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Original Article

First Report of Entomopathogenic Nematode Heterorhabditidae *(Rhabditida)* in Organic Vegetable Farms in Cebu, Philippines

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ABSTRACT

In the Philippines, there is little or no information on indigenous Entomopathogenic nematodes (EPNs) occurring in different habitats. Its significance can be more emphasized in Cebu, Philippines, due to reports of high biodiversity index in the area. Thus, a study was conducted to identify indigenous EPNs from different organic vegetable farms. Soil samples from 17 farms within 10-15 cm deep during wet and dry season were collected from eight municipalities across southern, central and northern Cebu for EPN morphological characterization and identification. Some ecological factors such as soil type, temperature, pH and land elevation were noted. All samples were positive of EPNs. These EPNs were found in elevations ranging from 22-884 m above sea level, under low light intensity, in wet soils with slightly acidic to slightly basic (6-8.0) soil pH. EPNs were found in all soil types surveyed (loam-clay). All isolates collected belong to genus *Heterorhabditis* of the family Heterorhabditidae. From these, four species were identified: *H. bacteriophora*, *H. indica*, *H. marelatus* and *H. taysearae*. *H. indica* was found to be widely distributed across southern, central and northern Cebu. On the other hand, both *H. bacteriophora* and *H. marelatus* were found only on samples taken from central Cebu, while *H. taysearae* was found in southern and central Cebu. Thus, the presence of indigenous EPNs and their distribution are influenced by environmental factors.

KEYWORDS: *entomopathogenic nematodes, habitat characterization, organic agriculture, taxonomy, Cebu Philippines*

1 INTRODUCTION

In developed countries like USA, Australia and Europe, entomopathogenic nematodes (EPNs) have been applied successfully against soil inhabiting insects (as soil application) as well as above-ground insects (foliar application) in cryptic habitats (Arthurs *et al.*, 2004; Shapiro-Ilan *et al.*, 2006). EPNs belong to the families Steinernematidae and Heterorhabditidae (*Rhabditida*) and are symbiotically associated with entomopathogenic bacteria *Photorhabdus* (Boemare *et al.*, 1993) and *Xenorhabdus* (Thomas and Poinar, 1979), respectively. Commercial nematode based products are available and are being utilized for biological control in these countries.

There have been rapid developments in the study and commercial application of EPNs as well as in the technology of mass producing nematodes (Grewal *et al.*, 2005). These recent advances, together with the need to reduce pesticide use, have resulted in a surge of scientific and commercial interests in EPNs in some countries in Asia like India, Thailand, Taiwan and Korea.

Using native biocontrol agents are mostly preferred due to its adaptability to local condition. In the Philippines, there is little or no information on indigenous EPNs occurring in different habitats. Novel species and strains may have more superior traits, making them suitable for direct commercial exploitation or as source of genetic diversity for breeding improved strains (Choo *et al.*, 1995). Great potential can be seen in the Province of Cebu due to reports of high biodiversity in the area and the pressure for organic vegetable production due to increased awareness of the benefits on organically grown produce. Thus, the identification of indigenous EPNs with its proper mapping together with their associated environmental conditions is important for EPN studies to progress in the Philippines in support to the organic agriculture programs in the country.

2 MATERIALS AND METHODS

Sampling and Collection of Indigenous EPNs

Soil samples were collected from 17 sampling sites based on the list of Organic Vegetable Farmers provided by the Department of Agriculture – Regional Field Unit VII across the province of Cebu. Soil samples representing each site were collected at 10-15 cm deep using a shovel. At each site, ten soil samples were randomly collected, placed in bucket and thoroughly mixed to come up with a composite soil sample for each farm. About one kilogram of soil was taken from the bucket and transferred to a plastic bag

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for processing. Samples were placed on coolers during transport and stored in refrigerator at 10°C. Ecological factors such as soil type, temperature, and pH (4 in 1 Sinokit KC300B ©) as well as coordinates and land elevation (Garmin GPS Map 62 SC ©) were noted. Soil type was also identified.

Nematode Isolation from Soil Samples

EPNs were recovered from soil samples using insect baiting methods described by Bedding and Akhurst (1975). Ten last instars of the identified test insect larvae (*Achroia grisella*) were placed in 300 ml plastic container with moist soil sample. The covered containers were placed at room temperature for two weeks. Water was added to the soil samples to keep them moist at any point during baiting. The traps were checked every two days starting day five. Dead larvae from each container was placed in white trap to collect emerging infective juveniles (IJs) from *A. grisella* cadavers and applied to fresh larvae (Fig. 1).

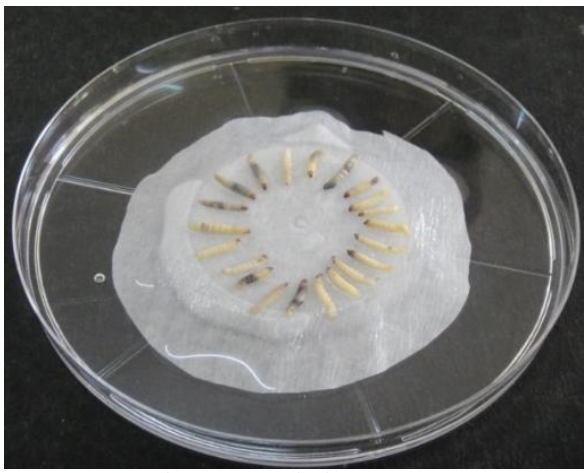

Figure 1.White trap for collection of infective juveniles (IJs).

