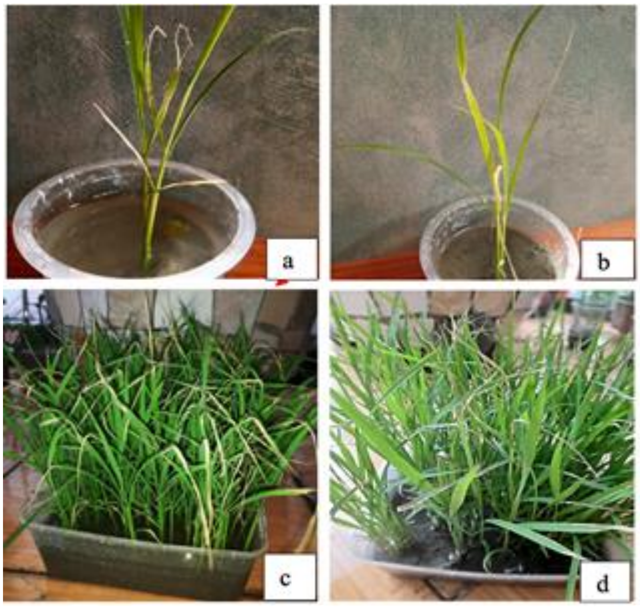
In the test in-plant rice seedlings, significant symptoms like discoloration and twisted leaves were observed. The nematodes become active again and infect the first leaves to complete one life cycle of 10 ± 2 days [2, 22]. The cycle continues to the second leaf and so on until it reaches the flag leaf and infects the rice grain. This nematode can survive for a long time in rice grains. It disrupts the development of the host plant by infecting the flowers and remaining at the bottom seed glumes as adults or stage four juveniles. This causes malformation in rice leaves and stems, and the leaf tips lose chlorophyll; so they are called white shoots.

Identification of *A. besseyi* Morphology and Morphometry. The body shape was vermiform with fine annulations (Figure 4a–e). Females were longer than males (Figure 4a–b). The anterior was characterized by a slender, needle-like stylet of the stoma style type, and a large median bulb (Figure 4c). The tail of the female had a protrusion (mucro) shaped like a star (star-shaped mucro) (2d). The female stylet was longer than the male one. Younger males were identified by their curved posterior and the presence of spicules measuring 15.77–22.02 µm (Table 1). *A. besseyi* were identified based on morphological and morphometric characters. The females were longer (457.07–738.86 µm) than the males (385.23–505.29 µm). The curved spicules were 17–21 µm long and had no bursae. The tail was ribbon-shaped with a tapered tip which bore a mucrone with 2–4 points.

Population density of *A. Besseyi*. *A. besseyi* were found in rice seeds only in their 4th juvenile and adult phases, majorly in the J4 phase (Table 2). The nematodes were extracted only from the seeds, but not the roots and the rhizosphere soil of the rice plants. Nematode development phases (four juveniles and adults) were higher in the Ciherang variety (32 adults and 7 J4 phase individuals) than in the Pandanwangi variety (20 adults and 3 J4 phase individuals). It is suspected that *A. besseyi* cannot survive for long in the soil under water and anaerobic conditions. It was active at an average humidity of 70%, in ripe grains with ~40% moisture content. Regarding movement, *A. besseyi* was very slow and went into dormancy at a water content of ~35%, whereas at ~27%, they dehydrated and died.

Their translocation through seeds could accelerate their spread across rice crops in Indonesia. *A. besseyi* was also found in several rice-growing areas in North Sumatra including: Deli Serdang Regency (Percut Sei Tuan District, Pagar Merbau, Sunggal), Batubara Regency (Madang Deras, Talawi, Sei Balai), Simalungun (Hutabayu Raja, Pematang Bandar, Siantar Marimbun), Langkat (Stabat, North Securai, Gebang), Serdang Bedagai (Sei Bamban, Dolok Masihul, Teluk Mengkudu). *A. besseyi* was detected on rice planting in Bogor, West Java Province [8–10].

Molecular analysis of seed-borne nematodes. Based on the identification of infected rice leaves and seeds along with the morphological and morphometric analysis results, these nematodes were confirmed as *A. besseyi*. DNA amplification using ITS-specific primer pairs produced a fragment ~750 nucleotides long (Figure 2). This analysis to confirm the symptoms appeared in leaves (Figure 3). Phylogenetic analysis identified the relationship between each isolate. In this study, all isolates from various countries were in the same group as the isolates from North Sumatra at the DNA level, which is per the rules of evolution in species relationships. The results of the phylogenetic analysis are shown in Figure 5 along with the phylogenetic analysis is presented in Table 3. In multiple sequence alignment, the varying nucleotides were marked with red letters. North Sumatra isolates were dissimilar to those from other countries at the 714th position. Apart from this analysis, Infected seeds commonly shows brownish spot on seeds surface indicating the entity of *A. basseyi* (Figure 6).



