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Research Note

First report of an important sheat nematode, *Hemicycliophora poranga*, associated with sugar beet (*Beta vulgaris* L.) in Vietnam

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Summary

Several species of the sheat nematodes, *Hemicycliophora* spp., have been known to cause significant damage to agricultural crops, including *Hemicycliophora arenaria*, *H. conida*, *H. parvana*, *H. poranga*, *H. similis*, and *H. typica*. Remarkably, our study reported on the presence of *H. poranga* for the first time in Vietnam. This species was found on 83.33% of the total samples with an average density of 270 individuals/100ml of soil (positive samples). In this study, the Vietnamese population of *H. poranga* was characterized based on both morphology and molecular characterization of D2-D3 expansion segment of 28S rRNA sequence. Besides, a molecular phylogenetic tree of the genus *Hemicycliophora* was also provided.
Keywords: Sheat nematode*;* molecular*;* D2-D3*;* 28S*;* Western Highlands*;* taxonomy

Introduction

Plant-parasitic nematodes belonging to the genus *Hemicycliophora* are commonly known as sheat nematodes that can parasitize various host plants including agricultural crops, fruit and nut trees, and ornamental plants. Strikingly, *Hemicycliophora* species have been reported from all continents and can induce serious symptoms such as root galls, stubby root, stunted growth, yellowing, and even death of host plants (Chitambar & Subbotin, 2014). Among *Hemicycliophora* spp., *H. poranga* (Monteiro & Lordello, 1978) was known as an important pest to agricultural crops and can cause significant damages to many crops such as celery in Argentina (Emilse *et al*., 2011); *Musa* sp., cabbage, cowpea, bean, okra, lettuce, and tomato in the USA (Chitambar, 1993); and other 21 crops from different localities listed by Chitambar and Subbotin (2014).
Currently, 135 valid species of the genus *Hemicycliophora* have been described over the world (Azimi *et al*., 2020; Chitambar & Subbotin, 2014; Nguyen *et al*., 2021). In Vietnam, *Hemicycliophora* spp. have been reported on 12 host plants that were black pepper, banana, cucurbits, grapefruit, orange, rice, tobacco, yard-long bean, bamboo, Malabar cardamom (Nguyen, 1983; Nguyen & Nguyen, 2001; Nguyen *et al*., 2021). However, only two endemic *Hemicycliophora* species have been recognized at species level including *Hemicycliophora vietnamensis* Nguyen and Nguyen (2001) and *H. cardamomi* Nguyen *et al*. (2021), therefore, a higher number of *Hemicycliophora* species is expected in the country. Herein, the first report of *H. poranga* associated with sugar beet (*Beta vulgaris* L.) in Vietnam is provided with the support of morphological and molecular characterizations.

Material and Methods

Soil samples were collected from the rhizosphere of sugar beet in Lam Dong, Vietnam (GPS coordinates N: 11°56'21", E: 108°26'6.5") in the winter season. The observation of plant symptoms was recorded and four samples around a host plant were collected at

