

# The morphological characteristics and phylogenetic analysis of *Pratylenchus vulnus* Taiwan strawberry isolate

Yu-po Lin,<sup>1</sup> Wan-chun Lee,<sup>2</sup>  
Pei-che Chung<sup>3</sup> and Jiue-in Yang<sup>1,2\*</sup>

<sup>1</sup>Plant Medicine Program, National Taiwan University, Taipei, 10617, Taiwan. No. 1 Roosevelt Rd., Sec. 4, Da-an Dist., Taipei City, 10617, Taiwan.

<sup>2</sup>Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, 10617, Taiwan. No. 1 Roosevelt Rd., Sec. 4, Da-an Dist., Taipei City, 10617, Taiwan.

<sup>3</sup>Miaoli District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan, Miaoli, 36346, Taiwan. No. 26, Guannan Rd., Gongguan Township, Miaoli County 36346, Taiwan.

\*E-mail: [jiueinyang@ntu.edu.tw](mailto:jiueinyang@ntu.edu.tw)

This paper was edited by Zafar Ahmad Handoo.

Received for publication June 24, 2019.

## Abstract

*Pratylenchus vulnus* was discovered in nematode-distributing fields from symptomatic seedling roots and corresponding rhizosphere soil on strawberry farms in Taiwan. Microscopic measurements and scanning electron microscope observations of both sexes of the nematode coincided with the general morphological descriptions of the species. Four different types of female tail termini were observed, including pointed, digitate, smooth and tapering. Molecular analysis of the ribosomal RNA sequence (SSU, ITS and LSU regions) and the mitochondria COI gene sequences confirmed the species identification. Phylogenetic analysis suggested no specific geographic linkage of the Taiwan population to other previously reported populations.

## Keywords

*Pratylenchus vulnus*, Morphology, SEM, Phylogenetic analysis, Strawberry, Root lesion nematode.

Root lesion nematodes (*Pratylenchus* sp.) are among the most economically damaging phytoparasitic nematodes on fruits, tree rootstocks and vegetables (Yu et al., 2012). *Pratylenchus vulnus* was first reported in 1951 as a pathogen of multiple trees and vines in California, USA (Allen and Jensen, 1951) and was later found to infect over 80 plant species (Castillo and Vovlas, 2007). In Uruguay, Sri Lanka and Australia, serious damage in strawberry (*Fragaria* × *ananassa*) fields caused by this nematode have been reported (Colbran, 1974; Minagawa and Maeso-Tozzi, 1990; Mohotti et al., 1997). We here report the discovery of one *Pratylenchus* sp. population in strawberry fields in Dahu township of Miaoli, Taiwan in 2017. The strawberry crops growing in the nematode-distributing fields GS and KDL were obviously stunted when discovered. Both fields had been cropping strawberry for over 10 years. The soil composition of the area was

characterized as sandy loam by hydrometer method (Bouyoucos, 1951). Nematodes were extracted from 100g±5% soil with modified Baermann funnel technique (Hooper, 1986). Morphological observations and measurements of adults (30 females and 30 males) were conducted with a compound microscope at magnification of up to 1000× (Table 2). The dorsal gland orifice of the female of our isolates fit the description of *P. vulnus* perfectly, with a range between 3.0 and 4.42 (means=3.55), rather than 1.9 to 3.0 characteristic of *P. penetrans* (Roman and Hirschmann, 1969). All nematodes observed had a lateral field composed of four incisures with wider inner band but two types of lateral field were observed. The first type had an evenly uplifted lateral field (Fig. 1E), while the second type carried uplifted and thinner outer bands (Fig. 1F). Four different types of female tail termini were observed, including pointed, digitate,

