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Aphelenchoides varicaudatus (Nematoda: Aphelenchoididae) and *Helicotylenchus erythrinae* (Nematoda: Hoplolaimidae) from Garlic Plantation in Magelang, Central Java, Indonesia

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Summary

There were two most found genera of plant parasitic nematodes from garlic plantation in Magelang, Central Java, Indonesia which suffered losses due to bulb rot, *Aphelenchoides* and *Helicotylenchus*. Polymerase Chain Reaction (PCR) was conducted using a pair of universal nematode primer (D2A/D3B) to determine the *Aphelenchoides* and *Helicotylenchus* species from those host. Both genera were amplified at ~780 bp. The Blast-N results for the *Aphelenchoides* showed high identity to *Aphelenchoides varicaudatus* from Yunnan China (HQ283353) with 99.47 % identity, while the *Helicotylenchus* showed 95.22 % identity to *Helicotylenchus erythrinae* from Colombia (MT321739). From morphological and molecular data, we confirm that the *Aphelenchoides* species is *A. varicaudatus.* Based on female morphological character, *Helicotylenchus* species refers to *H. erythrinae*. Which is also supported by its nucleotide alignment which has same region character as *H. erythrinae* (MT321739). This is the first report of molecular characterization of *H. erythrinae* in Indonesia.
Keywords: *Aphelenchoides varicaudatus*; *Helicotylenchus erythrinae*; identification; molecular; morphological

Introduction

Many nematodes can be associated to garlic plants. Twenty genera of nematodes have been recorded to be associated with the roots of garlic plants in Yemen; *Antarctenchus, Aphelenchoides, Aphelenchus, Basiria, Boleodorus, Ditylenchus, Helicotylenchus, Heterodera, Hoplolaimus, Meloidogyne, Microposthonia, Nothanguina, Pratylenchus, Rotylenchulus, Rotylenchus, Scutellonema, Tetylenchus, Tylenchorhynchus, Tylenchus,* and *Zygotylenchus* found in garlic (Mohamed, 2015). While in Indonesia, from 2019 to 2021, several plant parasitic nematodes have been found accompanying garlic plants in Central Java and East Java, Indonesia, such as *Helicotylenchus, Aphelenchoides, Rotylenchulus, Aphelenchus, Criconemoides,* and *Tylenchus* (Wulandari & Indarti, 2019; Wulandari *et al.,* 2021; and Kusuma *et al.,* 2020). Several species of plant parasitic nematodes have wide host range, such as *Ditylenchus dipsaci* (Musyarofah & Indarti, 2019). Amritha and Budijastuti (2018) stated that *Ditylenchus dipsaci, Aphelenchoides* sp. and *Xiphinema* sp. were found in imported and local garlic in East Java. Therefore, identification of the most common plant parasitic nematode associated with garlic is needed for further management and avoid yield losses on garlic.

The most common nematode genera associated with garlic plantations in Magelang, Central Java, were *Aphelenchoides* and *Helicotylenchus* (Wulandari *et al.,* 2021). The taxonomic determination of *Aphelenchoides* at the suborder level is quite controversial between "Tylenchid" and "Aphelenchid" (Sanchez-Monge *et al*., 2015). Many species are not fully described and do not have

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Fig. 1. Plant damaged symptoms. (A) plant symptoms on the ground (B) rot bulb associated with fungi (C) root rot damaged.

molecular data for taxonomic studies (Zhao, 2006; Sanchez-Monge *et al.,* 2015). Some species of *Helicotylenchus* have very similar diagnostic characters, and boundaries between species are not well established (Subbotin *et al.,* 2015). The existence of complex species in the genera makes them morphologically almost indistinguishable but may be quite distant from each other by their phylogeny (Subbotin *et al.,* 2015). The combination of morphological and molecular identification shown to be most effective method for species identification (De Ley *et al.,* 2005)

Nucleotides data for *Aphelenchoides* and *Helicotylenchus* genera are mostly limited in The National Center for Biotechnology Information (NCBI). At the time this research was conducted, there were only 1237 and 758 nucleotides records for *Aphelenchoides* and *Helicotylenchus,* consecutively (NCBI accessed in November 2021). However, molecular data at species level are obviously rare. Several molecular studies on *Aphelenchoides* were reported in Indonesia, such as *A. besseyi* on rice (Rahman *et al.,* 2018; Sembiring *et al.,* 2019) and *A. varicaudatus* on garlic plants (Kusuma *et al.,* 2020), but there was no information about the molecular study of Genus *Helicotylenchus* in Indonesia. This study aimed to characterizing the most dominant nematodes from garlic plants in Magelang, Central Java, Indonesia, based on morphological characters and DNA sequences.

Materials and Methods

Nematodes collection

The nematodes were extracted from garlic roots, bulbs, and soil

Table 1. The nematode population found extracted from root, soil and bulb of garlic plants.

Nematodes Genera	Site 1 (Lumbu Kuning Variety)			Site 2 (Tawangmangu Baru Variety)		
	Roots (0.5 g)	Bulbs (3 g)	Soil (100 g)	Roots (0.5 g)	Bulbs (3 g)	Soil (100 g)
Aphelenchoides	50.8	38	2.5	8	0.8	0
Helicotylenchus	0.6	0	7.5	0.2	0	19.7

