Zootaxa 3821 (4): 465–475
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3821.4.5
http://zoobank.org/urn:lsid:zoobank.org:pub:3D6C9C52-B778-4387-95FD-83945478EBF9

Morphological and molecular characterization of *Zygotylenchus gansuensis* n. sp. (Nematoda: Pratylenchinae) from China

HONGHONG WANG[1,2,3], KAN ZHUO[1,2,3] & JINLING LIAO[1,2,4]

[1]*Laboratory of Plant Nematology, South China Agricultural University, Guangzhou 510642, China*
[2]*Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou, China*
[3]*These authors contributed equally to this work*
[4]*Corresponding author. E-mail: jlliao@scau.edu.cn*

Abstract

Zygotylenchus gansuensis **n. sp.** is described and illustrated from the rhizosphere of jujube (*Zizyphus jujuba* Mill.) based on morphology and molecular analyses. This new species is characterized by a low and flattened labial region with three annuli, stylet 14.1±0.5 (13.0–14.9) µm long, deirids absent, five lateral lines in the vulval region, pharyngeal glands overlapping ventrally or ventrolaterally, V = 56.4±1.6 (54.0–60.8), indistinct spermatheca, subcylindrical tail with smooth and rounded terminus, and males absent. Molecular analyses show that the species has unique partial SSU, LSU D2D3 and ITS rRNA sequences. Phylogenetic relationships of *Z. gansuensis* **n. sp.** with other nematodes in Pratylenchinae are analysed using SSU and LSU D2D3.

Key words: *Zygotylenchus*, new species, morphology, molecular, morphometrics, phylogeny

Introduction

Siddiqi (1963) proposed the genus *Zygotylenchus* with a new species *Z. browni* Siddiqi, 1963; this species was later synonymized with *Z. guevarai* (Tobar-Jiménez, 1963) Braun & Loof, 1966. In the same year, two other species, *Pratylenchoides guevarai* Tobar-Jiménez, 1963 and *Mesotylus gallicus* de Guiran, 1964 were described (Tobar-Jiménez 1963, de Guiran 1964). Tarjan and Weischer (1965) later suggested that these three species were the same and synonymized the genera *Zygotylenchus* and *Mesotylus* de Guiran, 1964 with *Pratylenchoides* Winslow, 1958. But Braun and Loof (1966) considered *Pratylenchoides* to be different from *Zygotylenchus* (syn. *Mesotylus*), supporting *Zygotylenchus* as a valid genus. De Guiran and Siddiqi (1967) agreed, citing differences in the basal part of the pharynx as a basis for separating *Zygotylenchus* and *Pratylenchoides*.

The genus *Zygotylenchus* is morphologically similar to the genus *Pratylenchus* Filipjev, 1936 except in vulva position and number of ovaries. Both genera were placed in the subfamily Pratylenchinae Thorne, 1949 within the family Pratylenchidae Thorne, 1949 according to the classification of Siddiqi (2000). At present, this genus includes only three valid species: *Z. guevarai* (Tobar-Jiménez, 1963) Braun & Loof, 1966 (syn. *Pratylenchoides guevarai* Tobar Jiménez, 1963, *Zygotylenchus browni* Siddiqi, 1963, *Mesotylus gallicus* de Guiran, 1964 and *Zygotylenchus gallicus* (de Guiran, 1964) Braun & Loof, 1966), *Z. taomasinae* (de Guiran, 1964) Braun & Loof, 1966 and *Z. natalensis* van den Berg & Tiedt, 2003 (Siddiqi 2000, van den Berg & Tiedt 2003). Among these species, the type species *Z. guevarai* is widely distributed in Europe and Asia (Siddiqi 2000, Urek *et al.* 2003, Majd Taheri *et al.* 2013), while the other two species are less commonly encountered. *Z. taomasinae* has been found in France and South Africa, and *Z. natalensis* has only been reported from the type locality in South Africa (de Guiran 1964, van den Berg 1986). In China, species of *Pratylenchus* are widespread, but *Zygotylenchus* species have not been reported to date.