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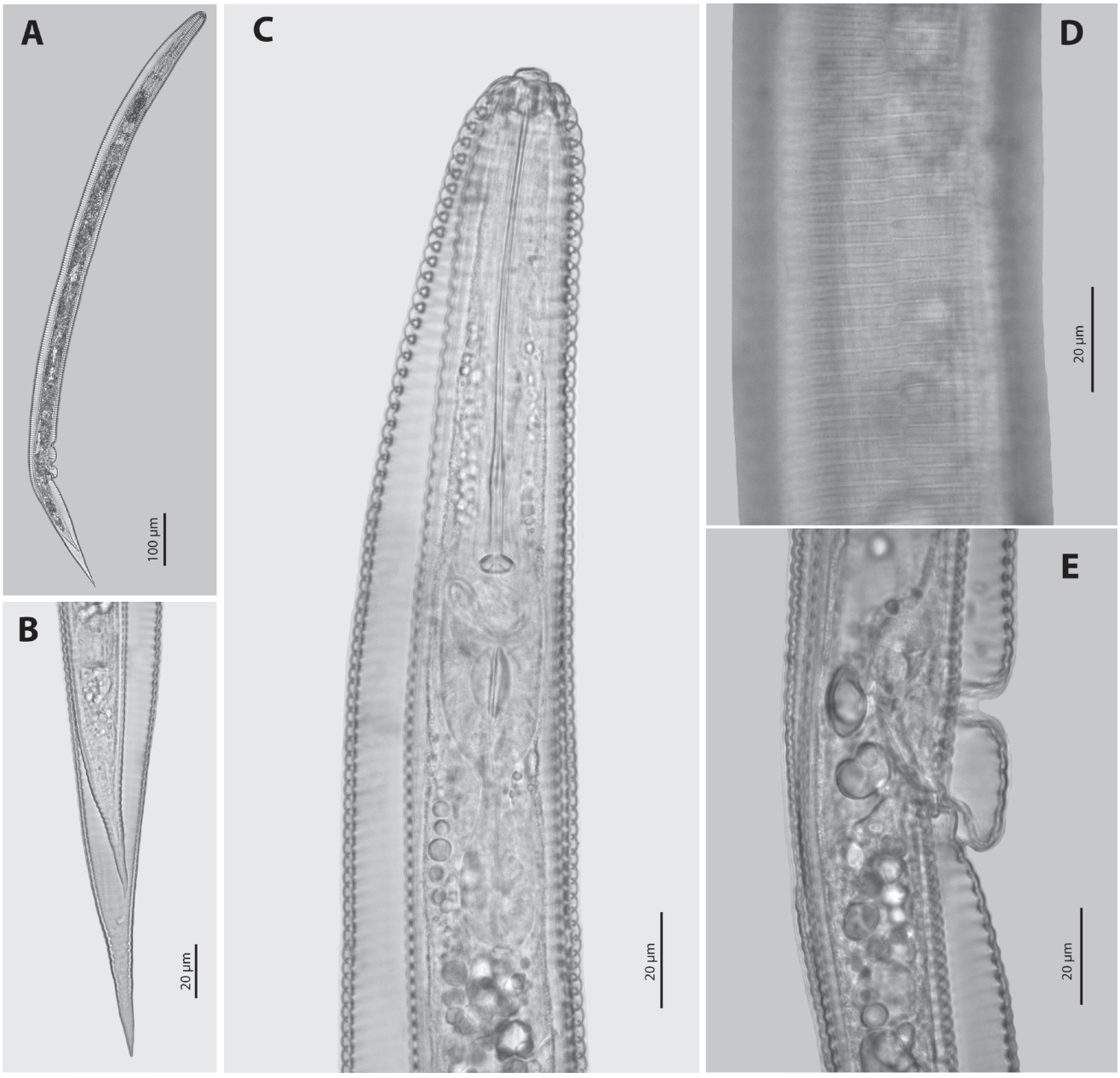

Fig. 1. Female of *Hemicycliophora poranga* from Vietnam.
A: entire body. B: posterior end region. C: anterior end region. D: lateral field region. E: vulva region

each sampling site (6 sites), after removing the detritus layer to create a bulk sample (collection of 24 soil samples using a core (5 × 25 cm) resulted in 6 bulk samples). Nematodes were extracted from soil samples using the modified Baerman tray method (Whitehead & Hemming, 1965). Permanent slides were made from fixed nematodes following Seinhorst (1959). Subsequently, measurements and pictures were taken using Carl Zeiss Axio Lab.A1 light microscope equipped with an Axiocam ERc5s digital camera.

For molecular analysis, DNA was extracted from a living nematode by cutting and transferring it to a PCR tube with 20 µl of WLB (50 mM KCl;10 mM Tris pH 8.3; 2.5 mM MgCl2; 0.45 % NP-40 (Tergitol Sigma); 0.45 % Tween-20). In the next steps, the sample was incubated at −20°C for at least 10 min, followed by adding 1 µl proteinase K (1.2 mg ml−1). Finally, sample was incubated in a PCR machine for 1 h at 65°C and 10 min at 95°C and finished by centrifugation for 1 min at 20 800 g.