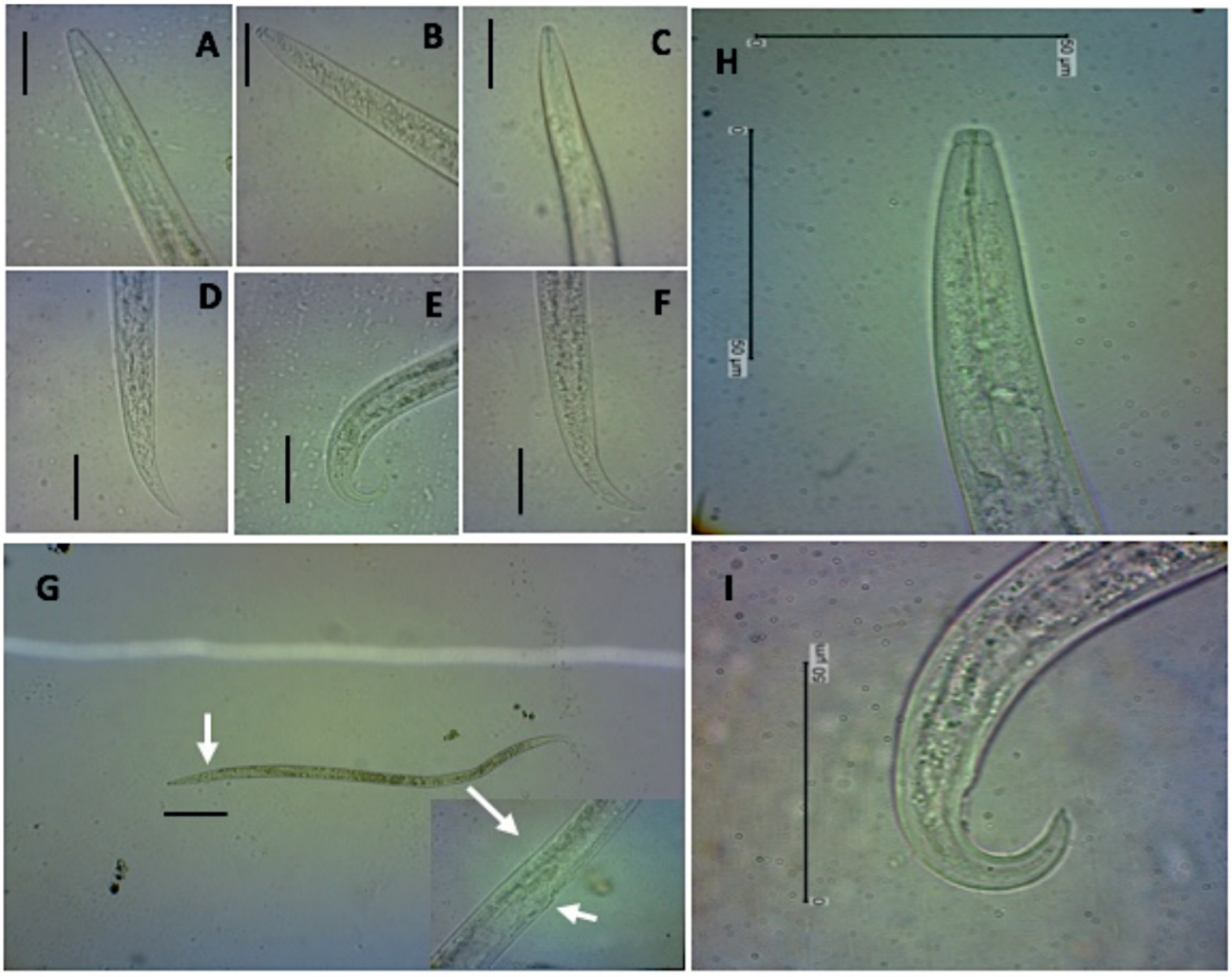

Fig. 2. *A.varicaudatus* (A-C) anterior region (D-F) tail shape variation (G) entire body with large metacorpus and vulva at ~60% of body length (H) lips, stylet, and large metacorpus (I) dorsally convex-conoid tail with little mucro (Scale bars: A-F, G-I: 50 µm; G: 100 µm)

obtained from garlic field in Kaliangkrik, Magelang, Central Java, Indonesia. Two main fields used as collection sites: the first site was planted Lumbu Kuning Variety and second site was planted Tawangmangu Baru Variety. Samples were collected using the direct sampling method on symptomatic plants (purposive sampling method). The symptoms shown on garlic plants were yellowing and wilted leaves, rotting bulbs, and disturbed plant growth.

Nematode extraction was carried out by directly soaking the plant's materials in the water. Without being washed, the roots and bulbs were cut into smaller parts. Then, the roots and bulbs were transferred to the petri-dish filled with sterile water and let sit for about 6 hours at 18 – 27°C. The water was filtered to remove the plant materials and collected to a becker glass (Ajri *et al.,* 2021). Nematode extraction from soil was carried out using the Oostenbrink dishes method (EPPO, 2013).

Nematode identification

Morphological identification

Morphological identification to genera was carried out by comparing the specimen to literature by Mai and Mullin (1996). The species identification observation compared with Uzma *et al.* (2015), Wouts and Yeats (1994) for *Helicotylenchus* identification and IPPC (2016) and Huang *et al.* (2012) for *Aphelenchoides* identification. Characters such as head region, stylet, tail, and the posisition of vulva were observed under a binocular microscope (Olympus CX31).

Molecular identification

DNA extraction. DNA extraction was perfomed using the CTAB method (Cseke *et al.,* 2003) with some modification. A total of 20 – 30 nematodes were cut into pieces using a needle in 25 µL of CTAB buffer on a glass object under a binocular microscope then

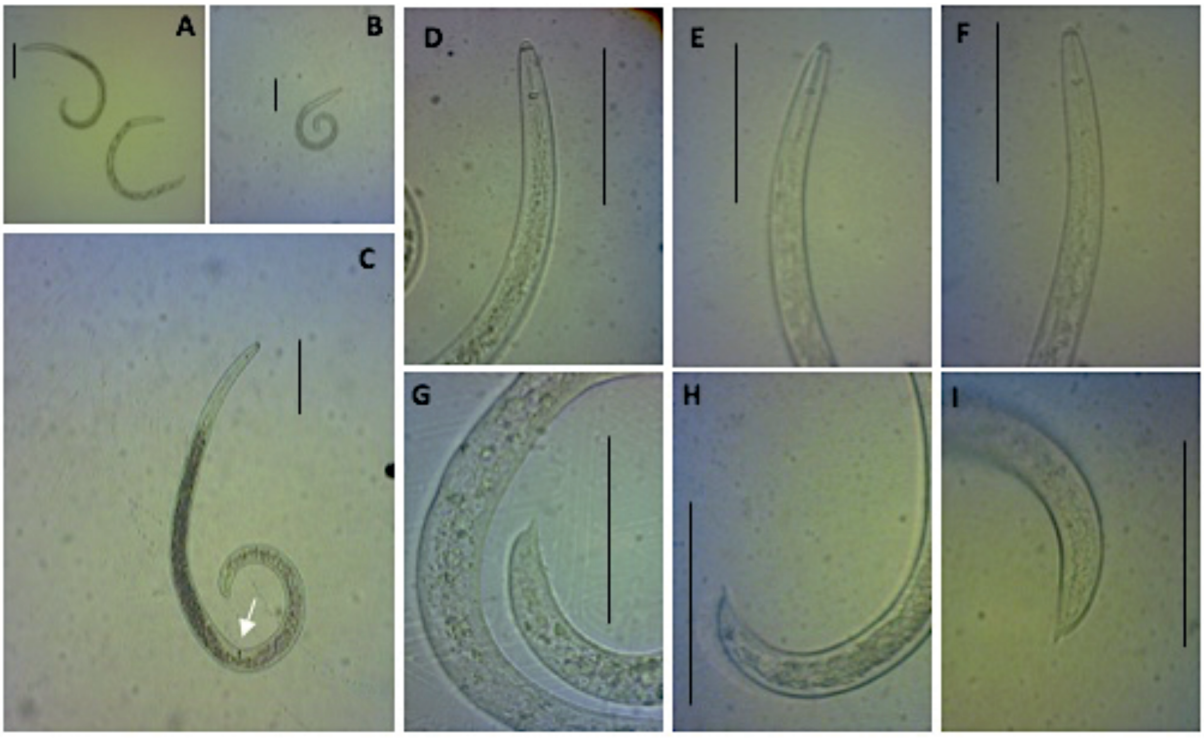

Fig. 3. *H.erythrinae* (A-C) spiral shape variation (C) entire body, vulva at ±60% of body length (D-F) anterior region, lips and stylet with knob flat or slightly anterior projection (G-I) conoid tail with variation of mucro (Scale bars: 100 µm)

transferred into the extraction tube. The tubes were incubated at 65°C for 30 minutes, inverted every 10 minutes followed by centrifugation for 5 minutes at 5000 RPM. The DNA suspension was added with chloroform: isoamyl alcohol (CIAA 24:1), then shaken until homogeneous (1 – 3 minutes). Samples were centrifuged at 10000 RPM for 15 minutes at room temperature. The supernatant was taken slowly with a micropipette and transferred to a new tube. Cold absolute ethanol was added with the proportion 1:2 for supernatant: ethanol. Samples were incubated at -20°C for 24 hours. Then, the samples were centrifuged at 10000 RPM for 15 minutes at room temperature. The supernatant was removed and then the pellets were added with 70 % cold ethanol. The solution

Table 2. Measurements of female *A. varicaudatus*. All measurements are in µm except for V in % and in the form mean±s.d (range).

