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MORPHOLOGICAL DIVERSITY OF *Meloidogyne* spp. FROM CARROT (*Daucus carota* subsp. *Sativus*) IN VIETNAM

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Carrot (*Daucus carota* subsp. *Sativus*) is known as one of the most widely cultivated and widely consumed vegetables in the world due to its nutritional and economic values. During a survey of nematodes parasites carrots from Vietnam, six populations of three species of root-knot nematodes, namely *M. incognita*, *M. arenaria*, and *M. graminicola* were found. The species specific primers were confirmed before morphological studies. By combining the morphology and morphometry of the females, males, and juveniles, this study provided useful references for classification of *Meloidogyne* on carrots in the future. Quantitative morphological studies reveal profound changes corresponding with the generation of morphological disparity at high taxonomic diversity. Especially, this study provided the first morphological and morphometric information of *M. graminicola*, that is known as aquatic root-knot nematodes, on carrots.

Keywords: Root-knot nematodes; *Meloidogyne*; carrot; Vietnam; morphological; new records.

1. INTRODUCTION

Currently, carrot (*Daucus carota* subsp. *Sativus*) is one of the most widely cultivated and widely consumed vegetables in the world, thanks to its nutritional and economic values [1,2]. Many reports indicated that carrots, which were infested by plant-parasitic nematodes (PPN), are often stunted, underdeveloped, or even the high density of PPN could trigger the death of seedlings.

Root-knot nematodes (*Meloidogyne* spp.) parasitize almost every species of higher plant and are the economically most important plant-parasitic nematodes that caused billions of dollars losses each year in the world [3,4,5]. At present, more than 104 species of this genus have been described [5,6,7,8,9]. *Meloidogyne* species are characterized primarily on morphological features of females, males and second-stage juveniles. The traditional identification of root-knot nematodes generally relies not only on close observation of three important taxonomic criteria, including the perineal patterns of females, male head shape, and stylet morphology [10]. Detailed diagnostic characters differentiating *Meloidogyne* species have been given by authors such as Eisenback

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et al., Eisenback, Hirschmann, Jepson, Taylor and Eisenback and Triantaphyllou [11,12,13,14,15,16]. Problematically, most descriptions are only based on a limited number of morphological features but so far, hardly any information pertaining to morphological and morphometrical [17]. The taxonomical work of of root-knot nematodes has been limited in Vietnam. The features of Vietnamese populations of root-knot nematodes is available, the intraspecific variability of *Meloidogyne* species are still lacking [18].

This study provides the morphological and morphometric characteristics of Vietnamese populations of *Meloidogyne* spp. on carrots, as well as their host and distribution around the world. These information are really necessary for the sustainable management and development of carrots.

2. MATERIALS AND METHODOLOGY

Sampling: Two hundred and forty samples were collected from 22 fields from 4 provinces (Lam Dong, Hai Duong, Ha Noi, Hung Yen) in Vietnam. Each sample consisted of 1kg soil from upper 30cm soil layer, and 1 tuber with roots that were sampled at equal intervals in a grid pattern (10×10m) at each field.

Nematode extraction: The vermiform nematodes were extracted by the modified Baermann tray method [19]. Swollen nematodes were extracted directly from the galls under a stereomicroscope, using a scalpel and forceps [20].

Nematode identification: The nematode populations were checked in Multiplex-PCR before morphological study following Kiewnick et al. [21]. Nematodes were fixed in TAF (91ml H_2O; 7ml Formalin 40%; 2ml Triethanolamine) and transferred to anhydrous glycerin to make permanent slides following Seinhorst (1959) [22]. Perineal patterns of *Meloidogyne* spp. were cut, cleaned, and mounted in glycerin following Hartman & Sasser [10]. Microphotographs were taken from permanent slides using a Carl Zeiss Axio Lab. A1 microscope equipped with a digital camera. The measurements were obtained by using the software ZEN lite. Classification was based on Eisenback, Jepson, Hewlett and Tarjan, Karssen, and Kazachenko & Mukhina [12,14,23,24,25]. The specific primers were used to confirm the morphological identification. The meaning for the indices *sensu* de Man [26,27] that were used: n = number of specimens; L = body length; V = % distance of vulva from anterior / body length; a = L / maximum body diameter; b = L / distance from anterior to pharyngo-intestinal junction; b' = L / distance from anterior to base of pharyngeal glands; c = L / tail length; c' = tail length / tail diameter at anus or cloaca; Stylet = stylet length; DGO = distance from dorsal gland orifice to basal knob of stylet; VBD = diameter of body at vulva; ABD = diameter of body at anus.

3. RESULTS AND DISCUSSION

3.1 *Meloidogyne incognita* (Kofoid& White, 1919) Chitwood, 1949

See Fig. 1 and Table 1.

Females: Pear-shaped, no posterior terminal protuberance. Stylet, basal knobs rounded, offset. Perineal pattern oval to rounded, typically with high, squared, dorsal arch, striae usually wavy, lateral field absent or weakly demarcated by forked striae. *Male*: Labial region not offset, labial disc elevated, lateral lips usually absent. Stylet, basal knobs offset, rounded to transversely elongate. *J2:* Hemizonid anterior or adjacent to excretory pore, tail long hyaline region, rounded tail tip.

Remarks: The measurements of *M. incognita* on carrots in Vietnam are mostly in agreement with the original description of Chitwood, (1949). Few variations were observed such as: slightly larger body length and stylet length (350 - 411 vs 360 - 393 µm; 10 – 12.2 vs 10 µm, respectively), smaller a and c values (23 - 33 vs 29 - 33; 7.8 - 9 vs 8 – 9.4, respectively). Compared to the description of Nguyen & Nguyen (2000) the a value is larger (43 - 59 vs 39 - 48) and Stylet length of the males is smaller (20 - 23 vs 23 - 26 µm; 14 - 16,6 vs 15 - 15 µm). [18].