Source: Suswati Documentation

Figure 3. Symptoms of *Aphelenchoides besseyi* Attack in Ciherang Variety Rice Seedlings; Remarks: a). Age 5 DAP, b). Fragrant Pandan Variety Aged 7 DAP, c). Ciherang Variety Aged 14 DAP, d). Pandanwangi Variety Aged 14 DAP

Figure 4. Morphology of *Aphelencoides besseyi*; Description a). Male Nematode at 200x Magnification, b). Female Nematode at 200x Magnification, c). Female Anterior, d). Mucron, e). Male Posterior, f). Female Posterior

Table 1. Morphometric Data of *Aphelenchoides bessseyi* Females and Males in Rice Seeds using the Method of De Man (1880)

Karakter*	Nematoda *Aphelenchoides besseyi*					
	Female			Male		
	Mean	Min	Maks	Mean	Min	Maks
n	20			20		
L (µm)	572.76	457.07	738.86	446.2	385.23	505.29
Diameter (µm)	13.17	10.90	17.86	12.73	9.90	16.32
Tail (µm)	34.47	28.72	43.34	31.12	24.71	36.51
Stylet (µm)	10.97	9.57	12.29	10.64	8.99	11.55
a	57.71	34.58	53.69	35.41	28.42	47.89
b	5.48	4.30	6.94	5.4	3.58	5.81
b'	4.94	4.00	5.98	4.94	4.00	5.98
c	16.68	13.75	20.41	16.68	13.75	20.41
c'	67.11	49.43	85.19	47.29	38.41	54.01
G1 (%)	43.9	30.09	53.75	63.69	57.99	68.48
G2 (%)	19.53	16.51	25.16	-	-	-
V (%)	70.77	69.13	72.51	-	-	-
V' (%)	75.51	73.37	79.37	-	-	-
PUS (µm)	111.9	85.52	153.29	-	-	-
T (%)	-	-	-	5.31	4.55	5.98
Spicule (µm)	-	-	-	18.21	15.77	22.02

Note*: L = body length, s = stylet length, a = total length/maximum width, b = total length/length of the esophagus (anterior end to the esophageal valve), b' = total length/length of the esophagus (anterior end to the base of the gland esophagus, c = total length/tail length, c' = body length/tail width, V = distance of vulva from anterior:body length, G1 = anterior female gonad length/body length, G2 = posterior female gonad length/body length, T = male gonad length:body length

Table 2. The Population Density of *Aphelenchoides besseyi* Juvenile 4 and Adults in the Seed Samples

No	Rice Plant Samples	Population Density of *A. besseyi* (tails)			
		Pandanwangi		Conference	
		Youth 4	Mature	Youth 4	Mature
1	Akar (5 g)	0	0	0	0
2	Soil (100 ml)	0	0	0	0
3	Rice seeds (10 g)	3	20	7	32

Table 3. Phylogenetic Analysis of the *Aphelenchoides besseyi* Isolates based on the ITS rDNA Nucleotide Sequence

Isolate Name	Accession Number	Country of Origin	1	2	3	4	5	6	7	8	9
Aphelenchoides besseyi Sumut	Na	Indonesia	ID								
Aphelenchoides besseyi isolate Millet	OQ711764.1	China	99.1	ID							
Aphelenchoides besseyi isolate Drr-Ab1	JF932290.1	India	99.1	100	ID						
Aphelenchoides besseyi isolate AB11	KJ009342.1	China	99	100	99.8	ID					
Aphelenchoides besseyi Kresnodar	EU186069.1	Rusia	99.1	100	99.7	100	ID				
Aphelenchoides besseyi isolate YLI	MF669503.1	Taiwan	99	100	99.5	100	100	ID			
Aphelenchoides besseyi isolate 1390-1	OR478689.1	Portugal	99.1	100	99.7	100	100	99.8	ID		
Aphelenchoides besseyi Hyderabad	JF826519.1	India	99	100	99.8	100	100	99.4	99.5	ID	
Hyeschmanniella oryzae isolate SMYX01	OM996113.1	China	43.7	44	43.7	44	44	43.8	44	44	ID