During a survey of plant nematodes in the family Pratylenchidae conducted in China beginning in 2010, an undescribed *Zygotylenchus* species was isolated from the rhizosphere of jujube (*Zizyphus jujuba* Mill.) in July 2013. Morphological observations and further molecular analyses indicated that this species is different from any known *Zygotylenchus* species. Therefore, it is described herein as a new species, *Zygotylenchus gansuensis* **sp. n.**

Accepted by P. Mullin: 21 May 2014; published: 24 Jun. 2014

the new species with the available ITS sequence of *Z. guevarai* (FJ717817, Palomares-Rius *et al.* 2010) at interspecific level showed divergence between 47.0–47.4%. A phylogenetic analysis was not conducted because of limited availability of comparable sequences.

Discussion

In China, the genus *Zygotylenchus* has not been reported to date, and the finding of *Z. gansuensis* **sp. n.** expands the geographic limits for this genus. *Z. guevarai* is a migratory endoparasite that feeds and reproduces in the root cortex, and induces large cavities (Vovlas *et al*. 1976, Siddiqi 2000). Whether *Z. gansuensis* **sp. n.** has the same parasitic behavior is still unknown. Preliminary attempts to raise a population on carrot discs failed, hindering further research on pathogenicity and host-parasite relationships of *Z. gansuensis* **sp. n.** on associated plants.

Siddiqi (2000) summarized the diagnostic morphological characters for the genus *Zygotylenchus*. Except for five lateral lines in the vulval region [(four lateral lines in the diagnostic morhological characters of Siddiqi (2000)], the other morphological characters of our new species conform to diagnostic characters by Siddiqi (2000). However, in some specimens of *Z. taomasinae* as decribed by van den Berg (1986), a fifth fainter and broken line was situated in between the two middle lateral lines. Therefore, *Zygotylenchus* may have both four and five lateral lines.

Interestingly, *Z. gansuensis* **sp. n.** and *Z. guevarai* do not form monophyletic groups in phylogenetic trees derived using SSU or LSU sequences, but instead show closer relationships with some *Pratylenchus* species. The paraphyly of the genus *Pratylenchus* had been proposed by several authors based on phylogenetic analyses inferred from SSU and/or LSU D2D3 (Al-Banna *et al.* 1997, Carta *et al.* 2001, De Luca *et al.* 2004, Holterman *et al.* 2009, Palomares-Rius *et al*. 2010). It seems that *Zygotylenchus* may be a paraphyletic assemblage, as reported for *Pratylenchus*.

However, except in vulval position and the number of ovaries, there are no other significant differences in morphology between *Zygotylenchus* and *Pratylenchus*. Intriguingly, in some females of *P. zeae*, the gonads follow a developmental pattern of uterus didelphy up to the fourth-stage juvenile, the posterior remnant ovary being formed with some differentiated cellular tissue (Román & Hirschmann 1969). In addition, a phylogenetic tree based on LSU D2D3 given by Majd Taheri *et al.* (2013) showed *Z. guevarai* was placed in the *Pratylenchus* clade. These results, in combination with the LSU D2D3 and SSU phylogenies in our study, suggest that *Zygotylenchus* may be a synonym of *Pratylenchus*, and the vulva position and number of ovaries may be not valid generic characters, as is the case in *Xiphinema* (Luc 1981). Unfortunately, only limited DNA sequence data are available for the genus *Zygotylenchus*. As more sequence data become available, phylogenetic relationships should be re-evaluated.

Acknowledgements

This research was supported by the Special Fund for Agro-scientific research in the Public Interest of China (grant number 201103018), National Key Basic Research Program of China (973 Program) (grant number 2013CB127501) and the Planning Project for Science and Technology in Guangzhou City (grant number 11A62100574)

References

Al-Banna, L., Williamson, V.M. & Gardner, S.L. (1997) Phylogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26S rDNA. *Molecular Phylogenetics and Evolution*, 7, 94–102.