D2-D3 expansion segment of 28S rRNA region was amplified using primers D2A (5'-ACAAGTACCGTGGGGAAA GTTG-3') and D3B (5'-TCGGAAGGAACCAGCTAC TA-3') (Nunn, 1992) with the following thermal profile: one cycle of 94°C for 4 min, followed by five cycles of 94°C for 30s, 56°C for 30s, 72°C for 2 min, and 45 cycles of 94°C for 30s, 54°C for 30s, 72°C for 1 min and finished at 10°C for 10 min (Nguyen *et al*., 2021). Successful PCR reactions were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin, USA) and sequenced commercially by Macrogen (Korea). The obtained forward and reverse sequences were assembled using Geneious R11 (www.geneious.com). Blast search was used to look for closely related sequences from GenBank (Altschul *et al*., 1997). MUSCLE in Geneious R11 was used to make multiple alignments for all sequences. The Bayesian phylogenetic analysis was carried out using MrBayes 3.2.6 add-in in Geneious R11 following Nguyen *et al*. (2019). GTR+G+I model was selected following Abadi *et al*. (2019).

Ethical Approval and/or Informed Consent

The result of this work has not been published previously and is not under consideration elsewhere.

Results and discussion

Measurements
See Table 1.

Morphological characterization
Females of *H. poranga* from Vietnam were characterized as following: body curved ventrally (Fig. 1A); cuticular sheath loosely fitting body (Fig. 1A); Lateral field mostly marked by breaks and anastomoses of transverse striae, sometimes also with two more or less distinct longitudinal lines; lip region rounded, not offset, with three annuli and a distinctly protruding labial disc (Fig. 1D); stylet long, slender, straight to slightly curved (Fig. 1C); stylet knobs sloping posteriorly with distinct cavity (Fig. 1C); opening of dorsal pharyngeal gland indistinct; excretory pore located posterior to level of pharyngo-intestinal junction (Fig. 1C); spermatheca indistinct or small without sperm; vulval lips modified, slightly elongate, vulval sleeve 2 – 3 annuli long (Fig. 1E); tail elongate conoid, tapering gradually to a finely rounded terminus; anus indistinct (Fig. 1B).

Molecular characterization
700 bp long D2-D3 expansion segment of 28S rRNA sequences of *H. poranga* was obtained with 0 – 0.01 % intraspecific variation (0 – 1 bp difference) compared to sequences of *H. poranga* from GenBank (accession number: MK348058, MG019816). In the phylogenetic tree based on D2-D3 expansion segment of 28S rRNA sequences, the sequences of *H. poranga* formed a maximally supported clade (100 % PP) with other sequences of *H. poranga* from GenBank (Fig. 2).

Host symptom
Sugar beet plants with presence of *Hemicycliophora poranga* were stunted following a patchiness pattern in comparison with healthy plants.