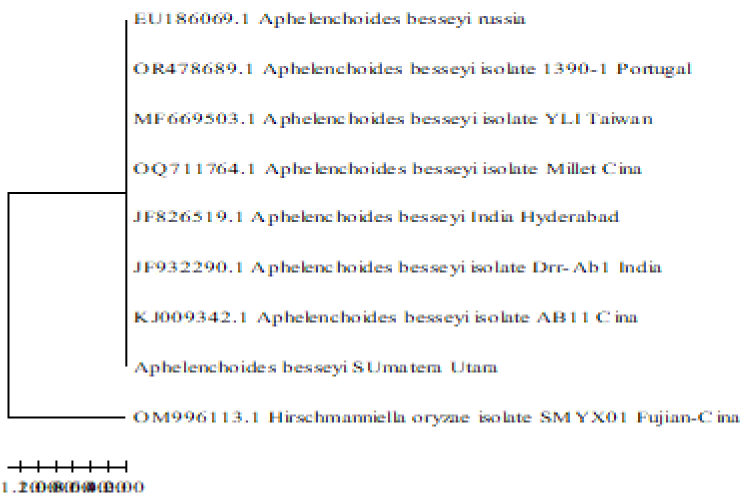


Figure 5. Phylogenetic Tree of the *Aphelenchoides besseyi* Isolates based on the ITS rDNA Nucleotide Sequence; *Hirschmaniella oryzae* was the Outgroup; The Phylogenetic Tree was Constructed using the MEGA v 11 Program with the UPGMA Approach

Source: Suswati Documentation, 2023

Figure 6. Rice Grains Infected with *Aphelenchoides besseyi*

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             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                 5          15         25         35         45         55
Aphelencho   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
OQ711764.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
JF932290.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
KJ009342.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
EU186069.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
MF669503.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
OR478689.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
JF826519.1   AGTGTCATCA ATCGCACTGC CGACATCCGA ACAACAACTC GCAAAGACCT TCTCAATAAC
OM996113.1   ACCGTGAGGG AAAGTTGCAA AGCACTTTGA AGAGAGAGTT AAAGAGGACG TGCCGATGAG
Clustal Co   *  ** *    *  *        *   *  ** * *   * *    * **  *  *  * ** *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                 65         75         85         95        105        115
Aphelencho   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
OQ711764.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
JF932290.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
KJ009342.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
EU186069.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
MF669503.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
OR478689.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
JF826519.1   AGCCAAGCAT CTCAATTGTG GAATCAAGTC ACTCAAAGCC GTACAGATCA AAAGCCAATC
OM996113.1   GTGGAAACGG ATAGATAGCG TATCTAACCT GTATTCAGCC ATG-AGTTTG TTGGTGCCTG
Clustal Co       *  *    *  ** * *  *   **          ****  *  ** *      *    *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                125        135        145        155        165        175
Aphelencho   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
OQ711764.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
JF932290.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
KJ009342.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
EU186069.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
MF669503.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
OR478689.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
JF826519.1   GAATCATGTT CCTTGCACAT GGACATCGTT CGAACAGTAC TAACAACGTA ATCACAGGCA
OM996113.1   GGGTGTTGAT CTCCAGATTG GGACGGCGTC TAGGTTACAT TTGTGGTGCA TTTGCAGGTA
Clustal Co   *  *  ** * *     *    ****  ***          *  *      * *  *  **** *
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             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                185        195        205        215        225        235
Aphelencho   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
OQ711764.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
JF932290.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
KJ009342.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
EU186069.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
MF669503.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
OR478689.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
JF826519.1   TACTCCGAAG AATACGCCAC CAAAGCGAAC ACGTCAACTG CATTCCGTGG GGAAGAGTCG
OM996113.1   TTGTGCGCCG AG-ATGCCAT CGGGATGGCG GCATTATCTT AGTTTTGAGG CCAGCTTGCT
Clustal Co   *  * **  * *  * **    *     *     * * * **    **  * **   *     *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                245        255        265        275        285        295
Aphelencho   ACGATTCGAT AGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
OQ711764.1   ACGATTCGAT AGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
JF932290.1   ACGATTCGAT AGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
KJ009342.1   ACGATTCGAT GGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
EU186069.1   ACGATTCGAT GGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
MF669503.1   ACGATTCGAT GGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
OR478689.1   ACGATTCGAT GGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
JF826519.1   ACGATTCGAT AGCGAAAACC ATTACACAAT GCATTTGGCT TCCCTTTC-C ATGAAAACAC
OM996113.1   GGTACCCGGT GGGGTAGTGC TGTTCATACT GGGTTTTGTA TGTTATTGAC ATGGCTACGG
Clustal Co      *  ** *  * * *   *   * ** * * *  *** *   *    **  * ***   **

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345        355
Aphelencho   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
OQ711764.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
JF932290.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
KJ009342.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
EU186069.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
MF669503.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
OR478689.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
JF826519.1   CCTGAGCTGC GTATGTCGAA GGGCAGAACC CGACGACGCA ATATGCAATC GAAAACAAGG
OM996113.1   GCTCGGGTGG GTTCGTCGGA CGGTCGCATG CGACGACGTG CTGTGCGGTC AGTTCGGTCT
Clustal Co    **  * **  **  **** *  **  * *   **** ***     * ***  **

