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Diversity of *Pratylenchus zeae* populations in sugarcane mills in the Brazilian Northeast region

Abstract – The objective of this work was to identity and characterize morphometric, molecular, and phylogenetic features of *Pratylenchus* spp. populations collected from sugarcane mills in the Brazilian Northeast region. Thirty populations of *Pratylenchus* were analyzed morphologically, morphometrically, and molecularly using the D2-D3 28S rDNA region. Samples were collected from sugarcane mills in the states of Paraíba, Pernambuco, and Rio Grande do Norte. Morphological analysis of *Pratylenchus* spp. collected from sugarcane mills suggests that the species present in the Brazilian Northeast region is *P. zeae*. Morphometric data show overlap with previous described *Pratylenchus* species but support the identification of *P. zeae* as the species present in the analyzed samples. Molecular analysis confirms that all *Pratylenchus* populations collected from sugarcane mills in the Brazilian Northeast region belong to the species *P. zeae*. Soil clay content and chemical characteristics affect the morphometry of *P. zeae* populations.

Index terms: *Saccharum*, PCR, phylogeny, root-lesion nematode, variability.

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Diversidade de populações de *Pratylenchus zeae* em usinas de cana-de-açúcar da região Nordeste do Brasil

Resumo – O objetivo deste trabalho foi identificar e distinguir as características morfométricas, moleculares e filogenéticas de populações de *Pratylenchus* spp. coletadas em usinas de cana-de-açúcar na região Nordeste do Brasil. Trinta populações de *Pratylenchus* foram analisadas morfológica, morfométrica e molecularmente utilizando a região D2-D3 do rDNA 28S. As amostras foram coletadas em usinas de cana-de-açúcar nos estados da Paraíba, Pernambuco e Rio Grande do Norte. A análise morfológica de *Pratylenchus* spp. coletados em usinas de cana-de-açúcar sugere que a espécie presente na região Nordeste do Brasil é *P. zeae*. Os dados morfométricos apresentam sobreposição com espécies de *Pratylenchus* descritas anteriormente, mas sustentam a identificação de *P. zeae* como a espécie presente nas amostras analisadas. A análise molecular confirma que todas as populações de *Pratylenchus* coletadas em usinas de cana-de-açúcar no Nordeste brasileiro pertencem à espécie *P. zeae*. O teor de argila no solo e as características químicas afetam a morfometria das populações de *P. zeae*.

Termos para indexação: *Saccharum*, PCR, filogenia, nematoide das lesões radiculares, variabilidade.

Introduction

An economically important crop in Brazil, particularly in the Northeast region (IBGE, 2025), sugarcane (*Saccharum* spp.) is highly susceptible to various diseases. Among these, plant-parasitic nematodes, especially those of the genus *Pratylenchus*, commonly known as root-lesion nematodes, are a significant threat. These nematodes are responsible for considerable yield losses, exceeding 50% in susceptible sugarcane cultivars (Ramouthar & Bhuiyan, 2018).

Despite the economic significance of sugarcane for Brazil and the substantial yield losses attributed to root-lesion nematode parasitism, few studies have been dedicated to characterizing *Pratylenchus* spp. populations detected in this industrial crop. Characterizing these nematode populations in Brazilian sugarcane regions is essential because their dynamics can shift over time, and new species may be introduced. For instance, *P. parazeae*, a recently described species parasitizing sugarcane in China (Wang et al., 2015), could similarly impact sugarcane productivity in Brazil.

Despite the need for accurate diagnosis of nematode species occurring in sugarcane fields in Brazil to develop appropriate management strategies (Ramouthar, 2022), most published studies on these populations are based on a small number of samples or locations (Noronha et al., 2017; Silva et al., 2017; Jesus et al., 2020). Globally, *P. brachyurus* and *P. zeae*, along with ten other species, have been found associated with sugarcane (Castillo & Vovlas, 2007). In Brazil, *P. zeae* is the most widespread root-lesion nematode in sugarcane fields, causing yield losses of 20% to 50% (Gabia, 2019).

The morphological diagnosis of these root-lesion nematode species relies mainly on female characters (Loof, 1991 cited by Castillo & Vovlas, 2007), which is complicated by their high interspecific overlapping and intraspecific variability (Araya et al., 2016). To mitigate these complications, molecular analysis of gene sequences, specifically D2-D3 expansion segments of 28S rRNA, ITS of 18S rRNA and *coxI* mtDNA, has been combined with morphological features to confirm species identifications (Oliveira et al., 2011; Kumari, 2015; Araya et al., 2016; Bonfim Junior et al., 2016). This integrative analysis is used for reliable identification of nematode populations and is crucial for revealing cryptic species, which are morphologically indistinguishable but molecularly distinct (Oliveira et al., 2011; Wang et al., 2015; Janssen et al., 2017).

The objective of this work was to identity and characterize morphometric, molecular, and phylogenetic features of *Pratylenchus* spp. populations collected from sugarcane mills in the Brazilian Northeast region.

Materials and Methods

The study was conducted in the Brazilian Northeast region, comprising the states of Pernambuco, Paraíba, and Rio Grande do Norte. According to the Köppen climate classification (Köppen & Geiger, 1928), the climate is humid tropical in Pernambuco and Rio Grande do Norte, while Paraíba features a warm and humid tropical climate. Soil samples were collected from sugarcane fields with a history of *Pratylenchus* sp. infestation at the following sugarcane mills: Miriri (MR) and Japungu (JP) in Paraíba; Olho d'Água (OD) and São José (SJ) in Pernambuco; and Baía Formosa (BF) and Estivas (ES) in Rio Grande do Norte (Table 1). A total of 180 soil samples (0–20 cm) were collected, with five areas sampled per mill, and six composite samples taken from each area. A subsample was sent to a specialized soil laboratory (Laborsolo Laboratórios, Londrina, Paraná, Brazil) for soil chemical analysis and textural characterization, as shown in Tables 2 and 3.

Nematodes were extracted from a 50 cm^3 soil subsample using Baermann funnel methodology (Machado & Silva, 2019). The extracted nematodes were killed by immersing them in hot water (60°C for 5 min) and then fixed in a TAF solution of 40% formalin, triethanolamine, and distilled water in a 7:2:91 v/v ratio (Courtney et al., 1955). For species identification and morphological and morphometrical characterization, adult female specimens of *Pratylenchus* sp. were mounted on microscope slides using TAF. The characteristics of ten females per sample were obtained following Castillo & Vovlas (2007) and Troccoli et al. (2016) methods.

The morphometric values of the studied populations were compared with those previously described for *P. zeae*. All observations and measurements, in µm, were taken using a light microscope Axio Scope A1 (Carl Zeiss, Oberkochen, Germany) with an attached camera

Zeiss Axiocam 105 Color (Carl Zeiss, Oberkochen, Germany). The measured variables included maximum body diameter (ØC), body diameter at vulva level (ØV), body diameter at anus level (ØA), distance from vulva to posterior end (V), stylet length (St), width of stylet knobs (Øbst), height of stylet knobs (Abst), dorsal esophageal gland aperture (DGO), distance from the anterior end to pharyngo-intestinal junction (Po), esophagus (F), distance from vulva to anus (v-a), tail length (T), and post-uterine branch length (PUB). Additionally, the following ratios were calculated: body length/maximum body diameter (a), body length/esophagus length (b), body length/tail length (c), tail length/body diameter at anus level (c'), percentual relationship between the distance from anterior end to vulva and body length (V%).