Braun, A.L. & Loof, P.A.A. (1966) *Pratylenchoides laticauda* n. sp., a new endoparasitic phytonematode. *Netherlands Journal of Plant Pathology*, 72, 241–245. http://dx.doi.org/10.1007/bf02650210

Carta, L.K., Skantar, A.M. & Handoo, Z.A. (2001) Molecular, morphological and thermal characters of 19 *Pratylenchus* spp. and relatives using the D3 segment of the nuclear LSU rRNA gene. *Nematropica*, 31, 193–207.

Castillo, P., Vovlas, N., Subbotin, S. & Troccoli, A. (2003) A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in Southern Spain. *Phytopathology*, 93, 1093–1102.
http://dx.doi.org/10.1094/phyto.2003.93.9.1093

Chizhov, V.N., Chumakova, O.A., Subbotin, S.A. & Baldwin, J.G. (2006) Morphological and molecular characterization of foliar nematodes of the genus *Aphelenchoides*: *A. fragariae* and *A. ritzemabosi* (Nematoda: Aphelenchoididae) from the Main Botanical Garden of the Russian Academy of Sciences. *Russian Journal of Nematology*, 14, 179–184.

De Guiran, G. (1964) *Mesotylus*: nouveau genre de Pratylenchinae (Nematoda: Tylenchoidea). *Nematologica*, 9 (1963), 567–575.
http://dx.doi.org/10.1163/187529263x00656

De Guiran, G. & Siddiqi, M.R. (1967) Characters differentiating the genera *Zygotylenchus* Siddiqi, 1963 (syn. *Mesotylus* de Guiran, 1964) and *Pratylenchoides* Winslow, 1958 (Nematoda: Pratylenchinae). *Nematologica*, 13, 235–240.
http://dx.doi.org/10.1163/187529267x00094

De Luca, F., Fanelli, E., Di Vito, M., Reyes, A. & De Giorgi, C. (2004) Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of *Pratylenchus* from the Mediterranean region. *European Journal of Plant Pathology*, 110, 949–957.
http://dx.doi.org/10.1007/s10658-004-0813-4

Feng, Z. (2001) *Plant Nematology*. Beijing, P.R. China, Chinese Agricultural Publishing, 170–175.

Filipjev, I.N. (1936) On the classification of the Tylenchinae. *Proceedings of the Helminthological Society of Washington*, 3, 80–82.

Holterman, M., Karssen, G., Van Den Elsen, S., Van Megen, H., Bakker, J. & Helder, J. (2009) Small subunit rDNA-based phylogeny of the Tylenchida sheds light on relationships among some high-impact plant-parasitic nematodes and the evolution of plant feeding. *Phytopathology*, 99, 227–235.
http://dx.doi.org/10.1094/phyto-99-3-0227

Huelsenbeck, J.P. & Ronquist, F. (2001) MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 1754–1755.
http://dx.doi.org/10.1093/bioinformatics/17.8.754

Larget, B. & Simon, D.L. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution*, 16, 750–759.
http://dx.doi.org/10.1093/oxfordjournals.molbev.a026160

Luc, M. (1981) Observations on some *Xiphinema* species with the female anterior genital branch reduced or absent (Nematoda: Longidoridae). *Revue de Nématologie*, 4, 157–167.

Majd Taheri, Z., Tanha Maafi, Z., Subbotin, S.A., Pourjam, E. & Eskandari, A. (2013) Molecular and phylogenetic studies on Pratylenchidae from Iran with additional data on *Pratylenchus delattrei*, *Pratylenchoides alkani* and two unknown species of *Hirschmanniella* and *Pratylenchus*. *Nematology*, 15, 633–651.
http://dx.doi.org/10.1163/15685411-00002707

Palomares-Rius, J.E., Castillo, P., Liebanas, G., Vovlas, N., Landa, B.B., Navas-Cortes, J.A. & Subbotin, S.A. (2010) Description of *Pratylenchus hispaniensis* n. sp. from Spain and considerations on the phylogenetic relationship among selected genera in the family Pratylenchidae. *Nematology*, 12, 429–451.
http://dx.doi.org/10.1163/138855409x12559479585043

Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.
http://dx.doi.org/10.1093/bioinformatics/14.9.817

Román, J. & Hirschmann, H. (1969) Embryogenesis and postembryogenesis in species of *Pratylenchus* (Nematoda: Tylenchidae). *Proceedings of the Helminthological Society of Washington*, 36, 164–174.

