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FIRST REPORT OF ROOT-KNOT NEMATODE *Meloidogyne enterolobii* INFECTING POMELO (*Citrus maxima* (Burm.) Merri) IN VIETNAM

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ABSTRACT

Meloidogyne enterolobii Yang & Eisenback, 1983 is amongst the most aggressive root-knot nematodes, causing significant annual losses worldwide to many crops and emerging in many countries recently. Although plants belonging to the citrus family such as grapefruit and citrus have been reported as non-hosts or poor hosts for *M. enterolobii*, our study recorded the heavy infection of a root-knot nematode that resembles *M. enterolobii* on pomelo, a species in the citrus family. The molecular data of D2-D3 of 28S rRNA region, morphometrics, and morphological features of second-stage juveniles, males, and females in the present study have confirmed that the root-knot nematode recovered from pomelo in Vietnam belongs to *M. enterolobii*. To the best of our knowledge, this represents the first report of *M. enterolobii* infecting pomelo, providing new insight into the host status of this important pest.

Keywords: Emerging pest, molecular, new host, root-knot nematode, taxonomy.

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INTRODUCTION

The guava root-knot nematode (*Meloidogyne enterolobii*) was described by Yang and Eisenback (1983). This species is recently reported as one of the most destructive plant-parasitic nematodes, causing significant yield loss to numerous host plants with a wide distribution in North, Central, and South America, Africa, and Asia (CABI, 2021; EPPO, 2021). Besides, it is able to reproduce on tomato genotypes carrying *Mi* resistance genes and can cause the highest yield loss (up to 65%) amongst all root-knot nematodes (Castagnone-Sereno, 2012; Kiewnick et al., 2009; Perry et al., 2009). Because of its damaging potential, *M. enterolobii* was listed in the A2 list of quarantine pests (EPPO, 2021).

Since *M. enterolobii* is morphologically relatively similar to other root-knot nematodes such as the tropical root-knot nematode group, its diagnosis based solely on morphology can be a problem. Fortunately, a recent study revealed that molecular analysis based on ribosomal genes (ITS, 28S rRNA) or mitochondrial genes (*COI*, *COII*/16S rRNA, and *Nad5* mtDNA) can be a sufficient and reliable tool in detecting *M. enterolobii* (Trinh et al., 2022a). Although being reported on a broad host range of more than 34 cultivated crops and weeds, several crops in the citrus family such as grapefruit, orange, and citrus have been reported as non-hosts or very poor hosts for *M. enterolobii* (EPPO, 2021; Philbrick et al., 2020). Currently, nine species of the genus *Meloidogyne*, including *Meloidogyne arenaria*, *Meloidogyne cynariensis*, *Meloidogyne daklakensis*, *Meloidogyne enterolobii*, *Meloidogyne graminicola*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne moensi*, have been confirmed as existing in Vietnam (Trinh et al., 2022b). Among these species, *M. enterolobii* has been only found associated with guava in the country (Iwahori et al., 2009; Trinh et al., 2022b). Interestingly, for the first time, our study recorded a serious infestation of *M. enterolobii* on pomelo (*Citrus maxima* (Burm.) Merr.) in Vietnam. The population of *M. enterolobii* recovered from this study was characterized using molecular data of D2–D3 of 28S rRNA region and morphological characters of second-stage juveniles, males, and females.

MATERIALS AND METHODS

Sampling and extraction

Soil and root samples were collected from the rhizosphere of pomelo (*Citrus maxima* (Burm.) Merr.) in Vietnam. Mature females and egg masses were extracted directly from the root galls of pomelo using a scalpel and forceps following the method of Perry et al. (2009). Juveniles and males of *M. enterolobii* were extracted from soil samples using the modified Baerman tray method (Whitehead & Hemming, 1965).

Morphological characterisation

For morphological characterisation, nematodes were fixed in TAF and transferred to glycerin to make permanent slides following Phan et al. (2020). In the next step, measurements and microphotographs were taken from nematodes in permanent slides using a Carl Zeiss Axio Lab. A1 light microscope equipped with a Zeiss Axiocam ERc5s digital camera.