The morphometric data was initially analyzed using one-way variance analysis (ANOVA). Before running the ANOVA, the necessary assumptions were verified using Shapiro-Wilk's test for normality and Bartlett's test for homogeneity of variance. To normalize data residuals, the Box-Cox procedure was applied, indicating that the specific transformations were required: the body diameter at vulva was transformed

Table 1. Geographical data, population code, and GenBank access code for *Pratylenchus zeae* populations collected from sugarcane mills in the Brazilian Northeast region.

Origin	Population code	Geographical coordinates	Altitude (m)	GenBank code[(1)]
Baía Formosa, RGN	BF1	6°28'22"S/ 35°2'7"W	34	MW362986
	BF2	6°29'23"S/ 35°8'44"W	87	MW362987
	BF3	6°29'0"S/ 35°1'59"W	37	MW362988
	BF4	6°27'6"S/ 35°4'21"W	49	MW362989
	BF5	6°24'54"S/ 35°2'29"W	45	MW362990
Estivas, RGN	ES1	6°13'37"S/ 35°12'55"W	73	MW362995
	ES2	6°13'37"S/ 35°12'55"W	73	MW363009
	ES3	6°13'37"S/ 35°12'55"W	73	MW363008
	ES4	6°12'56"S/ 35°12'47"W	87	MW363004
	ES5	6°12'56"S/ 35°12'43"W	87	MW363005
Japungu, Paraíba	JP1	6°52'20"S/ 34°59'57"W	42	MW362991
	JP2	6°52'17"S/ 35°0'4"W	63	MW363006
	JP3	6°51'18"S/ 35°0'33"W	64	MW362992
	JP4	6°52'57"S/ 35°5'21"W	81	MW362993
	JP5	6°52'56"S/ 35°5'25"W	80	MW362994
Miriri, Paraíba	MR1	6°52'24"S/ 34°58'11"W	54	-
	MR2	6°51'25"S/ 34°58'13"W	44	-
	MR3	6°51'24"S/ 34°58'27"W	47	MW363003
	MR4	6°51'24"S/ 34°58'26"W	46	MW362996
	MR5	6°51'5"S/ 34°58'37"W	50	MW362997
Olho D'Água, Pernambuco	OD1	7°15'27"S/ 35°6'43"W	152	MW362998
	OD2	7°15'26"S/ 35°6'50"W	142	MW362999
	OD3	7°15'27"S/ 35°6'47"W	146	MW363000
	OD4	7°15'25"S/ 35°7'9"W	132	MW363001
	OD5	7°15'26"S/ 35°7'7"W	132	MW363002
São José, Pernambuco	SJ1	7°47'55"S/ 35°0'48"W	125	MW363007
	SJ2	7°45'54"S/ 34°59'32"W	119	-
	SJ3	7°44'35"S/ 35°1'18"W	156	-
	SJ4	7°49'31"S/ 34°59'40"W	109	-
	SJ5	7°52'21"S/ 35°1'54"W	69	-

[(1)]Poor quality sequences that were removed from the analysis are indicated with a hyphen (-). RGN, Rio Grande do Norte.

Table 2. Physical analysis of soil samples collected from six sugarcane mills in the Brazilian Northeast region.

Sample[(1)]	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})
BF1	570	240	190
BF2	740	150	110
BF3	430	60	510
BF4	450	60	490
BF5	500	70	430
JP1	420	80	500
JP2	470	100	430
JP3	470	100	430
JP4	410	90	500
JP5	400	100	500
OD1	660	80	260
OD2	650	90	260
OD3	650	80	270
OD4	650	90	260
OD5	590	100	310
ES1	540	60	400
ES2	510	70	420
ES3	480	50	470
ES4	470	70	460
ES5	450	70	480
SJ1	610	110	280
SJ2	600	110	290
SJ3	570	200	230
SJ4	570	200	230
SJ5	590	230	180
MR1	590	100	310
MR2	560	90	350
MR3	540	100	360
MR4	600	90	310
MR5	600	100	300

[(1)]BF, Baía Formosa; ES, Estivas; JP, Jupungu; MR, Miriri; OD, Olho d'Água; SJ, São José.

using log(y), dorsal gland opening was transformed using √(y+0.5), and post-uterine branch was transformed using √y. Means were compared using the Scott-Knott's test with a significance level of $p \leq 0.05$, and multivariate techniques were also used to analyze the morphometrical data. A hierarchical clustering analysis, using the Ward's method (Ward, 1963), was performed based on a principal component analysis (PCA) obtained a priori using average values for each morphometrical variable. Furthermore, morphometrical data were correlated to physical and chemical characteristics of soil samples (Tables 2 and 3) through multivariate canonical correspondence analysis. All the analyzes were carried out using R 2.15.2 software (R Core Team, 2015), ExpDes (Ferreira et al., 2014), and vegan (Oksanen et al., 2017) packages.

For molecular analysis, DNA was extracted from *Pratylenchus* sp. A single adult female from each sample was isolated and sectioned in two parts in 25 µL of Worm Lysis Buffer (WLB). This initial extraction was performed using a thermocycler under the following conditions: 4°C for 3 hours, 60°C for 1 hour, and 95°C for 15 min. Subsequent DNA extraction and molecular analyzes were carried out using ten females per sample. The D2-D3 rDNA amplification was performed using the universal primers D2Ab (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') for

Table 3. Chemical analysis of soil samples collected from six sugarcane mills in the Brazilian Northeast region[1].

