DOI 10.2478/s11687-014-0252-5

HELMINTHOLOGIA, 51, 4: 352 – 354, 2014

Research Note

Pratylenchus brachyurus (Nematoda: Pratylenchidae) in Guariroba in the state of Goiás, Brazil

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Summary

Roots of *Syagrus oleracea* were collected in two growing areas in the municipality of Rio Verde, state of Goiás (Brazil). Morphological, morphometric and molecular (ITS1 sequences) approaches allowed us to identify the infecting nematode as *Pratylenchus brachyurus*. To our knowledge, this is the first record of *P. brachyurus* parasitizing guariroba elsewhere (new host).

Keywords: Brazil; guariroba; molecular approaches; *Pratylenchus brachyurus*; taxonomy

Introduction

Guariroba (*Syagrus oleracea* Becc) is a very important native palm tree in the Brazilian Cerrado, mainly in the state of Goiás. This palm is widely appreciated and used in the local cuisine (Melo, 2003). Nevertheless, as an emerging and small crop, information on guariroba diseases and/or parasites is scarce. In this sense, only fungal diseases have been mentioned until now (Oliveira *et al*., 2014).
Despite the wide guariroba distribution in the region of Brazilian Cerrado, no record on guariroba-nematode interactions was surveyed for us. Here, our objective was to describe morphologically and molecularly, a *Pratylenchus brachyurus* population infecting guariroba plants.

Materials and methods

In December 2012, samples of feeder roots of stunted plants, exhibiting small and inconspicuous lesions, were collected in municipality of Rio Verde (17° 47' 52" S 50° 55' 40" O), state of Goiás (Brazil). Previously, roots were washed with tap water and the extraction of specimens was carried out according to Coolen and D'Herde (1972). Later, nematode population was estimated.
Morphological, morphometric and molecular characterization were conducted to identify the specimens. Nematodes for morphological and morphometric studies (n=10) were heat killed and mounted on slides (formalin 2 %) (Cobb, 1918). Photomicrographs were recorded with a digital camera linked to a computer. We measure the following parameters: female body (*L*), maximum body diameter (*W*), stylet (*S*), the pharyngeal length (*Pl*), vulva position in relation to body length (*V*%) and tail length (*Tl*). The de Man's ratios (a, b and c) were also obtained (Siddiqi, 2000).
Foremost, specimens were preserved in 1M NaCl for DNA extraction. Total genomic DNA was obtained according to NaOH method (Stanton *et al*., 1998) and stored at -20 °C. Amplification of the 18S-ITS1-28S region of ribosomal RNA were performed using the Kit Taq PCR Master Mix (Promega) and the nematode universal primers rDNA2 (5'-

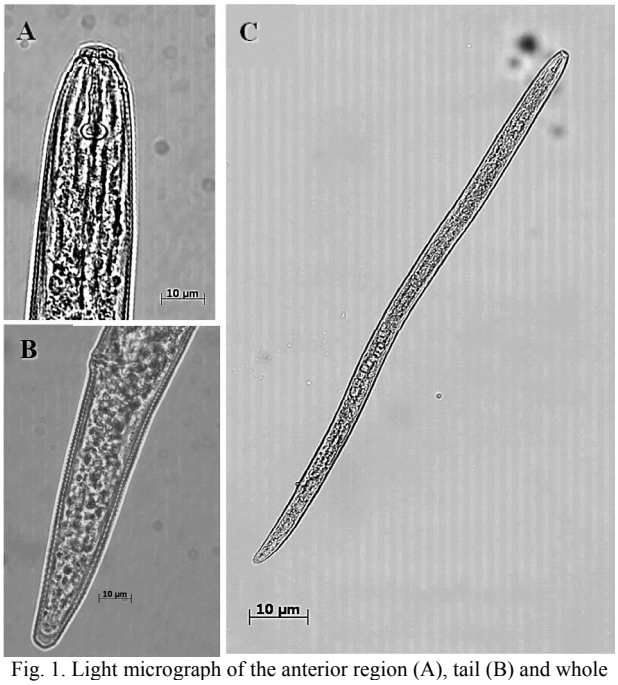

Fig. 1. Light micrograph of the anterior region (A), tail (B) and whole body (C) of female specimens of *Pratylenchus brachyurus*