Host: following CABI (2018) [28].

Distribution: following CABI (2018) [28].

3.2 *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949

See Fig. 2, Table 2.

Female: Pear-shaped, no posterior terminal protuberance; stylet cone curved dorsally, gradually tapering to blunt tip anteriorly; shaft broad, cylindrical, gradually widening posteriorly; basal knobs rounded to teardrop-shaped, offset; perineal pattern variable, rounded to ovoid with fine to coarse striae; dorsal arch low, flattened with striae smooth or slightly wavy, continuous or broken, slightly bent towards tail tip at lateral line; generally forming shoulders on lateral portion of arch; dorsal and ventral striae often meeting at an angle at lateral lines; lateral field distinct, slightly irregular. *Male*: Labial region

Table 1. Morphometrics of juveniles, females and males of *Meloidogyne incognita* from different populations. All measurements in µm (except for ratio) and in the format: mean ± S.D. (range)

Measurements	*Meloidogyne incognita*											
	M4859 (Ha Noi)			M3656 (Hai Duong)			M4907 (Hung Yen)			M5269 (Lam Dong)		
	Female	Male	Juvenile	Female	Male	Juvenile	Female	Male	Juvenile	Female	Male	Juvenile
n	10	3	10	10	3	10	10	5	10	10	5	10
L	574 ± 30 (521 - 612)	1208 ± 121 (1081 - 1323)	385 ± 11.4 (379 - 409)	596 ± 84 (494 - 857)	1218 ± 194 (1037 - 1423)	402 ± 14.5 (380 - 412)	627.8 ± 37.3 (594 - 668)	1398 ± 64 (1309 - 1474)	370 ± 10.2 (350 - 386)	687 ± 61 (510 - 705)	1504 ± 94 (1430 - 1667)	392 ± 12.2 (362 - 409)
a	1.3 ± 0.2 (1 - 1.6)	49 ± 4.6 (44 - 53)	27.8 ± 1.5 (25 - 29.4)	2 ± 0.2 (1.7 - 2.2)	52 ± 6.4 (47 - 59)	31 ± 2.2 (26.4 - 33)	1.3 ± 0.3 (1.2 - 1.6)	52 ± 5.2 (43 - 57)	25.6 ± 1.8 (23 - 28.7)	1.6 ± 0.2 (1.4 - 2)	47 ± 2.8 (43 - 50)	29.3 ± 1.5 (26.3 - 31)
b	-	10.4 ± 0.7 (9.7 - 11)	5.4 ± 0.1 (5.2 - 5.6)	-	10.5 ± 1.2 (9.5 - 11.8)	5.7 ± 0.1 (5.4 - 6)	-	12.4 ± 1 (10.8 - 13.5)	5.2 ± 0.4 (5 - 5.4)	-	13.3 ± 1.2 (12.4 - 15.3)	5.5 ± 0.1 (5.4 - 5.7)
b’	-	4.4 ± 0.4 (4 - 4.6)	2 ± 0.1 (2 - 2.3)	-	4.5 ± 0.7 (3.8 - 5.3)	2.2 ± 0.1 (2 - 2.5)	-	5.2 ± 0.3 (4.8 - 5.5)	2 ± 0.2 (2 - 2.4)	-	6 ± 0.4 (5.3 - 6.5)	2 ± 0.1 (2 - 2.2)
c	-	100 ± 7.6 (91 - 106)	8.3 ± 0.4 (7.8 - 8.8)	-	112 ± 22 (98 - 138)	8.2 ± 0.4 (7.8 - 8.7)	-	117 ± 17.7 (92 - 131)	8 ± 0.2 (7.7 - 8.2)	-	129 ± 14 (112 - 144)	8.3 ± 0.4 (7.7 - 9)