Sample[2]	P	C	pH	H+AL	Ca	Mg	K	SB	T	V
	(mg dm^{-3})	(g dm^{-3})	$CaCl_2$	----------(cmol$_c$ dm^{-3} of soil)----------						(%)
BF1	8.6	13.8	4.0	6.2	1.2	0.4	0.2	2.2	6.8	32.8
BF2	21.6	8.9	4.7	3.6	1.2	0.3	0.2	1.8	5.5	33.3
BF3	73.4	7.2	5.3	2.7	1.7	0.6	0.1	2.5	5.2	48.1
BF4	42.6	10.3	4.5	3.9	1.0	0.4	0.3	1.7	5.7	30.7
BF5	46.6	9.4	4.4	3.6	0.9	0.2	0.2	1.5	5.1	28.9
JP1	75.2	7.9	5.0	3.1	1.6	0.2	0.1	1.9	5.1	38.3
JP2	190.2	11.7	5.1	3.6	3.1	0.4	0.2	3.7	7.4	50.5
JP3	21.4	7.7	4.2	3.9	0.8	0.2	0.1	1.2	5.1	23.5
JP4	5.9	8.0	4.2	3.6	0.7	0.1	0.2	1.1	4.8	4.2
JP5	8.1	8.0	4.2	3.6	0.8	0.2	0.2	1.2	4.9	25.3
OD1	53.5	8.7	5.1	2.9	1.8	0.4	0.1	2.3	5.3	44.8
OD2	165.5	8.8	5.4	2.5	2.2	0.5	0.2	3.0	5.5	54.3
OD3	102.8	10.1	5.4	2.9	2.2	0.6	0.1	3.0	6.0	51.2
OD4	40.2	9.2	5.0	3.4	2.3	0.8	0.1	3.3	6.7	49.3
OD5	173.2	9.0	5.8	2.1	3.7	0.6	0.2	4.6	6.8	68.2
ES1	129.7	11.2	5.6	2.5	3.1	1.1	0.3	4.6	7.1	64.6
ES2	92.3	10.9	5.5	2.7	2.0	0.8	0.3	3.1	5.9	53.7
ES3	248.6	11.5	5.8	2.5	3.4	1.1	0.3	4.9	7.5	66.1
ES4	223.8	16.9	5.8	2.7	3.6	1.2	0.4	5.2	8.0	65.9
ES5	244.7	14.6	6.1	2.5	3.5	1.3	0.2	5.1	7.6	66.7
SJ1	40.2	13.5	4.7	4.2	1.9	0.6	0.6	3.1	7.3	42.2
SJ2	101.8	13.1	5.0	3.9	2.6	0.6	0.3	3.6	7.6	47.9
SJ3	295.7	15.2	6.1	2.5	4.0	1.2	0.6	5.8	8.4	69.7
SJ4	16.9	17.2	4.8	4.9	2.6	1.0	0.3	4.0	9.0	44.8
SJ5	11.7	13.8	4.0	6.2	1.2	0.6	0.2	2.0	8.2	25.1
MR1	116.8	19.5	4.6	5.7	2.7	0.8	0.2	3.8	9.6	40.0
MR2	20.0	14.0	5.7	2.9	2.9	1.3	0.2	4.6	7.5	61.0
MR3	127.0	11.2	5.2	3.1	2.4	0.6	0.2	3.2	6.4	50.8
MR4	25.4	16.0	5.1	4.2	2.5	0.9	0.2	3.7	8.0	46.8
MR5	6. 0	11.6	4.4	4.6	1.2	0.5	0.2	2.0	6.6	30.8

[1]P, phosphorus; C, carbon; Al, aluminum; H + Al, potential acidity; Ca, calcium; Mg, magnesium; K, potassium; SB, sum of bases; T, cation exchange capacity; V, base saturation; P – K, Mehlich I; Ca – Mg – Al, KCl M; pH, $CaCl_2$ 0.01 M; C, Walkley – Black. [2]BF, Baía Formosa; ES, Estivas; JP, Jupungu; MR, Miriri; OD, Olho d'Água; SJ, São José.

D2/D3 expansion segments of the LSU rDNA (De Ley et al., 1999), which were synthesized by Sigma-Genesys (Foster City, California, USA). The PCR mixture contained 21 µL of the master mix Platinum PCR Supermix (Invitrogen, Waltham, MA USA), 2 µL of DNA, and 1 µL of each primer added to a 0.2 µL centrifuge tube. Amplification conditions were as follows: 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min, and a final extension of 72°C for 7 min. Finally, the PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), and sequenced using the Sanger method (Sanger et al., 1977) (Myleus Biotechnology, Belo Horizonte, MG, Brazil).

The DNA sequences were analyzed using the Bioedit program (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and were compared to other *Pratylenchus* spp. sequences from GenBank (https://blast.ncbi.nlm.nih.gov) using BLASTN 2.2.19+ (Zhang et al., 2000). The sequences generated in this study were deposited into GenBank (Table 1). To confirm the identity of *P. zeae* isolates, their position in Bayesian phylogenetic tree was evaluated using both obtained sequences and sequences of *P. zeae* from Genbank (topotype KU198950; AB933457-AB933463; KU198951; MT227821; MH359159; EU130894; KT032999; KY424256; JN020930; KF765436; KP903446; KP903447) and *P. parazeae* (KF765433-KF765435; KP903440-KP903444). The investigation of genetic relationships were performed by two phylogenetic techniques: bayesian inference (BI) and maximum likelihood (ML). The ML tree was constructed and edited using MEGA v6.2 with 10,000 bootstrap replicates. Bayesian analysis was performed with MrBayes v.3.1.2 and four Markov chain Monte Carlo (MCMC) (Metropolis et al., 1953). The chain was run simultaneously for 1 x 10^7 generations. The first 25% of sample trees were discarded as burn-in, and the consensus tree, along with posterior probability was assessed from the remaining trees.

Results and Discussion

The study revealed that the morphological and morphometric characteristics of the females of all populations studied were consistent with established scientific descriptions cited in the literature (Graham, 1951 cited by Castillo & Vovlas, 2007; Sher & Allen, 1953 cited by Castillo & Vovlas, 2007; Troccoli et al., 2016). Males were not observed in any of the samples. A slender body that remained almost straight after death, with a not offset labial region, characterized the females (Figure 1 A). The body length (L) varied from 355 to 555 µm. Further analysis of variance showed that populations BF5, JP3, MR2, MR5, OD2, and SJ2 had the highest values of L for females, while ES3 and ES4 had the lowest values (Table 4).

Body diameter was measured at three points, showing variation. At the anus (BDA), it ranged from 7.58 to 15.15 µm; at the vulva (BDV), from 12.62 to 22.72 µm; and in the middle of the body (BD), from 10.10 to 22.72 µm. The stylet length (St) was found to be robust, varying from 13.20 to 17.60 µm. Its basal structure was characterized by well-developed and flat basal knobs (Figure 1 A), confirmed by the measurements of the knob height (HStk), which varied from 2.02 to 5.05 µm and the knob width (WStk), ranging from 2.02 to 3.03 µm. Figure 2 shows the anterior end of a female from each sugarcane mill.

The identification of *Pratylenchus* spp. is often challenging due to the high degree of interspecific overlap in some morphological characteristics. However, the morphological and morphometric characteristics found in the females evaluated in this study were consistent with previous reports for *P. zeae* (Troccoli et al., 2016). Although the values for body length and stylet length varied, they fit the descriptions for *P. zeae* (Castillo & Vovlas, 2007). The presence of well-developed and flat stylet knobs is considered a diagnostic and characteristic feature of this particular nematode species (Loof, 1991 cited by Castillo & Vovlas, 2007).

The final morphological analysis revealed that the tail shape (Figures 1 B and C) varied from a pointed shape (Figure 1 B) to more rounded and subacute shapes (Figure 1 C), consistent with other *P. zeae* populations in Brazil described by Jesus et al. (2020). The tail length (T) varied from 15.15 to 40.40 µm, with the highest values observed in populations BF2, BF5, JP1, JP2, and JP3 (Table 4). Females' anatomy showed an overlapping of the esophageal glands over the intestine and an anterior position of the vulva (Figure 1 D). The vulva position, measured from the labial region, expressed as a percentage of the total body length (V%), varied from 63.59 to 77.80 µm

(Table 5), and post-uterine branch (PUB) length was noted to be highly variable across samples (Table 4).

The lowest values for vulva position expressed as a percentage of total body length (V%) were found in the populations BF3, BF4, BF5, ES2, ES5, JP1, JP2, JP3, JP4, MR2, OD2, OD3, OD4, SJ4, and SJ5 (Table 5). V% was found to be a more stable variable for diagnosing *P. zeae*, since the species is characterized by low values of this variable (Castillo & Vovlas, 2007). Therefore, the overall morphometric values of *P. zeae* populations studied were in agreement with previous reports by Graham (1951) cited by Castillo & Vovlas, 2007 and revised values by Sher & Allen (1953) cited by Castillo & Vovlas, 2007 and the measurements from topotype specimens analyzed by Troccoli et al. (2016) (Table 6).