Location Publication	Magelang. Indonesia (This Study)	Tegal. Indonesia (Kusuma *et al.*. 2020)	Yunnan. China (Huang *et al.*. 2012)
Number of Specimen	5	7	20
Body Length	645.20 ± 54.44	567.35 ± 74.74	780 ± 56.70
	(565.39 – 710.57)	(494.51 – 704.59)	(634 – 900)
Stylet Length	17.14 ± 0.53	12.39 ± 1.01	14.20 ± 0.50
	(16.67 – 18.05)	(11.17 – 14.01)	(13.50 – 15.00)
Tail Length	55.66 ± 3.13	36.63 ± 2.96	49.00 ± 2.90
	(52.02 – 59.85)	(32.61 – 42.34)	(43.00 – 53.00)
V (%)	61.74 ± 1.25	69.72 ± 1.15	69.20 ± 0.75
	(60.30 – 63.18)	(68.32 – 71.2)	(68.20 – 71.20)
a	22.30 ± 2.08	28.71 ± 3.98	30.40 ± 1.68
	(20.02 – 25.03)	(24.29 – 35.8)	(26.50 – 33.20)
c	11.59 ± 0.68	15.47 ± 1.26	16.11 ± 0.58
	(10.87 – 12.65)	(13.81 – 16.90)	(15.00 – 17.10)

was homogenized and then centrifuged for 15 minutes at 10.000 RPM. The ethanol solution was discarded and left to dry. The DNA pellets were added with 20 µL of TE buffer. The DNA suspension was stored at -20°C for molecular identification.

Polymerase chain reaction. Molecular characterization was conducted by polymerase chain reaction (PCR) and sequencing nucleotides using universal primer targeting the 28S rRNA gene (D2A/D3B). PCR was carried out by *Bio-Rad T100 Thermal Cycler* in a total volume of 25 µL (12.5 µL MyTaq HS RedMix BioLine, 2.5 µL of each primer (10 pmol/µL), 5 µL DNA template, 2.5 µL nuclease-free water). The D2-D3 expansion region of LSU was amplified with forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse primer D3B (5'-TCG GAA GGAACC AGC TAC TA-3') (Nunn, 1992). Initial denaturation was carried out at 94°C for 2 minutes, followed by 35 cycles with the following steps: denaturation at 94°C for 15 seconds, annealing at 50°C for 30 seconds, extension at 68°C for 60 seconds. The final synthesis was carried out at 68°C for 5 minutes with a final temperature of 4°C. PCR products were analyzed on 2 % agarose gel electrophoresis and visualized on a UV transilluminator.

Phylogenetic tree. Amplified D2-D3 expansion region were sequenced to obtain DNA base sequence data. Sequence data were edited by *BioEdit 7.2* and submitted into the BLAST-N program through the NCBI (National Center for Biotechnology Information) website (https://www.ncbi.nlm.nih.gov). Several sequences within species and other groups were selected as a comparison and aligned using *BioEdit 7.2*. The phylogenetic tree was constructed by selecting the best fit model using *Mega X program*.

Ethical Approval and/or Informed Consent

For this study formal consent is not required.

Results

Nematode population

Garlic plants associated with some nematodes were looked smaller and wilted than other healthy plants. Their leaves turned yellow (Fig. 1.A). The root area looked unhealthy with rotting bulbs when the plants were uprooted. Bulbs rot usually comes with fungi (white rot) that causes the soil cover up the bulb (Fig. 1.B).

In this study, *Aphelenchoides* and *Helicotylenchus* were the most found population of nematodes genera on garlic plantations, potentially suppressing the plant's yield. The nematode population is shown in Table 1.

Nematode identification

Morphological character

Based on the identification to the nematode genera (Mai & Mullin, 1996), two most found genera were identified as genus *Aphelenchoides* and *Helicotylenchus* (Fig. 2 and Fig. 3). Morphological and morphometric data lead to *Aphelenchoides varicaudatus* and *Helicotylenchus erythrinae* (Fig. 2, 3 and Tables 2, 3).

The first genus identified as *Aphelenchoides* (Fig. 2, Table 2.) from its large metacorpus which clearly shown at low magnification and conoid tail. Vulva at ±60 % body length (Fig. 2.G), the lip region rounded and set off, metacorpus clearly shown round to square-shaped, stylet with small basal swelling (Fig. 2.H), dorsally convex-conoid tail with terminating step like mucro at end (Fig. 2.D-F,I).

The second genus population identified as *H. erythrinae* (Fig. 3, Table 3.). The general characteristic of the Genus *Helicotylenchus* is a ventrally curved or becoming spiral shape at rest. *Helicotylenchus* population from Magelang, Central Java had various spiral-shaped when it relaxed (Fig. 3.A-C). The spiral form did not reach 1.5 spiral and sometimes in C shapes. Vulva at ±60 % of the body (Fig. 3.C), lip region rounded/hemispherical (not truncate), stylet clearly observed with basal knob flat to intended anteriorly

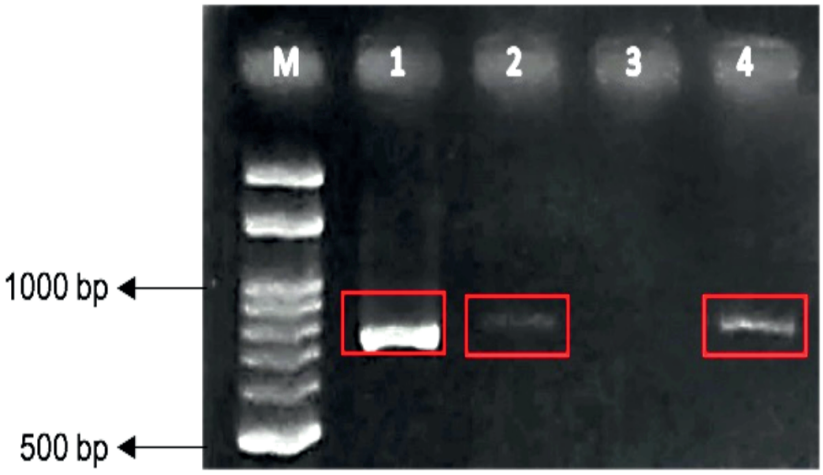

Fig. 4. Visualization PCR electrophoresis (M) Marker (DNA ladder 100 bp; (1) *H.erythrinae*; (2) *A.varicaudatus*; (3) negative control; (4) positive control (*Meloidogyne graminicola*)

(Fig. 3.D-F), tail asymmetrical and dorsally curved with a slight terminal projection to quite pronounced but not longer than the width of the nematode anal portion (Fig. 3.G-I)

