



## *Paratylenchus shenzhenensis* n. sp. (Nematoda: Paratylenchinae) from the rhizosphere soil of *Anthurium andraeanum* in China

KE WANG, HUI XIE<sup>1</sup>, YU LI, CHUN-LING XU, LU YU & DONG-WEI WANG

Laboratory of Plant Nematology and Research Center of Nematodes of Plant Quarantine, Guangdong Province Key Laboratory of Microbial Signals and Disease Control / Department of Plant Pathology, College of Nature Resources and Environment, South China Agricultural University, Guangzhou 510642, China

<sup>1</sup>Corresponding author. E-mail: [xiehui@scau.edu.cn](mailto:xiehui@scau.edu.cn)

### Abstract

*Paratylenchus shenzhenensis* n. sp. was collected from the rhizosphere soil of *Anthurium andraeanum* in Shenzhen, Guangdong Province, China. The new species is characterized by having a female with a small body (249–302  $\mu\text{m}$ ), well developed stylet (17–21  $\mu\text{m}$ ), rounded head with four submedian lobes and lip-region with a slight depression at the oral area, small post-vulval uterine sac with a few vestigial cells; male with body dorsally curved behind the cloacal opening, stylet absent, pharynx degenerate, prominent penial sheath; and juveniles with a stylet. It is morphologically similar to *P. minutus*. The internal transcribed spacer sequences of ribosomal DNA (ITS-rDNA) of the new species only have 72–73% identity with *P. minutus*, confirming its status as a separate species. The D2/D3 region of 28S ribosomal DNA (28S rDNA) and 18S small subunit ribosomal DNA (18S rDNA) from *P. shenzhenensis* n. sp. were also amplified and sequenced in this study.

**Key words:** new species, morphology, molecular, taxonomy

### Introduction

The pin nematodes, *Paratylenchus* Micoletzky, 1922 are root ectoparasites of various plants, such as grasses, crops, trees, ornamentals, perennial plants, etc and are found world-wide (Raski 1991). They are especially common in orchard soils and can reach huge numbers (Siddiqi 2000). At present, the taxonomy of *Paratylenchus* is based on morphological characterization only, and molecular data has rarely been explored for identification of species of this genus. More than 100 species have been described (Brzeski & Hanel 2000; Siddiqi 2000), and many are known pathogens of their hosts (Brzeski 1995). For example, *P. bukowinensis* parasitizes parsley and carrot and causes malformed, shorter or forked roots (Brzeski 1976, 1995). *Paratylenchus microdorus* inhibits the growth of red clover and lettuce, causing smaller leaves and fewer lateral roots (Andrassy 1985).

In China, a survey of plant nematodes around the roots of ornamental plants was carried out in 2012. A *Paratylenchus* population was collected from rhizosphere soil of *Anthurium andraeanum*, and is here described as a new species, *Paratylenchus shenzhenensis* n. sp. The internal transcribed spacer sequences of ribosomal DNA (ITS-rDNA), D2/D3 region of 28S ribosomal DNA (28S rDNA) and 18S small subunit ribosomal DNA (18S rDNA) from *P. shenzhenensis* n. sp. were amplified and sequenced. Scanning electron micrographs of it are presented in this study.

### Material and methods

**Nematode population.** Soil samples were obtained from the rhizosphere of *Anthurium andraeanum* growing in a nursery in Shenzhen, Guangdong Province. Nematodes were extracted using the modified Baermann funnel method and the pin nematodes were picked out by hand for examination.

Nine sequences were obtained from the D2/D3 region of 28S rDNA of *Paratylenchus shenzhenensis* n. sp. Intraspecific variation of the sequences from the D2/D3 region (GenBank KF668516–KF668524) for *P. shenzhenensis* n. sp. was 0–6 bp. There was at least 145 bp difference between the new species and two other sequenced *Paratylenchus* species, *P. bukowinensis* Micoletzky, 1922 and *P. nanus* Cobb, 1923 (GenBank AY780943 and AY780946) in D2/D3 region of 28S rDNA and the sequence identity was 73–74%.

Eleven newly obtained 18S rDNA sequences of *Paratylenchus shenzhenensis* n. sp. were 1731 bp in length and the intraspecific variation for this species was 0–10 bp. At present, only six 18S rDNA sequences from other *Paratylenchus* species are available: one from *P. neoamblycephalus* Geraert, 1965 (GenBank AY284634), two from *P. microdorus* (GenBank AY284632 and AY284633), two from *P. straeleni* (de Coninck, 1931) Oostenbrink, 1960 (GenBank AY284630 and AY284631) and one from *P. dianthus* Jenkins & Taylor, 1956 (GenBank AJ966496). The 18S rDNA sequences of *P. shenzhenensis* n. sp. (GenBank KF668494–KF668504) differed from other sequenced *Paratylenchus* species by 119–148 bp and the sequence identity between the new species and the morphologically similar species *P. microdorus* was 92–93%.