Molecular characterisation

For molecular characterisation, DNA was extracted from single juveniles hatched from egg masses. The D2–D3 of the 28S rRNA region was amplified using D2A/D3B primers (Nunn, 1992). The amplification process involved a thermal profile consisting of one cycle of 94 ^{o}C for 4 minutes, followed by 5 cycles of 94 ^{o}C for 30 seconds, 56 ^{o}C for 30 seconds, and 72 ^{o}C for 2 minutes. Subsequently, 45 cycles of 94 ^{o}C for 30 seconds, 54 ^{o}C for 30 seconds, and 72 ^{o}C for 1 minute were carried out, and the process finalised at 10 ^{o}C for 10 minutes (Trinh et al., 2018).

Raw forward and reverse sequences were assembled and edited using Geneious R11

(www.geneious.com). For alignment, closely related sequences were selected using Blast in GenBank (Altschul et al., 1997). The MUSCLE in Geneious R11 was used to make multiple alignment and a Bayesian phylogenetic tree was constructed following Nguyen et al. (2021). The best fit model was selected using Mega 7 based on the BIC criterion (Nguyen et al., 2019).

RESULTS AND DISCUSSION

Measurements

All measurements of *M. enterolobii* from Vietnam are provided in Tables 1–3.

Table 1. Second-stage juveniles of *Meloidogyne enterolobii* from different hosts. All measurements are in μm (except for ratio) and in the form: mean ± sd. (min-max)

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
Source and locality	This study, Vietnam	Yang and Eisenback (1983) China	Trinh et al. (2022) Vietnam	Rammah and Hirschmann (1988) Puerto Rico	Brito et al. (2004) US
Host	Pomelo	Pacara earpod tree	Guava	Eggplant	Ornamental plant
Number of specimens (n)	15	30	20	35	20
Body length (L)	376 ± 16 (336–398)	436 ± 17 (405–473)	396 ± 13.6 (374–438)	454 ± 28 (390–528)	461 ± 15.9 (433–481)
Lip height	2.3 ± 0.3 (1.9-2.8)	-	2.0 ± 0.2 (1.8–2.3)	-	-
Lip width	3.6 ± 0.3 (2.7–4.1)	-	3.5 ± 0.3 (2.9–3.8)	-	-
Stylet length	9.9 ± 0.9 (8.2–11.5)	11.7 ± 0.5 (10.8–13)	11 ± 0.7 (10.4–12.5)	11.6 ± 0.3 (11.1–12.2)	10.9 ± 0.3 (10.4–11.5)
Stylet base to dorsal gland orifice (DGO)	3.4 ± 0.3 (2.8–3.7)	3.4 ± 0.3 (2.8–4.3)	3.2 ± 0.7 (2.1–4.2)	3.9 ± 0.2 (3.3–4.3)	3.8 ± 0.3 (2.9–4.1)
Pharynx	52 ± 2.2 (48–56)	-	53 ± 1.9 (48–57)	-	78 ± 3.5 (73–89)
Anterior end to nerve ring	64 ± 3 (59–68)	-	(66 ± 2.3) (59–70)	-	-
Anterior end to pharyngo-intestinal junction	75 ± 5.3 (64–87)	-	79 ± 2.4 (75–84)	-	-
Anterior end to secretary-excretory pore	76 ± 4.7 (66–84)	92 ± 3.3 (84–99)	89 ± 4.4 (81–97)	88 ± 3.3 (80–98)	92 ± 4.0 (88–98)
Max body diam. (MBD)	14.3 ± 1.5 (11.7–16.5)	15.3 ± 0.9 (13.9–17.8)	15.6 ± 1.1 (13.5–17.7)	14.7 ± 0.5 (13.8–15.8)	15 ± 0.4 (14.5–16.1)
Body diam. at anus (ABD)	9.9 ± 1.3 (8–12.3)	-	11 ± 1 (9.4-12.5)	10.9 ± 0.5 (10.2-12.2)	10.6 ± 0.3 (10.0–11.2)
Tail length	50 ± 3.1 (45–55)	56 ± 4.5 (42–63)	41 ± 3.6 (37–47)	54 ± 3.6 (49–63)	56 ± 2.9 (51–61)
Hyaline	19.9 ± 6.2 (14.1–33)	-	25.1 ± 1.8 (21.4–26.7)	-	11.1 ± 2.6 (5.0–14.7)