Molecular Characterization

PCR electrophoresis result showed that the *Helicotylenchus* and *Aphelenchoides* population from Magelang, Central Java was successfully amplified at ~780 bp (Fig. 4). The expansion region of D2-D3 28S rDNA was sequenced for molecular analysis. The BLAST-N results for the *Aphelenchoides* sample showed high identity to *Aphelenchoides varicaudatus* from Yunnan, China (HQ283353) with 99.47 % identity, while *Helicotylenchus* sample showed 95.22 % identity to *H. erythrinae* from Colombia (MT321739). Sequence results for the *Aphelenchoides* sample from Magelang Indonesia were submitted to GenBank with accession number OK055306, while OL757653 for *Helicotylenchus* sample from Magelang Indonesia. Multiples sequence alignment of *Aphelenchoides* species showed that *A. varicaudatus* Magelang (OK055306) and *A. varicaudatus* Yunnan (HQ283353) had 0.58 % differences. The differences were showed at length of 130, 212, 530, 628. While, differences with *A. varicaudatus* Tegal (MN587128) were only 0.29 % at length of 212 and 530. Moreover, the closest species, *A. xui* (FJ643488) had more than 100 different nucleotides from our sample. Multiple sequence alignments for these species are presented in Figure 6.

The phylogenetic analysis of D2-D3 28S rRNA gene showed that our sample (OK055306) *Aphelenchoides* from Magelang, Indonesia clustered well with the species *A. varicaudatus* from Yunnan, China (HQ283353) and *A. varicaudatus* from Tegal, Indonesia (MN587128). *A. varicaudatus* Magelang, Central Java, Indonesia separated from other *Aphelenchoides* species. Phylogenetic relationship for species representatives within *Aphelenchoidea* Superfamily from partial 28S rRNA gene sequences are presented in Figure 5.

Multiple sequences alignment for species representatives within Genus *Helicotylenchus* are presented in Figure 8. Nucleotides at 37, 39, 47,53, 56, 57, 58, 59, 60, 61, 63, 64, 65, 66, 78, 79, 80, 83, 85, 86, 87, 88, 106,118, 176,177, 182,183, 231, 232, 235, 236, 237, 257, 259, 278, 281, 323, 325, 328, and 495 showed specific differences from other species, which separated them from other species and clustered well with *H. erythrinae* (MT321739). However, *Helicotylenchus* from Magelang Indonesia (OL757653) had 28 different nucleotides from 569 nucleotides length (4.9 %) with *H. erythrinae* (MT321739). Different nucleotides were observed more for other species compared.

Phylogenetic tree for *Helicotylenchus* showed *Helicotylenchus* from Magelang, Indonesia (OL757653) separated well from other species within genus except for *H. erythrinae* (MT321739). Phylogenetic relationships for species representatives within *Helicotylenchus* genus from the partial 28S rRNA gene sequences are presented in Figure 7.

Discussion

Common symptoms caused by nematodes include yellowing, stunted growth, and accompanied by a decrease in yield. Plant

Table 3. Measurements of female *H.erythrinae*. All measurements are in µm except for V in % and in the form mean±s.d (range).

Location	Magelang. Indonesia	Palmerston. New Zealand	Haast. New Zealand	Calarcá. Quindío. Colombia	La Celia. Risaralda. Colombia	
Publication	(This Study)	(Wouts & Yeates. 1994)		(Riascoz-Ortiz *et al.*. 2020)		(Uzma. *et al.*. 2015)
Number of Specimen	7	10	10	15	25	
Body Length	605.35 ± 68.33	873 ± 63.90	690 ± 49.80	530 ± 100	600 ± 100	
	(500.30 – 690.54)	(761 – 938)	(631 – 758)	(400 – 700)	(460 – 680)	480 – 610
Stylet Length	28.19 ± 2.57	28.30 ± 1.23	27.90 ± 0.97	22.70 ± 1.00	22.80 ± 0.83	
	(22.64 – 29.87)	(26.00 – 30.00)	(25.50 – 29.00)	(21.00 – 25.00)	(21.00 – 24.00)	(23 – 26)
Tail Length	23.69 ± 6.22	20.60 ± 2.80	14.70 ± 2.71	20.53 ± 2.20	20.65 ± 2.39	
	(19.39 – 30.57)	(16.00 – 24.00)	(9 – 18)	(17.00 – 25.00)	(17.00 – 26.00)	
V (%)	66.55 ± 4.08	62 ± 2.85	63 ± 2.22	63.80 ± 1.80	63.70 ± 2.16	
	(62.95 – 72.87)	(58 – 68)	(59 – 65)	(60.10 – 67.60)	(60.82 – 68.54)	(60 – 65)
a	21.79 ± 1.64	29.70 ± 1.64	28.20 ± 1.87	25.03 ± 2.00	25.90 ± 2.20	23 – 26
	(18.86 – 23.64)	(27.00 – 32.00)	(24.00 – 30.00)	(22.70 – 29.70)	(22.86 – 30.91)	
c	23.73 ± 2.92	43 ± 5.99	48 ± 10.55	25.93 ± 4.10	28.09 ± 3.00	27 – 34
	(18.79 – 27.13)	(31 – 51)	(35 – 74)	(19.20 – 31.50)	(21.82 – 32.94)	

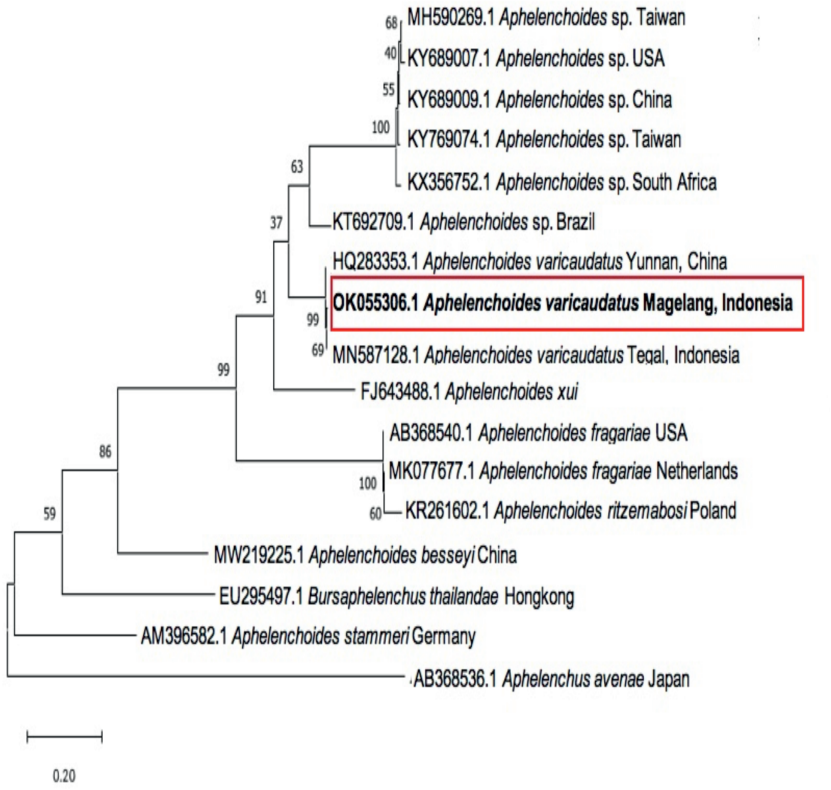

Fig. 5. Maximum-likelihood phylogenetic tree with Hasegawa-Kishino-Yano model of *Aphelenchoides* spp. and other represented species based on 28S rDNA gene sequences. Substitution models with the lowest Bayesian Information Criterion (BIC) scores. Bootstrap values are shown at the nodes.