Principal component analysis (PCA), based on morphometric data and on the de Man's indices, showed that 33.97% of the total variation was associated with the ratios tail length/ tail diameter at anus (c') and 20.22% with the V%. Subsequent cluster analysis (Ward), derived from PCA scores, allowed the separation of the populations into three distinct and well-defined groups: ES3 and ES4; MR5, MR2, OD2, BF5, and JP3; and all the other populations (Figure 3 A). This intraspecific variation was also reported for other *Pratylenchus* species, such as *P. brachyurus* (Machado et al., 2015) and *P. coffeae* (Lira et al., 2014). Such morphological and morphometrical intraspecific variations within a species are often linked to environmental factors, with geographical isolation playing a critical role (Doucet et al., 2001). However, the results indicated that there is not a clear relationship between the morphometry of *P. zeae* populations and the localities where they were collected, since populations originating from the same sugarcane mill were grouped in distinct clusters.

The multivariate canonical correspondence analysis was utilized to link the *P. zeae* morphometric data with both physical (Table 2) and chemical characteristics of the soil samples (Table 3). The results showed that 77.30% was associated with sand and 62.27% with

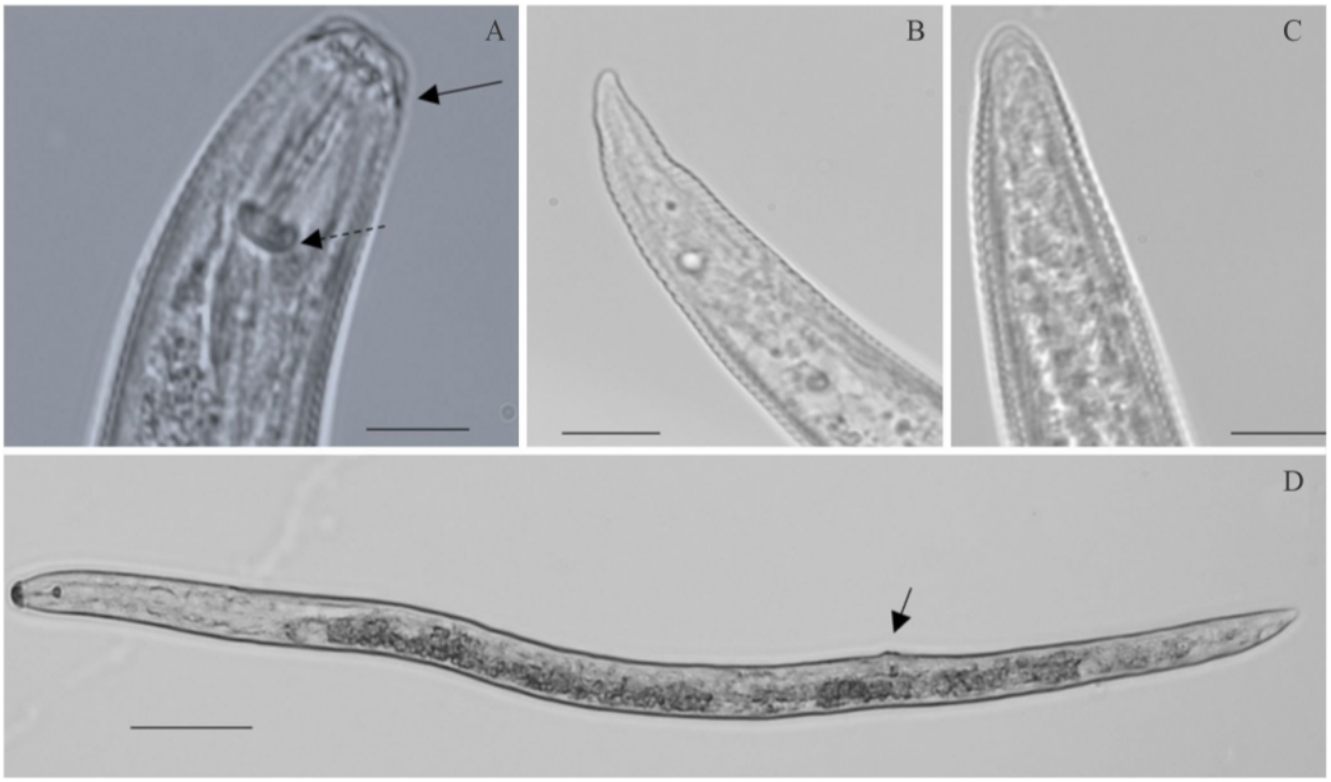

Figure 1. *Pratylenchus zeae* female morphology: labial region not offset from the body (continuous arrow) and well-developed and flat stylet knobs (discontinuous arrow) (A); tail tip shapes (B and C); and entire female body, evidencing the anterior position of the vulva (arrow) (D). Scale bars correspond to 10 µm.

Table 4. Morphometric characters (µm) of *Pratylenchus zeae* populations collected from sugarcane mills in the Brazilian Northeast region[1].