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
a=L/MBD	27 ± 2.8 (23–31)	28.6 ± 1.9 (24–33)	25.4±1.8 (22.3–29.7)	30.9 ± 1.9 (26–35)	31 ± 1.2 (28.3–33)
b = L/distance from anterior end to pharyngo-intestinal valve	5 ± 0.3 (4.6–5.8)	-	4.5 ± 0.2 (4.1–4.9)	-	5.9 ± 0.3 (5.2–6.3)
b′ = L/distance from anterior end to posterior end of pharyngeal glands	2.4 ± 0.3 (2–3)	-	-	-	-
c = L/Tail length	7.6 ± 0.6 (6.1–8.4)	7.8 ± 0.7 (6.8–10.1)	7.8 ± 0.5 (6.9–9.1)	8.3 ± 0.4 (7.0–9.2)	8.2 ± 0.4 (7.6–8.6)
c′ = Tail length/ABD	5.1 ± 0.6 (4.3–6)	-	4.6 ± 0.4 (4.1–5.7)	-	5.3 ± 0.3 (4.8–5.9)

Table 2. Males of *Meloidogyne enterolobii* from different hosts. All measurements are in μm (except for ratio) and in the form: mean ± sd (min-max)

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
Source and locality	This study, Vietnam	Yang and Eisenback (1983) China	Trinh et al. (2022) Vietnam	Rammah and Hirschmann (1988) Puerto Rico	Brito et al. (2004) US
Host	Pomelo	Pacara earpod tree	Guava	Eggplant	Ornamental plant
Number of specimens (n)	6	20	10	30	20
Body length (L)	1,406 ± 68 (1,338–1,474)	1,600 ± 160 (1,378–1,913)	1,505 ± 107 (1,397–1,612)	1,503 ± 142 (1,175–1,742)	996 ± 97 (857–1,141)
Testis length	823 ± 90 (733–913)	810 ± 140 (597–1,055)	-	-	-
Lip height	4.9 ± 0.2 (4.7–5.1)	-	5.7 ± 0.5 (5.2–6.2)	-	-
Lip width	8.3 ± 0.4 (8–8.7)	-	6.7 ± 0.5 (6.2–7.2)	-	-
Stylet length	17.3 ± 1.3 (16–18.6)	23.4 ± 1.0 (21.2–25.5)	16 ± 0.5 (15.6–16.6)	22.9 ± 0.8 (20.7–24.6)	19.7 ± 0.8 (17.5–20.8)
Stylet base to dorsal gland orifice (DGO)	6.3 ± 0.8 (5.5–7)	4.7 ± 0.4 (3.7–5.3)	3.9 ± 0.2 (3.6–4.1)	4.1 ± 0.4 (3.3–5.0)	4.6 ± 0.4 (3.9–5.0)
Anterior end to median bulb	-	-	88 ± 2.6 (85–91)	92 ± 4.3 (85–102)	86 ± 3.8 (78–93)
Pharynx	82 ± 0.7 (81–83)	-	-	-	-
Anterior end to nerve ring	95 ± 4.8 (90–100)	-	-	-	-
Anterior end to secretary-excretory pore	143 ± 6.4 (137–150)	178 ± 11.2 (160–206)	136 ± 7.8 (128–143.5)	166 ± 8.8 (147–181)	138.3 ± 14.8 (118–183)

