Korean Journal of Environmental Biology

Note

https://doi.org/10.11626/KJEB.2023.41.4.657

Korean J. Environ. Biol.
41(4) : 657-665 (2023)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Morphological and molecular characterization of root-lesion nematode *Pratylenchus hippeastri* from Korea

Sungchan Huh[1], Namsook Park[2], Heonil Kang[3], Changhwan Bae[4] and Insoo Choi[1,2,*]

[1]Department of Plant Bioscience, Pusan National University, Miryang 50463, Republic of Korea
[2]Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Republic of Korea
[3]Forest Entomology and Pathology Division, National Institute of Forest Science, Seoul 02455, Republic of Korea
[4]Biological and Genetic Resources Assessment Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea

Contribution to Environmental Biology

- We report the taxonomic aspects of an unrecorded species that have been reported abroad but not yet reported in Korea.
- *Pratylenchus hippeastri* is an important plant-parasitic nematode that can affect agricultural crops.

***Corresponding author**
Insoo Choi
Tel. 055-350-5504
E-mail. ichoi@pusan.ac.kr

Received: 30 November 2023
Revised: 15 December 2023
Revision accepted: 22 December 2023

Abstract: The root-lesion nematode *Pratylenchus* spp. is the most important plant-parasitic nematode due to its worldwide distribution, wide host ranges, and migratory endoparasitic characteristics. One population of *Pratylenchus* collected from the giant pussy willow (*Salix chaenomeloides* Kimura) in the Andong area as part of a nematode survey in Korea was characterized morphologically and by molecular methods. The analysis of morphological measurements and morphometric characteristics, as well as DNA sequencing of the rRNA large subunit (LSU) D2/D3 expansion segments and the internal transcribed spacer (ITS) gene sequence, confirmed the identity of this population as *P. hippeastri*. This study is the first report of *P. hippeastri* associated with *Salix chaenomeloides* in Korea and worldwide. Further studies on distribution and pathogenicity in different *P. hippeastri* host crops, such as grapevines, strawberries, and apples, are necessary. The taxonomic keys to 16 *Pratylenchus* species in Korea are provided.

Keywords: *Pratylenchus hippeastri*, *Salix chaenomeloides*, root-lesion nematode, D2/D3 expansion segments, ITS gene sequence

1. INTRODUCTION

The root-lesion nematode, *Pratylenchus* spp. is a migratory endoparasitic nematodes with over 350 hosts, including import crops such as soybeans, potatoes, corn, bananas, wheat, and cabbage. The genus *Pratylenchus* Filipjev, 1936 contains approximately 100 diverse species (Handoo *et al.* 2021), with 16 species recorded in the Republic of Korea (Park 1996; Park *et al.* 2002; Choi *et al.* 2006; Kim *et al.* 2006; Park *et al.* 2009; Kim and Chun 2014).

Research on nematodes in Korean agricultural environments is being conducted (Ko *et al.* 2021). In addition, nematodes are an important factor in investigating the microbial environment in the Korean plant ecosystem (Eo *et al.* 2018). The large number of hosts as well as its migratory endoparasitic habits make this genus a very important root-lesion nematode in

crops. During the survey of soil nematodes, *Pratylenchus hippeastri* was isolated from Giant pussy willow (*Salix chaenomeloides* Kimura). *Pratylenchus hippeastri*, also known as the amaryllis lesion nematode, has been previously reported from the USA (De Luca *et al.* 2010; Handoo *et al.* 2020), China (Wang *et al.* 2016), and South Africa (Knoetze *et al.* 2019; Shokoohi 2019). The host range of this nematode includes amaryllis, bromeliads, grapevines, strawberry and apples (De Luca *et al.* 2010; Wang *et al.* 2016; Knoetze *et al.* 2019; Handoo *et al.* 2020; Brenes-Campos *et al.* 2022), as well as Acer (Chen *et al.* 2014) and cape willow trees, *Salix mucronata* (Shokoohi 2019). This study reports the first detection of this species in Korea from Giant pussy willow, or *S. chaenomeloides*.

2. MATERIALS AND METHODS

Collection methods of nematode. Soil and roots were collected from a Giant pussy willow tree in Andong (36°25′48.0″N 128°38′20.0″E), Gyeongsangbuk-do, Korea. Nematodes were separated from soil and roots using the modified Baermann funnel method (Kang 2016).