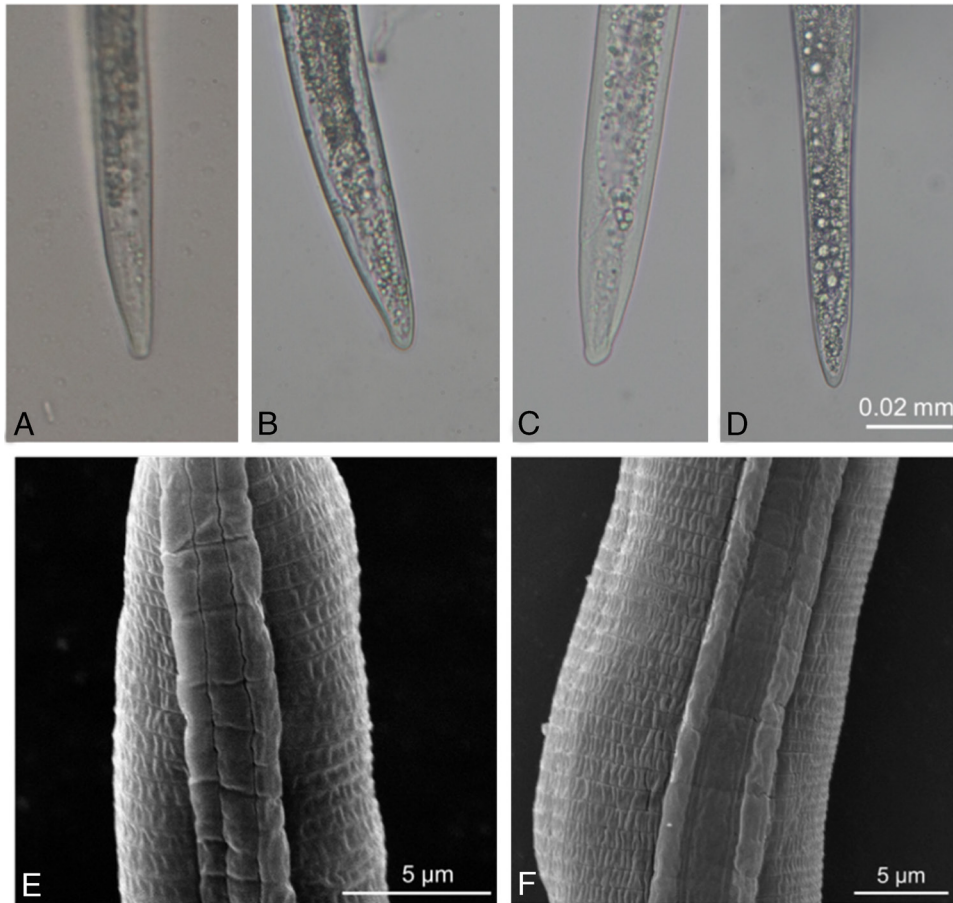


Figure 1: The morphological variations within the *P. vulnus* Taiwan strawberry population. Two lateral field types and four tail termini were observed. Photographs were taken at 1000X magnification with compound microscope and SEM.

smooth and tapering (Fig. 1A-D). *P. vulnus* is known to express morphological differences when cultured under different temperature conditions (Doucet et al., 2001). A previous report also characterized five *P. vulnus* groups in Japan by tail morphology (Mizukubo 1990). The fact that four different tail terminus types were observed in the *P. vulnus* Taiwan population implies this population may have been established in the region rather than being recently imported.

Molecular analysis of the ribosomal RNA and mitochondrial gene sequences of the extracted nematodes confirmed their species as *P. vulnus* (Table 1). The rDNA LSU region (D2A/D3B: ACAAGTACCCTGAGGGGAAAGTTG/TCGGAAGGAACCAGCTACTA) (Nunn, 1992), rDNA ITS region (TW81/AB28: GTTCCGTAGGTGAACCTGC/ATATGCTTAAGTTCA GCGGGT) (Amiri et al., 2002; Subbotin et al., 2001), rDNA SSU region (SSU18A/SSU26R: AAAGATTAAGCCATGCATG/CATTCTTGGCAAATGCTTTTCG)

(Eyuaem and Blaxter, 2003) and mtDNA COI region (JB3/JB4.5: TTTTTTGGGCATCCTGAGGTTTAT/TAAAGAAAGAACATAATGAAAATG) (Derycke et al., 2010) of the Pv-GS and Pv-KDL are 99.04, 95.96, 99.66 and 99.24% identical, respectively. Both Pv-GS and Pv-KDL isolates had maximum 100% similarity of their rDNA LSU sequences to *P. vulnus* (GenBank accession: U47547.1). The Pv-KDL mtDNA COI region sequences of isolates were 99.74 to 100% similar to other sequences of *P. vulnus* available in the database (GenBank accessions KY424094, KY424095, KY828312, KY828317, KY424096-7 and KX349427), and only 81.08% identical to the second most closely related species, *P. scribneri* (GenBank accession: KY424089.1). The rDNA SSU region sequence analysis provided a similar result as Pv-KDL and are 99.42% (GenBank accession: KY424163) to 99.88% (GenBank accession: KY424164) identical to all available *P. vulnus* sequences in GenBank, and only 94.48%

**Table 1. GenBank sequence deposit information of multiple gene regions of *P. vulnus* Taiwan isolates from strawberry fields GS and KDL.**

<i>P. vulnus</i> sequences GenBank deposit information				
Isolate location	rDNA			mtDNA
	LSU (730 bp)	ITS (669 bp)	SSU (883 bp)	COI (441 bp)
GS	MG372808	MG372806.1	MG372807	MN431203
KDL	MK713641	MK713613	MK713614	MK764689

similar to *P. kumamotoensis* (GenBank accession: AB905295.1). The rDNA ITS sequences also separated the Pv-KDL from *P. kumamotoensis* (GenBank accession: KT175521.1) clearly with a very low 86.22% similarity. Phylogenetic analysis of

the rDNA ITS and LSU regions combined suggested the nematodes from the two fields belong to one population (Fig. 2) and are isolated from the rest of the previous populations from other geographic regions (Table 2).

