

***Pratylenchus* spp.: morphological, molecular characterization and population density in banana (*Musa* spp.) in Pernambuco**

Mayara Castro Assunção, Francisco Jorge Carlos de Souza Junior, Jaime Corbiniano dos Santos Neto, Arielena Augusta Rodrigues Mello, Liany Regina Bezerra de Oliveira Silva

Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil. E-mails: mayara_castroa@hotmail.com, jorgesouza@alu.ufc.br, jaime.corbiniano@hotmail.com, arielenaAugusta71@gmail.com, lianyregina@gmail.com

Abstract: The objective of this study was to determine the identity, morphological characteristics, molecular phylogenetic, and *Pratylenchus* populations and population density in areas grown with banana trees. Root collections were carried out at each point in banana orchards in Recife. Subsequently, the extractions and morphological and molecular identification of the nematodes were carried out. Two species were identified: *P. coffeae* and *P. brachyurus*, with a predominance of *P. brachyurus*. The occurrence of these species of nematodes, and the prevalence of *P. brachyurus* inform new data on parasitism *Pratylenchus* banana, and occasioning severe damage to the crop.

Keywords: Morphometry, Phylogeny, Nematode.

***Pratylenchus* spp.: caracterização morfológica, molecular e densidade populacional em bananeira (*Musa* spp.) em Pernambuco**

Resumo: O objetivo desse estudo foi determinar a identidade, características morfométricas, moleculares e filogenéticas de populações de *Pratylenchus* e densidade populacional em áreas cultivadas com bananeira. Foram realizadas coletas de raízes, em cada ponto, nos pomares de banana em Recife. Posteriormente, procedeu-se as extrações e identificação morfológica e molecular dos fitonematoides. Houve a identificação de duas espécies: *P. coffeae* e *P. brachyurus*, com predominância de *P. brachyurus*. A ocorrência dessas espécies de fitonematoides, e a prevalência de *P. brachyurus*, informam um novo dado sobre o parasitismo de *Pratylenchus* em banana, além de ocasionarem danos severos à cultura.

Palavras chave: Morfometria, Filogenia, Nematóide.

The banana (*Musa* spp. L.) has great economic and social importance, being the most consumed fruit in the world and the staple food of millions of people. Banana farming is highlighted in the world and Brazilian agribusiness, according to the Food and Agriculture Organization of the United Nations [FAO] (2017) the Brazil is the fourth largest producer. According to the Brazilian Institute of Geography and Statistics [IBGE] (2018) the Brazilian northeast region has 73% of national production, and the state of Pernambuco occupies the second position in this ranking.

As in other cultures, the banana is also subject to the occurrence of several diseases that limit its production, among those caused by nematodes, which can significantly reduce its productivity. Among the nematodes important for culture, the genus *Pratylenchus* Filipjev stands out, with the species *P. coffeae* (Zimmerman) Filipjev and Schuurmans Stekhoven being the most frequent (Kubo et al., 2013).

Nematodes of the genus *Pratylenchus*, are known as nematodes of root lesions, presenting wide geographic distribution, present in tropical and subtropical regions, and polyphages, being the second genus of economic importance in the world (Jones et al., 2013). These organisms are responsible for the destruction of the primary roots, which directly interferes with the plant fixation and results in its tipping (Sikora et al., 2018).

The correct diagnosis of the species is fundamental for the choice of effective management strategies, and for this reason, the association of morphometric studies and molecular techniques has been an important tool in this process. Thus, the objective of this study was to determine the identity, morphometric, molecular and phylogenetic characteristics of populations of *Pratylenchus* and population density in areas cultivated with banana trees.

During October 2019 to February 2020 the collections of populations of *Pratylenchus* were carried out in banana orchards (8°17'S 34°56'48"W) in the city of Recife, located in the metropolitan mesoregion of the state of Pernambuco. It collected 72 samples in two varieties moderately resistant to the nematode.

Nematodes were extracted from root samples (Coolen & D'Herde, 1972). The nematode suspensions obtained from the samples were kept refrigerated (4 to 6 °C), counting and identifying *Pratylenchus* spp. with

the aid of Peters slides under an optical microscope, using the average of two readings.