Maintenance of Insect Host Cultures

Lesser wax moth larvae (*A. grisella*) were used in culturing nematodes for mass production since maintenance of these larvae using artificial diet is well established. Insect cultures were maintained using artificial diet following the method described by van Zyl and Malan (2015), with modifications, containing 200 g rice powder, 100 ml honey, 100 ml glycerol and 50 g beer yeast in aerated plastic containers. Approximately 200-300 eggs were placed in artificial diet in plastic container and kept at room temperature (Fig. 2). Eggs hatched after 3-4 days; after two weeks larvae were given fresh diet. After three weeks, late instars were collected and those not immediately utilized were stored on paper shavings for 2-3 weeks at 10°C.

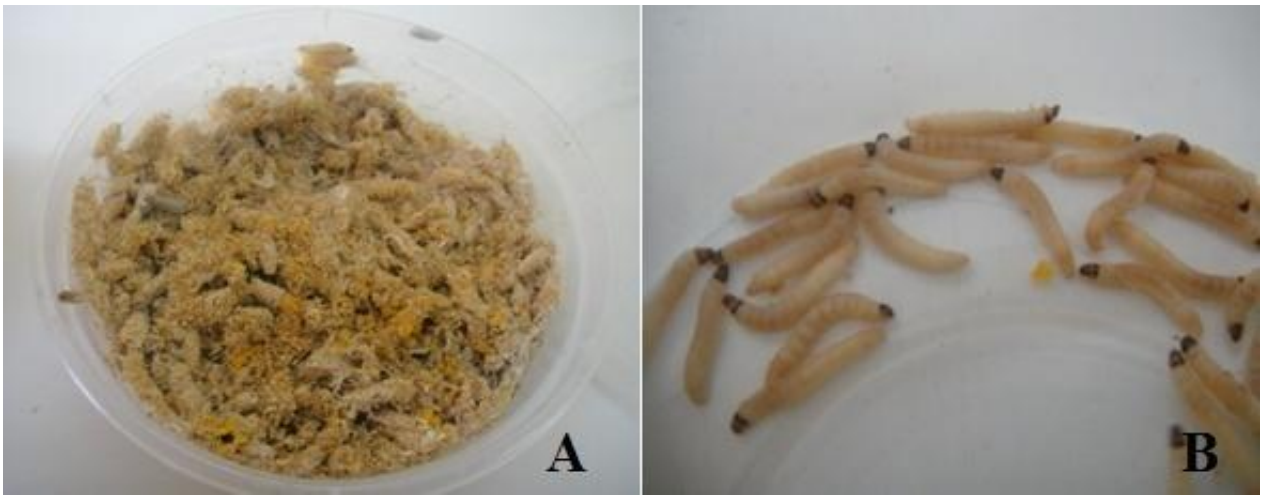

Figure 2. Culture media (A) of *Galleria mellonella* larvae (B).

Identification of Indigenous EPNs

Nematodes recovered were identified to families, genera and species level by morphological characterization. Nematodes were examined live or heat killed in Ringer's solution heated to 60°C. Ten *A. grisella* larvae were exposed to approximately 1000 IJ in a petri dish lined with two moistened filter papers at room temperature. In isolating mature females and males of the first and second generations, the infected larvae were dissected in Ringer's solution four and seven days after infection, respectively. Heat-killed nematodes were placed in triethanolaminformalin (TAF) fixative (Kaya and Stock, 1997) and were processed to anhydrous glycerine for mounting by slow evaporation method (Poinar, 1976). Morphology and morphometric studies were conducted using an Olympus BX41 microscope equipped with differential interference elements and drawing tube.

The following characters were measured in males and IJ: total body length; maximum body diameter; anal body diameter; excretory pore position; distance from anterior end to base of pharynx; gubernaculums length; spicule length (measured along the curvature in a line along the center of the spicule); gubernaculums length divided by spicule length (%); distance from anterior end to nerve ring position; ratio a (total body length divided by maximum body diameter); ratio b (total body length divided by distance from anterior end to base of pharynx); ratio c (body length divided by tail

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length); ratio D (excretory pore position divided by distance from anterior end to base of pharynx); ratio E (excretory pore position divided by tail length); spicule length divided by anal body diameter and tail length (measured with consideration of the extra cuticular sheath of the second-stage juvenile). Morphological identification was made using taxonomic criteria suggested by Stock and Kaya (1996) and Hominick *et al.* (1997).

3 RESULTS AND DISCUSSION

Morphological Identification of EPN Isolates

EPN isolates were identified to genus and species level by morphological characteristics of infective juveniles (Fig. 3), females (Fig. 4) and males (Fig. 5). Genus identification revealed that all EPN isolates belong to genus *Heterorhabditis* of the family *Heterorhabditidae* (Kaya and Stock, 1997). Light microscopy indicated that the excretory pore of nematodes is located posterior to the nerve ring and the presence of bursa in males. Both are distinguishing characters of the genus *Heterorhabditis* (Fig. 6).

Four species of *Heterorhabditis* were identified namely: *H. bacteriophora* (Poinar, 1976), *H. indica* (Poinar *et al.*, 1992), *H. taysearae* (Shamseldean *et al.*, 1996), and *H. marelatus* (Liu *et al.*, 1997). Species identification was mainly based on the body length of IJs, spicule and gubernaculum length, and bursa of males. Morphometric data of 17 infective juveniles and males of the 17 EPN isolates are shown in Table 1 and Table 2, respectively.