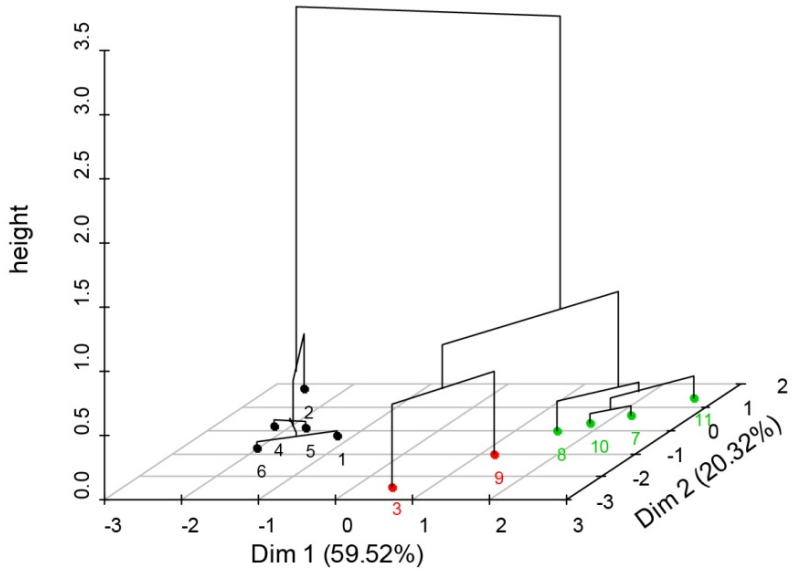

Fig. 2. Scarterplot obtained from morphometric data of female *Pratylenchus* spp. subjected to PCA. 1 – 6 (*P. brachyurus*); 7 (*P. coffeae*); 8 (*P.jaehni*); 9 (*P.zeae*); 10 (*P. penetrans*) and 11 (*P. vulnus*)

TTGATTACGTCCCTGCCCTTT-3') and rDNA1.58S (5'-ACGAGCCGAGTGATCCACCG-3'). In a microcentrifuge tube were added 25 µl of the Kit Taq PCR Master Mix, 1.5 µl (0.3 microM) from each primer, 18 µl water mili-Q and 4 µl total DNA. The DNA was subjected to a PCR with the following specifications: 94 °C (2 min); followed by 40 cycles at 94 °C (1 min), 57 °C (1 min) and 72 °C (2 min) (Cherry *et al*., 1997).

The sequence obtained was analyzed by BLASTn (www.ncbi.nlm.nih.gov/blast/Blast.cgi), deposited in the GenBank (accession number KC782525) and aligned with other *Pratylenchus* sequences using CLUSTAL W (Tompson *et al*., 1994). Finally, phylogenetic analysis was obtained by MEGA 5.1 (Tamura *et al*., 2011) using the algorithm UPGMA with Maximum Likelihood model and complete deletion (1000 replications). *Xiphinema rivesi* (Dorylaimida: Longidoridae; accession number JX912152.1) was included as outgroup.

Phenotypic characterization was obtained using multivariate techniques. For this purposes, hierarchical clustering analysis (Ward's method), based in a principal component analysis (*PCA*) obtained *a priori*, was performed using mean values of our measurements and some obtained in literature for *Pratylenchus* species (Román & Hirschmann, 1969; Café-Filho & Huang, 1978; Torres *et al*., 2004; Gonzaga, 2006; Machado *et al*., 2007). We used R 2.15.2 (R Development Core Team, 2012) for this analysis.