Morphological analysis. In the analysis, twelve nematodes with fully grown and confirmed genital organs were used. Individual nematodes were mounted in water on temporary glass slides, measured, and digitally photographed using an Olympus, BX53 light microscope with differential interference contrast optics and an Olympus DP73 digital camera. Additional measurements were taken from specimens processed by either the lactophenol method (Franklin and Goodey 1949) or the quick glycerol method (Seinhorst 1959).

Molecular analysis. Each nematode individuals was picked and thirty nematodes were transferred to a 2.0 mL microcentrifuge tube. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., CA, USA). Two rRNA fragments, namely LSU D2/D3 and ITS region, were amplified, respectively. The primers for D2/D3 expansion segments amplification were D2A (5′-ACAAGTACCGTGGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Subbotin *et al.* 2006). The primers for ITS amplification were TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Subbotin *et al.* 2008). The genomic DNA was used as a template for PCR as follows: after an initial 5-minute denaturation step at 94°C, a 40-cycle amplification (94°C for 1 minute, 56°C [D2/D3 expansion segments] to 58°C [ITS region] for 1 minute, and 72°C for 1 minute) was conducted. The final extension step was continued for 10 minute at 72°C. To confirm the successful amplification of DNA by PCR, electrophoresis was performed using 0.5× TAE buffer on a 1% agarose gel. The PCR product was subsequently purified using a PCR purification kit (Bioneer Co., Korea). The amplicons were cloned into pGEM-T Easy Vector system (Promega, USA), and the resultant plasmid DNA was isolated using a Plasmid Miniprep System (Bioneer Co., Korea). Both strands of the PCR amplicons were cycle-sequenced with an ABI PRISM BigDye Terminator version 1.1 Cycle Sequencing kit and ABI Prism ABI 377 Genetic analyzer (PE Applied Biosystems, CA, USA).

Phylogenetic analysis. The LSU D2/D3 expansion segments and ITS sequences of *P. hippeastri* were compared using the BLAST sequence alignment software of the NCBI database (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/). Sequences were also aligned using the ClustalW implemented MEGA 11.0 software. Results of closest nucleotide sequences were selected to BLASTn analysis. The seqences of the ITS region exhibited 99% similarity to *P. hippeastri* (FN554886.1) and 28S rDNA D2/D3 expansion segment were found to be 99% identical to those of *P. hippeastri* (MK749422.1). Phylogenetic analyses were performed using MEGA version 11.0 (Tamura *et al.* 2021) with the neighbor-joining (NJ) statistical method and the Jukes-Cantor model.

3. RESULTS

***Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn, and Duncan, 2007**
왕버들뿌리썩이선충 (신칭) (Figs. 1-4; Table 1)

Description. Female (n = 12) : Body slender, slightly curved ventrally after relaxation with length = 424.1 ± 16.0 µm and maximum body diameter = 19.0 ± 3.8 µm (Figs. 1A, 2A). Lip region flatten with two annuli. The stylet is strong with length = 15.3 ± 0.4 µm, basal knobs ellipsoidal or slightly concave with a height = 1.8 ± 0.1 µm and width = 2.9 ± 0.1 µm (Fig. 1B). Pharyngeal

Fig. 1. Light microscopy photos of *Pratylenchus hippeastri* females. A. Entire female body, B. anterior region and stylet, and C. lateral view of the vulva and tail (scale bar: A. 50 µm; B, C. 20 µm).

glands overlapping intestine ventrally (Fig. 2B). Spermatheca rectangular without sperm. Vulval lips usually slightly raised in some specimens. Vulval body diameter = 16.9 ± 0.7 µm and distance between vulva to anus = 73.0 ± 16.6 µm, anal body width = 11.5 ± 1.0 µm. Tail conoid with bluntly pointed or subhemispherical, smooth terminus with length = 24.7 ± 2.7 µm (Figs. 1C, 2C). De Man ratios are a = 22.8 ± 2.0 µm, b = 5.7 ± 0.2, b′ = 4.1 ± 0.1, c = 16.7 ± 1.5, c′ = 2.2 ± 0.1, V = 79.3 ± 0.4 (Table 1).