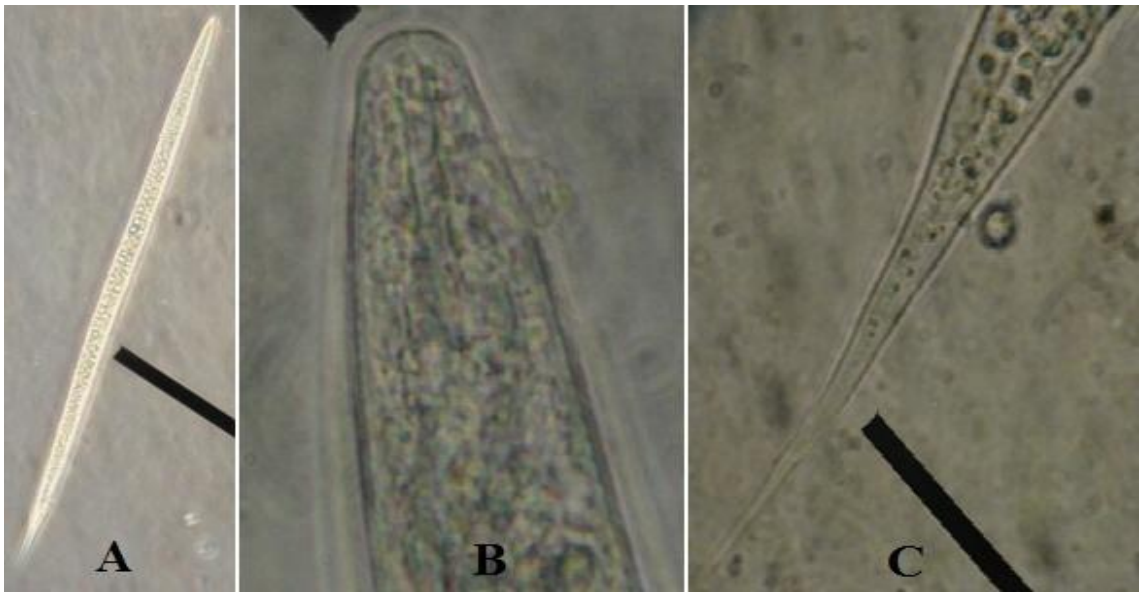

Figure 3. Infective juvenile showing its entire body (A), anterior region with its mouth closed (B) and tail, short tapering to a small spike-like tip (C).

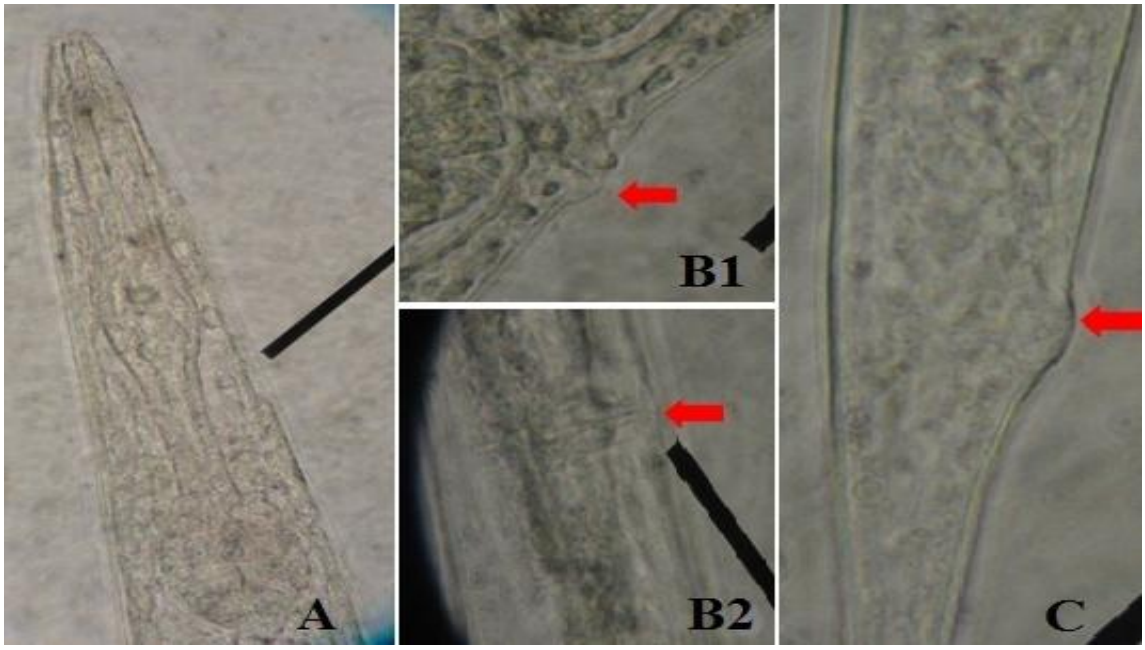

Figure 4. Female nematode showing its anterior end (A), protruding (B1) and non-protruding (B2) vulva lips, and tail showing post-anal (C