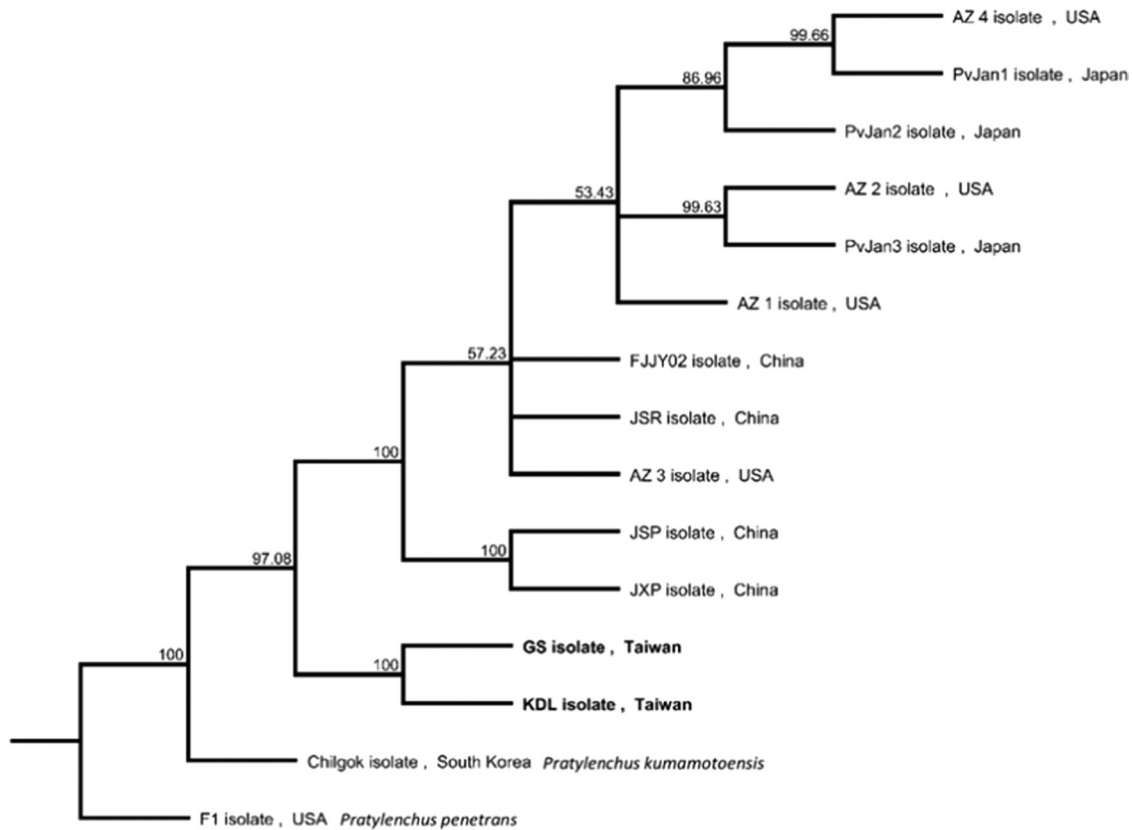


Figure 2: The phylogenetic tree of the combined partial 28S D2-D3 region and complete ITS region of the *P. vulnus* rDNA sequences. Morphologically similar species, *Pratylenchus penetrans* F1 isolate and of *P. kumamotoensis* Chilgok isolate, were used as out groups. Each node is marked with the isolate code, species and origin. The number at the fork represents the percentage of the bootstrap tested with 10,000 times for the indicating result.