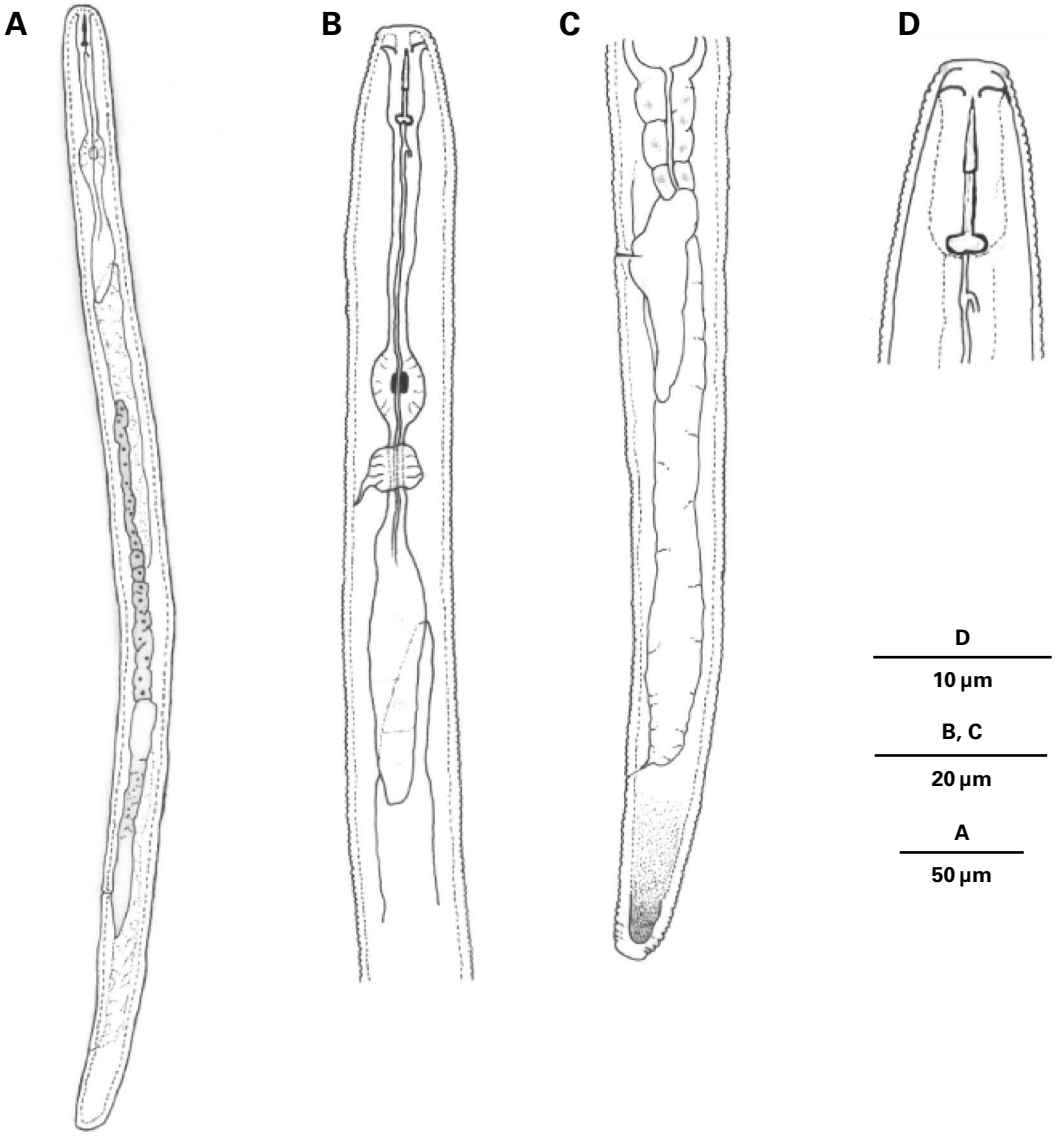

Fig. 2. Drawings of *Pratylenchus hippeastri*. A. Entire female body, B. basal bulb and esophagus, C. lateral view of the vulva and tail, and D. anterior region with stylet (scale bar: A. 50 µm; B, C. 20 µm; D. 10 µm).

Male : Not found.