damage due to nematodes alone is difficult to observe because the aboveground symptoms are similar to symptoms of nutrient deficiency and they also can be associated with other pathogens. It is necessary to carry out laboratory tests to see the presence of important plant-parasitic nematodes that can potentially reduce yields (Schmitt & Sipes, 2000; William *et al.,* 2018). *Aphelenchoides* found in each extracted part (roots, bulbs, soil) in each field surveyed. The highest population for *Aphelenchoides* was found in the first field, which showed severe bulb rot (causing soil cover up the bulbs) with the presence of white fungi. The presence and interaction between nematodes and fungi in plants could be antagonistic interactions or synergistic interactions. Antagonistic interactions can occur through competition for space and food. The synergistic interactions can result in more severe damage to the host plant (Zhang *et al.,* 2020). In other hand, *Helicotylenchus* mostly found from the soil for both field.

The *Aphelenchoides* samples had characters as described by Huang *et al.* (2012) as *A. varicaudatus*. This species with a rounded set-off head, multireflexed or outstretched ovary, posterior ovary with V%=±60, conoid convex tail with variable tail terminus shape, one or two mucronate structures on the tail terminus (Huang *et al.,* 2012). *Aphelenchoides* sample from Magelang have the same body length range as *A.varicaudatus* Yunnan, China.

The molecular analysis supported *Aphelenchoides* Magelang Indonesia as *A.varicaudatus* species. All sequences comparison in this study were picked from large subunit (LSU) 28S rRNA gene region. This region has conserved domains and expansion segments, such as the D2-D3 expansion region. This region is useful for designing of diagnostics tool for the identification of nematodes to the species level (Cunha *et al.,* 2018). In this study, *A. varicaudatus* Magelang Indonesia separated from unidentified *Aphelenchoides* species (*Aphelenchoides* sp.) from various country based on its 28S gene region. *A. varicaudatus* from Yunnan, China is closely related to *Aphelenchoides* sp. from ginger plant (*Zingiber* sp.) in Indonesia from its small subunit (SSU) and very different to *A. besseyi* and *A. fragariae* (Huang *et al.,* 2012). The phylogenetic tree in this study also revealed that *A. varicaudatus* separated from other species of *Aphelenchoides* such as *A. besseyi*, *A. fragariae, A. ritzemabosi* based on 28S region as Huang *et al.* (2012) reported.

Morphology and molecular identification of *Aphelenchoides* from garlic plants in Magelang showed the species indicated to *A. varicaudatus*. *A. varicaudatus* is not a common plant-parasitic nematode reported species from Genus *Aphelenchoides* like *A.besseyi, A.fragariae*, and *A.ritzemabozi*. The status of *A.varicaudatus* was still unclear. However, study about this species is also important, remembering that this species was also found in the rose root and pine xylem (Huang *et al.,* 2012; Ibrahim & Hooper, 1994). A

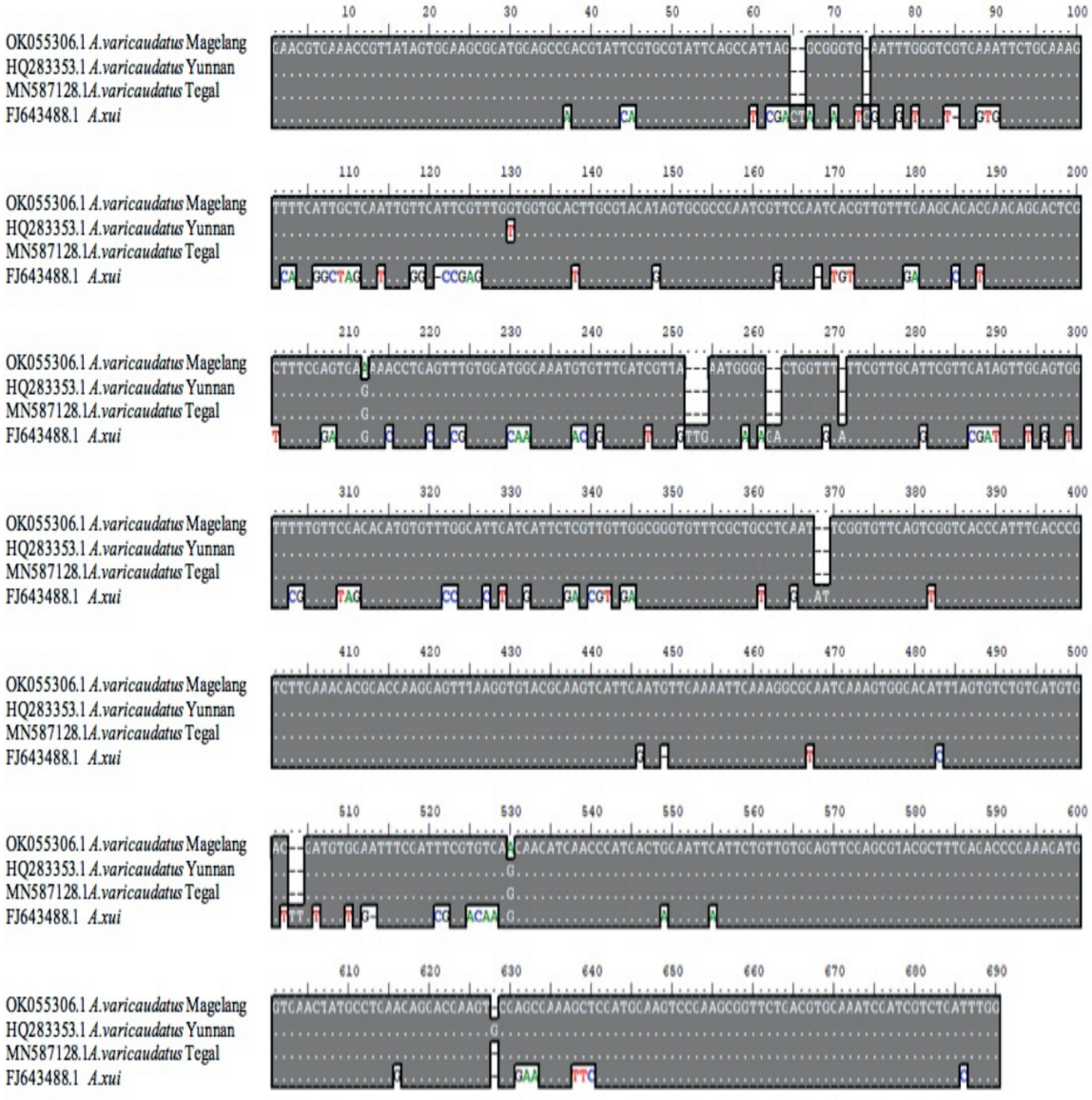

Fig. 6. Multiple sequences alignment of *Aphelenchoides varicaud*atus species and *Aphelenchoides xui* from D2-D3 expansion region.