c’	-	0.6 ± 0.2 (0.6 - 0.7)	4.5 ± 0.3 (4 - 5)	-	0.6 ± 0.1 (0.6 - 0.7)	4.8 ± 0.4 (4.3 - 5.6)	-	0.6 ± 0.2 (0.5 - 0.6)	4.8 ± 0.7 (3.8 - 6)	-	0.5 ± 0.1 (0.5 - 0.6)	4.6 ± 0.3 (4 - 5)
Stl	15 ± 0.5 (14.6 - 15)	20.4 ± 0.7 (20 - 21.2)	10.2 ± 0.4 (10 - 11)	15.5 ± 1 (15 - 16.2)	21.2 ± 0.5 (20.8 - 21.8)	11 ± 0.6 (10.4 - 12)	15.8 ± 0.4 (15.2 - 16.6)	22.6 ± 0.8 (21.5 - 23.3)	10.8 ± 0.4 (10 - 11.6)	14.6 ± 0.8 (14 - 16.4)	21.7 ± 0.3 (21.2 - 22)	10.5 ± 0.5 (10 - 11.2)
E.P.(%)	-	-	19.8 ± 0.3 (19 - 20.3)	-	-	20 ± 0.5 (19.3 - 21)	-	-	20.3 ± 1.2 (19 - 22.6)	-	-	20.2 ± 0.4 (19.7 - 21)
Lip width	4.4 ± 0.4 (3.8 - 4.8)	10.8 ± 0.5 (10.4 - 11.3)		5 ± 1 (3.8 - 6.5)	11 ± 0.2 (10.8 - 11)		4.7 ± 0.1 (4.5 - 4.8)	11.2 ± 0.5 (10.6 - 11.6)		4.7 ± 0.7 (3.7 - 5.5)	12 ± 0.6 (11.3 - 13)	
Lip height	2 ± 0.2 (1.7 - 2.3)	6 ± 0.3 (5.6 - 6)	-	3 ± 0.2 (2.8 - 3.2)	6 ± 0.3 (5.8 - 6.4)	-	2 ± 0.2 (1.7 - 2.1)	5.8 ± 0.7 (5 - 6.7)	-	2.6 ± 0.4 (2.2 - 3.2)	7 ± 0.3 (6.5 - 7.3)	-
DGO	4 ± 0.8 (3 - 5)	3 ± 0.2 (2.8 - 3.2)	2.6 ± 0.3 (2.3 - 3)	3.6 ± 0.1 (3.6 - 3.8)	2.7 ± 0.2 (2.5 - 3)	3 ± 0.1 (2.7 - 3.2)	4.8 ± 0.3 (4.5 - 5)	3 ± 0.2 (2.7 - 3.3)	2.5 ± 0.2 (2.2 - 3)	5.5 ± 0.3 (5.2 - 6)	3 ± 0.3 (2.6 - 3.3)	2.8 ± 0.3 (2.5 - 3.2)
Median bulb width	33 ± 3.4 (28 - 38)	8.8 ± 1 (8.2 - 10)	7.3 ± 0.3 (6.8 - 7.8)	36 ± 4.7 (31 - 41)	9.2 ± 0.3 (9 - 9.6)	7.6 ± 0.6 (7 - 8.6)	35 ± 2.3 (33 - 38)	9.7 ± 1 (8.4 - 11)	7.4 ± 0.7 (6 - 8.3)	41 ± 4.2 (37 - 47)	10 ± 0.6 (9.4 - 11)	7.2 ± 0.3 (6.8 - 7.8)
Median bulb length	35 ± 3 (30 - 40)	16.2 ± 0.5 (15.8 - 16.7)	11 ± 0.6 (10 - 12)	31 ± 3.1 (30 - 38)	17.7 ± 0.7 (17 - 18.2)	11.6 ± 0.7 (10.8 - 13)	34 ± 3.7 (30 - 37)	16.7 ± 1 (15.8 - 18.2)	11.6 ± 0.5 (12 - 12.4)	51 ± 6.0 (43 - 58)	18.3 ± 1.7 (16.7 - 21)	11 ± 0.7 (10 - 12)
Median bulb valve length	12.2 ± 1.4 (10.5 - 14)	-	-	30 ± 0.7 (29.3 - 31)	-	-	11 ± 1 (10.5 - 12.4)	-	-	12.7 ± 0.4 (12.2 - 13)	-	-
Median bulb valve width	10.3 ± 1.3 (8 - 12)	-	-	27 ± 5.5 (20 - 31.5)	-	-	9.7 ± 2 (8 - 12)	-	-	10 ± 0.3 (9.7 - 10.3)	-	-
Distance from anterior end to median bulb	74 ± 11 (58 - 87)	76 ± 1.2 (75 - 77)	52 ± 1.5 (50 - 55)	79 ± 6.6 (72 - 85)	77 ± 4 (72 - 80)	52 ± 1.7 (50 - 55)	76 ± 12.2 (63 - 87)	80 ± 3.4 (75 - 83)	50 ± 2.6 (46 - 55)	86 ± 12.4 (69 - 97)	85 ± 2.3 (82 - 88)	53 ± 1.8 (50 - 55)
Distance from anterior end to nerve ring	-	98 ± 3.3 (94 - 100)	66 ± 1.6 (62 - 68)	-	98 ± 4.2 (94 - 103)	64 ± 2 (61 - 66)	-	100 ± 5.3 (92 - 106)	63 ± 2.6 (60 - 69)	-	103 ± 6.4 (92 - 108)	67 ± 2.5 (62 - 71)