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
Max body diam. (MBD)	32 ± 0.4 (31–33)	43 ± 3.6 (37–48.3)	34 ± 2.6 (31–37)	38 ± 3.1 (32–44)	27.0 ± 1.8 (24.1–31.5)
Body diam. at anus (ABD)	25 ± 3.5 (22–29)	-	24.4 ± 1.5 (22.9–26)	-	-
Tail length	30 ± 6.4 (24–37)	12.5 ± 2.2 (8.6–20.2)	12 ± 1.5 (10.4–13.5)	14.3 ± 1.1 (11.3–16.3)	11.2 ± 1.0 (9.8–13.5)
Phasmid to tail tip	6.6 ± 0.5 (6.1–7)	-	-	-	-
Spicule length	31 ± 5.4 (26–37)	30 ± 1.2 (27–32)	22 ± 3 (19.4–25)	28.3 ± 1.5 (24.4–31.3)	26.0 ± 1.6 (23.5–29.4)
Spicule width	3.4 ± 0.8 (2.6–4.2)	-	-	-	-
Gubernaculum	8 ± 0.2 (7.7–8.2)	6.2 ± 1.0 (4.8–8)	8.8 ± 1.5 (7.2–10.4)	7.1 ± 0.6 (6.1–9.3)	6.9 ± 0.4 (6.1–7.7)
a=L/MBD	44 ± 1.6 (42–45)	38 ± 3.2 (34–46)	45 ± 1 (44–46)	40 ± 3.9 (31–50)	37 ± 2.6 (32–41)
b = L/distance from anterior end to pharyngo-intestinal valve	13.3 ± 0.5 (12.9–13.8)	-	17.1 ± 1.7 (15.4-18.9)	-	-
b′ = L/distance from anterior end to posterior end of pharyngeal glands	6.1 ± 0 (6.1–6.2)	-	-	-	-
c=L/Tail length	49 ± 12.7 (37–62)	132 ± 24.2 (72–173)	-	106 ± 10 (86–124)	89 ± 10.0 (73–103)
c′ =Tail length/ABD	1.2 ± 0.1 (1.1–1.3)	-	0.5 ± 0.1 (0.4–0.6)	-	-

Table 3. Females of *Meloidogyne enterolobii* from different hosts. All measurements are in μm (except for ratio) and in the form: mean ± sd (min-max)

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
Source and locality	This study, Vietnam	Yang and Eisenback (1983) China	Trinh et al. (2022) Vietnam	Rammah and Hirschmann (1988) Puerto Rico	Brito et al. (2004) US
Host	Pomelo	Pacara earpod tree	Guava	Eggplant	Ornamental plant
Number of specimens (n)	7	20	10	35	14
Body length (L)	486 ± 54 (419–580)	735 ± 93 (541–926)	707 ± 98 (548–914)	651 ± 53 (518–770)	-
Neck length	210 ± 68 (148–369)	218 ± 74 (114–446)	220 ± 68 (129–365)	171 ± 73 (81–526)	-
L/neck length	2.5 ± 0.6 (1.4–3.1)	-	3.4 ± 0.7 (1.9–4.4)	-	-
Lip height	1.4 ± 0.4 (0.9–2.2)	-	2.5 ± 0.2 (2.3–2.8)	-	-
Lip width	3.8 ± 0.9 (2.6–5.5)	-	5 ± 0.5 (4.7–6.1)	-	-

Character	*Meloidogyne entorolobii* Yang and Eisenback, 1983				
Stylet length	9.8 ± 2 (6.8–13.5)	15.8 ± 0.8 (13.8–16.8)	14.6 ± 3.3 (11–18)	15.8 ± 0.8 (13.8–16.8)	14.31 ± 0.36 (13.9–15.0)
Stylet base to dorsal gland orifice (DGO)	3.1 ± 0.6 (2–3.9)	4.8 ± 0.8 (3.5–6.7)	4.4 ± 0.5 (4–5.3)	4.8 ± 0.8 (3.5–6.7)	4.3 ± 0.33 (3.9–4.9)
Median Bulb width	26.1 ± 6.4 (18.7–39)	-	37 ± 3.3 (29–40)	-	-
Median bulb length	35 ± 6.9 (25–47)	-	45 ± 3 (43–51)	-	-
Anterior end to secretary-excretory pore	34 ± 9.9 (22–50)	63 ± 10.5 (43–81)	50 ± 6.8 (40–61)	48 ± 13.6 (25.9–87)	-
Max body diam. (MBD)	271 ± 78 (148–405)	606 ± 120 (376–810)	433 ± 120 (194–645)	501 ± 44 (413–599)	-
Vulva-slit length	17 ± 2.2 (14.5–20.2)	28.7 ± 2.0 (25.3–32)	28.1 ± 3.2 (21–32)	26.1 ± 1.9 (20.9–30.4)	26.5 ± 1.6 (23.5–29.4)
Vulva-anus distance	14.6 ± 2.4 (12.1–20)	22.2 ± 1.8 (19.7–26.6)	21 ± 1.9 (18–24)	18.4 ± 1.5 (12.7–21.1)	-
Anus-tail tip distance	9.9 ± 1.4 (7.6–11.6)	-	12.5 ± 2.8 (8.8–16.4)	-	-
Interphasmidial distance	16.4 ± 2.8 (11–19.7)	31 ± 1.1 (22.2–42)	25.6 ± 1.7 (24–29)	23.2 ± 2.5 (18.1–29.6)	-
a=L/MBD	2 ± 0.6 (1.4–3.4)	1.3 ± 0.2 (1.0–1.9)	1.7 ± 0.6 (1.3–3.6)	1.3 ± 0.1 (1.1–1.6)	-