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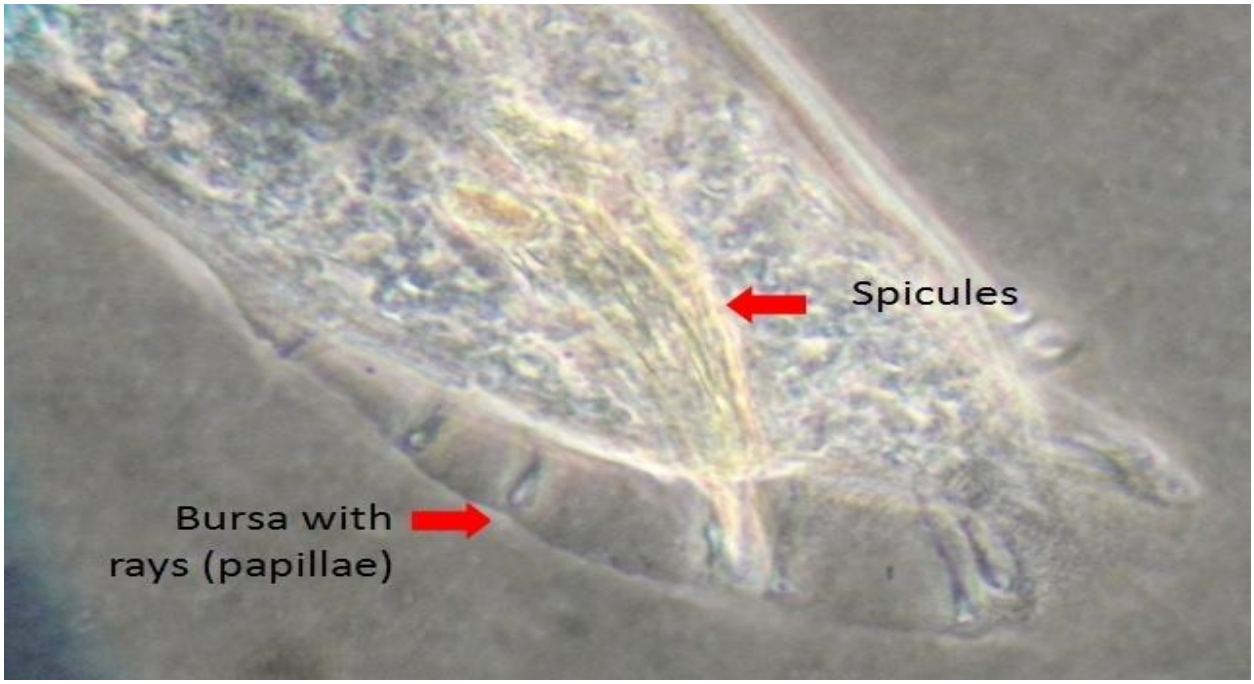

Figure 5. Male nematode reflecting its spicules and bursa with rays (papillae).

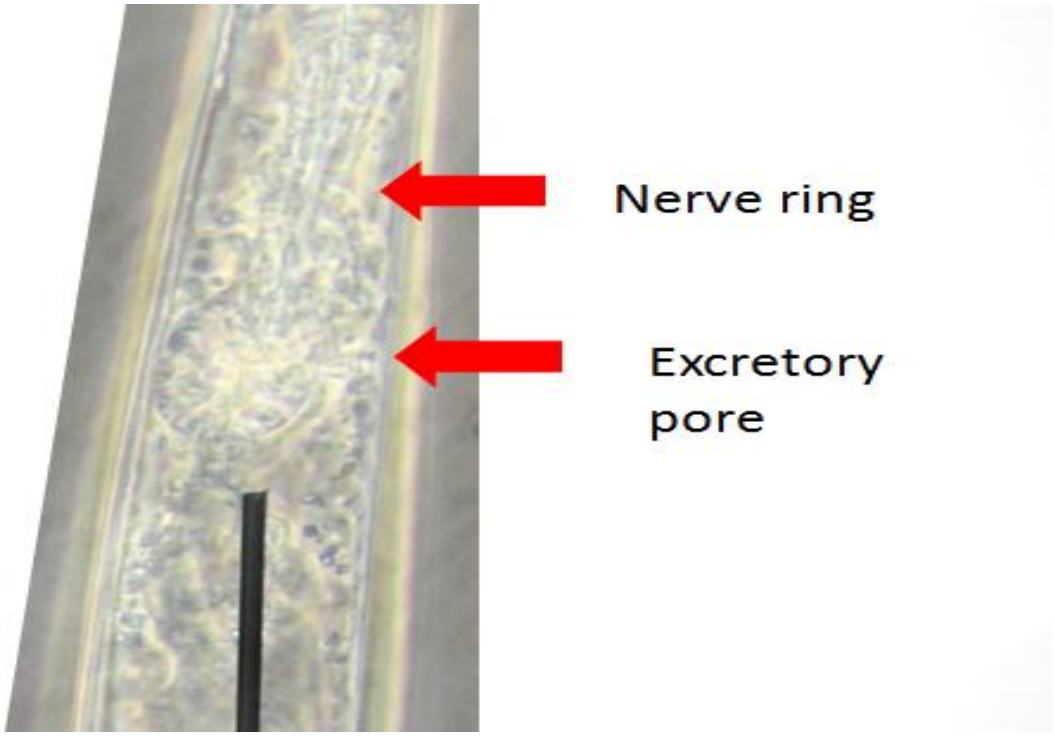

Figure 6. Distinguishing features of the genus *Heterorhabditis* reflecting its nerve ring and excretory pore