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                365        375        385        395        405        415
Aphelencho   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
OQ711764.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
JF932290.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
KJ009342.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
EU186069.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
MF669503.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
OR478689.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
JF826519.1   CACTCGTAAT ATCTGTAATT CGTGCTTATT AACGCAATTC ACTGCGTTCT TCATCGACCC
OM996113.1   TGTTCGAGCT CTCTGTCTTT TCTCGGTGTA AAAGCTGGTC ATTCCGACCC GTCTTGAAAC
Clustal Co      ***   *  *****  **   *   * *  ** **   ** * * **  *      * **  *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                425        435        445        455        465        475
Aphelencho   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA CGAATTGAAA
OQ711764.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA CGAATTGAAA
JF932290.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA TGAATTGAAA
KJ009342.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA TGAATTGAAA
EU186069.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA CGAATTGAAA
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MF669503.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA CGAATTGAAA
OR478689.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA CGAATTGAAA
JF826519.1   ACGAGCCAAG TGATTCACCG ACATAACTTG CTCGATTAGT TGGCATTGAA TGAATTGAAA
OM996113.1   ACGGACCAAG GAGTTTATCG GCGCGAGTCA TTGGGTTGAA AACCCAAAGG CGAAATGAAA
Clustal Co   ***  *****    ** * **  *   * *   * * **       *        *** *****

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                485        495        505        515        525        535
Aphelencho   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
OQ711764.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACATCGC CGAAGCGGTG
JF932290.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
KJ009342.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
EU186069.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
MF669503.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACATCGC CGAAGCGGTG
OR478689.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
JF826519.1   ATGGGTTGTT GGTTCTGGGT GCCCGACCGG GTCATCAACT CTAACACCGC CGAAGCGGTG
OM996113.1   GTGAATTATC CGTATGGAAC TGACGTGCGA CCCGTCATTT CGATGTCCGG GAGAGCATGG
Clustal Co    **  ** *   **   *        **  **    * ***  * * *    **       ***   *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                545        555        565        575        585        595
Aphelencho   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
OQ711764.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
JF932290.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
KJ009342.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
EU186069.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
MF669503.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
OR478689.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAC AACTGCTCAA TCCAACATAG
JF826519.1   TCTACTATTG GACATCGTCG CAGCCACGGA CGTGGAACAA AACTGCTCAA TCCAACATAG
OM996113.1   CCCCATCCTG ACTGCTTGCA GTGGGGTGGA GGTAGAGCGT AAGCGGTGAG ACCAAGATGG
Clustal Co    *   *  **          *     *    ***  ** ** *   **  * * *    **   ** *

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645        655
Aphelencho   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAA CGATCGGGAA
OQ711764.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
JF932290.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
KJ009342.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
EU186069.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
MF669503.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
OR478689.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
JF826519.1   AGGCCCAGAG AAGCCGTTTG TGAAAACTGA AATCTCATTG AAGAGACCAG CGATCGGGAA
OM996113.1   TGAACTATTC GAGCAAGATG AAGAGAGGAA ACTCTGATGG AAGTCTGAAG CGATTGGTGC
Clustal Co    *  * *     ***    **    * *   * * *** ** * ***     *   **** **

             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                665        675        685        695        705        715
Aphelencho   ATACCGAGTG CTTCACGACG ACCCTTCAAA TATCCGCACC ACGGCACTCC CAATTCTATT
OQ711764.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
JF932290.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
KJ009342.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
EU186069.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
MF669503.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
OR478689.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
JF826519.1   ATACCGAGTG CTTCACGACG ACCGTTCGAA TATCCGCACC ACGGCACTCC CAACTCGATT
OM996113.1   AAATCGATCG TCTGACTAGG GGCGAAAGAC TAATCGAACC ATCTAGTAGC TGGTTCTTCC
Clustal Co   * * ***  *   * ** * *   *     *   **  ** *** *        *     **
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In this study, the isolates from various countries were in the same group as those from North Sumatra. Hence, it can be interpreted that the Indonesian isolate (Deli Serdang/North Sumatra) had the same origin as those from China, India, Russia, Taiwan, and Portugal.

Conclusions

The white tip nematode that caused symptoms in the Pandanwangi and Ciherang variety rice plants cultivated in Pasarkawat Keltan, Beringin District, Deli Serdang, North Sumatra was confirmed to be *A. Beseyi*. Morpho-logically, the females were longer than the males. The nematode was successfully identified using the ITS gene and using PCR to obtain a 750 bp amplicon. Based on phylogenetic analysis *A. besseyi* isolates from North Sumatra were found to be similar to those from China, India, Russia, Taiwan, and Portugal.

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