Population[2]	Body length	ØC	ØV	ØA	V	St	Øbst	Abst	DGO	Po	F	v-a	T	PUB
BF1	463.50±36.14b	17.42±1.86a	17.42±2.51a	11.36±1.33a	133.08±16.23b	14.41±0.81a	3.73±0.83b	2.12±0.32b	2.82±0.64b	72.92±4.70b	114.93±12.45b	121.71±15.78b	26.76±4.47b	28.38±11.89a
BF2	466.00±37.47b	16.16±2.12b	16.66±2.12b	10.86±1.70b	132.82±14.54b	14.96±0.57a	3.93±0.32b	2.32±0.49a	2.22±0.42b	75.14±7.36a	114.93±12.97b	121.97±14.08b	29.18±5.37a	20.30±5.70a
BF3	438.50±37.71c	16.41±1.33b	16.41±1.78b	10.35±0.79b	131.31±17.85b	14.19±1.51a	4.04±0.47b	2.02±0.00b	2.12±0.32b	72.21±3.66b	116.35±8.32b	120.96±17.91b	25.85±4.15b	20.40±8.31b
BF4	453.50±25.71b	16.66±1.76b	16.66±1.76b	10.60±1.06b	132.06±6.41b	14.52±0.46a	3.93±0.31b	2.72±0.48a	2.82±0.79b	73.12±3.60b	118.17±13.14b	121.46±5.76b	25.95±7.49b	17.06±6.14b
BF5	492.00±31.46a	18.18±1.99a	17.93±2.21a	11.61±1.30a	146.71±10.34a	14.96±0.57a	4.54±0.53a	2.32±0.48a	2.82±0.63a	75.75±5.77a	123.52±4.59a	135.10±9.46a	29.99±4.44a	29.49±8.48a
ES1	431.00±33.61c	16.66±1.38b	16.16±1.38b	11.11±1.38b	122.72±4.58c	14.52±1.14a	4.04±0.00b	2.22±0.45b	3.03±0.00a	67.46±6.03b	112.11±8.26b	111.61±4.51c	24.84±4.98b	17.77±5.83b
ES2	437.00±27.20c	15.65±1.59b	15.40±0.79b	10.35±0.79b	127.77±5.73b	14.41±0.81a	4.04±0.00b	2.32±0.48a	3.03±4.68a	76.55±3.52a	118.17±6.02b	117.42±5.61b	24.84±3.33b	11.61±1.28c
ES3	419.30±35.52d	15.15±2.06b	15.15±2.06b	10.82±1.23b	121.57±10.45c	14.74±0.57a	4.32±0.49a	2.16±0.38b	2.88±0.38b	74.45±3.62a	109.08±7.37b	110.75±9.60c	24.09±2.76b	14.71±4.97c
ES4	408.50±33.08d	15.40±1.43b	14.89±1.43b	10.35±0.79b	115.15±10.79c	14.52±0.49a	4.14±0.31b	2.02±0.00b	3.03±4.68a	74.53±4.89a	120.89±4.92a	104.80±10.59c	24.03±2.37b	16.05±4.13b
ES5	446.50±29.25c	15.65±1.06b	15.65±1.06b	10.60±1.06b	131.31±11.10b	14.30±0.52a	4.34±0.48a	2.02±0.00b	3.03±0.68a	73.93±4.53a	115.84±5.87b	120.70±11.40b	25.95±4.59b	13.73±5.04c
JP1	461.00±29.98b	16.92±1.70b	16.66±1.76b	11.87±1.21a	135.35±12.72a	14.41±0.81a	4.34±0.48a	2.32±0.48a	2.92±0.74b	72.82±5.15b	116.75±12.90b	123.48±12.62b	30.50±3.83a	17.47±5.08b
JP2	465.00±26.56b	17.67±1.68a	17.42±1.86a	11.11±1.76b	137.62±8.27a	14.96±0.93a	4.04±0.47b	2.52±0.53a	2.62±0.70b	75.75±3.62a	119.88±6.91a	126.51±7.47a	31.00±4.54a	19.19±7.88b
JP3	488.00±28.00a	17.93±1.86a	17.42±0.79a	11.36±1.33a	144.44±14.27a	14.63±0.74a	4.34±0.48a	2.32±0.48a	2.12±0.31b	69.89±3.80b	116.45±5.92b	133.08±13.67a	32.42±3.93a	23.43±6.86a
JP4	435.00±25.49c	16.66±1.38b	16.16±1.38b	11.61±1.38a	130.30±6.58b	14.63±0.74a	3.33±0.45b	2.22±0.45b	2.22±0.45b	72.11±7.23b	122.21±13.81a	118.68±5.92b	23.63±3.24b	17.77±2.82b
JP5	435.00±19.92c	15.90±2.67b	15.65±1.06b	10.10±0.00b	122.47±9.83c	14.63±0.53a	4.04±0.00b	2.12±0.31b	2.12±0.31b	71.71±3.49b	127.15±8.82a	112.37±9.83c	23.23±2.56b	24.84±7.96a
MR1	449.50±26.18c	17.42±1.43a	16.92±1.21a	11.87±1.21a	128.28±11.95b	14.74±0.60a	4.14±0.31b	2.32±0.48a	3.03±4.68a	72.41±5.15b	112.81±13.36b	116.41±12.10b	23.83±4.10b	16.16±4.56b
MR2	477.00±35.05a	18.68±2.12a	18.43±2.07a	11.87±1.70a	138.88±11.90a	14.52±0.46a	4.14±0.31b	2.02±0.00b	2.92±0.31a	74.03±3.65a	119.98±6.41a	127.01±10.71a	26.05±3.11b	18.68±5.04b
MR3	453.50±18.26b	17.42±1.79a	17.17±1.59a	11.61±1.30a	129.54±5.71b	14.30±1.92a	4.14±0.31b	2.02±0.00b	2.82±0.42b	71.81±1.87b	113.62±6.96b	117.93±6.30b	26.26±2.93b	15.65±4.47b
MR4	453.50±21.73b	18.18±1.06a	17.67±1.19a	11.11±1.30b	130.55±8.07b	14.63±0.53a	4.04±0.00b	2.12±0.31b	2.92±0.31a	75.54±2.84a	120.19±6.40a	119.44±7.43b	25.55±2.81b	15.15±5.36c
MR5	475.00±26.56a	19.95±1.86a	19.44±2.07a	12.37±1.43a	140.65±11.04a	14.41±0.35a	4.44±0.52a	2.22±0.42b	3.03±4.68a	73.42±3.86a	111.60±6.46b	128.28±11.02a	27.67±2.94b	17.37±2.68b
OD1	458.50±21.08b	17.17±2.32b	16.92±1.70a	11.11±1.30b	130.80±10.56b	14.52±0.46a	4.14±0.31b	2.02±0.00b	2.62±0.52b	71.20±3.34b	116.95±12.34b	119.69±9.90b	23.33±2.71b	17.97±5.03b
OD2	478.50±38.88a	18.43±1.70b	17.93±1.43a	10.85±1.21b	140.40±13.63a	14.41±0.35a	4.14±0.88b	2.22±0.42b	2.62±0.52b	72.92±4.19b	121.80±8.34a	129.54±13.57a	26.76±2.74b	19.39±4.80b
OD3	445.50±27.73c	16.66±1.30b	16.41±1.33b	10.60±1.06b	132.57±9.97b	14.85±0.58a	4.14±0.31b	2.42±0.52a	2.62±0.52b	72.41±3.47b	116.15±9.71b	121.96±10.17b	25.55±3.65b	17.27±5.22c
OD4	443.00±23.11c	16.41±2.14b	16.16±1.76b	10.10±1.19b	128.78±11.35b	14.96±0.77a	4.14±0.31b	2.12±0.31b	2.52±0.53b	73.02±5.21b	120.19±13.08a	118.68±10.97b	25.04±4.61b	16.76±5.26c
OD5	463.00±20.57b	18.18±2.86a	17.67±1.68a	11.36±1.33a	132.82±6.20b	14.41±0.35a	4.04±0.00b	2.02±0.00b	2.22±0.42b	73.02±4.12b	117.46±8.20b	121.46±6.00b	25.45±3.83b	15.04±3.85c
SJ1	463.00±12.73b	18.43±1.70a	17.17±1.99a	11.11±1.30b	129.54±7.34b	14.41±0.35a	4.04±0.00b	2.12±0.31b	2.82±0.42b	74.94±4.09a	115.44±7.89b	118.43±6.88b	23.83±2.19b	17.77±4.97b
SJ2	471.00±20.78a	19.44±2.07a	18.43±1.70a	12.12±1.59a	132.82±5.48b	14.52±0.46a	4.04±.00b	2.12±0.31b	2.92±0.31a	74.43±3.36a	115.74±13.19b	120.70±5.29b	24.84±2.48b	13.23±4.34c
SJ3	456.00±13.29b	17.42±1.86a	17.67±1.68a	11.61±1.30a	127.77±13.05b	14.30±0.12a	4.14±0.31b	2.12±0.31b	3.03±4.68a	73.73±3.62a	125.64±8.43a	116.16±12.82b	25.95±3.01b	16.76±5.87b
SJ4	433.50±20.28c	16.92±1.70b	16.92±1.21a	10.85±1.21b	131.81±5.02b	14.41±0.35a	4.04±0.00b	2.22±0.42b	2.82±0.42b	73.83±2.20a	120.99±7.43a	120.95±4.97b	24.44±2.00b	12.22±4.77c
SJ5	450.50±28.42c	18.18±1.06a	17.42±1.43a	11.61±1.30a	134.84±13.79a	14.63±0.53a	4.04±0.00b	2.02±0.00b	2.52±0.53b	74.43±3.95a	118.67±7.04a	123.23±13.19b	23.93±3.68b	15.45±8.98c