Distribution of Indigenous EPNs from Organic Vegetable Farms in Cebu

A total of 17 EPN isolates were obtained from organic vegetable farms across north (Bogo, Tuburan, and Asturias), central (Cebu City and Aloguinsan) and south Cebu (Barili, Alegria, and Dalaquete). All vegetable farms surveyed during wet (January) and dry season (May) were positive of EPNs (Fig. 7). EPNs were found to occur in both lowland and highland vegetable organic farms with an elevation that ranged from 22 to 884 m ASL. Light intensity in most of the sites was low during the wet season and ranged from normal to low during the dry season collection. Soil moisture in most of the sites was described as wet during wet season, while in dry season there were areas that were described as wet, normal and dry. Majority of the vegetable farms had near to neutral soil pH and clay to loam soil type (Table 3). These similarities could account to the prevalence of indigenous EPNs in all sampling areas. Soil temperature, moisture and physical properties among others (Tangchitsomkid *et al*., 1998; Tabassum *et al.*, 2005) all affects the distribution of EPNs.

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Table 1. Infective juveniles morphometrics of EPN isolates from different farms. Measurements are in µm, except the indexes, in form: mean (range).

Characters	F-01	F-O2	F-O3	F-O4	F-O5	F-O6	F-O7	F-O8	F-O9	F-O10	F-O11	F-O12	F-O13	F-O14	F-O15	F-O16	F-O17
n	10	10	10	10	10	10	10	10	10	10	15	10	10	7	10	9	10
L	537 (500-590)	585 (550-630)	498 (432-560)	368 (340-408)	481 (468-500)	587 (560-615)	463 (376-512)	438 (400-468)	560 (495-600)	585 (485-715)	648 (550-725)	458 (428-484)	570 (520-630)	447 (384-472)	504 (472-544)	455 (408-512)	456 (384-504)
MBD	22 (21-25)	19 (18-23)	21 (17-25)	18 (16-19)	19 (18-20)	23 (19-30)	23 (20-24)	18 (16-20)	23 (19-29)	23 (16-29)	26 (24-29)	23 (16-72)	23 (21-27)	17 (13-19)	24 (22-26)	23 (20-25)	23 (19-26)
EP	110 (102-115)	120 (114-133)	107 (100-117)	88 (80-102)	105 (100-108)	119 (112-130)	105 (85-116)	96 (92-102)	112 (99-125)	123 (108-139)	124 (111-139)	136 (87-108)	120 (111-130)	103 (99-109)	110 (107-113)	99 (92-117)	98 (87-107)
NR	94 (89-100)	99 (92-110)	88 (77-94)	71 (65-77)	85 (77-88)	96 (92-104)	84 (72-90)	79 (72-84)	92 (82-99)	98 (89-112)	104 (94-111)	82 (72-86)	98 (90-102)	82 (73-90)	87 (84-90)	79 (69-88)	81 (76-97)
ES	137 (129-145)	158 (150-164)	133 (128-140)	107 (99-121)	133 (125-138)	149 (145-156)	126 (115-130)	123 (117-128)	135 (121-149)	146 (134-167)	152 (135-158)	133 (123-139)	149 (142-157)	131 (124-134)	132 (127-135)	123 (113-142)	122 (116-141)
T (w/sheath)	74 (62-85)	86 (78-92)	85 (58-107)	63 (55-83)	77 (74-84)	90 (85-99)	84 (62-97)	72 (61-77)	79 (66-92)	81 (59-100)	87 (77-96)	78 (68-85)	87 (68-99)	71 (62-75)	97 (81-103)	85 (76-98)	87 (69-108)
a (L/MBD)	24 (22-26)	31 (28-34)	24 (20-27)	21 (19-23)	25 (23-27)	26 (20-30)	21 (19-230	25 (22-28)	25 (19-31)	24 (22-29)	25 (22-28)	25 (6-28)	25 (22-26)	26 (24-30)	21 (20-22)	20 (18-21)	20 (18-22)
b (L/ES)	3.9 (3.6-4.5)	3.7 (3.5-4.1)	3.7 (3.2-4.1)	3.5 (3.3-3.6)	3.6 (3.5-3.9)	3.9 (3.6-4.1)	3.7 (3.3-4.0)	3.6 (3.4-3.7)	4.2 (3.9-4.8)	4.0 (3.6-4.4)	4.3 (3.9-4.9)	3.8 (3.1-3.7)	3.8 (3.4-4.4)	3.6 (2.9-3.6)	3.8 (3.6-4.0)	3.7 (3.4-4.1)	3.7 (3.2-4.0)
c (L/T)	7.3 (6.4-8.1)	6.8 (6.3-7.5)	6.0 (4.9-7.8)	5.9 (4.3-6.8)	6.3 (5.9-6.7)	6.5 (6.0-7.1)	5.6 (4.3-7.5)	6.2 (5.3-7.4)	7.1 (6.3-8.1)	7.3 (6.6-8.3)	7.4 (6.4-8.5)	6.5 (5.2-7.2)	6.6 (5.6-8.7)	6.5 (6.0-7.0)	5.2 (5.0-6.0)	5.4 (4.9-5.8)	5 (4.6-6.8)
D% = EP/ES x 100	80 (77-84)	76 (71-83)	80 (75-87)	82 (78-85)	79 (75-84)	80 (74-87)	83 (74-90)	79 (76-83)	83 (79-87)	85 (77-95)	82 (76-88)	79 (67-348)	80 (77-86)	79 (75-83)	83 (81-86)	81 (76-85)	80 (74-88)
E% = EP/T x 100	151 (128-180)	139 (127-157)	129 (93-182)	140 (101-160)	137 (127-144)	133 (120-145)	127 (98-174)	135 (122-169)	142 (132-153)	154 (127-184)	143 (116-171)	136 (104-157)	138 (122-176)	146 (134-166)	114 (103-135)	116 (107-132)	114 (94-149)
ABD	14 (13-16)	12 (10-15)	12 (11-14)	10 (8-11)	11 (10-12)	13 (9-13)	12 (11-12)	10 (9-11)	13 (11-16)	14 (10-16)	16 (13-18)	11 (10-12)	13 (11-13)	11 (9-12)	13 (12-13)	12 (11-12)	12 (11-13)
T	52 (41-62)	65 (61-68)	59 (47-69)	44 (39-48)	57 (55-61)	68 (58-74)	56 (52-63)	55 (50-62)	61 (54-67)	67 (53-80)	70 (56-79)	85 (59-288)	67 (59-75)	59 (50-60)	61 (55-68)	57 (52-65)	57 (53-61)