Molecular characters and phylogenetic analyses. The sequenced LSU D2/D3 expansion segments of *P. hippeastri* were 748 bp in length. A Blastn search of the LSU D2/D3 expansion segments sequence revealed high matches with the originally reported sequences of *P. hippeastri* (KP161608.1 and MK749422.1). The identities of the LSD D2/D3 expansion segments and the originally reported sequence were 99.5% without insertions or deletions. The phylogenetic tree based on the LSU D2/D3 expansion segments sequences showed that *P. hippeastri* was nested within the same clade with all the reported *P. hippeastri* (Fig. 3). The sequenced ITS segments of *P. hippeastri* were 932 bp in length. A Blastn search of the ITS sequence revealed high matches with the originally reported sequences of *P. hippeastri* (FJ713000.1). The identities of ITS segments and the originally reported sequence were 99.6% without insertions/deletions. The phylogenetic tree based on the ITS sequence showed that the sequence of *P. hippeastri* obtained in this study and all the reported *P. hippeastri* sequences formed a high de-

Table 1. Morphometric measurements of *Pratylenchus hippeastri*

	Korea	Wang *et al.* (2016)	Gu *et al.* (2014)	De Luca *et al.* (2012)	Inserra *et al.* (2007)
n	12	10	16	10	22
L	424.14 ± 16.0	447.8 ± 28.2	479.9 ± 31.9	614 ± 22.4	590 ± 21.8
a	22.8 ± 2.0	27.7 ± 1.4	28.0 ± 1.9	25.2 ± 2.0	25.5 ± 1.2
b	5.7 ± 0.2	5.4 ± 0.3	6.2 ± 0.3	6.6 ± 0.4	6.5 ± 0.4
b′	4.1 ± 0.1	3.2 ± 0.2	3.9 ± 0.3	4.5 ± 0.4	3.9 ± 0.2
c	16.7 ± 1.5	17.9 ± 1.5	16.5 ± 1.3	18.6 ± 2.0	16.1 ± 1.0
c′	2.2 ± 0.1	2.3 ± 0.2	2.7 ± 0.3	2.2 ± 0.2	2.6 ± 0.2
V	79.3 ± 0.4	78.2 ± 1.3	77.2 ± 1.0	77.7 ± 1.2	77 ± 0.8
Stylet length	15.3 ± 0.4	14.9 ± 0.4	15.8 ± 0.8	15.8 ± 0.4	15.5 ± 0.4
Stylet knob width	2.9 ± 0.1	3.2 ± 0.2	–	–	4.7 ± 0.3
Stylet knod height	1.8 ± 0.1	1.8 ± 0.1	–	–	2.1 ± 0.3
Max. body diam	19.0 ± 3.8	16.2 ± 0.9	17.2 ± 1.7	24.4 ± 0.7	23.2 ± 1.4
Vulva body diam	16.9 ± 0.7	14.9 ± 1	15.6 ± 1.7	21.6 ± 1.6	20.5 ± 1.1
Anal body diam	11.5 ± 1.0	11 ± 0.7	11.4 ± 1.2	15.3 ± 0.4	14.4 ± 0.8
Tail length	24.7 ± 2.7	25 ± 1.9	29.5 ± 3.1	33.3 ± 3.0	36.8 ± 2.2
Vulva to anus distance	73.0 ± 16.6	74.4 ± 8.6	77.0 ± 9.8	103 ± 5.4	98 ± 6.1

All measurements are in μm and in the form of mean ± standard deviation (L, overall body length; a, body length/greatest body diameter; b, body length/distance from the anterior to the esophago-intestinal valve; b′, body length/distance from the anterior to the base of the esophageal glands; c, body length/tail length; c′, tail length/tail diameter at the anus or cloaca; V, % distance of vulva from anterior).

gree of similarity (Fig. 4). The result was indicated as *P. hippeastri* with a high probability, but the number of matching data was small. Compared with the results of the LSUD2/D3 expansion segments sequence, it is assumed that it is simply the result of a small number of data.

4. DISCUSSION

In this study, the morphometric and morphological characteristics of *P. hippeastri* from Korea were found to be consistent with the original report, except for a shorter body and tail length (424.14 ± 16.0 μm and 24.7 ± 2.7 μm vs. 614 ± 22.4 μm and 33.3 ± 3.0 μm, respectively) (De Luca *et al.* 2010). The body and tail length of *P. hippeastri* from Korea were identified to be similar to those from China (Wang *et al.* 2016) and Japan (Gu *et al.* 2014). The body and tail length of *P. hippeastri* from China was 447.8 ± 28.2 μm and 25 ± 1.9 μm, respectively. While those from Japan were 479.9 ± 31.9 μm and 29.5 ± 3.1 μm, respectively.