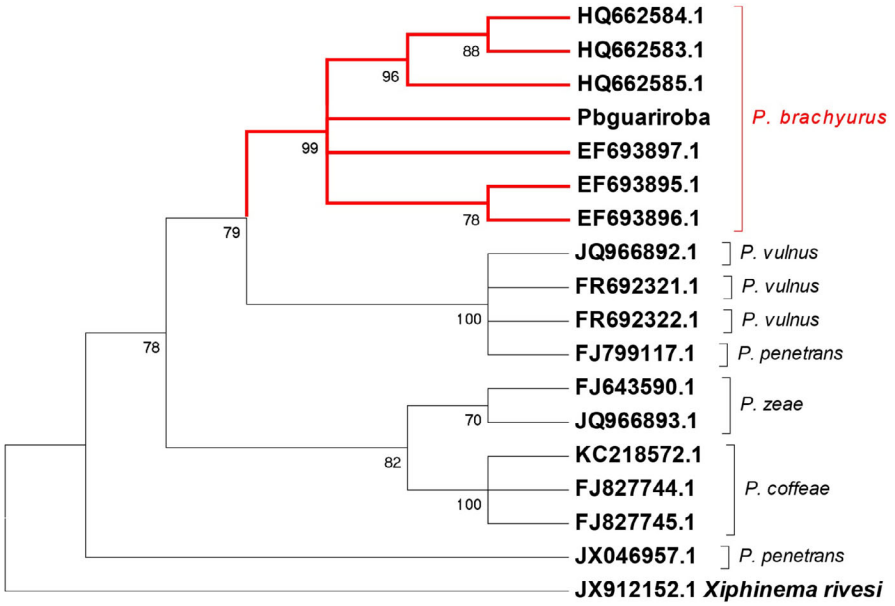

Fig.3. Phylogenetic tree (UPGMA) resulting from alignment of the partial sequences of the 18S-ITS1-5.8S of populations of *Pratylenchus* spp. Bootstrap values were obtained from 1,000 replications. Population isolated from Guariroba plants is indicated as $Pb_{guariroba}$

Results and discussion

The population density in the samples was 114 specimens per gram of roots. Characters observed were consistent with those described for *P. brachyurus* (Godfrey, 1929) Filipjev & S. Stekhoven, 1941 (Castillo & Vovlas, 2007); the labial region showed two annuli, *L* was 526.1 (±56.7) µm long, *W* was 21.9 (±3.4) µm long, *S* was 19.0 (±1.1) µm long, *Pl* was 86.9 (±7.4) µm long and V% was 85.4 % (±1.2). Males were not found. The *Tl* (29.9 ± 4.1 µm) was broadly conoid, smooth, with a broadly rounded, truncate tip (Fig. 1). The de Man's ratios obtained were: a = 24.4 ± 3.1; b = 6.1 ± 0.3 and c = 17.6 ± 1.6. The PCA performed here resulted in inertia of nearly 79.80%; by the hierarchical clustering analysis, our population belongs to the same cluster of others *P. brachyurus* populations and differed from other *Pratylenchus* species (Fig. 2).

Amplicon of ca. 519 pb in length obtained showed 93 to 99 % identity with known sequences of *P. brachyurus* (accession numbers HQ662584.1, EF693897.1 and EF693896.1). Phylogenetic analysis with maximum likelihood of those sequences placed the *Pratylenchus* sp. from Rio Verde in a clade (99 % bootstrap support) which included only *Pratylenchus* sequences available in the GenBank database thus confirming its identity (Fig. 3).

To our knowledge, this is the first report of *P. brachyurus* parasitizing guariroba roots in Brazil and elsewhere (new host). This findings have a great importance not only for the inclusion of these parasites in guariroba pathological scenario, but also for predicting possible damage in plant species associated with guariroba crop. Additional work is necessary in order to elucidate the losses caused by *P. brachyurus* on guariroba plants.

Acknowledgments

JVAF, MFBJ and FRCM thank CNPq (National Council for Scientific and Technological Development) and FAPESP (São Paulo Research Foundation) for their academic grant.