Morphological characterization (Fig. 1)

Second-stage juveniles. The body of juveniles is vermiform in shape, gradually tapering towards both the anterior and posterior ends. The lip region is slightly set off from the general contour of the body. At the mid-body level, the lateral field displays four prominent lines (Fig. 1I). The stylet of the organism is slender and possesses small knobs (Fig. 1F). The median bulb is oval-shaped, with a discernible valve (Fig. 1F); The pharyngeal gland is of variable length and partially overlaps the intestine ventrally. The tail end is characterized by a hyaline at rounded tail tip (Fig. 1G).

Females. The body is pearly white in color and exhibits significant variability in size, ranging from pear-shaped to globular with a noticeable neck (Fig. 1A). The lip region is continuous with the body contour (Figs. 1A, 1B). The stylet of the organism is robust, either straight or slightly curved, and has distinct knobs (Fig. 1B). Typically, the perineal pattern is oval-shaped with visible phasmids, and the dorsal arch is high and rounded. The striae are smooth and coarse, and the perivulval region is free of striae. The lateral lines of the organism are indistinct, and the tail tip is visible (Fig. 1C).

Males. The body is vermiform and slightly tapers at its anterior end (Fig. 1D). The lateral field of the body has four lines at the mid-body region. The head cap of the organism is high and is situated off from the general contour of the body (Fig. 1E). The stylet of the organism is robust, possessing a straight and pointed cone, cylindrical shaft, and large, rounded knobs (Fig. 1E). The median bulb is prominent and oval-shaped with a distinct valve. The pharyngeal glands are of variable length. The spicules of the organism are arcuate, with a rounded base, and the gubernaculum is short and indistinct. The tail of the organism is short and rounded (Fig. 1H). The phasmids are pore-like and are located at the level of the cloaca.

