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Phylogenetic position of *Pratylenchus vulnus* species (Nematoda: Pratylenchidae) associated with banana using mtDNA (cox1 region) from South Africa

MEHRNOUSH AMINISARTESHNIZI*

Aquaculture Research Unit, School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa
**(e-mail: mehrnoush.aminisarteshnizi@ul.ac.za)*

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ABSTRACT

Plant parasitic nematodes are the main nematodes that live in the soil, causing yield loss for various crops. *Pratylenchus* species is endoparasite and known as root lesion nematode, therefore, can feed on the roots of the banana, resulting in necrosis and less production. This species is indicated to be highly risky to plants and must be identified correctly, and it is associated with bananas as one of the most important crops in Limpopo Province in South Africa. Therefore, this molecular study was conducted in 2023 at the Limpopo University to identify the *Pratylenchus* from South Africa's soils using the cox1 region of mtDNA marker. The recovered nematode was extracted using the tray method, and then its DNA was extracted using the Chelex method. The nematode was identified as *Pratylenchus vulnus*. Afterwards, cox1 of mtDNA was amplified using specific primers to identify the nematode. The Nblast analysis based on the mtDNA showed that South African *P. vulnus* had 99% similarity (KY424096; KY424097) with Chinese populations. Phylogenetic analysis using maximum likelihood placed this species with those molecularly identified as *P. vulnus* in the same clade with highly supported (100) bootstrap values. In conclusion, this species was determined using cox1 of mtDNA; however, other rDNA markers, such as ITS, and rDNA, were recommended for a better understanding of *Pratylenchus* phylogeny.

Key words: mtDNA, phylogeny, plant parasitic nematode, *Pratylenchus*

INTRODUCTION

The soil nematodes are divided into free-living bacterivores (Abolafia and Shokoohi, 2017) and plant-parasitic nematodes (Shokoohi, 2022a, b and c). However, the free-living nematode's importance is because of their ecological diversity, and parasitism arose multiple times within this bacterivorous group (Shokoohi *et al.*, 2013; Shokoohi and Abolafia, 2019). On the other hand, plant-parasitic nematode, such as *P. vulnus* is an endoparasite resulting in the yield loss of various crops. The genus *Pratylenchus* is a well-distributed genus across all continents and is known as root lesion nematodes which are highly dangerous for plant health and production (Castillo and Vovlas, 2007).

Additionally, *P. vulnus* is associated with several crops in South Africa (Shokoohi, 2022c). Because *P. vulnus* can feed on several crops, it becomes an important plant-parasitic nematode (Castillo and Volvlas, 2007). Therefore, mtDNA markers, such as cox1 (Shokoohi, 2022a), have been used for plant-parasitic identification.

Identification of the species in the genus *Pratylenchus* is based on morphology and molecular characteristics. However, the identification of *Pratylenchus* only based on the morphology is challenging. Therefore, molecular DNA barcodes such as mtDNA (Shokoohi, 2022a,b) are essential for identifying this group of nematodes.

MATERIALS AND METHODS

The present investigation was conducted in 2023 at the University of Limpopo to find out the *Pratylenchus* nematodes. The objective of the study was to identify the nematode based on the mtDNA gene. All the samples were processed at the Aquaculture Research Unit.

Nematode Extraction and Processing

Rhizosphere soil samples of bananas were collected from Limpopo Province (GPS coordinates: S: 23°48'3.8622"; E: 30° 7'40.0332") of South Africa. Nematode extraction was achieved using the Shokoohi and Abolafia (2022a,b) technique.

Molecular Analysis

The DNA extraction was done using the Chelex method (Aminisarteshnizi, 2021a). Five specimens of *Pratylenchus* were hand-picked with a fine-tip needle and transferred to a 1.5 ml Eppendorf tube containing 20 µl double distilled water. The nematodes in the tube were crushed with the tip of a fine needle and vortexed. Ten microliters of 5% Chelex® 50 and 2 µl of proteinase K were added to each of the microcentrifuge tubes that contained the crushed nematodes and mixed. These separate microcentrifuge tubes with the nematode lysate were incubated at 56°C for 2 h and then incubated at 95°C for 10 min to deactivate the proteinase K and finally spin for 2 min at 16000 rpm (Aminisarteshnizi, 2021b). The supernatant was then extracted from each of the tubes and stored at -20°C. Following this step, the forward and reverse primers, JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT–3′), JB4.5 (5′-TAA AGA AAG AAC ATA ATG AAA ATG–3′) (Derycke *et al.*, 2010) were used in the PCR reactions for partial amplification of the cox1 of mtDNA. The PCR was conducted with 8 µL of the DNA template, 12.5 µL of 2X PCR Master Mix Red (Promega, USA) for the Botswanan specimens, 1 µl of each primer (10 pmol/µl), and ddH_2O for a final volume of 30 µL. The amplification was processed using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), with the following program: initial denaturation for 3 min at 94°C, 37 cycles of denaturation for 45 s at 94°C; 54°C annealing temperatures for mtDNA; extension for 45 s to 1 min at 72°C, and finally an extension step of 6 min at 72°C followed by a temperature on hold at 4°C. After DNA amplification, 4 µl of product from each tube was loaded on a 1% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid and 1 mM EDTA) for evaluation of the DNA bands. The bands were stained with RedGel and visualized and photographed on a UV transilluminator. The amplicons of each gene were stored at –20°C (Aminisarteshnizi, 2021c). Finally, the PCR products were purified for sequencing by Inqaba Biotech (South Africa). The phylogenetic analysis was done using neighbour-joining implemented in Mega-X software (Kumar *et al.*, 2018).