The variability in body length within a species can be affected by nutritional or environmental factors (Castillo and Vovlas 2007). The body lengths of *P. hippeastri* from different region varied, while the ratio of V (%, distance of vulva from anterior) is very similar: among specimens form Korea (79.3 ± 0.4 μm), China (78.2 ± 1.3 μm) (Wang *et al.* 2016), Japan (77.2 ± 1.0 μm) (Gu *et al.* 2014), California (77 ± 0.8 μm) (Inserra *et al.* 2007), and Florida (77.7 ± 1.2 μm) (De Luca *et al.* 2012), respectively. *Pratylenchus hippeastri* may be better adapted to warm tropical regions (Wang *et al.* 2016). Consequently, *P. hippeastri* found in tropical ornamentals such as amaryllis and bromeliads (Inserra *et al.* 2007; De Luca *et al.* 2012) were larger than those found on apple trees from China (Wang *et al.* 2016). Significant variation in length has previously been observed in *Pratylenchus* species. Bogal *et al.* (2021) reported that the body length of seven populations of *P. penetrans* collected in Europe ranged from 381 to 625 μm. *P. hippeastri* was previously identified as an unrecorded species of *Pratylenchus* in Korea (NIBR 2010). The identification relied on 28S rDNA analysis along with four photos. However, the specimens lacked information on morphological measurements, locality, or host plant

Fig. 3. Bayesian phylogenetic tree of the genus *Pratylenchus*. The analysis was conducted using 30 *Pratylenchus hippeastri* specimens. Bayesian 50% majority rule consensus tree from two runs, as inferred from the analysis of D2D3 of 28S rDNA gene sequences under the GTR + G model. Posterior probability values of more than 50% are given in the appropriate clades.

details. Therefore, this is the first comprehensive report of *P. hippeastri* in Korea. *P. hippeastri*, a root-lesion nematodes, can damage apples, strawberries, and grapevines as well as willows native to Korea, also a possibility of infection with other unknown species of plants, requiring further research and quarantine.

Key to *Pratylenchus* Species in Korea

1. Lip region composed of two annules 2
− Lip region composed of three annules 7
2. Tail terminus crenate ..... *P. flakkensis* Seinhorst, 1968
− Tail terminus smooth 3
3. Males are common; spermatheca is filled with sperm 4
− Males are rare; spermatheca is empty 5
4. Tail terminus is narrowly rounded to subacute ..... *P. loosi* Loof, 1960