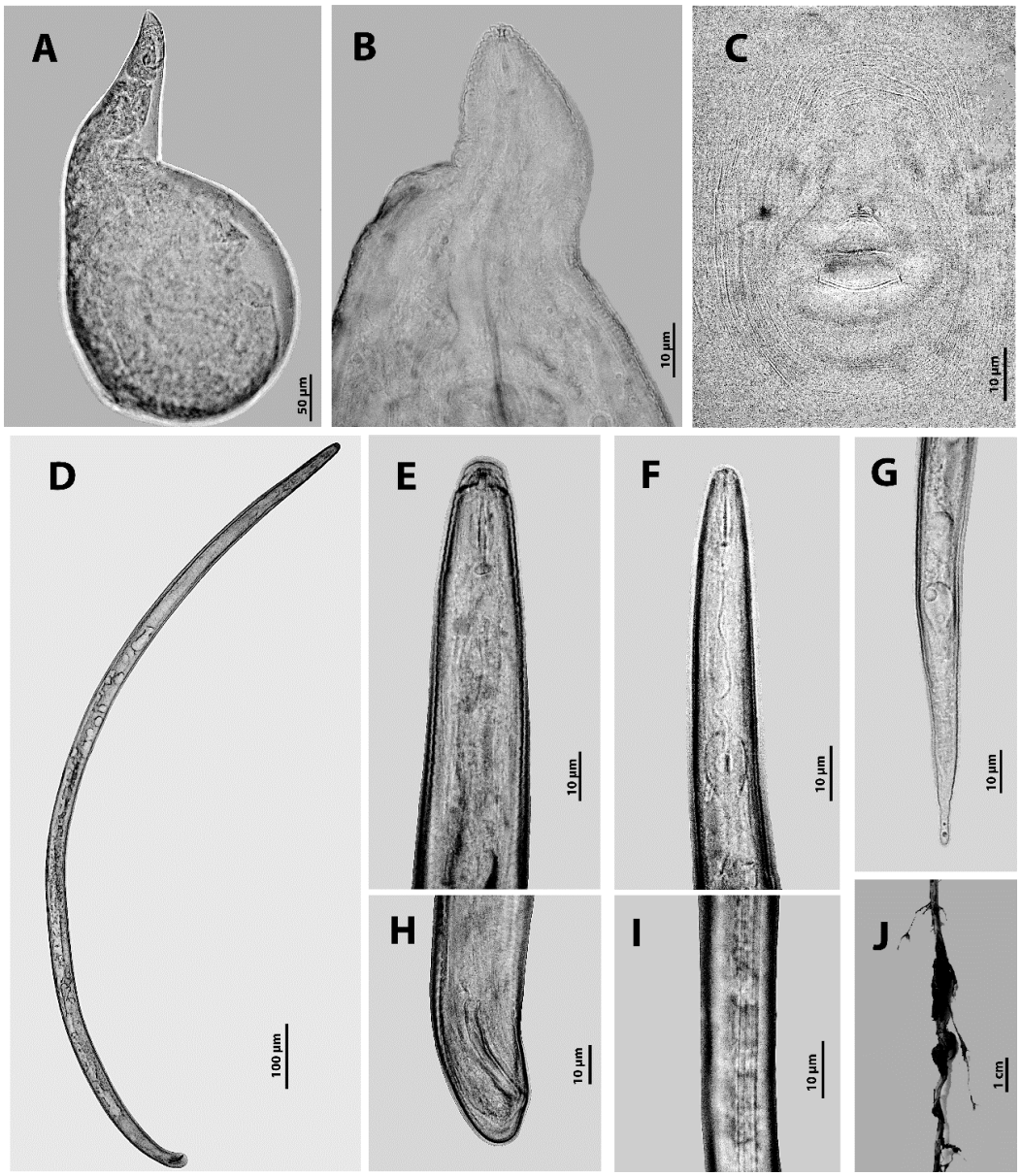

Figure 1. Microphotograph of *Meloidogyne enterolobii* from pomelo in Vietnam. A-C: Female. A: Entire body; B: Anterior end region; C: Perineal pattern. D, E, H: Male. D: Entire body; E: Anterior end regions; H: Tail region. F, G, I: Second-stage juvenile. F: Anterior end region; G: Tail region; I: Lateral field; J: Root galls

Molecular characterisation

Two D2–D3 of 28S rRNA sequences of *M. enterolobii* from Vietnam (accession number: OP216773, OP216774) were obtained with 0.5% intrapopulation variation (3 bp difference), 663–702 bp long. The sequences of *M. enterolobii* is 99.5–99.9% similar to other sequences of *M. enterolobii* from Genbank. The phylogenetic tree based on D2–D3 of 28S rRNA sequences showed that the sequences of *M. enterolobii* from Vietnam were embedded in a maximally-supported clade

of *M. enterolobii* (PP 1), clearly separated from other species. These sequences have a sister relationship to the sequences of *Meloidogyne hispanica*, *Meloidogyne luci*, *Meloidogyne lopezi*, and *Meloidogyne ethiopica* (PP 0.75) (Fig. 2).