n: number of specimens; L: body length; W: greatest body diam.; T: tail length without sheath; ABD: anal body diam.; EP: distance from anterior end to excretory pore; NR: distance from anterior end to nerve ring; ES: distance from anterior end to end of esophagus.

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Table 2. Male morphometrics of EPN isolates from different farms. Measurements are in µm, except the indexes, in form: mean (range).

Character	F-O1	F-O2	F-O3	F-O4	F-O5	F-O6	F-O7	F-O8	F-O9	F-O10	F-O11	F-O12	F-O13	F-O14	F-O15	F-016	F-17
n	10	10	3	5	10	8	2	10	10	9	5	10	7	10	3	2	5
L	978 (837-1130)	972 (810-1148)	875 (801-917)	983 (935-1032)	872 (765-988)	938 (828-1050)	1041 (952-1130)	1104 (846-1291)	946 (801-1068)	937 (739-1095)	958 (819-1068)	891 (757-1041)	1048 (979-1121)	881 (748-970)	1044 (1015-1068)	1175 (1139-1210)	983 (935-1032)
W	58 (48-77)	42 (40-46)	38 (31-46)	63 (51-68)	35 (26-44)	47 (35-68)	50 (46-53)	50 (44-62)	57 (51-66)	41 (35-48)	61 (55-66)	48 (37-59)	43 (37-57)	56 (33-90)	55 (48-62)	63 (62-64)	50 (46-53)
T	27 (18-30)	35 (30-43)	26 (25-28)	36 (31-44)	16 (13-18)	39 (31-45)	34 (33-36)	12 (8-15)	31 (26-40)	31 (24-34)	35 (26-48)	40 (32-47)	40 (36-45)	34 (27-41)	38 (34-40)	41 (40-43)	34 (33-36)
ABD	9 (7-11)	23 (21-25)	8 (8-9)	24 (21-27)	8 (7-9)	21 (13-27)	21 (20-23)	18 (18-20)	22 (18-26)	21 (18-23)	23 (20-26)	23 (20-26)	23 (20-26)	24 (20-28)	22 (20-25)	28	18 (18-20)
EP	169 (150-187)	174 (152-202)	147 (145-150)	172 (158-185)	174 (158-191)	163 (130-194)	155 (154-156)	178 (167-187)	173 (161-205)	165 (147-176)	167 (150-183)	158 (145-172)	199 (180-213)	150 (136-161)	189 (174-198)	194	178 (167-187)
NR	132 (97-154)	144 (125-167)	128 (125-130)	139 (130-143)	141 (119-169)	134 (110-152)	142 (141-143)	139 (123-156)	129 (119-141)	136 (121-143)	128 (121-136)	130 (119-145)	152 (141-161)	128 (117-134)	150 (145-154)	151 (150-152)	139 (123-156)
ES	190 (174-209)	216 (191-242)	194 (187-200)	207 (194-218)	210 (176-242)	185 (150-202)	209 (205-213)	192 (167-207)	189 (176-216)	209 (165-240)	186 (176-196)	202 (189-222)	203 (189-213)	200 (187-224)	205 (200-213)	208 (198-218)	202 (189-222)
SP	37 (29-44)	42 (37-45)	34 (33-35)	41 (37-46)	38 (35-44)	41 (36-45)	38 (36-40)	46 (42-51)	34 (29-37)	42 (33-50)	39 (37-42)	38 (36-42)	49 (41-54)	42 (38-44)	49 (42-54)	54 (51-57)	38 (36-42)
GL	16 (13-20)	20 (18-22)	16 (15-18)	19 (17-20)	18 (15-22)	20 (18-22)	18 (18-19)	20 (18-24)	15 (11-20)	21 (18-24)	18 (18-20)	18 (16-19)	24 (21-25)	20 (17-23)	23 (23-24)	22 (21-23)	18 (16-19)
a (L/W)	17 (13-23)	23 (20-29)	23 (20-26)	16 (15-18)	25 (22-29)	20 (15-26)	21	22 (15-26)	17 (13-19)	23 (15-29)	16 (15-17)	19 (16-21)	25 (20-29)	17 (10-25)	19 (17-21)	19 (18-20)	19 (16-21)
b (L/ES)	5.1 (5-6)	4.5 (4-5)	4.5 (4-5)	4.8 (4-5)	4.2 (4.0-4.4)	5.1 (4.4-5.7)	5.0 (4.5-5.5)	5.8 (4.4-6.7)	5.0 (4.4-5.6)	4.5 (3.7-5.2)	5.2 (4.2-6.1)	4.4 (4.0-5.3)	5.2 (4.8-5.7)	4.4 (4.0-4.7)	5.1 (5.0-5.2)	5.7 (5.2-6.1)	4.4 (4.0-4.7)
c (L/T)	37 (28-57)	28 (24-34)	34 (28-37)	27 (23-31)	55 (46-65)	24 (21-30)	31 34-27)	94 (63-150)	30 (26-36)	30 (25-35)	29 (20-40)	23 (19-29)	26 (24-29)	26 (20-36)	28 (25-32)	28 (27-30)	26 (20-36)
D% = EP/Es x 100	89	81 (77-84)	76 (75-78)	83 (77-92)	84 (73-98)	88 (79-96)	74 (73-75)	93 (88-108)	91 (86-95)	80 (69-91)	90 (80-96)	78 (75-82)	98 (92-103)	75 (70-79)	93 (81-99)	93 (89-98)	75 (70-79)
SW%=SP/ABDx100	412 (346-494)	181 (157-200)	416 (396-439)	176 (140-200)	469 (420-549)	528 (371-824)	179 (154-205)	250 (211-288)	160 (125-213)	197 (168-225)	173 (142-190)	167 (145-183)	211 (170-255)	176 (148-213)	224 (214-242)	195 184-206)	195 184-206)
GS%=GU/SP x100	45 (35-53)	48.476811 (46-53)	46.80556 (44-50)	45 (43-47)	47 (40-50)	50 (44-58)	48 (44-53)	44 (35-53)	43 (31-60)	50 (43-56)	46 (42-53)	46 (40-53)	49 (43-57)	48 (41-55)	48 (44-55)	41 (41-42)	41 (41-42)

