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

T. D. NGUYEN1,2, Q. P. TRINH1,2*

1Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Cau Giay, 100000 Hanoi, Vietnam, *E-mail: tqphap@gmail.com; 2Graduate University of Science and Technology, Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Cau Giay, 100000 Hanoi, 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, yardlong 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
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’-ACAAGTACGTGGGAAAA 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).

Results and discussion

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

The authors declare that they have no conflict of interest.

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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).

| Locality | *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) |
|-----------|-------------------------|---------------------------------|--------------------------------|---------------------------------|
| Vietnam   | 10                      | 10                              | 19                              | 10                              |
| Brazil    | 1217 ± 42 (1167-1286)   | 1040 (960-1130)                 | 1046 ± 59.9 (952-1165)         | 1015 ± 37 (972-1084)           |
| South Africa | 99 ± 2 (97-102) | 92 ± 7 (79-100) | 91 ± 3.7 (86-96) |
| Italy     | 82 ± 1 (79-84)          | 7.0 ± 0.6 (6.0-8.0)            | 5.2 ± 0.3 (5.0-5.5)           |
| L'        | 1152 ± 41 (1093-1205)   | 8.1 ± 0.7 (7.0-9.1)            | 7.2 ± 0.3 (7.0-7.5)           |
| L         | 155 ± 6 (143-162)       | 4.7 ± 0.39 (4.0-5.3)           | 5.0 ± 0.5 (5.0-5.5)           |
| Pharynx length | 190 ± 6 (178-197) | -                              | 188 ± 8 (176-212)           |
| Pharynx length | 199 ± 5 (191-205) | 184 (160-206) | 176 ± 7 (170-183) |
| Pharynx length | 114 ± 8 (100-129) | -                              | 120 ± 12 (92-140)           |
| Pharynx length | 20 ± 1 (17-21) | -                              | 33 ± 2.3 (30-39)           |
| Body diam. at vulva | 32 ± 2 (29-35) | -                              | 41 ± 4 (33-49)           |
| Vulva-anus distance | 38 ± 7 (48-67) | -                              | 57 ± 8 (49-77)           |
| Body diam. at vulva | 25 ± 1 (23-27) | -                              | 33 ± 2.3 (30-39)           |
| Tail length | 37 ± 3 (34-41) | -                              | 46 ± 4 (37-50)           |
| 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 vulva | 25 ± 1 (23-27) | -                              | 33 ± 2.3 (30-39)           |
| Tail length | 120 ± 12 (92-140) | -                              | 98 ± 8 (86-109)           |
| Rst       | 30 ± 0.8 (29-31)       | 28 ± 2 (26-33)                 | 31 ± 1.5 (29-34)            |
| Roes      | 112 ± 7 (88-128)       | 57 ± 2.5 (52-63)               | 63 ± 2.9 (59-67)            |
| Rex       | 61 ± 2 (58-64)         | 60 ± 2.1 (56-65)               | 65 ± 2.8 (60-68)            |
| Rv        | 68 ± 3 (62-73)         | 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)            |
| Rvan      | 20 ± 1 (17-21)         | 23 (20-26)                     | 19 ± 2.2 (15-23)            |
| R         | 317 ± 7 (304-326)      | 302 (286-333)                  | 313 ± 9.2 (297-330)         |
| V         | 85 ± 0.7 (84-86)       | 85 (84-86)                     | 83 ± 0.9 (81-85)            |
| a         | 31 ± 1 (29-32)         | 29 (24.3-32.1)                 | 23 ± 1.9 (20-26)            |
| b         | 6.1 ± 0.2 (5.9-6.3)    | 6.3 (5.6-8.4)                  | 5.6 ± 0.3 (5.0-5.9)         |
| c         | 10.1 ± 0.6 (9.3-10.9)  | 10.1 (8.9-11.2)                | 8.7 ± 0.7 (7.1-10.0)        |
| Case      | 5.4 ± 0.3 (4.8-5.8)    | 4.5 (3.9-4.9)                  | 4.5 ± 0.4 (3.8-5.3)         |
| VL/VB     | 3.7 ± 0 (3.1-3.7)      | 3.7 (3.0-3.9)                  | 3.7 ± 0.2 (3.1-3.5)         |
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