study by Santos *et al.* (2021) revealed that *Aphelenchoides pseudogoodeyi,* which also has unclear information about its pathogenicity, is an opportunistic pathogen as the injury was required to induce symptoms in Bird's-Nest Fern and Oriental Lily. It was showed that a mainly mycophagus *Aphelenchoides* could become phytophagous under stress conditions (Santos *et al.,* 2021). *A. varicaudatus* was first described by Ibrahim and Hooper (1994) from rose roots plants. Furthermore, *A. varicaudatus* was found in Yunnan, China from Simao Pine plant (*P. kesiya* var. *langbianensis*) (Huang *et al.,* 2012). *A. varicaudatus* could be found in bark/moss/lichens and able to grow on fungi media (Sanchez-Monge *et al*., 2015). This indicated that *A. varicaudatus* has a wide distribution and different host associations (Huang *et al.,* 2012). The species in *Aphelenchoides* are very diverse and have different types of feeding. Most species in *Aphelenchoide*s are mycophagous (Kanzaki, 2012). There are 13 other species known as plant parasites. Most of them are generalists because they have a broad host range (Sanchez-Monge *et al.,* 2015). Only a few species are economically important (Nickel & Hopper, 1991).

The morphological characters of *Helicotylenchus* are quite complicated to observe between species. Our sample had descriptions as *H. erythrinae* in Uzma *et al.* (2015) based on lips and tail observations and also description by Riascos-Ortiz *et al.* (2020). Based on morphometrical data, our specimens close to *H. erythrinae*

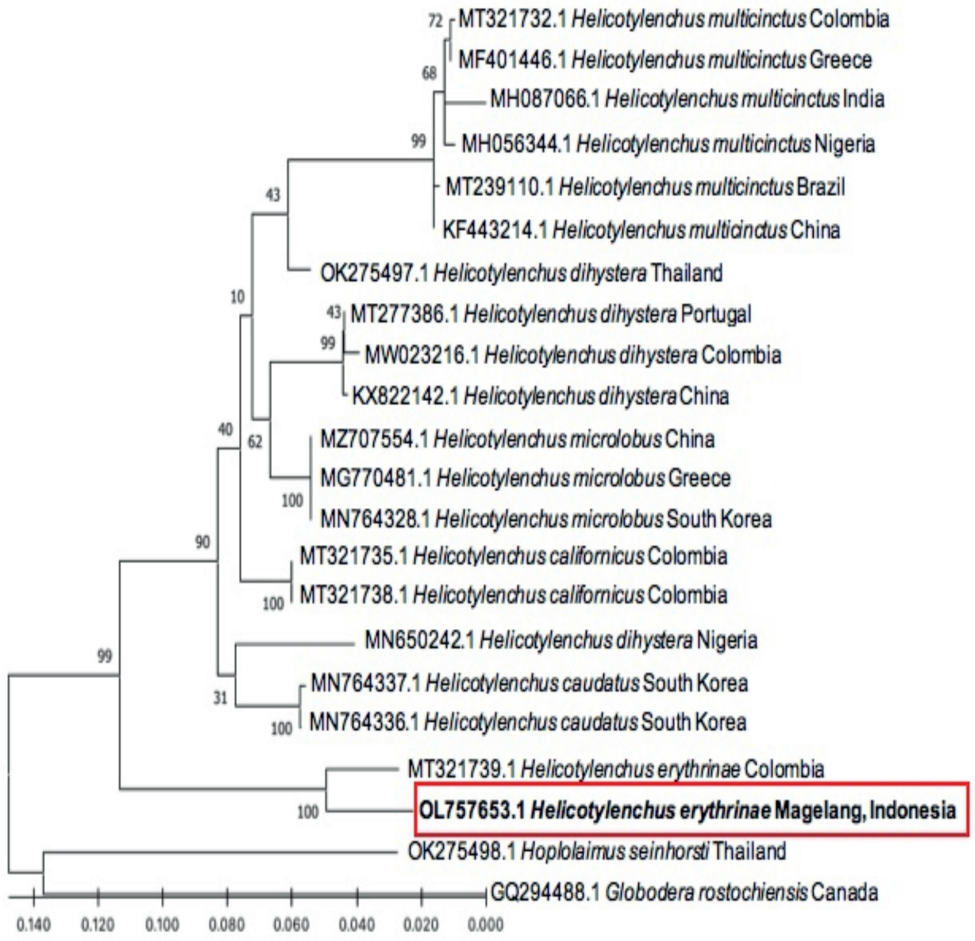

Fig. 7. Maximum-likelihood phylogenetic tree general time-reversible model and gamma-shaped distribution (GTR+G) of *Helicotylenchus* spp. and other represented species based on 28S rRNA gene sequences. Substitution models with the lowest Bayesian Information Criterion (BIC) scores. Bootstrap values are shown at the nodes.

from Calarcá, Quindío, Colombia by Riascos-Ortiz *et al.* (2020) with high variability observed in some characters. The existence of intraspecific variation and limited character information makes identification difficult. However, no male nematodes were found in this study. According to Riascos-Ortiz *et al.* (2020), female *H. erythrinae* species have functional spermatheca with sperm in it and there are male nematodes. Species *H. erythrinae* has a loose spiral post-mortem habitus, a hemispherical lip region with an indented or flattened anterior knob, a tail with long ventral projections (Riascos-Ortiz *et al.,* 2020) which these morphological characteristics were found in our specimens.

The phylogenetic tree supported that *Helicotylenchus* from Magelang (OL757653) clustered well with *H. erythrinae* (MT321739) from Colombia. Percent identification with this species was only 95.66 %, indicating the Magelang specimen had some nucleotide variations with *H. erythrinae* (MT321739). However, there was only one sequence submitted for *H. erythrinae* out of a total of 758 submitted sequences in the Genus *Helicotylenchus* (data accessed in November, 2021). Nucleotide sequence observations showed specific characters with *H. erythrinae* (MT321739) at some orders of nucleotides length, such as at 56 – 66, and 78 – 89 of 569 nucleotides compared with other representative species in the Genus *Helicotylenchus.*

Based on morphological observations and molecular characters, *Helicotylenchus* found from garlic plantations in Magelang, Central Java, refers to the *H. erythrinae* species. Molecularly, *H. erythrinae* was first reported by Riascos-Ortiz *et al.* (2020) from banana plantain in Colombia. *H. erythrinae* has a fairly wide host range, including dadap (Sher, 1966), pepper, ginger (Koshi *et al.,* 2005), tea (Nalini *et al*., 2005), cocoa, olive (El-Borai & Duncan, 2005), cassava, rice (Bridge *et al.,* 2005). In addition to being found in some cultivated plants, *H. erythrinae* was also found in uncultivated jungle soils (Sauer & Winoto, 1975). *H. erythrinae, H. dihystera* and three undescribed *Helicotylenchus* species were found from Java (Sher, 1966). Systematics within the genus of *Helicotylenchus* is in a very unsatisfactory state and identification to species is difficult (Wouts & Yeates, 1994).