n: number of specimens; L: body length; W: greatest body diam.; T: tail length ; ABD: anal body diam.; EP: distance from anterior end to excretory pore; NR: distance from anterior end to nerve ring; ES: distance from anterior end to end of esophagus; SP: spicule; GL: gubernaculum;

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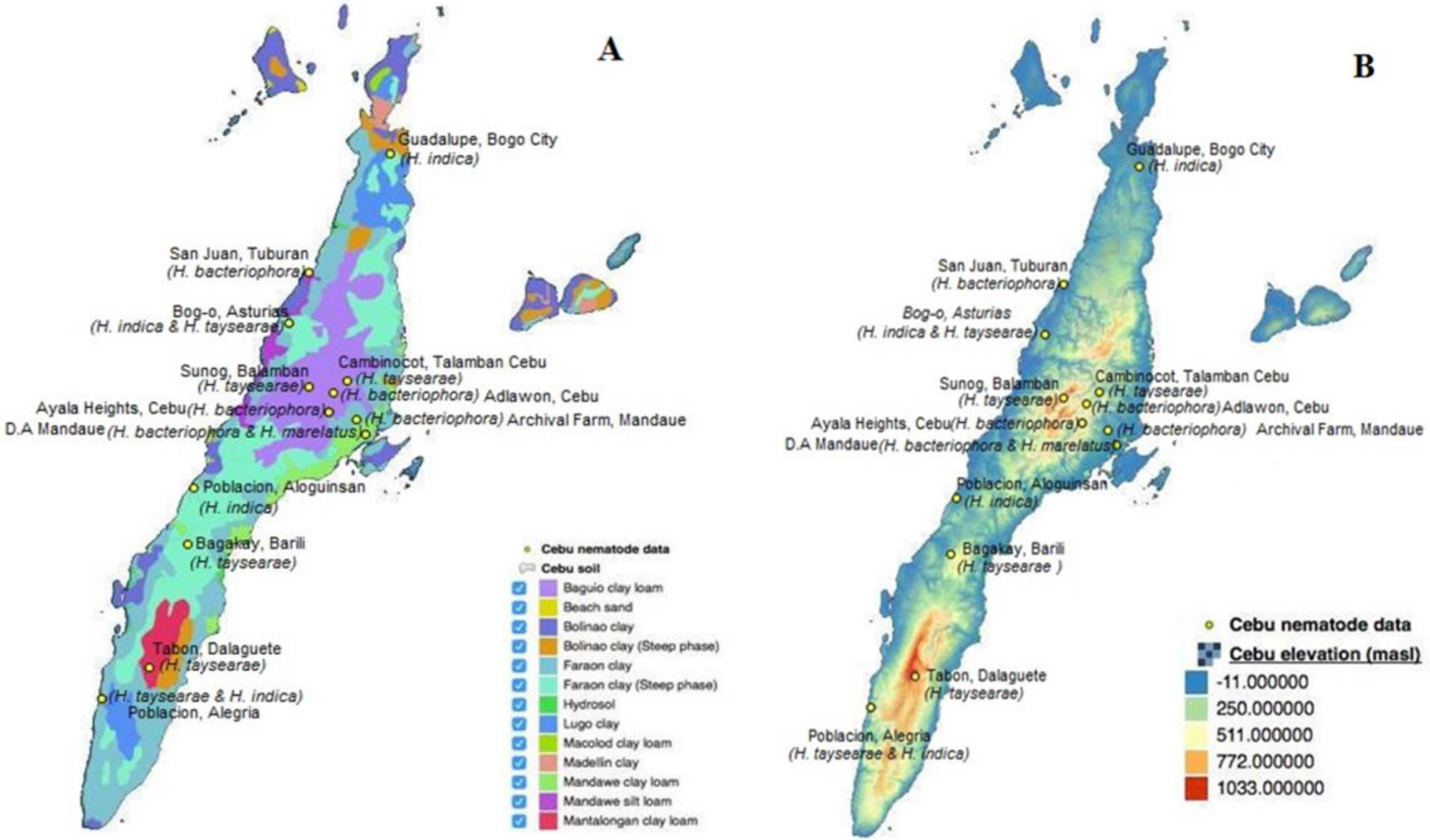