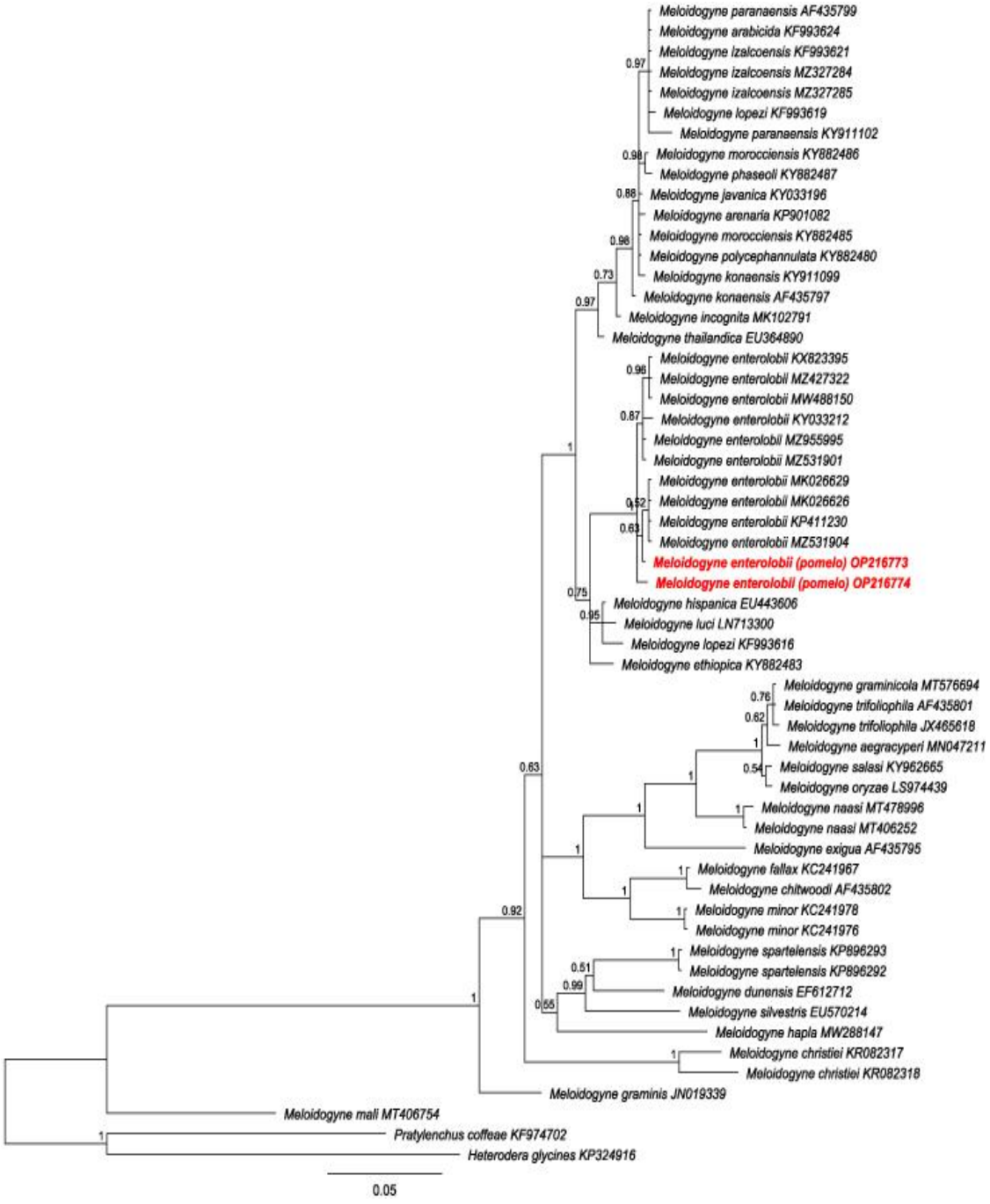

Figure 2. Phylogenetic tree generated from D2-D3 of 28S rRNA sequences under GTR+G model. Posterior probability (in percentage) was given next to each node. Sequence of *Meloidogyne enterolobii* from Vietnam was marked by red color and bold font

DISCUSSION

The morphology of *M. enterolobii* recovered from pomelo is generally in agreement with the original description by Yang and Eisenback (1983). In this study, we recorded that the population of *M. enterolobii* in our study is smaller than the type population in size of juveniles, males, and females, however, the variation in morphometrics was also observed in other populations of *M. enterolobii* from the world (Tables 1–3).

Traditionally, root-knot nematode species have been identified using morphological characterisations of juveniles, adult females, and males with a major focus on the structure of female perineal patterns (Perry et al., 2009). Nevertheless, it is known that the morphology of *M. enterolobii* is highly similar to other species in the tropical root-knot nematode group (Philbrick et al., 2020; Trinh et al., 2022a; Yang & Eisenback, 1983). Therefore, a polyphasic approach is needed in identifying this species. The study of Trinh et al. (2022a) using detailed morphological characterisations and molecular analyses of five gene regions has indicated that molecular analysis based on a single gene of ribosomal genes (ITS, 28S rRNA) or mitochondrial genes (*COI*, *COII*/16S rRNA, and *Nad5* mtDNA) can clearly separate *M. enterolobii* from other *Meloidogyne* species. Importantly, our molecular analysis based on D2-D3 of the 28S rRNA region confirmed that our nematode population belongs to *M. enterolobii*.