For the morphological characterization, observations were made based on the morphometric characteristics (Castillo & Vovlas, 2007). In molecular identification, genomic DNA was extracted for each isolate with the kit AxyPrep Multisource Genomic DNA Miniprep (Axygen®), according to the protocol described by the producer, and the quality and quantity of DNA were estimated respectively on an agarose gel 0,8% and NanoVue™ (GE Healthcare®).

The ribosomal DNA fragments (rDNA) (D2-D3 of 28S rRNA and ITS region) were sequenced. The D2-D3 region of 28S rDNA segment was amplified using primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Nadler, S et al., 1999); and ITS was amplified using primers 18S (5'-TTGATTACGTCCCTGCCCTT-3') and Vrain2R (5'-TTTCACTCGCGTTACTAAGGGAATC-3') (Vrain et al., 1992).

The PCR amplifications were performed in a final volume of 25 µl using the PCR Master Mix (2X) (Fermentas®) according to the producer recommendations, the reactions were performed in a thermocycler Biocycler MJ 96+ (Biosystems®) (Powers & Harris, 1993). The PCR products were visualized on an agarose gel 2%, purified with the kit AxyPrep™ PCR Cleanup (Axygen®) and sequenced in both directions with the same primers used in the amplification.

The sequencing was performed by the company Ludwig Biotec (Alvorada, Rio Grande do Sul, Brazil). Sequences of ex-type isolates were used as a reference, obtained from GenBank (Benson et al., 2012) and multiple sequence alignments were performed with the MAFFT (Katoh & Toh, 2013) manually adjusted to allow maximum alignment and maximum similarity between sequences. The ambiguously aligned regions were excluded from the analyzes.

The Maximum Likelihood (ML) analyzes were performed for phylogenetic relationships using the RAxML-HCP2 v.8.2.8 (Stamatakis, 2014) implemented in CIPRES Portal v.2.0 (Miller et al., 2010).

According to the morphological and molecular characterization, two species of *Pratylenchus* were identified: *P. coffeae* and *P. brachyurus*; with a predominance of *P. brachyurus*, corresponding to 88% of all

specimens, and *P. coffeae* with only 12% of the total.

The females of *P. brachyurus* showed the stiletto measured 20,9 µm; vulva position was 88,5% of body length; and tail measurements were $c = 18,32$ µm and $c' = 2,21$ µm. Total body length was 591,2 µm, with $a = 24,4$ µm, $b = 8,42$ µm and $b' = 4,03$ µm. *P. coffeae* females showed the stiletto measured 20,8 µm; vulva position was 83,6% of body length; and tail measurements were $c = 18,27$ µm and $c' = 2,13$ µm. Total body length was 790,64 µm, with $a = 21,7$ µm, $b = 7,6$ µm and $b' = 3,12$ µm.

Four isolates were sequenced: three from *P. brachyurus* (CN0017-CN0019) and one from *P.*

coffeae (CN0020). The sequences of the studied rDNA regions were submitted to GenBank (ITS: MT230582-MT230585 and D2–D3 of 28S: MT230586-MT230589). The CN0017-CN0019 populations used for molecular analysis showed a high degree of sequence identity (99%) with *P. brachyurus* from Brazil for the ITS region and CN0020 showed coverage of 99% homology and 99% in consultation with the *P. coffeae* sequences (Figure 1.). The identity of the D2/D3 sequence of CN0017-CN0019 was 100% with isolates of *P. brachyurus* from Brazil and CN0020 showed 99% identity with *P. coffeae* sequences from Australia and China (Figure 2.).

Figure 1 - Tree of the phylogenetic relationships of *Pratylenchus brachyurus* and *P. coffeae* and populations with other *Pratylenchus* spp. as inferred from the Maximum Likelihood analysis of STIs. GenBank access numbers are shown before taxonomic names.