Measurements	*Meloidogyne incognita*											
	M4859 (Ha Noi)			M3656 (Hai Duong)			M4907 (Hung Yen)			M5269 (Lam Dong)		
	Female	Male	Juvenile	Female	Male	Juvenile	Female	Male	Juvenile	Female	Male	Juvenile
Distance from anterior end to secretory-excretory pore	21 ± 4 (15.6 - 22)	128 ± 5.3 (122 - 131)	78 ± 2 (74 - 81)	17.8 ± 0.3 (17.5 - 18)	126 ± 5.3 (120 - 131)	79 ± 1.5 (78 - 83)	20.6 ± 5.8 (15.6 - 24)	126 ± 3.8 (121 - 130)	76 ± 3 (72 - 81)	18.5 ± 1 (18 - 20.2)	125 ± 6 (118 - 134)	79 ± 2.7 (74 - 83)
Distance from anterior end to pharyngo-intestinal junction	-	116 ± 4.4 (111 - 120)	74 ± 2 (69 - 78)	-	116 ± 5.7 (109 - 120)	77 ± 2.5 (73 - 81)	-	113 ± 7 (105 - 121)	76 ± 6.7 (67 - 92)	-	114 ± 3.2 (109 - 117)	75 ± 2.6 (69 - 77)
ES	-	278 ± 10 (267 - 287)	190 ± 10.5 (178 - 205)	-	271 ± 2 (269 - 273)	176 ± 8 (163 - 186)	-	267 ± 9.3 (252 - 277)	175 ± 13 (157 - 195)	-	257 ± 9.4 (248 – 272)	187 ± 8 (178 - 199)
OPL	-	162 ± 10 (150 - 168)	117 ± 10 (104 - 131)	-	155 ± 5.4 (149 - 160)	99 ± 7.3 (86 - 108)	-	153 ± 12.2 (140 - 160)	100 ± 11.7 (78 - 118)	-	143 ± 8.4 (133- 155)	112 ± 8 (104 - 123)
W	454 ± 56 (349 - 529)	24.6 ± 1.7 (23 - 26.4)	14.3 ± 0.7 (13 - 15.2)	311 ± 95 (237 - 494)	23.4 ± 1 (22.2 - 24)	13 ± 1 (12 - 14.8)	423 ± 67 (349 - 479)	27 ± 2.2 (25 - 30)	14.7 ± 0.8 (13.5 - 16)	403 ± 73 (262 - 465)	32 ± 1.5 (29.3 - 33)	13.4 ± 0.5 (12.7 - 4.2)
ABD	-	19.5 ± 2 (18.2 - 21.7)	10.7 ± 0.7 (9.4 - 12)	-	17 ± 0.4 (16.5 - 17.2)	10 ± 0.6 (8.8 - 10.8)	-	21.3 ± 2 (18 - 23.3)	10 ± 1.2 (7.5 - 11.2)	-	22.7 ± 1 (22 - 24.7)	10.2 ± 0.6 (9.2 - 11)
T	-	12 ± 0.4 (11.8 - 12.5)	47 ± 0.8 (46 - 49)	-	11 ± 0.8 (10.3 - 11.8)	48 ± 2.6 (44 - 52)	-	12 ± 1.7 (10.3 - 14.3)	47 ± 2.3 (42 - 50)	-	11.8 ± 1.2 (10.4 - 13)	47 ± 1 (46 - 49)
H	-	-	11.2 ± 1 (9 - 12.6)	-	-	11.4 ± 1 (10.2 - 13)	-	-	10.8 ± 0.6 (10 - 11.7)	-	-	11.5 ± 1 (9 - 13)
Testis length	-	730 ± 88 (630 - 799)	-	-	724 ± 94 (651 - 830)	-	-	760 ± 70 (702 - 868)	-	-	851 ± 43 (783 - 896)	-
Spicule length	-	31 ± 1.2 (29.2 - 34)	-	-	31 ± 1.4 (29 - 31)	-	-	34 ± 1 (32 - 36)	-	-	33 ± 1 (30 - 36)	-
Spicule width	-	3.8 ± 0.3 (3.6 - 4)	-	-	4 ± 0.5 (3.6 - 4.7)	-	-	3.4 ± 0.3 (3.0 - 3.8)	-	-	3.9 ± 0.5 (3.0 - 4.4)	-
Gubernaculum length	-	8.6 ± 0.6 (8 - 9.2)	-	-	8 ± 0.4 (7.8 - 8.5)	-	-	8.8 ± 0.6 (8 - 9.3)	-	-	9 ± 0.6 (8.4 - 9.7)	-
Neck length	127 ± 29.5 (75 - 172)	-	-	259 ± 77.4 (139 - 369)	-	-	158 ± 19.7 (137 - 175)	-	-	208 ± 30 (158 - 245)	-	-
L/ neck length	4.8 ± 1 (3.6 - 7)	-	-	2.4 ± 0.6 (2 - 3.6)	-	-	4.0 ± 0.4 (3.5 - 4.3)	-	-	3.1 ± 0.5 (2.5 - 4)	-	-
Vulva slit length	18.8 ± 1 (17 - 20.5)	-	-	18.2 ± 1.8 (16 - 19.8)	-	-	18.5 ± 2 (16 - 19.7)	-	-	17.8 ± 2.2 (14.8 - 21)	-	-
Vulva-anus distance	17.2 ± 1 (16 - 18.7)	-	-	18.2 ± 0.2 (18 - 18.5)	-	-	18.2 ± 0.2 (18 - 18.5)	-	-	15 ± 2.4 (12.2 - 20)	-	-
Anus-tail tip distance	11.7 ± 1.2 (9.7 - 13.4)	-	-	14.3 ± 0.7 (13.5 - 15)	-	-	14 ± 0.6 (13.5 - 14.5)	-	-	12.3 ± 1.4 (10 - 14)	-	-
Distance between two plasmids	19.5 ± 4 (14 - 24.2)	-	-	21 ± 3.2 (17 - 23.5)	-	-	22.2 ± 1 (21.5 - 23.5)	-	-	20 ± 2 (17 - 22.3)	-	-
Distance from anterior end to secretory-excretory pore/Stylet	1.6 ± 0.3 (1.4 - 1.8)	-	-	1.8 ± 0.4 (1.3 - 2)	-	-	1.7 ± 0.5 (1.4 - 2)	-	-	1.3 ± 0.1 (1.2 - 1.4)	-	-