Future study for *A. varicaudatus* and *H. erythrinae* found from

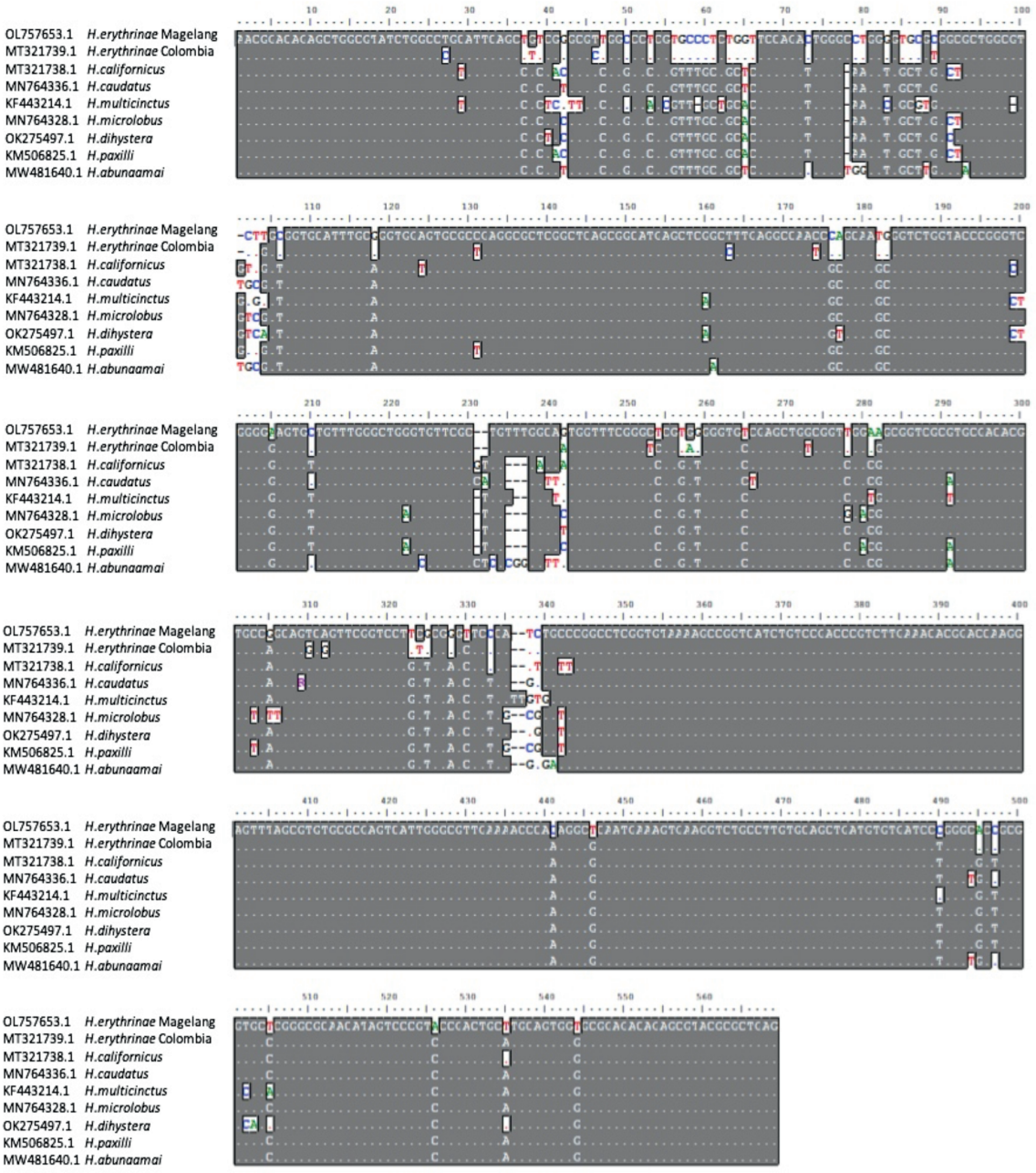

Fig. 8. Multiple sequences alignment of *Helicotylenchus* species within genus from D2-D3 expansion region.

garlic plantation is needed since the sequences data in NCBI for both species were limited. Molecular characterization from other DNA genes target is needed to perform more comprehensive molecular characterization.

Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia.

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References

Ajri, M., Indarti, S., Soffan, A., Huu, N.N. (2021): Morphological and phylogenetic characteristics of *Ditylenchus dipsaci* among

garlic plants. *Jordan J. Biol. Sci*, 14(4): 769 – 773. DOI: 10.54319/jjbs/140418

Amritha, M.L., Budijastuti, W. (2018): Tingkat serangan nematoda parasit pada bawang putih (*Allium sativum*) impor dan lokal di Jawa Timur [The Infection Rate Parasitic Nematode On Import And Local Garlic In East Java]. *LenteraBio*, 7(3): 214 – 220 (In Indonesian)

Bridge, J., Plowright, R.A., Peng, D. (2005): Nematode Parasites of Cereals. In: Luc, M., Sikora, R.A., Bridge, J. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture 2nd Edition.* Wallingford, CAB International.

Cseke, L.J., Kaufman, P.B., Podila, G.K., Tsai, C.J. (2003): *Handbook of Molecular and Cellular Methods in Biology and Medicine*. Second Edition. CRC Press, United States of America.

Da Cunha, T.G., Visotto, L.E., Lopes, E.A., Oliviera, C.M.G., God, P.I.V.G. (2018): Diagnostic methods for identification of root-knot nematodes species from Brazil. *Crop Protection, Cienc. Rural*, 48(02). DOI: 10.1590/0103-8478cr20170449

De Ley, P., De Ley, I.T, Morris, K., Abebe, E., Mundo-Ocampo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Jay Burr, A.H., Baldwin, J.G., Thomas, W.K. (2005): An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. *Philos Trans R Soc B,* 360: 1945 – 1958. DOI: 10.1098/rstb.2005.1726

EPPO (2013): *PM 7/119 (1) Nematode extraction.* EPPO Bull 43: 471 – 495. DOI: 10.1111/epp.12077

El-Borai, F.E., Duncan, L.W. (2005): Nematode Parasites of Subtropical and Tropical Fruit Tree Crops. In: Luc, M., Sikora, R.A. Bridge, J. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture 2nd Edition.* Wallingford, CAB International.

Huang, Ren-E., Ye, W., Liang, J., Lu, Q., Zhang, X. (2012)*: Tylaphelenchus jiaae* n. sp. and *Aphelenchoides varicaudatus* (Nematoda: Aphelenchoidinae) from Simao pine in Yunnan Province, China. *Nematology*, 14(1): 93 – 108. DOI: 10.1163/138855411X579418

Ibrahim, S.K., Hooper, D.J. (1994): *Aphelenchoides varicaudatus* sp. n. (Nematoda: Aphelenchoididae). *AfroAsian J Nematol*, 4: 210 – 214

IPPC (2016): DP 17: *Aphelenchoides besseyi, A. fragariae* and *A. ritzemabosi*. Retrieved from https://www.ippc.int/static/media/files/publication/en/2016/11/DP_17_2016_En_2016-11-01_iaK6Hls.pdf

Kanzaki, N., Giblin-Davis, R. (2012): Chapter 7: Aphelenchoidea. In: Manzanilla-Lopez, R. Mendoza, N. *Practical Plant Nematology.* Biblioteca Básica de Agricultura, Guadalajara, México.