## Discussion

It is a challenging task to identify species of the genus *Paratylenchus* accurately, given their small size and the overlap of many measurements and characters between different species (Raski 1975b). Over the past 20 years, many studies have suggested that sequences of rDNA are a valuable marker for the taxonomy of nematodes (Powers *et al.* 1997; Uehara *et al.* 1998; Gasser & Newton 2000; Holterman *et al.* 2006; Meldal *et al.* 2007). Based on morphological features and morphometrics, *P. shenzhenensis* n. sp. is closest to *P. minutus*. However, there are big differences in the ITS sequences of *P. shenzhenensis* n. sp. and *P. minutus*. The sequence identity between the two species was only 72–73% and there was at least 347 bp difference between the two species in ITS region, while the intraspecific sequence identity for *P. shenzhenensis* n. sp. was at least 97% and at least 99.6% in *P. minutus* (Chen *et al.* 2009). Thus, both the morphological and molecular data support *P. shenzhenensis* n. sp. as a new species.

In addition, molecular sequence data is useful in the identification of closely similar species or cryptic species (Chilton *et al.* 1995). However, little molecular data for *Paratylenchus* is available and there are not enough sequenced species of *Paratylenchus* to support a phylogenetic analysis. Molecular data should be included in future species descriptions and may be invaluable for identification of *Paratylenchus* species.

## Acknowledgement

This work was supported by a Special Project of Scientific and Technological Basis of the Ministry of Science and Technology of the People's Republic of China to Hui Xie (Grant no. 2006FY120100).

## References

- Andrássy, I. (1959) Neue und wenig bekannte Nematoden aus Jugoslawien. *Annales Historico Naturales Musei Nationalis Hungarici*, 51, 259–275.
- Andrássy, I. (1985) *Paratylenchus microdorus*. *CIH Descriptions of Plant-parasitic Nematodes. Set 8, No. 107*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, 2 pp.
- Bernard, E.C. (1982) Criconematina (Nematoda: Tylenchida) from the Aleutian Islands. *Journal of Nematology*, 14, 323–331.
- Brzeski, M.W. (1976) *Paratylenchus bukowinensis*. *CIH Descriptions of Plant-parasitic Nematodes. Set 6, No. 79*. Commonwealth Agricultural Bureaux, Farnham Royal, UK, 2pp.
- Brzeski, M.W. (1995) Paratylenchinae: Morphology of some known species and descriptions of *Gracilacus bilineata* sp. n. and *G. vera* sp. n. (Nematoda: Tylenchulidae). *Nematologica*, 41, 535–565.  
<http://dx.doi.org/10.1163/003925995x00495>
- Brzeski, M.W. & Háněl, L. (2000) Paratylenchinae: evaluation of diagnostic morpho-biometrical characters of females in the genus *Paratylenchus* Micoletzky, 1922 (Nematoda: Tylenchulidae). *Nematology*, 2, 253–261.  
<http://dx.doi.org/10.1163/156854100509097>
- Chen, D.Y., Ni, H.F., Yen, J.H. & Tsay, T.T. (2009) Identification of a new recorded pin nematode *Paratylenchus minutus* (Nematoda: Criconematoidea, Tylenchulidae) in Taiwan. *Plant Pathology Bulletin*, 18, 167–174.