M. enterolobii is reported to be one of the most destructive root-knot nematodes and is able to cause the highest yield loss (up to 65%) compared to other *Meloidogyne* spp. (Castagnone-Sereno, 2012; Philbrick et al., 2020). Recently, this species has been found in an increasing number of countries over the world and was listed in the A2 list of quarantine pests (Castagnone-Sereno, 2012; EPPO, 2021; Philbrick et al., 2020). Therefore, its profile, including damage, host status, and distribution, needs to be updated and monitored carefully for better management. Our study reports on a new threat of *M. enterolobii* damaging pomelo in Vietnam.

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REFERENCES

Altschul S. F., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. & Lipman D. J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25: 3389–3402.

CABI, 2021. Invasive Species Compendium. https://www.cabi.org/isc. In*:* International, C. (Ed.). Wallingford, UK.

Castagnone-Sereno P., 2012. *Meloidogyne enterolobii* (= *M. mayaguensis*): profile of an emerging, highly pathogenic, root-knot nematode species. *Nematology*, 14: 133–138.

EPPO, 2021. PQR-EPPO database on quarantine pests (available online). http://www.eppo.int.

Iwahori H., Truc N. T. N., Ban D. V. & Ichinose K., 2009. First report of root-knot nematode *Meloidogyne enterolobii* on guava in Vietnam. *Plant disease*, 93: 675–675.

Kiewnick S., Dessimoz M. & Franck L., 2009. Effects of the Mi-1 and the N root-knot nematode-resistance gene on infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. *Journal of Nematology*, 41: 134–139.

Nguyen H. T., Le T. M. L., Nguyen T. D., Liebanas G., Nguyen T. A. D. & Trinh Q. P., 2019. Description of *Geocenamus vietnamensis* sp. n. (Nematoda: Merliniidae) from Vietnam. *Journal of nematology*, 51: e2019–25.

Nguyen H. T., Trinh Q. P., Couvreur M., Nguyen T. D. & Bert W., 2021. Description of *Hemicycliophora*

cardamomi sp. n. (Nematoda: Hemicycliophoridae) associated with *Amomum longiligulare* T. L. Wu and a web-based key for the identification of *Hemicycliophora* spp. *Journal of Helminthology*, 95: e2.

Nunn G. B., 1992. Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences (Doctoral dissertation). University of Nottingham.

Perry R. N., Moens M. & Starr J. L., 2009. Root-knot nematodes.CABI, pp. 488.

Phan K. L., Le T. M. L., Nguyen H. T. & Duyen T. D., 2020. First report and new molecular and morphological characterizations of root-knot nematode, *Meloidogyne javanica*, infecting ginger and long coriander in Vietnam. *Journal of Nematology*, 53: e2021–11.

Philbrick A. N., Adhikari T. B., Louws F. J. & Gorny A. M., 2020. *Meloidogyne enterolobii*, a major threat to tomato production: current status and future prospects for its management. *Frontiers in plant science*, 11: 1–13.

Trinh Q. P., Le T. M. L., Nguyen T. D., Le T. T. T. & Nguyen H. T., 2022a. Integrative taxonomy of the aggressive pest *Meloidogyne enterolobii* and molecular phylogeny of *Meloidogyne* spp. based on five gene regions. *Australasian Plant Pathology*, 51: 345–358.

Trinh Q. P., Le T. M. L., Nguyen T. D., Le T. T. T. & Nguyen H. T., 2022b. Integrative taxonomy of the aggressive pest *Meloidogyne enterolobii* and molecular phylogeny of *Meloidogyne* spp. based on five gene regions. *Australasian Plant Pathology*: 1–14.

Trinh Q. P., Le T. M. L., Nguyen T. D., Nguyen H. T., Liebanas G. & Nguyen T. A. D., 2018. *Meloidogyne daklakensis* n. sp. (Nematoda: Meloidogynidae), a new root-knot nematode associated with Robusta coffee *(Coffea canephora* Pierre ex A. Froehner) in the Western Highlands, Vietnam. *Journal of Helminthology*: 1–13.

Whitehead A. G. & Hemming J. R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annales of Applied Biology* 55: 25–38.

Yang B. & Eisenback J. D., 1983. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. *Journal of Nematology*, 15: 381–391.