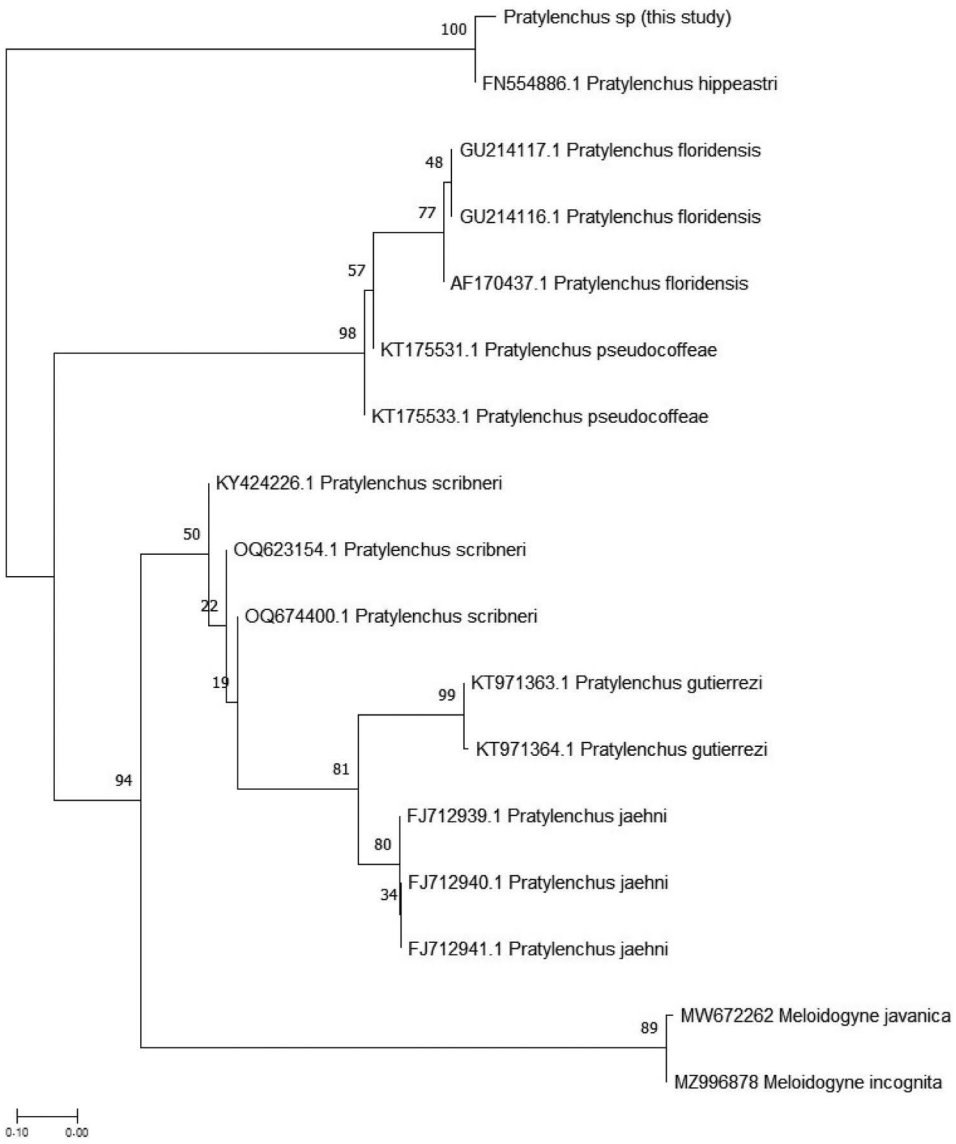

Fig. 4. Bayesian phylogenetic tree of the genus *Pratylenchus*. The analysis was conducted using 30 *Pratylenchus hippeastri* specimens. Bayesian 50% majority rule consensus tree from two runs, as inferred from the analysis of ITS sequences under the GTR + G model. Posterior probability values of more than 50% are given in the appropriate clades.

– Tail terminus is slender, broadly to conically rounded, truncate or indented ····· *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhovem, 1941
– (closely resembles *P. coffeae*), but is distinguished by its divided pattern when viewed en face, a short distance from the spear base to DGO (1.8–2.3 µm vs. 2.6–3.3 µm in mean), narrower spear knobs (3.4–3.7 µm vs. 3.7–4.2 µm in mean), and frequent 3 lip annuli (28.5% on either side and 5.5% on both sides: n = 91) ····· *P. pseudocoffeae* Mizukubo, 1992

5. Vulva at 80–87%; tail terminus rounded or slightly oblique ····· *P. neglectus* (Rensch, 1924)
– Vulva at 72–80%; tail conoid to broad ····· 6
6. Post-uterine branch = 19.8 (12–43) ····· ····· *P. scribneri* Steiner, 1943
– Post-uterine branch = 37–45 µm long (usually more than twice of vulval body diameter); Tail conoid ····· ····· *P. kumamotoensis* Mizukubo, Sugimura & Uesugi, 2007
– Post-uterine branch = 30 ± 4.9 (21–45); Female tail

tip with areolated lateral field ..... *P. hippeastri* Inserra,Troccoli, Gozel, Bernard, Dunn & Duncan, 2007