Figure 7. Distribution of *Heterorhabditis* based on soil type (A) and elevation (B)

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Table 3. Locations, GPS coordinates, elevation, light density, soil moisture, soil pH, and soil type of organic vegetable farms surveyed during wet and dry season in Cebu.

Sample	Isolates	Location	GPS Coordinate	Elevation	Light Intensity	Soil moisture			Soil pH	Soil Type	Associated Vegetables
					WS	DS	WS	DS			
0-1	*H. indica*	Guadalupe, Bogo	N 11°E E 124°	47m	Low	Normal	Wet+	Dry+	7.5	Lugo Clay	string beans, okra
0-2	*H. bacteriophora*	San Juan, Tuburan	N10°43.705' E 123°36.56'	22m	Low-	Normal	Wet+	Dry+	7.5	Bolinao Clay	eggplant, bitter gourd
0-3	*H. indica*	Bogo, Asturias	N10°36.56' E123 46.343'	212m	Low	Normal	Wet+	Dry+	7.0	Faroan Clay (steep phase)	okra, eggplant
0-4	*H. taysearae*	Bogo, Asturias	N10° 12.964' E123° 46.343'	212m	Low	Low+	Wet+	Normal	7.0	Faroan Clay (steep phase)	carrots, leaf onion
0-5	*H. indica*	Poblacion, Aluguinsan	N 10° 12.964' E 123° 33.038	310m	Low-	Low+	Wet+	Wet +	6.5	Faroan Clay	lettuce
0-6	*H. bacteriophora*	Ayala Heights, Cebu	N10° 12.964' E 123° 51.49'	590-600m	Low	Low+	Wet+	Wet+	7.0	Mandaue Silt Loam	lettuce, various herbs
0-7	*H. taysearae*	Ayala Heights, Cebu	N10° 12.964' E123° 51.49'	590-600m	Low	Normal	Wet+	Wet+	7.0	Mandaue Silt Loam	lettuce, various herbs
0-8	*H. taysearae*	Sunog, Balamban	N10° 27.19 E123° 51.49'	781-790m	Low	Normal	Normal	Normal	7.5	Mandaue Silt Loam	lettuce
0-9	*H. bacteriophora*	Mandaue, Cebu	N10° 22.478' E 120° 47.387'	40m	Low-	Normal	Wet+	Dry	7.5	Faroan Clay	various herbs
0-10	*H. bacteriophora*	Mandaue, Cebu	N10° 20.207' E123° 56.715'	24m	Low+	Normal	Normal	Dry+	8.0	Mandaue Clay Loam	various herbs, sweet corn
0-11	*H. marelatus*	Mandaue, Cebu	N10° 26.293' E123° 52.225'	24m	Low+	Normal	Wet+	Wet+	8.0	Mandaue Clay Loam	various herbs, lettuce
0-12	*H. taysearae*	Cambinocot, Cebu	N10° 27.944' E123° 54.132'	200m	Low	Normal	Normal	Dry+	6.0	Mandaue Silt Loam	sweet potato, bell peppers
0-13	*H. bacteriophora*	Adlawon, Cebu	N10° 26.293' E123° 20.637'	395m	Low	Normal	Wet+	Wet+	7.0	Mandaue Silt Loam	lettuce
0-14	*H. taysearae*	Poblacion, Alegria	N9° 43.20'	56m	Low-	Normal	Wet+	Wet+	7.5	Faroan Clay	carrots, pechay
0-15	*H. indica*	Poblacion, Alegria	E123° 20.637'	56m	Low-	Normal	Wet+	Wet+	7.5	Faroan Clay	lettuce, tomato
0-16	*H. taysearae*	Tabon, Dalaguete	N9° 43.20' E 123° 20.637'	884m	Low+	Low+	Wet+	Wet+	6.0	Mantalongon Clay	cabbage, potato
0-17	*H. taysearae*	Bagacay, Barili	N10° 6.276' E123° 23.092'	201m	Low-	Normal	Wet+	Wet+	7.0	Faroan Clay (steep phase)	lettuce, kale, carrots

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4 CONCLUSION

Four species of nematodes were identified: *H. bacteriophora*, *H. indica*, *H. marelatus* and *H. taysearae*. *H. indica* species were found to be widely distributed across south, central and northern Cebu. On the other hand, both *H. bacteriophora* and *H. marelatus* (found only in DA Mandaue) were found only on samples taken from central Cebu, while *H. taysearae* species were found in southern and central Cebu. These were found in elevations ranging from 22-884 m above sea level, under low light intensity, in wet soils with slightly acidic to slightly basic (6-8.0) soil pH. They were found in loamy to clayey soils.

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