[1]Means followed by equal letters, in the rows, do not differ from each other by Scott-Knott's test, at 5% of significance. Measurements (µm) are represented by the mean of ten adult females ± standard deviation. ØC, maximum body diameter; ØV, body diameter at vulva level; ØA, body diameter at anus level; V, distance from vulva to posterior end; St, stylet length; Øbst, width of stylet knobs; Abst, height of stylet knobs; DGO, dorsal esophageal gland aperture; Po, distance from the anterior end to pharyngo-intestinal junction; F, esophagus; v-a, distance from vulva to anus; T, tail length; PUB, post-uterine branch length. (2)BF, Baía Formosa; ES, Estivas; JP, Jupungu; MR, Miriri; OD, Olho d'Água; SJ, São José.

H+Al of the total variation, and only 22.70% was associated with clay and 23.70% with SB. Subsequent the cluster analysis based on morphometric data and physical characteristics of the soil, populations were divided into four distinct and well-defined groups: i) BF2, with the highest clay content in soil samples (74%); ii) SJ3, SJ4, BF1, and SJ5, with high silt content in the soil samples (> 20%); iii) OD3, OD1, OD2, OD4, MR5, MR4, OD5, MR1, SJ1, and SJ2, with high clay content of in soil samples (< 59%); and iv) all the other populations with high sand content (> 35%) (Figure 3 B). The clay content was the critical factor, separating population BF2 into an individual group due to its highest clay content. The effect of soil on the morphometric characteristics was observed in BF2 by its high value of the ratio body length/ greatest body diameter ('a'), indicating that the clay content may have influenced the nematode's body diameter.

The clustering analysis based on morphometric data and chemical characteristics of the soil (Table 3) separated populations into three groups: i) SJ3, ES4, ES3, and ES5, with the highest values of V and pH in the soil samples; ii) JP2, OD2, OD5, ES1, MR3, MR1, ES2, OD3, and SJ2, with high content of calcium; and III) all the other populations with the lowest values of V (< 49%) (Figure 3 C). Based on these results, the chemical characteristics of soil, V, pH, and calcium content, may be responsible for the morphological clustering.

Apparently, soil characteristics that influence host nutrition can affect the morphometry of *P. zeae* populations. Soils that provide adequate levels of

Figure 2. Anterior end morphology of *Pratylenchus zeae* females from six sugarcane mills: Baía Formosa (A); Japungu (B); Olho d´Água (C); Estivas (D); São José (E); Miriri (F). Scale bars correspond to 5 µm.

nutrition, pH and V allow host plants to develop better, which, in turn, influence nematode characteristics. Olowe & Corbett (1984) reported that the body length of *P. zeae* was larger when the nematodes fed on host roots grown on White's nutrient medium than those on Krusberg's or Carew's media.

The phylogenetic analysis, comparing the studied sequences with other populations of *P. zeae* from GenBank, collected from various hosts and localities, suggested that the clustering was driven by the host, since the *P. zeae* populations collected in sugarcane fields were grouped together, independently of the locality.

BLAST analysis revealed homology (> 90%) with another *P. zeae* populations collected from sugarcane available in GenBank (Figure 4). Additionally, the amplifications resulted in fragments of 750 bp for D2-D3. Based on these sequences, a Bayesian phylogenetic tree was constructed in which all presently groups could be recognized with 89% to 100% support in their posterior probability. The tree inferred from the alignment of D2-D3 sequences (430 bp) of *P. zeae* populations, along with 19 D2-D3 sequences

Table 5. Morphometric ratios and standard deviation of *Pratylenchus zeae* populations collected from sugarcane mills in the Brazilian Northeast region[1].

Population[2]	a	b	C	c'	V%
BF1	26.71±1.83a	4.08±0.58a	17.82±3.73a	2.37±0.43c	71.27±2.82a
BF2	29.14±3.41a	4.09±0.49a	16.35±2.66b	2.71±0.43a	71.50±1.83a
BF3	26.78±2.23a	3.77±0.32b	17.22±2.26a	2.51±0.45b	70.10±2.49b
BF4	27.35±1.78a	3.88±0.53a	19.05±6.58a	2.43±0.58b	70.84±0.98b
BF5	26.05±1.53b	3.83±0.34a	16.12±2.85b	2.62±0.56b	70.17±1.35b
ES1	26.01±3.00b	3.85±0.38a	17.77±2.94a	2.24±0.37c	71.39±2.38a
ES2	28.07±2.25a	3.70±0.27b	17.93±3.16a	2.41±0.37b	70.69±1.64b
ES3	27.88±2.27a	3.86±0.44a	17.57±2.29a	2.24±0.31c	70.99±0.80a
ES4	26.77±3.85a	3.37±0.23b	17.08±1.67a	2.33±0.29c	71.79±1.75a
ES5	28.57±1.87a	3.86±0.34a	17.55±2.29a	2.46±0.46b	70.59±1.40b
JP1	27.47±3.12a	4.00±0.57a	15.32±2.01b	2.58±0.33b	70.66±1.61b
JP2	26.45±2.21b	3.89±0.36a	15.26±2.16b	2.83±0.46a	70.38±1.80b
JP3	27.43±2.67a	4.20±0.40a	15.25±1.99b	2.88±0.44a	70.44±1.61b
JP4	26.15±1.05b	3.58±0.26b	18.57±1.61a	2.03±0.14c	70.02±1.01b
JP5	28.10±5.17a	3.44±0.31b	19.00±2.63a	2.30±0.25c	71.90±1.31a
MR1	25.90±1.85b	4.02±0.37a	19.41±4.08a	2.01±0.30c	71.48±1.66a
MR2	25.65±1.47b	3.98±0.38a	18.46±1.89a	2.21±0.22c	70.89±0.96b
MR3	26.07±1.44b	4.00±0.34a	17.43±1.77a	2.28±0.32c	71.42±1.03a
MR4	24.98±1.27b	3.78±0.30b	17.92±1.97a	2.31±0.27c	71.21±1.17a
MR5	23.95±2.06b	4.27±0.39a	17.28±1.49a	2.25±0.29c	70.40±1.14b
OD1	27.00±2.60a	3.96±0.51a	19.82±1.89a	2.11±0.25c	71.49±1.29a
OD2	26.04±2.37b	3.93±0.36a	17.96±1.66a	2.47±0.23b	70.53±2.96b
OD3	26.84±2.22a	3.85±0.36a	17.80±3.04a	2.41±0.31b	70.16±2.57b
OD4	27.30±2.94a	3.72±0.46b	18.12±2.81a	2.50±0.50b	70.92±2.11b
OD5	25.99±3.80b	3.95±0.29a	18.51±2.57a	2.25±0.30c	71.30±0.89a
SJ1	25.28±2.10b	4.03±0.33a	19.57±1.84a	2.16±0.27c	72.02±1.41a
SJ2	24.41±2.18b	4.12±0.59a	19.18±2.68a	2.07±0.31c	71.77±1.17a
SJ3	26.45±3.09b	3.64±0.22b	17.77±2.02a	2.25±0.32c	71.97±2.74a
SJ4	25.79±2.18b	3.59±0.30b	17.82±1.33a	2.27±0.29c	69.54±1.70b
SJ5	24.82±1.67b	3.80±0.20b	19.42±4.63a	2.08±0.39c	70.09±2.11b

[1]Means followed by the same letter in columns did not differ according to Scott-Knott test at 5% of significance. [2]BF, Baía Formosa; ES, Estivas; JP, Jupungu; MR, Miriri; OD, Olho d'Água; SJ, São José. a, ratio of body length and maximum body diameter; b, ratio of body length and esophagus length; c, ratio of body length and tail length; c', ratio of tail length and body diameter at anus level; V%, percentual relationship of the distance from anterior end to vulva and body length.