RESULTS AND DISCUSSION

Pratylenchus vulnus studied (Fig. 1) resembles the previously published materials (Castillo and Vovlas, 2007). The Nblast result of *P. vulnus* showed 99% similarity (KY424096; KY424097) with Chinese populations. Compared with another *P. vulnus* from the USA (EU280787; DQ328753), it showed 98% similarity. Compared with another population of *P. vulnus* from China (KX349427), it showed 99% similarity. Phylogenetic analysis using maximum likelihood placed this species with those molecularly identified as *P. vulnus* in the same clade with highly supported 100 bootstrap values (Fig. 2).

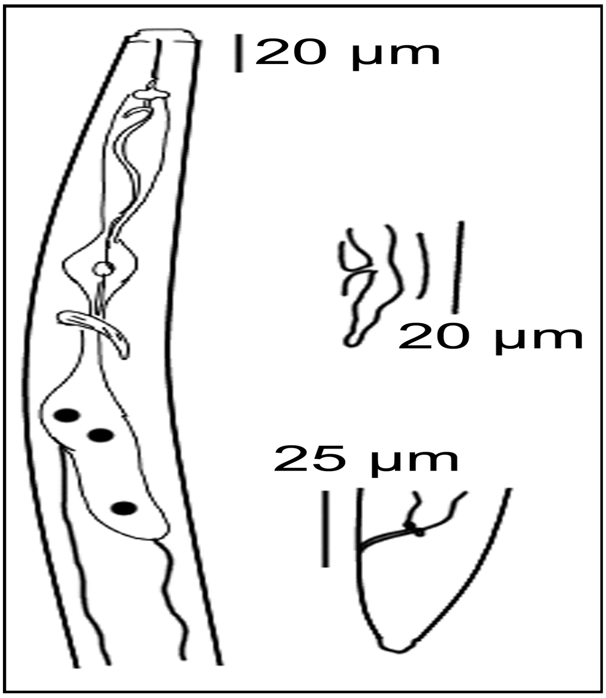

Fig. 1. Line illustration of the South African *Pratylenchus vulnus*.

Regarding *P. vulnus*, the result obtained here agrees with the result obtained by Handoo *et al.* (2021). In the present study, several sequences for *P. vulnus* populations were used. The result indicated a genetic variation among

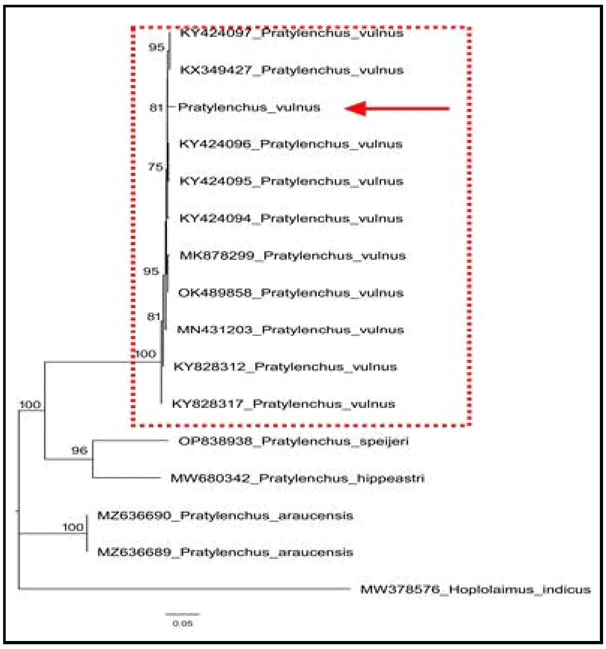

Fig. 2. Maximum likelihood tree of *Prtylenchus vulnus* from South Africa (red arrow color) and other related taxa based on the sequence of the mtDNA, cox1 region.