- Chilton, N.B., Gasser, R.B. & Beveridge, I. (1995) Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the *Hypodontus macropi* complex (Nematoda: Strongyloidea). *International Journal for Parasitology*, 25, 647–651.  
[http://dx.doi.org/10.1016/0020-7519\(94\)00171-j](http://dx.doi.org/10.1016/0020-7519(94)00171-j)
- Esser, R.P. (1992) A diagnostic compendium to species included in Paratylenchinae Thorne, 1949 and *Tylenchocriconema* Raski & Siddiqi, 1975 (Nematoda: Criconematoidea). *Nematologica*, 38, 146–163.  
<http://dx.doi.org/10.1163/187529292x00135>
- Gasser, R.B. & Newton, L.A. (2000) Genomic and genetic research on bursate nematodes: significance, implications and prospects. *International Journal for Parasitology*, 30, 509–534.  
[http://dx.doi.org/10.1016/S0020-7519\(00\)00021-7](http://dx.doi.org/10.1016/S0020-7519(00)00021-7)
- Geraert, E. (1965) The genus *Paratylenchus*. *Nematologica*, 11, 301–334.  
<http://dx.doi.org/10.1163/187529265x00221>
- Harris, T.S., Sandall, L.J., & Powers, T.O. (1990) Identification of single *Meloidogyne* juveniles by polymerase chain reaction amplification of mitochondrial DNA. *Journal of Nematology*, 22, 518–524.
- Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution*, 23, 1792–1800.  
<http://dx.doi.org/10.1093/molbev/msl044>
- Jenkins, W.R. & Taylor, D.P. (1956) *Paratylenchus dianthus* n. sp. (Nematoda: Criconematidae) a parasite of carnation. *Proceedings of the Helminthological Society of Washington*, 23, 124–127.
- Khan, E., Prasad, S.K. & Mathur, V.K. (1967) Two new species of the genus *Paratylenchus* Micoletzky, 1922 (Nematoda: Criconematidae) from India. *Nematologica*, 13, 79–84.  
<http://dx.doi.org/10.1163/187529267x00959>
- Linford, M.B., Oliveira, J.M. & Ishii, M. (1949) *Paratylenchus minutus* n. sp., a nematode parasitic on roots. *Pacific Science*, 3, 111–119.
- Meldal, B.H., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., Austen, M.C., Blaxter, M.L., Rogers, A.D. & Lamshead, P.J.D. (2007) An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Molecular Phylogenetics and Evolution*, 42, 622–636.  
<http://dx.doi.org/10.1016/j.ympev.2006.08.025>
- Mohilal, N. & Dhanachand, Ch. (2004) Two new species of Paratylenchidae from Manipur. *Uttar Pradesh Journal of Zoology*, 24, 173–177.
- Powers, T.O., Todd, T.C., Burnell, A.M., Murray, P.C.B., Fleming, C.C., Szalanski, A.L., Adams, B.A. & Harris, T.S. (1997) The rDNA internal transcribed spacer region as a taxonomic marker for nematodes. *Journal of Nematology*, 29, 441–450.
- Pramodini, M. & Mohilal, N. (2009) One new and three known species of *Paratylenchus* (Nematoda: Tylenchulidae) from Manipur, India. *Journal of Threatened Taxa*, 1, 177–179.  
<http://dx.doi.org/10.11609/jott.o1744.177-9>
- Raski, D.J. (1975a) Revision of the genus *Paratylenchus* Micoletzky, 1922 and descriptions of new species. Part I. *Journal of Nematology*, 7, 15–34.
- Raski, D.J. (1975b) Revision of the genus *Paratylenchus* Micoletzky, 1922, and descriptions of new species. Part II. *Journal of Nematology*, 7, 274–295.
- Raski, D.J. (1991) Tylenchulidae in Agricultural. In: Nickle, W.R. (Ed.), *Manual of Agricultural Nematology*. Marcel Dekker, Inc., New York, pp. 761–794.
- Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67–69.  
<http://dx.doi.org/10.1163/187529259x00381>
- Siddiqi, M.R. (2000) *Tylenchida parasites of plants and insects*, 2nd edition. CABI Publishing, Wallingford, UK, 833 pp.
- Subbotin, S.A., Vovlas, N., Crozzoli, R., Sturhan, D., Lamberti, F., Moens, M. & Baldwin, J.G. (2005) Phylogeny of Criconematina Siddiqi, 1980 (Nematoda: Tylenchida) based on morphology and D2/D3 expansion segments of the 28S-rRNA gene sequences with application of a secondary structure model. *Nematology*, 7, 927–944.  
<http://dx.doi.org/10.1163/156854105776186307>
- Thorne, G. & Malek, R.B. (1968) *Nematodes of the Northern Great Plains. Part I. Tylenchida (Nemata: Secernentea)*. Tech. Bull. S. Dakota agric. Exp. Stn. 31, 111 pp.
- Uehara, T., Mizukubo, T., Kushida, A. & Momota, Y. (1998) Identification of *Pratylenchus coffeae* and *P. loosi* using specific primers for PCR amplification of ribosomal DNA. *Nematologica*, 44, 357–368.  
<http://dx.doi.org/10.1163/005525998x00034>
- Vrain, T.C., Wakarchuk, D.A., Levesque, A.C. & Hamilton, R.I. (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology*, 15, 563–573.
- Wang, J.L., Zhang, J.C. & Gu, J.F. (2011) Method of extract DNA from a single nematode. *Plant Quarantine*, 25, 32–35.
- Xie, H. (2005) *Taxonomy of plant nematodes*, 2nd edition. Beijing, P.R. China, Higher Education Press, pp. 38–50.
- Xu, C.L., Xie, H. & Li, Y. (2012) Description of *Neodolichodorus hainanensis* n. sp. (Nematoda: Dolichodoridae) from rhizosphere soil of golf turf in China. *Zootaxa*, 3236, 62–68.