Table 6. Comparative morphometrics (µm) of *Pratylenchus zeae* populations from Brazilian Northeast region with those of the original and revised descriptions.

Character[1]	BF[2]	ES[2]	JP[2]	MR[2]	OD[2]	SJ[2]	*P. zeae*[3]	*P. zeae*[4]	*P. zeae*[5]
N	50	42	45	50	50	50	11	-	-
Body length (L)	360-555	355-485	405-535	405-545	415-535	400-505	350-470	396-660	360-580
a	23.2-35.6	21.5-35.6	22.5-41.6	20.9-29.0	20.0-34.4	20.2-31.0	20.3-29.3	20-25	25-30
b	2.9-5.0	2.9-4.5	3.1-5.0	3.3-5.1	2.9-4.9	3.1-5.3	5.1-7.3	-	5.4-8.0
c	11.1-31.3	13.6-26.3	10.9-23.9	14.3-30.3	12.6-23.0	15.2-31.7	12.1-18.5	-	17-21
c'	1.4-3.7	1.6-3.3	1.8-3.8	1.5-2.9	3.5-1.6	1.2-2.7	2.5-3.4	-	-
V%	64.7-77.3	67.9-74.7	68.1-73.9	68.6-74.1	63.6-74.4	65.9-77.8	68.9-77.3	-	68-76
Stylet length (St)	12.1-16.1	13.1-14.1	12.1-16.1	13.1-14.1	12.1-15.1	13.1-15.1	15.0-16.0	16-18	15-17
WStk	2.0-5.0	4.0-5.0	3.0-5.0	4.0-5.0	2.0-5.0	4.0-5.0	3.9-4.5	3.9-5.1	-
HStk	2.0-3.0	2.0-3.0	2.0-3.0	2.0-3.0	2.0-3.0	2.0-3.0	1.8-2.4	1.9-3.4	-
DGO	2.0-4.0	2.0-3.0	2.0-4.0	2.0-3.0	2.0-3.0	2.0-3.0	1.8-2.1	-	-
BD	12.6-22.7	12.6-17.6	10.1-20.2	15.1-22.7	12.6-22.7	15.1-22.7	13.9-18.8	-	-
BDV	15.1-22.7	12.6-17.6	15.1-20.2	15.1-22.7	12.6-20.2	12.6-20.2	13.4-17.8	-	-
BDA)	7.5-12.6	10.1-12.6	7.5-12.6	10.1-15.1	7.5-12.6	10.1-15.1	9.0-11.4	-	-
PUB	10.1-50.5	10.1-26.2	11.1-36.3	10.1-30.3	10.1-26.2	6.0-33.3	20.3-27.7	-	-
Tail length (T)	15.1-40.4	13.1-33.3	18.2-38.4	15.1-33.3	20.2-35.3	15.1-29.3	23.5-33.6	-	-

[1]N, number of specimens measured; a, ratio of body length and maximum body diameter; b, ratio of body length and esophagus length; c, ratio of body length and tail length; c', ratio of tail length and body diameter at anus level; V%, percentual relationship between the distance from anterior end to vulva and body length; WStk, width of stylet knobs; HStk, height of stylet knobs; DGO, dorsal esophageal gland aperture; BD, maximum body diameter; BDV, body diameter at vulva level; BDA, body diameter at anus level; PUB, post-uterine branch length. [2]BF, Baía Formosa; ES, Estivas; JP, Jupungu; MR, Miriri; OD, Olho d'Água; SJ, São José. [3]Topotype from Troccoli et al. (2016). [4]Topotype from Graham (1951). [5]Topotype from Sher & Allen (1953).

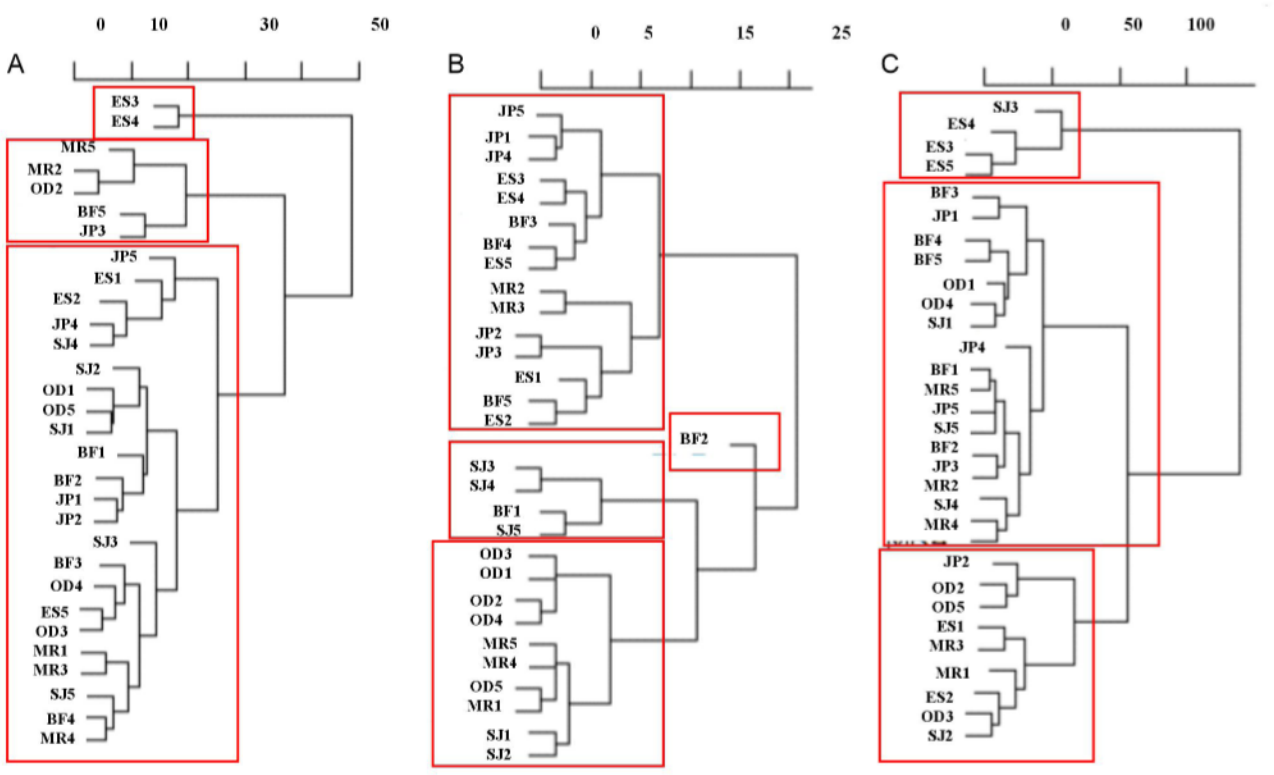

Figure 3. Dendrograms illustrating the dissimilarity among *Pratylenchus zeae* populations determined by Ward algorithm, based on Euclidean distance, calculated from: the average of morphometrical characteristics (A), morphometrical characteristics and physical properties of soil samples (B), and morphometrical characteristics and chemical analysis of soil samples (C). The samples were collected from sugarcane mills in the Brazilian Northeast region: Baía Formosa-BF, Estivas-ES, Japungu-JP, Miriri-MR, Olho d'Água-OD, and São José-SJ.