**Table 2. Measurements of the Taiwan *P. vulnus* population morphological characteristics.**

Characteristics	Female (n=30)			Male (n=30)		
	Mean	SD m.	Range	Mean	SD m.	Range
L (µm)	704.01	74.85	573.46–839.91	587.02	46.83	492.76–678.1
K (µm)	25.97	4.06	17.76–36.18	18.47	1.47	15.49–21.50
Stylet (µm)	14.5	1.1	12.6–16.5	13.76	0.73	12.18–15.40
Tail (µm)	32.87	3.93	24.35–41.10	28.74	4.07	22.28–39.80
a (ratio)	27.47	3.15	22.74–34.30	31.85	2.53	26.83–36.62
b (ratio)	6.1	0.6	4.87–7.16	5.7	0.47	4.88–6.95
b' (ratio)	4.86	0.42	4.07–5.59	4.51	0.37	3.92–5.26
c (ratio)	21.57	2.38	17.83–27.46	20.71	2.68	13.51–23.99
c' (ratio)	2.29	0.31	1.59–2.86	2.29	0.38	1.77–3.83
V (%)	78.34	1.67	73.84–81.09	–	–	–
Spicules (µm)	–	–	–	16.88	1.89	12.06–20.15
DGO (µm)	3.55	0.32	3.00–4.42	3.41	0.33	2.77–4.31

Our discovery of the existence and population dynamics of *P. vulnus* in Taiwan implies a new threat to the 1,639 million NTD (ca. \$52 million) strawberry industry. Currently, no nematicide is registered for root-lesion nematode suppression in strawberry. Related cultural and physical control options are currently undergoing evaluation by agricultural extension agencies to prevent serious damage.

## Acknowledgments

The authors thank Dr. Zeng-Yei Hseu (Soil Survey and Contamination Remediation Laboratory, National Taiwan University) for the soil characterization assistance. The authors also thank Dr. Shiu-Feng Shiao (Laboratory of Insect Systematics, National Taiwan University) and Ms. Shiang-Jiun Chen (Department of Life Science, National Taiwan University) for SEM assistance. This research was funded by the Council of Agriculture, Executive Yuan, Taiwan, Grant No. 107AS-1.2.7-ST-aA. The authors declare no conflict of interest.

## References

Allen, M. and Jensen, H. 1951. *Pratylenchus vulnus*, new species (Nematoda Pratylenchinae), a parasite of trees and vines in California. Proceedings of the Helminthological Society of Washington 18:47–50.

Amiri, S., Subbotin, S. A. and Moens, M. 2002. Identification of the beet cyst nematode *Heterodera schachtii* by PCR. European Journal of Plant Pathology 108:497–506.

Bouyoucos, G. J. 1951. A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy Journal 43:434–8.

Castillo, P. and Vovlas, N. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management, Brill, Leiden.

Colbran, R. 1974. Nematodes in strawberries. Queensland Agriculture Journal 100:522–5.

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. and Moens, T. 2010. Exploring the use of cytochrome oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes. PLoS One 5:e13716.

Doucet, M., Baujard, P., Pinochet, J., Di Rienzo, J. and Lax, P. 2001. Temperature-induced morphometrical variability in an isolate of *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida). Nematology 3:1–8.

Eyuaem, A. and Blaxter, M. 2003. Comparison of biological, molecular, and morphological methods of species identification in a set of cultured *Panagrolaimus* isolates. Journal of Nematology 35:119.

Hooper, D. 1986. Extraction of free-living stages from soil, in Southey, J. F. (Ed.), Laboratory methods for work with plant and soil nematodes, HMSO, London, pp. 5–30.

Minagawa, N. and Maeso-Tozzi, D. 1990. Plant-parasitic nematodes of Uruguay: a preliminary report. Japanese Journal of Nematology 20:44–50.

Mizukubo, T. 1990. Pictogram analysis of spear length, lip region diameter and tail morphology in cohabiting *Pratylenchus penetrans* and *P. vulnus* (Tylenchida: Pratylenchidae). Japanese Journal of Nematology 20:51–5.

Mohotti, K., Navaratne, N. and Nugaliyadda, M. 1997. New record on the occurrence of walnut root lesion nematode, *Pratylenchus vulnus* on strawberry in Sri Lanka. International Journal of Nematology 7:225–6.

Nunn, G. B. 1992. Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences. University of Nottingham, Nottingham.

Roman, J. and Hirschmann, H. 1969. Morphology and morphometrics of six species of *Pratylenchus*. Journal of Nematology 1:363.

Subbotin, S. A., Vierstraete, A., De Ley, P., Rowe, J., Waeyenberge, L., Moens, M. and Vanfleteren, J. R. 2001. Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the its regions of ribosomal DNA. Molecular Phylogenetics and Evolution 21:1–16.

Yu, Y.-T., Liu, H. L., Zhu, A. G., Zhang, G., Zeng, L. B. and Xue, S. D. 2012. A review of root lesion nematode: identification and plant resistance. Advances in Microbiology 2:411.