Kusuma, M.D., Supramana, Giyanto (2020): Phytonematodes community and polyphasic character of *Aphelenchoides varicaudatus* on garlic plants in Tegal Regency, Central Java. *Jurnal Perlindungan Tanaman Indonesia*, 24(2):216 – 223. DOI: 10.22146/jpti.49779

Mai, W.F., Mullin, P.G. (1996): *Plant-Parasitic Nematodes: A Pictorial Key to Genera.* Comstock Pub. Associates, Cornell University Press. ISBN: 08-014-31166

Mohamed, R.M. Saeed (2015): Plant–parasitic nematodes associated in garlic in Yemen. *Egypt. J. Agronematol*, 14(1): 37 – 44. DOI: 10.21608/ejaj.2015.57489

Musyarofah, A., Indarti, S. (2020): Host Range of Stem and Bulb Rot Parasite Nematode (*Ditylenchus dipsaci*). In: Tutik D.R., Roto, R., Rohana, A., Laurent, C., Kuwat, T., Indriana, K., Julius, M., Dwi, S. (Eds) *Key Engineering Materials.* Trans Tech Publications Ltd, Switzerland. Vol. 840: 137 – 141. DOI: 10.4028/www.scientific.net/KEM.840.137

Nalini, C.G., Mohotti, K.M. (2005): Nematode Parasites of Tea. In: Luc, M., Sikora, R.A. Bridge, J. (Eds) *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture 2nd Edition.* Wallingford, CAB International.

Nickle, W.R., Hooper, D.J. (1991): The Aphelenchina: Bud, Leaf and Insect Nematodes. In: Nickle, W.R. (Ed) *Manual of Agricultural Nematology,* New York, NY, USA, Marcel Dekker.

Nunn, G. (1992): *Nematode molecular evolution. An investigation of evolutionary patterns among nematodes based upon DNA sequences.* Ph.D. dissertation, University of Nottingham, UK.

Rahman, R.M., Munif, A., Kurniawati, F. (2018): Deteksi dan Identifikasi Nematoda Aphelenchoides besseyi dari Benih Padi [Detection and identification of *Aphelenchoides besseyi* from rice seeds]. *Jurnal Fitopatologi Indonesia*, 14(2): 39 – 46. DOI: 10.14692/jfi.14.2.39

Riascos-Ortiz, D., Mosquera-Espinosa, A.T., De Agudelo, F.V., De Oliveira, C., Muñoz-Florez, J.E. (2020): An integrative approach to the study of *Helicotylenchus* (Nematoda: Hoplolaimidae) Colombian and Brazilian populations associated with *Musa* crops. *J Nematol*, *52*, 1 – 19. DOI: 10.21307/jofnem-2020-054

Sanchez-Monge, A., Flores, L., Salazar, L., Hockland, S., Bert, W. (2015): An updated list of the plants associated with plant-parasitic *Aphelenchoides* (Nematoda: *Aphelenchoididae)* and its implications for plant-parasitism within this genus. *Zootaxa* 4013(2):207. DOI: 10.11646/zootaxa.4013.2.3

Santos, E., Silva, I., Rosa, R., Oliveira, C., Buonicontro, D. (2021): Pathogenicity of *Aphelenchoides pseudogoodeyi* on the ornamental plants *Asplenium nidus* and *Lilium speciosum* in Brazil. *Helminthologia,* 58(1): 74 – 84. DOI: 10.2478/helm-2021-0003

Sauer, M.R., Winoto, R. (1975): The genus *Helicotylenchus* Steiner 1945 in West Malaysia. *Nematologica,* 341 – 350. DOI: 10.1163/187529275x00077

Schmitt, D.P., Sipes, B.S. (2000): Plant-Parasitic Nematodes and Their Management. In: Silva, J.A., Uchida, R. (Eds) *Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

Sembiring, A.I.M., Kurniawati, F., Supramana. (2019): Karakterisasi internal transcribed spacer (ITS) rDNA nematoda pucuk putih (*Aphelenchoides besseyi* Christie) [(Characterization of the internal transcribed spacer (ITS) rDNA of white tip nematode (*Aphelenchoides besseyi* Christie)]. *Agrovigor*,12(1): 16 – 25. DOI: 10.21107/agrovigor.v12i1.5085 (In Turkish)

Sher, S.A. (1966): Revision of the *Hoplolaiminae (*Nematoda) *VI. Helicotylenchus Steiner, 1945. Nematologica,* 12: 1 – 56

Subbotin, S.A., Volvas, N., Yeates, G.W., Hallmann, J., Kiewnich, S., Chizhov, V.N., Manzanilla-Lopez, R.H., Inserra, R.N.,Castaillo, P. (2015): Morphological and molecular characterization of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology*, 17: 27 – 52. DOI: 10.1163/15685411-00002850

Uzma, I., Nasira, K., Firoza, K., Shahina, F. (2015): Review of the genus *Helicotylenchus* Steiner, 1945 (Nematoda: *Hoplolaimidae*) with updated diagnostic compendium. *Pak J Nematol,* 33: 115 – 160. DOI: 10.18681/2015.V33.I02.P01201507310001

William-A.M., Collins, A., Esker, P. D. (2018): *Plant Parasitic Nematode Explained*. College of Agricultural Science The Pennsylvania State University. Retrieved from https://extension.psu.edu/plant-parasitic-nematodes-explained

Wouts, W.M., Yeates, G.W. (1994): *Helicotylenchus* species (Nematoda: Tylenchida) from native vegetation and undisturbed soils in New Zealand. *N Z J Zool*, 21: 213 – 224. DOI: 10.1080/03014223.1994.9517988

Wulandari, A.S., Indarti, S. (2020): Distribution and abundance of a new pest "root and bulb parasitic nematode" at different elevation levels and soil abiotic factors garlic growing centres in Central Java. In: Tutik, D.W., Roto, R., Rohana, A., Laurent, C., Kuwat, T., Indriana, K., Julius, M., Dwi, S. (Eds) *Key Engineering Materials*. Vol. 840. Trans Tech Publications Ltd, Switzerland, pp. 124 – 130. DOI: 10.4028/www.scientific.net/KEM.840.124

Wulandari, A.S., Indarti, S., Massadeh, M.I., Van Minh, N.V. (2021): The effect of abiotic factors and elevation on the diversityof plant parasitic nematodes in garlic on Central Java, Indonesia. *Sarhad J. Agric.*, 37 (Special issue 1): 75 – 83. DOI: 10.17582/journal.sja/2021/37.s1.75.83

Zhang, Y., Li, S., Li, H., Wang, R., Zhang, K., Xu, J. (2020): Fungi-Nematode Interactions: Diversity, Ecology, and Biocontrol Prospects in Agriculture. *J Fungi (Basel)*, 6(206): 1 – 24. DOI: 10.3390/jof6040206

Zhao, Z.Q. (2006): *Occurrence, taxonomy, biology and pathogenicity of aphelenchid nematodes associated with conifers in south-eastern Australia.* Ph.D. Thesis, The University of Adelaide, South Australia.