Table 2. Morphometrics of *Meloidogyne arenaria* and *Meloidogyne graminicola* from different populations. All measurements in µm (except for ratio) and in the format: mean ± S.D. (range)

Measurements	*Meloidogyne arenaria*			*Meloidogyne graminicola*		
	M4806 (Hai Duong)			M4856 (Ha Noi)		
	Female	Male	Juvenile	Female	Male	Juvenile
n	10	**6**	10	10	**3**	10
L	676 ± 41.2 (630 - 757)	1396 ± 110 (1227 - 1542)	407 ± 11.8 (388 - 428)	559 ± 61.4 (460 - 694)	1288 ± 394 (881 - 1668)	378 ± 11.4 (366 - 401)
a	2.2 ± 0.3 (1.6 - 2.6)	50 ± 3.7 (46 - 57)	28.7 ± 1.6 (25.7 - 31)	1.8 ± 0.3 (1.6 - 2.2)	45 ± 6 (39 - 51)	29.4 ± 0.7 (28.4 - 30)
b	-	12 ± 1 (10.6 - 13.2)	5.3 ± 0.2 (5 - 5.6)	-	12.2 ± 3.4 (8.7 - 15.4)	5.4 ± 0.2 (5 - 5.6)
b’	-	5 ± 0.5 (4.3 - 5.5)	2.3 ± 0.1 (2 - 2.4)	-	6.5 ± 1.8 (4.5 - 8)	2 ± 0.1 (2 - 2.2)
c	-	121 ± 9.7 (107 - 132)	8 ± 0.3 (7.7 - 8.3)	-	113 ± 21.6 (80 - 130)	6.5 ± 0.5 (5.4 - 7)
c’	-	0.5 ± 0.1 (0.5 - 0.6)	5 ± 0.3 (4.5 - 5.5)	-	0.6 ± 0.1 (0.5 - 0.7)	5 ± 0.3 (4.7 - 5.6)
Stylet	15.5 ± 2 (13.8 - 16.7)	22.7 ± 0.7 (21.5 - 23.6)	12.2 ± 0.5 (11.4 - 13)	12.8 ± 1.2 (11.4 - 13.2)	20 ± 2 (18.6 - 22.4)	11 ± 0.4 (10 - 12.2)
Lip width	5.5 ± 1 (4 - 6.2)	11.6 ± 0.3 (11.2 - 12)	-	5 ± 0.4 (4.5 - 5.5)	11.3 ± 0.3 (11 - 11.6)	-
Lip height	2.3 ± 0.7 (1.3 - 3)	6 ± 0.4 (5.3 - 6.5)	-	2.8 ± 0.3 (2.4 - 3.2)	5.7 ± 0.8 (5 - 6.5)	-
DGO	5 ± 0.7 (4 - 5.8)	4.8 ± 0.3 (4.4 - 5)	3 ± 0.2 (2.7 - 3.3)	3 ± 0.3 (2.8 - 3.3)	3 ± 0.3 (2.8 - 3.4)	2.8 ± 0.2 (2.5 - 3)
Median bulb width	30 ± 4.7 (27 - 39)	9.2 ± 0.6 (8.4 - 10)	7.5 ± 0.3 (7.2 - 8.3)	39 ± 5.3 (33 - 47)	10.8 ± 0.5 (10.2 - 11)	7.8 ± 0.4 (7.2 - 8.8)
Median bulb length	46 ± 5.8 (38 - 51)	17 ± 1.2 (15.8 - 18.4)	12 ± 0.7 (10.6 - 13)	50 ± 5.4 (43 - 58)	16.7 ± 0.8 (15.8 - 17.3)	11.5 ± 1 (9.8 - 13.2)
Median bulb valve width	8.5 ± 0.6 (7.8 - 9.6)	-	-	10 ± 0.5 (9.3 - 10.4)	-	-
Median bulb valve length	12.4 ± 2 (9.5 - 14.3)	-	-	13.6 ± 0.8 (12.7 - 14.6)	-	-
Distance from anterior end to median bulb	80 ± 4.8 (73 - 83)	85 ± 2 (83 - 88)	52 ± 2.5 (49 - 56)	97 ± 5.6 (90 - 105)	75 ± 3 (72 - 78)	49 ± 1.2 (48 - 51)
Distance from anterior end to nerve ring	-	101 ± 3.3 (97 - 106)	66 ± 2.3 (62 - 69)	-	91 ± 1.6 (90 - 93)	59 ± 1.8 (56 - 61)
Distance from anterior end to secretory-excretory pore	34 ± 1.6 (32 - 37)	128 ± 3 (124 - 131)	80 ± 2.3 (78 - 86)	32 ± 5.5 (26 - 42)	120 ± 9 (111 - 129)	75 ± 1.6 (72 - 77)

Measurements	*Meloidogyne arenaria*			*Meloidogyne graminicola*		
	M4806 (Hai Duong)			M4856 (Ha Noi)		
	Female	**Male**	**Juvenile**	**Female**	**Male**	**Juvenile**
Distance from anterior end to pharyngo-intestinal junction	-	116 ± 3 (111 - 120)	77 ± 2.4 (73.2 - 81.3)	-	105 ± 3 (102 - 108)	70 ± 2 (67 - 74)
Distance from anterior end to end of pharynx	-	277 ± 6.5 (268 - 286)	178 ± 6.3 (166 - 187)	-	197 ± 11.7 (185 - 209)	181 ± 6 (170 - 188)
Maximum body diameter	322 ± 61 (247 - 426)	28 ± 3 (24 - 32)	14.2 ± 0.7 (13.2 - 15)	276 ± 41 (227 - 353)	28.0 ± 5.2 (22.6 - 33)	13 ± 0.6 (12.2 - 13.5)
Neck length	241 ± 25.6 (193 - 270)	-	-	218 ± 71 (128 - 349)	-	-
Body length/ neck length	3 ± 0.5 (2.3 - 4)	-	-	2.8 ± 0.8 (1.4 - 4.4)	-	-
Vulva slit length	20.2 ± 2.4 (16 - 23.2)	-	-	20.8 ± 0.6 (20 - 21.8)	-	-
Vulva-anus distance	18.4 ± 2.3 (14.6 - 21)	-	-	17 ± 0.7 (16 - 17.8)	-	-
Anus-tail tip distance	15.5 ± 4 (12 - 23.2)	-	-	14.2 ± 1.4 (12.7 - 16)	-	-
Distance between two phasmids	17.2 ± 3 (13 - 23.8)	-	-	16.0 ± 1.5 (14.3 - 18.3)	-	-
Distance from anterior end to secretory-excretory pore/Stylet length	2.2 ± 0.3 (2 - 3)	-	-	2.3 ± 0.4 (1.9 - 2.9)	-	-
ABD	-	22.3 ± 1.7 (20 - 23.8)	10.4 ± 0.5 (9.5 - 11)	-	18.8 ± 3.6 (14.7 - 21)	9 ± 0.4 (8.3 - 9.6)
Tail length	-	11.6 ± 1 (10.7 - 13.5)	51 ± 1.3 (50 - 54)	-	11.3 ± 2.2 (10 - 13.8)	57 ± 1.6 (52 - 59)
Hyaline length	-	-	12.5 ± 0.7 (11.3 - 13.5)	-	-	16 ± 1 (14 - 18.5)
Testis	-	837 ± 54 (787 - 932)	-	-	722 ± 243 (462 - 943)	-
Spicule length	-	31 ± 1.2 (29.4 - 33)	-	-	34 ± 1 (33 - 34)	-
Spicule width	-	4 ± 0.2 (3.7 - 4.4)	-	-	3.7 ± 0.7 (3.2 - 4.5)	-
Gubernaculum length	-	8 ± 0.6 (7.4 - 9)	-	-	8.8 ± 0.8 (8 - 9.6)	