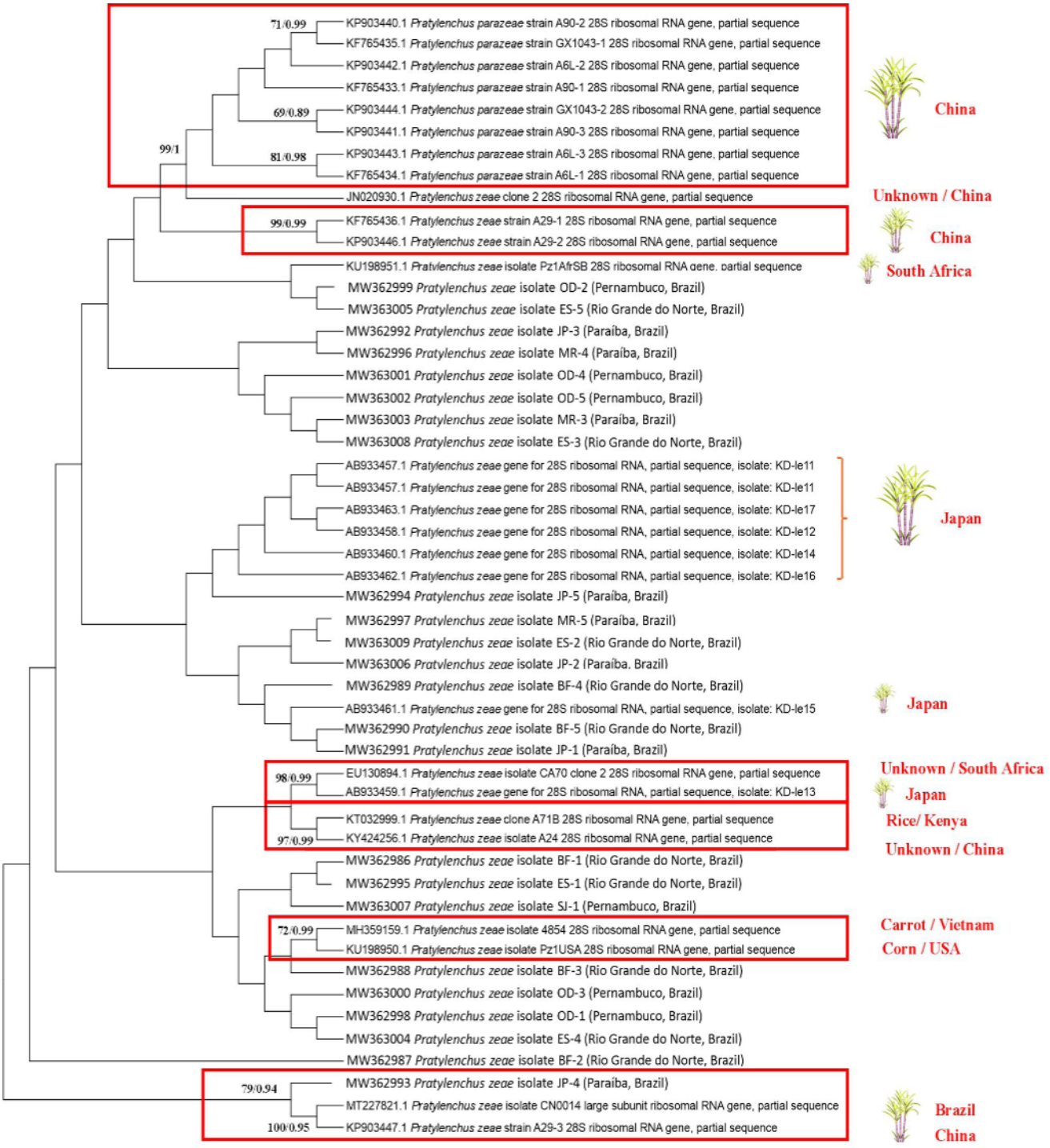

Figure 4. Phylogenetic tree generated by Bayesian inference and maximum likelihood, resulting from the alignment of the D2-D3 sequences of *Pratylenchus zeae*. The nematodes were found in soil samples collected in sugarcane mills from the Brazilian Northeast region: Baía Formosa-BF, Estivas-ES, Japungu-JP, Miriri-MR, Olho d'Água-OD, and São José-SJ. The *P. zeae* and *P. parazeae* D2-D3 sequences are from GenBank. Numbers on the branches are percentages of 10,000 replicates of MEGA6-maximum likelihood /Mr Bayes' Bayesian posterior probabilities. The tree is unrooted.

(430 bp) of *P. zeae* found in GenBank (AB933457-AB933463; KU198951; MT227821; MH359159; EU130894; KT032999; KY424256; JN020930; KF765436; KP903446; KP903447), including the topotype sequence KU198950 (Troccoli et al. 2016), and eight D2-D3 sequences (430 bp) of *P. parazeae* (KF765433-KF765435; KP903440-KP903444) from Wang et al. (2015). The tree indicated that all populations of *P. parazeae* grouped separately from *P. zeae* populations. The difficulty in distinguishing these species underscores the need to prevent the introduction of *P. parazeae* into Brazilian sugarcane growing regions, as its symptoms are similar to those caused by *P. zeae* or *P. brachyurus*, complicating diagnosis and, consequently, increasing the damage to the crop.

Moreover, all *P. zeae* populations from sugarcane clustered together in the largest group, except MT227821 + KP903447, KP903446 + KF765436, and AB933459, which clustered together in separate groups. Furthermore, *P. zeae* populations collected from different hosts clustered in different groups, such as carrot (MH359159) and corn (KU198950), and rice (KT032999) and an unknown host (KY424256) (Figure 4). The basal branches separating these groups were supported by Bayesian posterior probabilities and booststrap, and some deeper internal branches of *P. parazeae* populations received strong support (99%/1.0).

Conclusions

1. Morphological analysis of *Pratylenchus* spp. collected from sugarcane mills suggests that the species present in the Brazilian Northeast region is *P. zeae*.

2. Morphometric data show overlap with previous described *Pratylenchus* species but support the identification of *P. zeae* as the species present in the analyzed samples.

3. Molecular analysis confirms that all *Pratylenchus* populations collected from sugarcane mills in the Brazilian Northeast belong to the species *P. zeae*.

4. Soil clay content and chemical characteristics affect the morphometry of *Pratylenchus zeae* populations.

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No generative artificial intelligence (AI) was used in this study.

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