P. vulnus populations from different geographic regions was detected, as reported by Handoo *et al.* (2021). The same result was obtained in the present study. Besides, the biological and morphological characters varied among populations of *P. vulnus*. This species of *Pratylenchis* is cosmopolitan species that feed on various crops. They are the most crucial soil nematode because they are distributed worldwide, especially in sandy soil, as indicated by Handoo *et al.* (2021). Therefore, they are getting important in crop production, as they are endoparasite nematodes. Here in the present study, a sequence of *P. vulnus* from South Africa is provided. The result indicated that the mtDNA sequence placed the present species within the relevant taxa and confirmed its identification and phylogenetic position. However, an ecological study is suggested to determine the role of *P. vulnus* in the banana fields of South Africa.

CONCLUSION

In conclusion, mtDNA can be a helpful marker for diagnosing the family Pratylenchidae, especially for the *Pratylenchus*. However, using other DNA markers to understand the phylogeny of *Pratylenchus* is recommended.

REFERENCES

Abolafia, J. and Shokoohi, E. (2017). Description of *Stegelletina lingulata* sp. n. (Nematoda, Rhabditida, Cephalobidae) from xeric environments in Iran. *Zootaxa.* **4358**: 462-70.

Aminisarteshnizi, M. (2021a). Phylogenetic position of *Aphelenchus avenae* (Nematoda: Aphelenchidae) using 28S rDNA from South Africa. *Res. Crop.* **22**: 692-95.

Aminisarteshnizi, M. (2021b). Lipid content in the juvenile, female and male of *Acrobeles complexus* nematode. *Res. Crop.* **22**: 167-70.

Aminisarteshnizi, M. (2021c). Phylogenetic position of *Diploscapter coronatus* (Nematoda: Rhabditida) using 28S rDNA from South Africa. *Res. Crop.* **22**: 696-99.

Castillo, P. and Vovlas, N. (2007). Pratylenchus (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. Nematology Monographs and Perspectives 6 (Series editors: Hunt, D. J. and Perry, R. N.) Leiden, The Netherlands, Brill. pp. 529.

Derycke, S., Vanaverbeke, G., Rigaux, A., Backljau, 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**: 1-9.

Handoo, Z. A., Yan, G., Kantor, M. R., Huang, D., Chowdhury, I.. A. Plaisance, A., Bauchan, G. R. and Mowery, J. D. (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.*

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**: 1547-49.

Shokoohi, E. (2022a). Observation on *Hemicriconemoides brachyurus* (Loos, 1949) Chitwood & Birchfield, 1957 associated with grass in South Africa. *Helminthologia* **59**: 210-16. *https://doi.org/10.2478/helm-2022-0019.*

Shokoohi, E. (2022b). First report of *Tripylina zhejiangensis* associated with grassland in South Africa. *Helminthologia* **59**: 311-16. *https://doi.org/10.2478/helm-2022-0025.*

Shokoohi, E. (2022c). Plant extracts and their effects on plant-parasitic nematodes, with case studies from Africa. In: *Sustainable Management of Nematodes in Agriculture*, Vol. 1: *Organic Management, Sustainability in Plant and Crop Protection*, Chaudhary, K. K. and Meghvansi, M. K. (eds.). Springer, Cham. International Publishing. pp. 189-216. *https://doi.org/10.1007/978-3-031-09943-4_8.*

Shokoohi, E. and Abolafia, J. (2019). *Soil and Freshwater Rhabditid Nematodes (Nematoda, Rhabditida) from Iran: A Compendium.* University of Jaen (UJA) Publishing. 226 pp.

Shokoohi, E. and Abolafia, J. (2022a). Morphological and molecular characterization of Hemicycliophora poranga Monteiro and Lordello, 1978 (Nematoda: Rhabditida: Hemicycliophoridae) from Iran and South Africa. *Biologia* **77**: 709-20. *https://doi.org/10.1007/s11756-021-00990-9.*

Shokoohi, E. and Abolafia, J. (2022b). Observation on the *Rotylenchus brevicaudatus* Colbran, 1962 from Botswana, with the first SEM of the species. *Biologia* **77**: 3501-09. *https://doi.org/10.1007/s11756-022-01240-2.*

Shokoohi, E., Mehrabi-Nasab, A., Abolafia, J. and Holovachov, O. (2013). Study of the genus *Plectus* Bastian, 1865 (Nematoda: Plectidae) from Iran. *Biologia* **68**: 1142-54. *https://doi.org/10.2478/s11756-013-0264-5.*