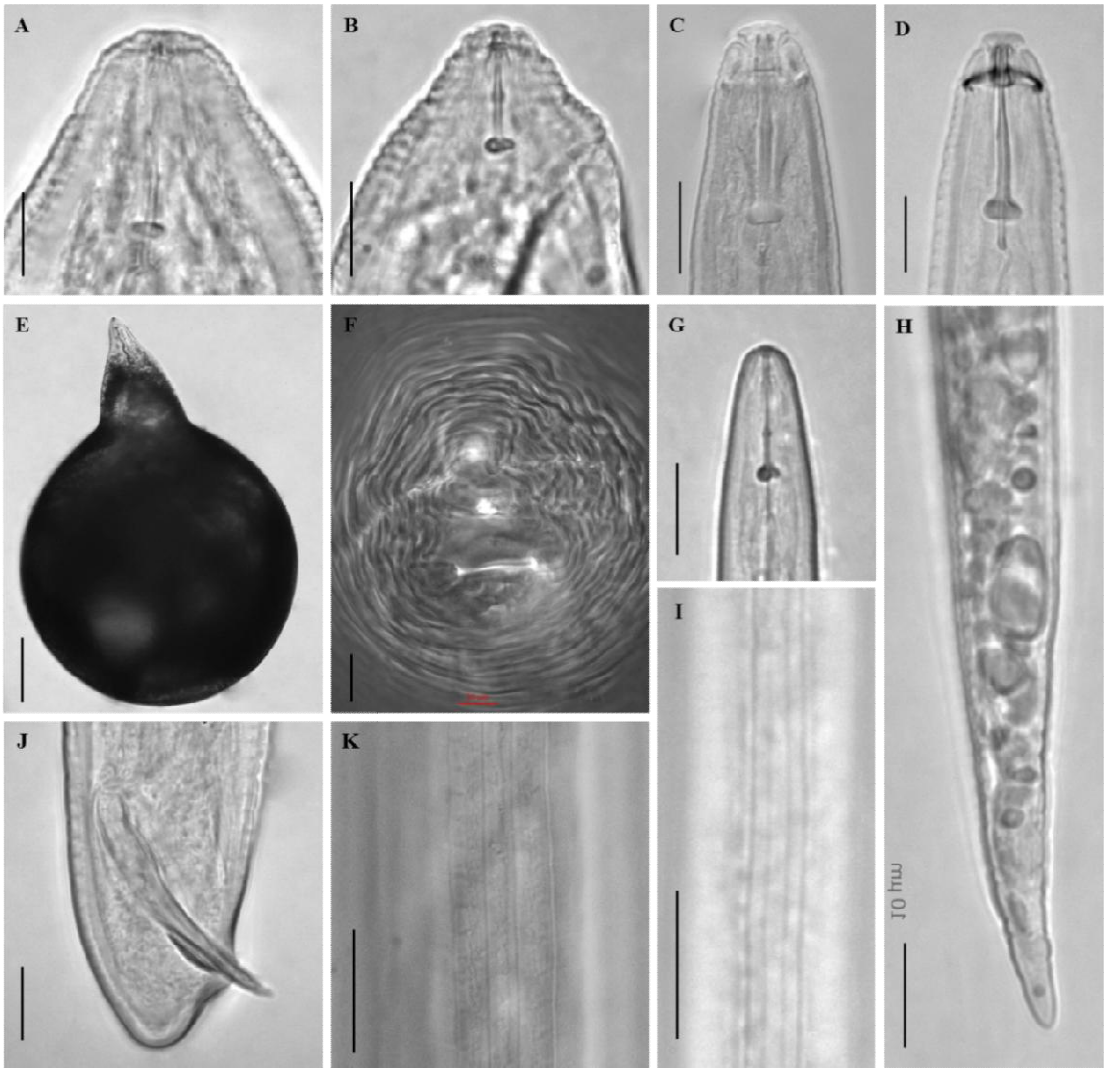

Fig. 1. *Meloidogyne incognita* (Kofoid& White, 1919) Chitwood, 1949
(LM ♀). A: Head region, B: Stylet, E: Entire body, F: Perriniel pattern. (LM ♂). C-D: Stylet, J: Tail region, K: Lateral field; (LM J2). G: Stylet, I: Lateral field, K: Tail region (Scalebar: A = 100 µm, B-H = 10 µm)

not offset, smooth, rarely with one or two incomplete annulations, labial disc more or less rounded, slightly raised above level of medial lips, lateral lips usually absent (remnants occasionally present); stylet basal knobs offset, angular or more amalgamated. *J2*: Hemizonid one to three annules anterior to excretory pore, tail rounded to pointed tail tip and indistinct long hyaline region.