Remarks and discussion
Although morphology of *H. poranga* in this study is in agreement with the type population, small variations in measurements were seen such as slightly higher L value (1152 (1092 – 1205) *vs* 1040 (960 – 1130)), smaller Rvan value (20 (17 – 21) *vs* 23 (20 – 26)), larger Ran value (48 (44 – 52) *vs* 33 (27 – 37)), larger VL/VB value (5.4 (4.8 – 5.8) *vs* 4.5 (3.9 – 4.9)). However, these variations were also present in other populations of *H. poranga* (Table 1).
Correct identification of *Hemicycliophora* spp. is of crucial importance since several species in this group have been reported to cause significant damage to agricultural crops. Our study provides the first report of *Hemicycliophora poranga*, a known harmful species on many hosts, on sugar beet in Vietnam based on morphological and molecular characterizations. This study also reports sugar beet as a new host of *H. poranga*. Since the nematodes have appeared with high density (average of 270 individuals/100 ml soil in positive samples) along with the stunting symptom in patchiness pattern of host plant, it is suggested that *H. poranga* could be a potential pest of sugar beet and management measures should be applied to control this pest.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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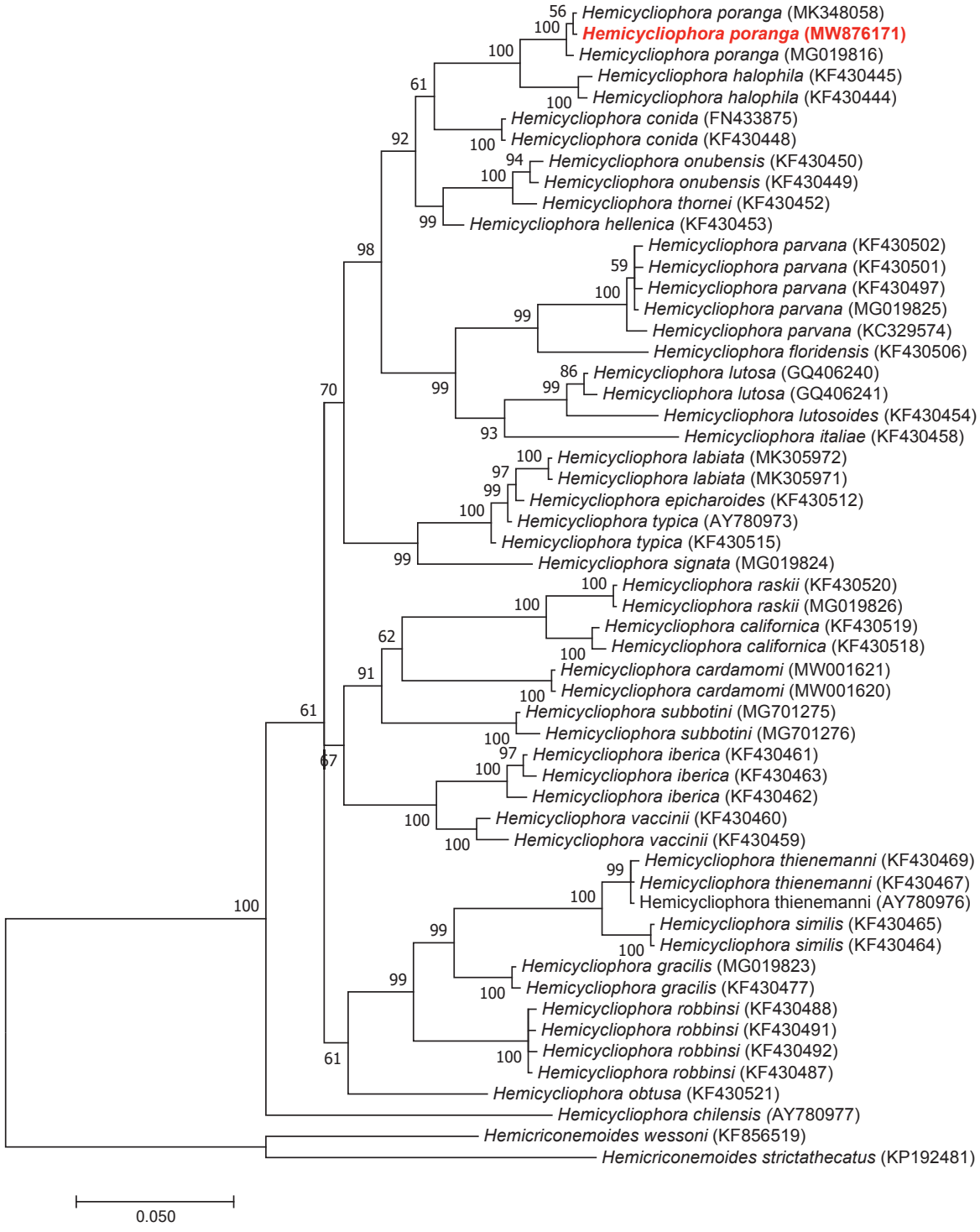

Fig. 2. Phylogenetic tree generated from D2-D3 expansion segment of 28S rRNA sequences using GTR+G+I model. Posterior probability (in percentage) was given next to each node. Sequence of *Hemicycliophora poranga* from Vietnam was marked by red color.