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. (1963) On the classification of the Pratylenchidae (Thorne, 1949) nov. grad. (Nematoda: Tylenchida), with a description of *Zygotylenchus browni* nov. gen. *et* nov. sp. *Zeitschrift für Parasitenkunde*, 23, 390–396.
http://dx.doi.org/10.1007/bf00331238

Siddiqi, M.R. (2000) Tylenchida parasites of plants and insects, 2nd edition. Wallingford, UK, CABI Publishing, 833 pp.

Subbotin, S.A., Ragsdale, E.J., Mullens, T., Roberts, P.A., Mundo-Ocampo, M. & Baldwin, J.G. (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. *Molecular Phylogenetics and Evolution*, 48, 491–505.
http://dx.doi.org/10.1016/j.ympev.2008.04.028

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology*, 8, 455–474.
http://dx.doi.org/10.1163/156854106778493420

Swofford, D.L. (1998) PAUP: Phylogenetic analysis using parsimony, version 4.0. Sunderland, Massachusetts: Sinauer.

Tanha Maafi, Z., Subbotin, S.A. & Moens, M. (2003) Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on the ITS sequences of rDNA. *Nematology*, 5, 99–111.

http://dx.doi.org/10.1163/156854102765216731
Tarjan, A.C. & Weischer, B. (1965) Observations on some Pratylenchinae (Nemata), with additional data on *Pratylenchoides guevarai* Tobar-Jimenez, 1963 (syn: *Zygotylenchus browni* Siddiqi, 1963 and *Mesotylus gallicus* de Guiran, 1964). *Nematologica*, 11, 432–440.
http://dx.doi.org/10.1163/187529265x00357
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121
Thorne, G. (1949) On the classification of the Tylenchida, new order (Nematoda, Phasmidia). *Proceedings of the Helminthological Society of Washington,* 16, 37–73.
Tobar-Jiménez, A. (1963) *Pratylenchoides guevarai* n. sp., nuevo nematode tylenchida, relacionado con el cipres (*Cupressus sempervirens* L.). *Revista Ibérica de Parasitología*, 23, 27–36.
Urek, G., Sirca, S. & Karssen, G. (2003) A review of plant-parasitic and soil nematodes in Slovenia. *Nematology*, 5, 391–403.
http://dx.doi.org/10.1163/156854103769224386
van den Berg, E. (1986) *Zygotylenchus taomasinae* (de Guiran, 1964) Braun & Loof, 1966 from South Africa with a note on *Pratylenchus crenatus* Loof, 1960 (Tylenchoidea: Nematoda). *Phytophylactica*, 18, 17–19.
van den Berg, E. & Tiedt, L.R. (2003) *Zygotylenchus natalensis* sp. n. (Nemata: Pratylenchidae) from a potato field in South Africa. *Journal of Nematode Morphology and Systematics*, 5, 145–151.
Vovlas, N., Inserra, R.N. & Lamberti, F. (1976) Observations on the pathology of *Zygotylenchus guevarai* (Tobar) Brown and Loof. *Nematologica Mediterranea*, 4, 183–193.
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.
Winslow, R.D. (1958) The taxonomic position of *Anguillulina obtusa* Goodey, 1932 and 1940. *Nematologica*, 3, 136–139.
http://dx.doi.org/10.1163/187529258x00210
Zhuo, K., Cui, R.Q., Ye, W.M., Luo, M., Wang, H.H., Hu, X.N. & Liao, J.L. (2010) Morphological and molecular characterization of *Aphelenchoides fujianensis* n. sp. (Nematoda: Aphelenchoididae) from *Pinus massoniana* in China. *Zootaxa*, 2509, 39–52.