Remarks: The morphology and morphometry of *M. arenaria* on carrots in Vietnam is mostly similar to the original description of Chitwood, (1949) except for the slightly smaller body length of females, males, and juveniles (630 - 767 vs 510 - 1000 µm; 1227 - 1542 vs 1270 - 2000 µm; 388 - 428 vs 450 - 490 µm, respectively), smaller b value in males and juveniles (10.6 - 13 vs 11 – 16, 5 – 5.6 vs 7.2 – 7.8, respectively), larger c value and stylet length in juveniles (7.7 – 8.3 vs 6 – 7.5, 11 - 13 vs 10 µm, respectively) [29]. Compared to the description of Nguyen & Nguyen (2000), the stylet of this population is smaller (11 - 13 vs 13 – 14.5 µm) [18].

Host: following CABI [28].

Distribution: following CABI [28].

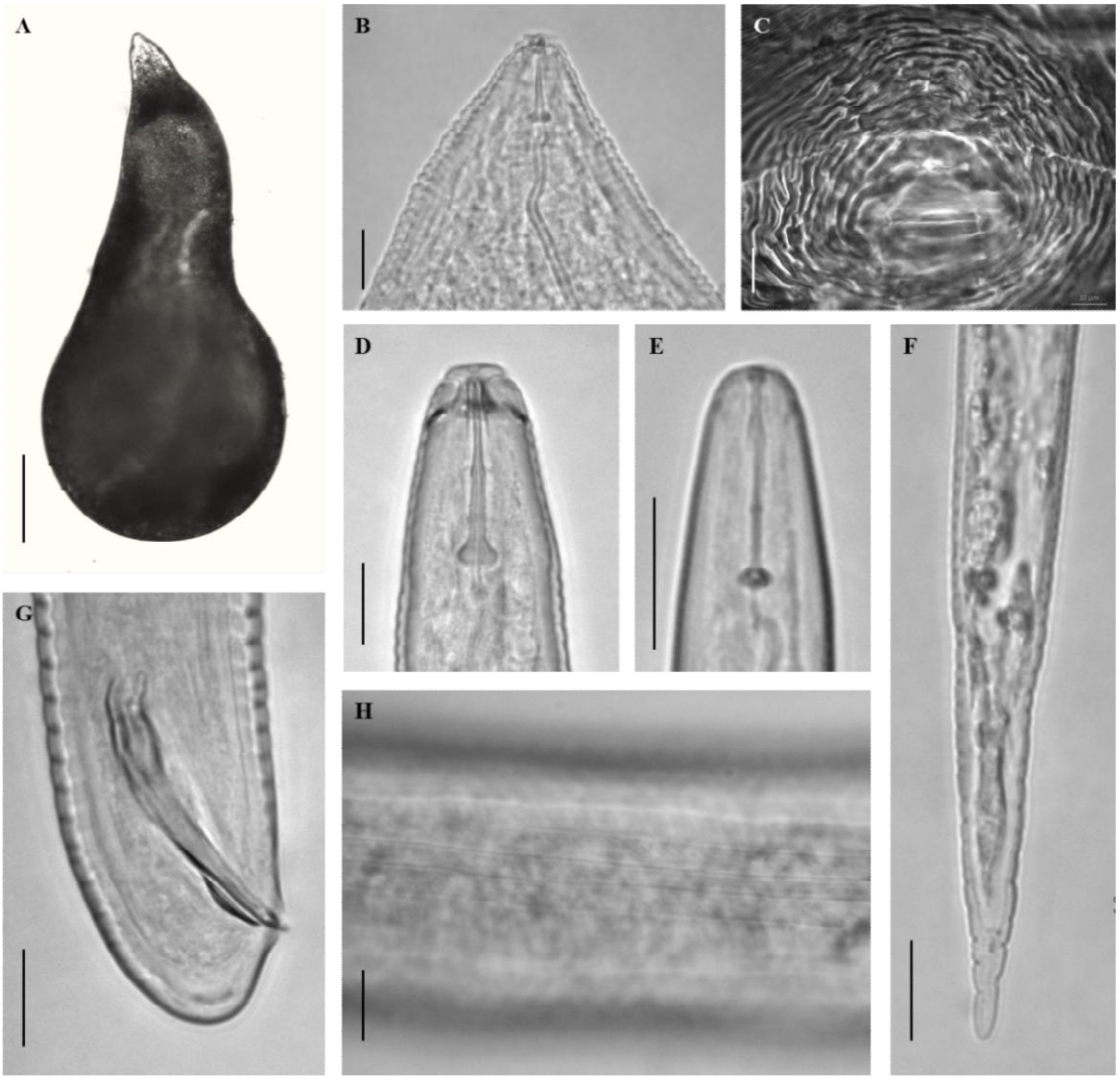

Fig. 2. *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949.
(LM ♀). A. Entire body; B. Stylet; C. Perineal pattern. (LM ♂). D: Stylet, G: Tail region, H: Lateral field. (LM J2). E: Stylet, F: Tail region (Scale bar: A = 100 µm, B - H = 10 µm)

3.3 *Meloidogyne graminicola* Golden & Birchfield, 1965

See Fig. 3, Table 2.