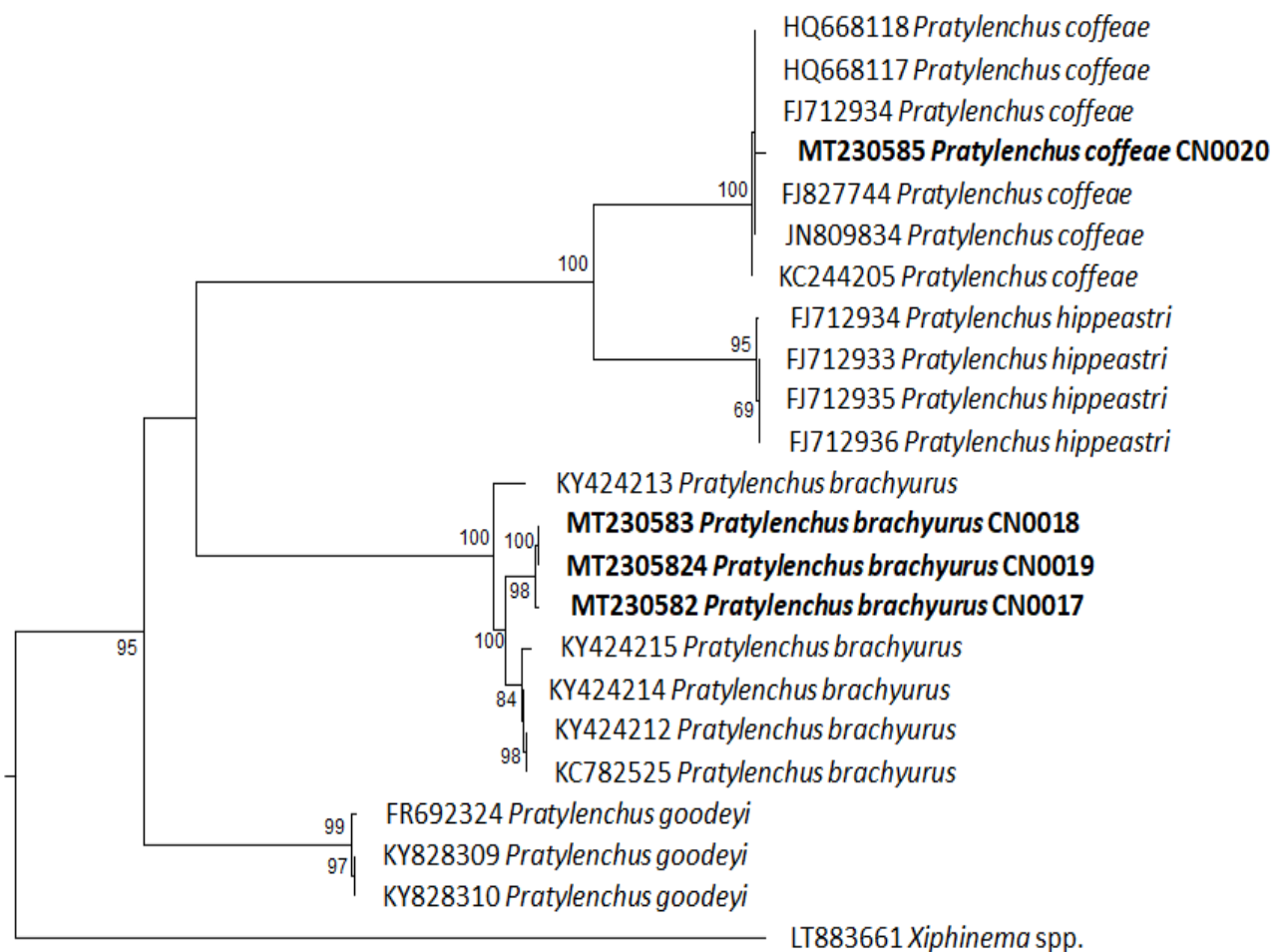
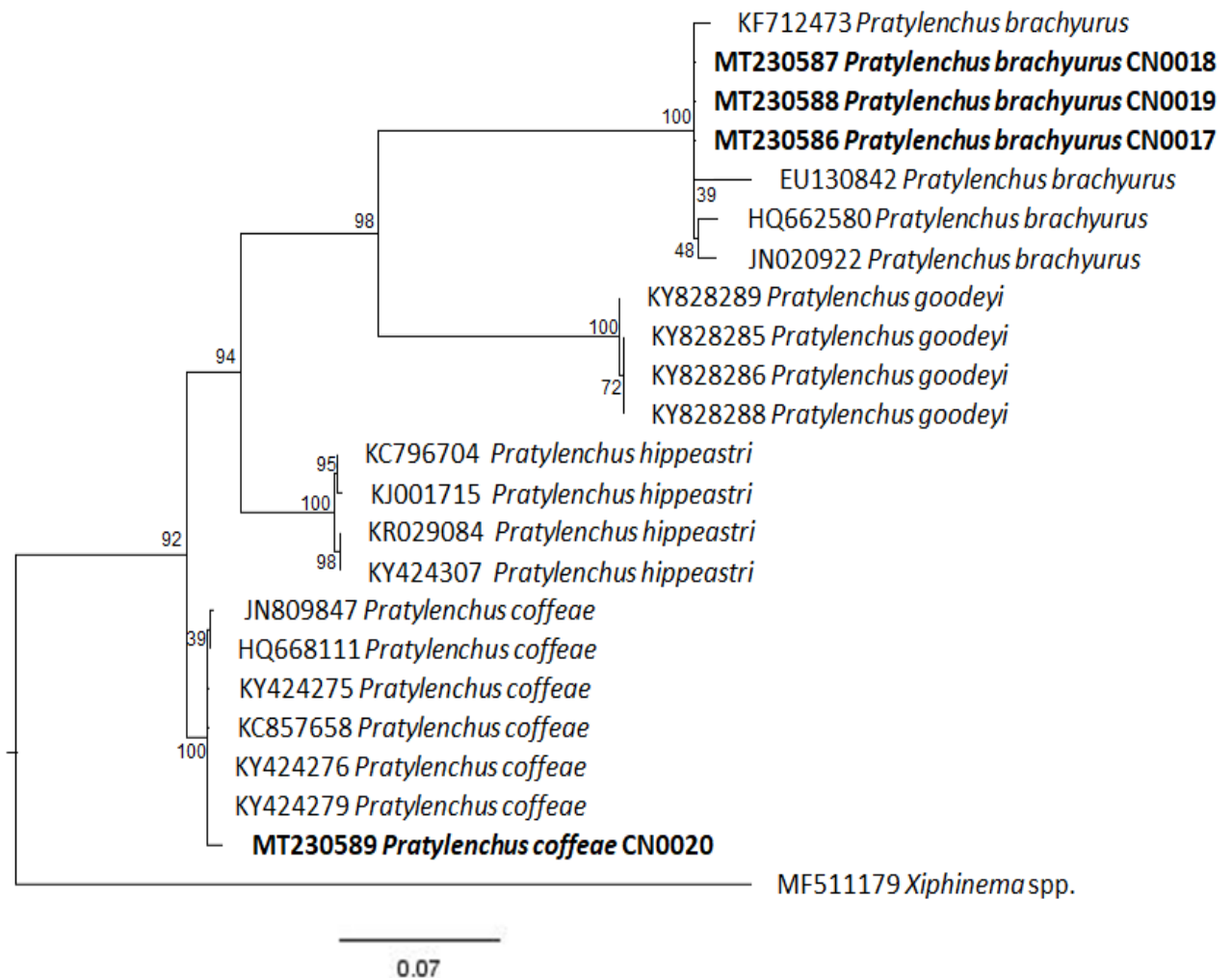


Figure 2. Tree of the phylogenetic relationships of *Pratylenchus brachyurus* and *P. coffeae* and populations with other *Pratylenchus* spp. as inferred from Maximum Likelihood analysis of 28S. The GenBank accession numbers are shown before the taxonomic names.



The prevalence of the species *P. brachyurus* found in this work brought a new paradigm about which species may be causing more damage to the banana. In studies by Jesus and Wilcken (2010) and Luambano, et al. (2019), *P. coffeae* is reported as the main species that occurs in banana production areas, being cited in several countries, such as India, Vietnam, Honduras and Tanzania, among others; which occurs the wide distribution of this nematode in the culture, mentioning the resistance of the specimens in different climatic conditions.

It is concluded that there are two species associated with banana in the state of Pernambuco: *P. coffeae* and *P. brachyurus*, showing a difference in population density in the culture, thus, this research informs a new data

about the parasitism of *Pratylenchus* in banana, however, such results must be ratified.

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