7. Tail terminus crenate or with a distinct sub-ventral or terminal projection 8
– Tail terminus smooth 10
8. Males unknown; spermatheca without sperm; Vulva at 80–86%; lateral field with four lines; annulation prominent ..... *P. crenatus* Loof, 1960
– Males common; spermatheca with sperm 9
9. Spermatheca large, elongate; tail terminus oblique, sometimes appearing more symmetrically conoid or slightly mucronate ..... *P. pratensis* (de Man, 1880) Filipjev, 1936
– Spermatheca round, square, or rectangular; Tail cylindrical or conical; tail terminus rounded and finely crenate ..... *P. fallax* Seinhorst, 1968
10. Males rare; spermatheca empty ..... *P. thornei* Sher & Allen 1953
– Males common; spermatheca filled with sperm 11
11. Spermatheca oval; body slender; average a = 29 or more ..... *P. vulnus* Allen & Jensen, 1951
– Spermatheca round, sometimes more square and subspherical 12
12. Posterior uterine branch longer with cellular distal part; body short and stout; average a = 25 or less ..... *P. subpenetrans* Taylor & Jenkins, 1957
– (closely resembles to those of *P. subpenetrans*) but body length is much longer (female: 518–626 µm vs. 330–481 µm; male: 514–581 µm vs. 329–472 µm) ..... *P. subpenetrans* Choi, Lee, Park, Han, & Choi, 2006
– Posterior uterine branch short, undifferentiated; body long and slender; average a = more than 25 13
13. Lip region low, slightly set off; stylet 15–17 µm long; a = 26 (21–31) ..... *P. penetrans* (Cobb, 1917) Filipjev & Shuurmans Stekhoven, 1941
– Lip region high, continuous; stylet 14–16 µm long; a = 27 (24–31) ..... *P. mediterraneous* Corbett, 1983

CRediT authorship contribution statement

S Huh: Investigation, Data curation, Formal analysis, Writing - Original draft, Writing - Review & editing. **N Park:** Conceptualization, Resources, Methodology. **H Kang:** Conceptualization, Resources, Methodology. **C Bae:** Conceptualization, Resources. **I Choi:** Supervision, Writing - Review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interest

ACKNOWLEDGEMENTS

This work was carried out with the support of "Cooperative research Program for Agriculture Science and Technology Department", Rural Development Administration (project number PJ01565402), Republic of Korea.

REFERENCES

Bogale M, B Tadesse, RH Nuaima, B Honermeier, J Hallmann and P DiGennaro. 2021. Morphometric and molecular diversity among seven European isolates of *Pratylenchus penetrans*. Plants 10:674. https://doi.org/10.3390/plants10040674

Brenes-Campos R, LA Nunez-Rodriguez, L Flores-Chaves and DA Humphreys-Pereira. 2022. New record of the amaryllis lesion nematode, *Pratylenchus hippeastri* associated with strawberry in the Central Region of Costa Rica. Nematropica 51:131–136.

Castillo P and N Vovlas. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Nematology, Monographs and Perspectives 6 (DJ Hunt and RN Perry eds.). Brill. Leiden, Netherlands. https://doi.org/10.1163/ej.9789004155640.i-523

Chen WJ, T Bian, R Wu, XJ Lin, MZ Zhang, Y Wu, XY Wu and X Chen. 2014. Root-lesion nematode, *Pratylenchus hippeastri*, on *Acer palmatum* imported from Japan. Acta Agric. Zhejiangensis 26:708–713.

Choi DR, JK Lee, BY Parte, HR Han and YE Choi. 2006. A new and one unrecorded species of *Pratylenchus* from Korea (Nematoda: Pratylenchidae). J. Asia-Pac. Entomol. 9:5–9. https://doi.org/10.1016/S1226-8615(08)60269-1

De Luca F, A Troccoli, LW Duncan, SA Subbotin, L Waeyenberge, DL Coyne, FC Brentu and RN Inserra. 2012. *Pratylenchus speijeri* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode pest of plantain in West Africa. Nematol. 14:987–1004. https://doi.org/10.1163/156854112X638424

De Luca F, A Troccoli, LW Duncan, SA Subbotin, L Waeyenberge, M Moens and RN Inserra. 2010. Characterisation of a pop-

ulation of *Pratylenchus hippeastri* from bromeliads and description of two related new species, *P. floridensis* n. sp. and *P. parafloridensis* n. sp., from grasses in Florida. Nematol. 12:847–868. https://doi.org/10.1163/138855410X495809

Eo J, MH Kim, HK Nam, SI Kwon and YJ Song. 2018. Effect of organic waste application on soil chemical properties and organisms under *Zelkova serrata* cultivation. Korean J. Environ. Biol. 36:471–478. https://doi.org/10.11626/KJEB.2018.36.4.471

Franklin MT and JB Goodey. 1949. A cotton blue-lactophenol technique for mounting plant-parasitic nematodes. J. Helminthol. 23:175–178. https://doi.org/10.1017/S0022149X0003251X

Gu JF, N Wang, J He and WJ Chen. 2014. Identification of *Pratylenchus hippeastri* from imported seedlings. Plant Quarantine 4:41–46.