References

CAFÉ FILHO, A. C., HUANG, C. S. (1978): Nematóides do gênero *Pratylenchus* no Brasil. *Fitopatol. Bras*., 3: 232 – 235

CASTILLO, P., VOVLAS, N. (2007): *Pratylenchus (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management*. Koninklijke Brill NV, Leiden, 529pp.

CHERRY, T., SZALANSKI, A. L., TODD, T. C., POWERS, T. O. (1997): The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *J. Nematol*., 29 (1): 23 – 29

COBB, N. A. (1918): *Estimating the nema population of the soil, with special reference to the sugar-beet and root-gall nemas, Heterodera schachtii Schmidt and Heterodera radicicola (Greef) Muller, and with a description of Tylencholaimus aequalis n.sp*. USDA, Agricultural Technology Circular, Bureau Plant Industry, United States Department of Agriculture, 48pp.

COOLEN, W. A., D'HERDE, C. J. (1972): *A method for the quantitative extraction of nematodes from plant tissue*. Ghent, Belgium, State Agricultural and Entomology Research Station, 77pp.

GONZAGA, V. (2006): Caracterização morfológica, morfométrica e multiplicação in vitro das seis espécies mais comuns de *Pratylenchus* Filipjev, 1936 que ocorrem no Brasil. PhD thesis, Brazil, Jaboticabal: Universidade Estadual de São Paulo.

MACHADO, A. C. Z., MOTTA, L. C. C., SIQUEIRA, K. M. S., FERRAZ, L. C. C. B., INOMOTO, M. M. (2007). Host status of green manures for two isolates of *Pratylenchus brachyurus* in Brazil. *Nematology*, 9: 799 – 805. DOI: 10.1163/156854107782331153

MELO, J. T. (2003): *Cultivo de guariroba (Syagrus oleracea Becc) em sistemas consorciados com espécies florestais no cerrado*. EMBRAPA Cerrados, Planaltina. *Comunicado Técnico,* 97, 2pp.

OLIVEIRA, R. R., AGUIAR, R. L., TESSMANN, D. J., NUNES, W. M. C., SANTOS, A. F., VIDA, J. B. (2014): First Report of leaf spot caused by *Cladosporium perangustum* on *Syagrus oleracea* in Brazil. *Plant Dis*., 98(2): 280. DOI: 10.1094/PDIS-06-13-0595-PDN

THE R DEVELOPMENT CORE TEAM (2012): *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Retrieved June 15, 2013 from http://www.R-project.org.

ROMÁN, J., HIRSCHMANN, H. (1969): Morphology and morphometrics of six species of *Pratylenchus*. *J. Nematol*., 1: 363 – 386

SIDDIQI, M. R. (2000): *Tylenchida parasites of plants and insects*. 2nd Edition. Wallingford, UK: CABI Publishing, 852pp.

STANTON, J. M., MCNICOL, C. D., STEELE, V. (1998): Non-manual lysis of second-stage *Meloidogyne* juveniles for identification of pure and mixed samples based on the polymerase chain reaction. *Australas. Plant Pathol.*, 27: 112 – 115. DOI: 10.1071/AP98014

TAMURA, K., PETERSON, D., PERTERSON, N., STECHER, G., NEI, M., KUMAR, S. (2011): Mega 5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol*., 28: 2731-2739. DOI: 10.1093/molbev/msr121

TOMPSON, J. D., HIGGINS, D. G., GIBSON, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22(22): 4673 – 4680

TORRES, G. R. C., PEDROSA, E. M. R., SIQUEIRA, K. M. S., MOURA, R. M. (2004): *Pratylenchus brachyurus* em *Cucumis melo* no Brasil. *Fitopatol. Bras*., 29(6): 668 – 669. DOI: 10.1590/S0100-41582004000600013

RECEIVED MAY 9, 2014

ACCEPTED AUGUST 4, 2014