Female: Elongate, slight terminal protuberance present; stylet basal knobs ovoid, offset; perineal pattern rounded/oval, striae smooth, lateral field absent. *Male*: Labial region not offset, labial disc not elevated, lateral lips usually present, stylet basal knobs ovoid, offset. *J2*: Hemizonid anterior or adjacent to excretory pore, tail = 60–80mm, tail tip finely rounded.

Remarks: Few variation were observed from the population of *M. graminicola* on carrots in Vietnam compared to the description of Golden & Birchfield [30] such as: smaller body length in females (460 - 694 vs 445 - 765 µm), larger stylet in females (11.4 – 13.2 vs 10 – 11.2 µm); larger body length and stylet length in males (881 - 1668 vs 1020 - 1428 µm; 18.6 – 22.4 vs 16.2 – 17.4 µm, respectively); smaller body length and stylet in juveniles (366 - 401 vs 415 - 484 µm).

Host: following CABI [28]. Carrot is a new host in this study.

Distribution: following CABI [28].

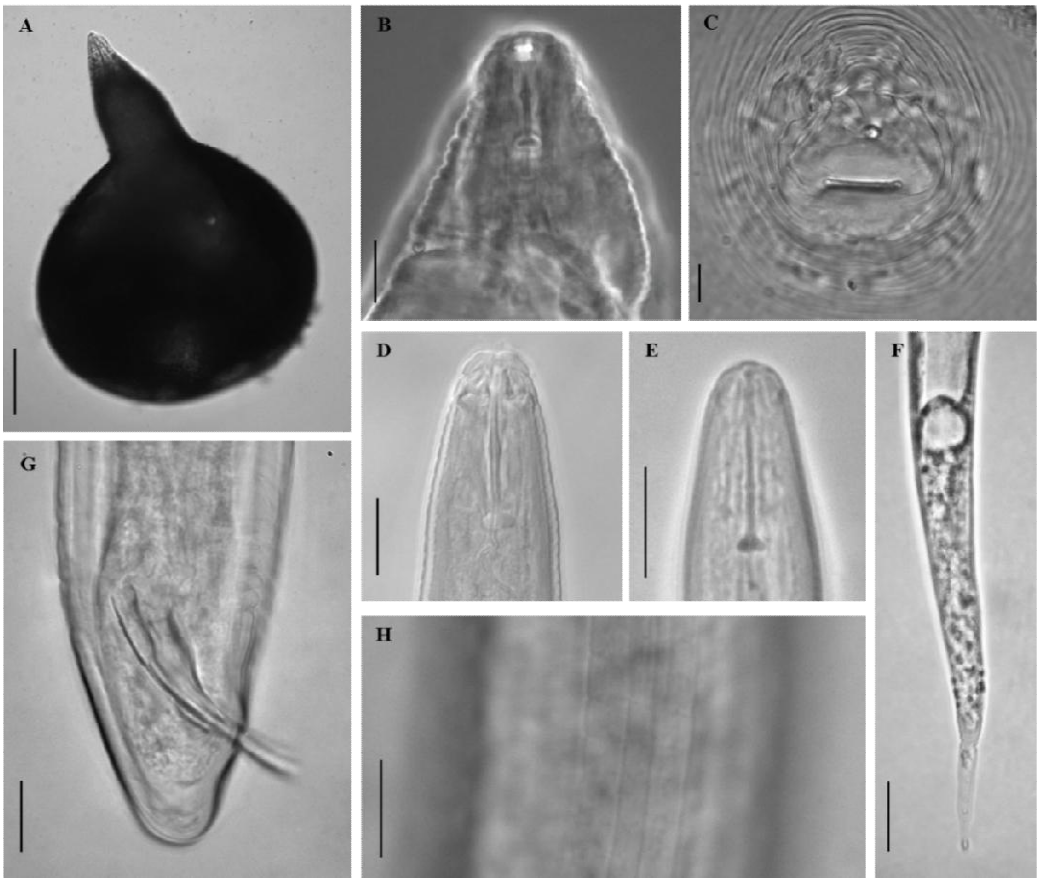

Fig. 3. *Meloidogyne graminicola* Golden & Birchfield, 1965
(LM ♀). A: Entire body, B: Stylet, C: Perineal pattern. (LM ♂). D: Stylet, G: Tail region, H: Lateral field. (LM J2). E: Stylet, F: Tail region (Scalebar: A = 100 µm; B-H = 10 µm)

4. CONCLUSION

This study provides the morphological and morphometric characterization of six populations from three species of the genus *Meloidogyne* namely *M. incognita*, *M. arenaria* and *M. graminicola*. This is the first time *M. graminicola* is reported to be present on carrots.

The morphology and morphometry of all the studied species were given and the classification was confirmed by PCR with specific primers following Kiewnick *et al.* (2013). The results of electrophoresis products of *M. incognita* was 300 bp long, *M. arenaria* species was 420 bp long, and *M. graminicola* was negative. Presently, the diversity of PPN on carrots is still poorly studied. The morphology and morphometry of the genus *Meloidogyne* in this study provide useful information for management and control of plant-parasitic nematodes on carrots. Furthermore, this study provides good references for identification of the genus Meloidogyne in future.

Meloidogyne graminicola is previously known as aquatic root-knot nematode because their life cycle is stick to aquatic host plants. However, we have found these nematodes on carrots proving the ability of *M. graminicola* to survive on other host plants on dry land.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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