Handoo ZA, AM Skantar, MR Kantor, SL Hafez and MN Hult. 2020. Molecular and morphological characterization of the amaryllis lesion nematode, *Pratylenchus hippeastri* (Inserra *et al.* 2007), from California. J. Nematol. 52:1–5. https://doi.org/10.21307/jofnem-2020-058

Handoo ZA, G Yan, MR Kantor, D Huang, IA Chowdhury, A Plaisance, GR Bauchan and JD Mowery. 2021. Morphological and molecular characterization of *Pratylenchus dakotaensis* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode species on soybean in North Dakota, USA. Plants 10:168. https://doi.org/10.3390/plants10010168

Inserra RN, A Troccoli, U Gozel, EC Bernard, D Dunn and LW Duncan. 2007. *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*, Nematol. 9:25–42. https://doi.org/10.1163/156854107779969754

Kang H, G Eun, J Ha, Y Kim, N Park, D Kim and I Choi. 2016. New cyst nematode, *Heterodera sojae* n. sp. (Nematoda: Heteroderidea) from soybean in Korea. J. Nematol. 48:280–289. https://doi.org/10.21307/jofnem-2017-036

Kim D and JY Chun. 2014. Identification of root-lesion nematode (Pratylenchidae: Pratylenchus) intercepted on imported plants. Korean J. Appl. Entomol. 53:51–57. https://doi.org/10.5656/KSAE.2013.10.1.083

Kim JH, YH Jeon, H Park, BD Lee, DH Cho, BY Park, Z Khan and YH Kim. 2006. The root-lesion nematode, *Pratylenchus subpenetrans*, on ginseng (*Panax ginseng*) in Korea. Nematol. 8:637–639. https://doi.org/10.1163/156854106778613949

Knoetze R, E van den Berg, C Girgan and L van der Walt. 2019. First report of the root lesion Nematode, *Pratylenchus hippeastri*, on apple in South Africa. J. Plant Dis. Prot. 126:607–609. https://www.jstor.org/stable/48699416

Ko HR, E Park, EH Kim, SK Park, H Kang and B Park. 2021. Screening for resistance of Brassicaceae plant resources to clover cyst nematode. Korean J. Environ. Biol. 39:329–335. https://doi.org/10.11626/KJEB.2021.39.3.329

NIBR. 2010. The Genetic Evaluation of Important Biological Resources. Pub. No. 11-1480592-000125-01. National Institute of Biological Resource. Incheon, Korea.

Park BY. 1996. Taxonomical study of *Pratyelchus* spp. in Korea. MS Thesis. Konkuk University. Seoul, Korea.

Park BY, DR Choi, JK Lee, YE Choi and GH Shin. 2002. Unrecorded species of *Pratylenchus loosi* Loof (Tylenchida: Pratylenchidae) from tea in Korea. Korean J. Appl. Entomol. 41:299–303.

Park SN, YN Youn, JK Lee and BY Park. 2009. Survey of plant parasitic nematode in Citrus fruit orchards in Korea. Korean J. Appl. Entomol. 48:535–539.

Seinhorst JW. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematol. 4:67–69. https://doi.org/10.1163/187529259X00381

Shokoohi E, J Abolafia, PW Mashela and N Divsalar. 2019. New data on known species of *Hirschmanniella* and *Pratylenchus* (Rhabditida, Pratylenchidae) from Iran and South Africa. J. Nematol. 51:1–26. https://doi.org/10.21307/jofnem-2019-041

Subbotin SA, D Sturhan, VN Chizhov, N Vovlas and JG Baldwin. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematol. 8:455–474.

Subbotin SA, EJ Ragsdale, T Mullens, PA Roberts, M Mundo-Ocampo and JG Baldwin. 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): Evidence from 18S and D2–D3 expansion segments of 28S ribosomal RNA genes and morphological characters. Mol. Phylogenet. Evol. 48:491–505. https://doi.org/10.1016/j.ympev.2008.04.028

Tamura K, G Stecher and S Kumar. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biol. Evol. 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Wang H, K Zhuo and J Liao. 2016. Morphological and molecular characterization of *Pratylenchus hippeastri*, a new record of root lesion nematode associated with apple in China. Pakistan J. Zool. 48:665–671.