Table 1. Measurements of females of *Hemicycliophora poranga* from different localities. All measurements are in µm (except for ratio).

	H. poranga in this study	*H. poranga* Monteiro and Lordello (1978)	*H. poranga* from Van den Berg *et al*. (2018)	*H. poranga* from Subbotin *et al*. (2014)
Locality	Vietnam	Brazil	South Africa	Italy
n	10	10	19	10
L'	1217 ± 42 (1167-1286)	-	-	-
L	1152 ± 41 (1093-1205)	1040 (960-1130)	1046 ± 59.9 (952-1165)	1015 ± 37 (972-1084)
Stylet	99 ± 2 (97-102)	94 (92-100)	92 ± 7 (79-100)	91 ± 3.7 (86-96)
cone	82 ± 1 (79-84)	-	-	-
Stylet knob width	8.1 ± 0.7 (7.0-9.1)	-	4.0 ± 0.3 (3.5-4.5)	7.2 ± 0.3 (7.0-7.5)
Stylet knob height	4.7 ± 0.39 (4.0-5. 3)	-	7.0 ± 0.6 (6.0-8.0)	5.2 ± 0.3 (5.0-5.5)
Distance from anterior end to nerve ring	155 ± 6 (143-162)	-	-	-
Pharynx length	190 ± 6 (178-197)	-	188 ± 8 (176-212)	172 ± 8 (158-181)
Distance from anterior end to Secretory-excretory pore	199 ± 5 (191-205)	184 (160-206)	202 ± 27 (182-284)	176 ± 7 (170-183)
Mid-body diam.	37 ± 3 (34-41)	-	46 ± 4 (37-50)	40 ± 3 (37-45)
Body diam. at vulva	32 ± 2 (29-35)	-	41 ± 4 (33-49)	-
Vulva-anus distance	58 ± 7 (48-67)	-	57 ± 8 (49-77)	-
Body diam. at anus (ABD)	25 ± 1 (23-27)	-	33 ± 2.3 (30-39)	-
Tail length	114 ± 8 (100-129)	-	120 ± 12 (92-140)	98 ± 8 (86-109)
Rst	30 ± 0.8 (29-31)	-	28 ± 2 (26-33)	31 ± 1.5 (29-34)
Roes	58 ± 2 (55-61)	-	57 ± 2.5 (52-63)	63 ± 2.9 (59-67)
Rex	61 ± 2 (58-64)	62 (59-67)	60 ± 2.1 (56-65)	65 ± 2.8 (60-68)
Rv	68 ± 3 (62-73)	56	71 ± 5.3 (62-81)	75 ± 5 (68-83)
RV(ant)	250 ± 6 (235-259)	246 (235-266)	-	254 ± 9 (236-267)
Ran	48 ± 2 (44-52)	33 (27-37)	52 ± 4.8 (43-62)	54 ± 4 (46-59)
Rvan	20 ± 1 (17-21)	23 (20-26)	19 ± 2.2 (15-23)	21 ± 2 (18-24)
R	317 ± 7 (304-326)	302 (286-333)	313 ± 9.2 (297-330)	329 ± 10 (317-345)
V	85 ± 0.7 (84-86)	85 (84-86)	83 ± 0.9 (81-85)	81 ± 2 (78-84)
a	31 ± 1 (29-32)	29 (24.3-32.1)	23 ± 1.9 (20-26)	26 ± 1 (24-27)
b	6.1 ± 0.2 (5.9-6.3)	6.3 (5.6-8.4)	5.6 ± 0.3 (5.0-5.9)	5.9 ± 0.3 (5.5-6.4)
c	10.1 ± 0.6 (9.3-10.9)	10.1 (8.9-11.2)	8.7 ± 0.7 (7.1-10.0)	10.4 ± 1 (9.2-12.5)
VL/VB	5.4 ± 0.3 (4.8-5.8)	4.5 (3.9-4.9)	4.5 ± 0.4 (3.8-5.3)	5.6 ± 0.4 (4.8-6.1)

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