MORPHOLOGICAL AND MORPHOMETRIC CHARACTERIZATION OF *PRATYLENCHUS* SPECIES IN SUGARCANE CROPPING AREAS OF PERNAMBUCO STATE[1]

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ABSTRACT - The root-lesion nematodes *Pratylenchus* spp. have a wide geographic distribution and a large number of host plants. These migratory endoparasites are found associated with sugarcane around the world causing diseases that induce permanent losses in productivity. Although this genus has ordinarily been reported in low-producing sugarcane areas in northeastern Brazil, studies of the species occurrence are few and restricted to a very low number of sites and samples. This study aimed to morphologically and morphometrically characterize *Pratylenchus* species from different sugarcane regions of Pernambuco state, where composite samples of soil and roots were collected on 30 farms from three sugarcane mills. The external morphological characters of the nematode body from different populations had a diagnostic value compatible with those obtained in the literature, showing the dominance of *P. zeae* (87%) in the region. Atypical populations of the genus *Pratylenchus* were also found.

Keywords: Lesion nematode. *Saccharum*. Morphological and morphometric variation.

CARACTERIZAÇÃO MORFOLÓGICA E MORFOMÉTRICA DE ESPÉCIES DE *PRATYLENCHUS* EM ÁREAS DE CULTIVO DE CANA-DE-AÇÚCAR DO ESTADO DE PERNAMBUCO

RESUMO - Os nematoides das lesões radiculares *Pratylenchus* spp. apresentam ampla distribuição geográfica e grande número de plantas hospedeiras. Esses endoparasitas migradores são encontrados associados à cana-de-açúcar em regiões canavieiras diversas do mundo causando doenças que induzem permanentes perdas em produtividade. A despeito deste gênero ser frequentemente relatado em áreas de baixa produtividade no nordeste do Brasil, estudos sobre espécies ocorrentes são poucos e restritos a um número muito baixo de amostras e áreas. O presente estudo teve como objetivo avaliar morfológica e morfometricamente espécies de *Pratylenchus* de diferentes regiões do estado de Pernambuco cultivadas com cana-de-açúcar. Amostras compostas de solo e de raízes foram coletadas de 30 fazendas de três usinas do Estado de Pernambuco. As características morfológicas externas do corpo dos nematoides das diferentes populações estudadas apresentaram valor diagnóstico compatível com aqueles obtidos na literatura nematológica, demonstrando a predominância de *P. zeae* (87%) na região. Populações atípicas de *Pratylenchus* também foram encontradas.

Palavras-chave: Nematoide das lesões radiculares. *Saccharum*. Variação morfológica e morfométrica.

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INTRODUCTION

Sugarcane (*Saccharum* sp.) is one of the best options among renewable energy sources in the agribusiness, with an important role in the energy matrix of Brazil and other countries (OLIVEIRA, 2018). The growing search for less polluting and renewable fuels has turned the sugarcane market into one of the world's most promising in agribusinesses, favoring the expansion of the sugarcane industry. However, despite the Brazilian tradition in sugarcane production, sugarcane productivity is regulated by several factors, with health problems reducing crop productivity in different producing regions, especially those caused by nematodes (BELLÉ et al., 2017; PORTO et al., 2018).

The damage caused by nematodes varies according to the population level, soil type, and crop variety (SILVA et al., 2016; BELLÉ et al., 2017; NORONHA et al., 2017; MARANHÃO et al., 2018). When at a high population density, nematodes cause severe damage to the root system, which becomes underdeveloped and inefficient, leading to decreased productivities (SILVA et al., 2017; PORTO et al., 2018). Two nematode genera are important for sugarcane in Brazil: the root-knot nematodes *Meloidogyne* spp. and the root-lesion nematode *Pratylenchus* spp. (CHAVES et al., 2009; BELLÉ et al., 2014, 2017). Damage caused by *Pratylenchus* species in sugarcane fields is reported with levels of up to 52.4% for susceptible varieties, accounting for 35% of production losses (RAMOUTHAR; BHUIYAN, 2018).

Most the *Pratylenchus* species are polyphagous. The knowledge of species and inter- and intraspecific variability of nematodes is essential for the management of infestations since, by means of diagnosis, the identification of species and races allows establishing adequate crops to be explored or that will compose a rotation plan (OVERSTREET et al., 2014). However, intraspecific variability in *Pratylenchus* spp. has been pointed out as one of the main difficulties in the taxonomy of this group since most of the characters used for identifying the species are very variable (YU et al., 2012; MOKRINI et al., 2013; NORONHA et al., 2017).

Despite the existing techniques for characterization, some nematologists find it difficult to identify *Pratylenchus* species (YU et al., 2012). Morphometric characters that define nematode species may vary not only between specimens of distinct subpopulations but also among individuals belonging to the same subpopulation. Morphometric differences between subpopulations of a species may be partially influenced by environmental factors, with geographical isolation being the most prominent, followed by the host plant and temperature (OLOWE; CORBETT, 1984; DOUCET et al., 2001).

Because species of the genus *Pratylenchus* can be differentiated only by means of minor morphological and morphometric differences, efforts to differentiate *Pratylenchus* species in sugarcane fields in Northeastern Brazil are scarce and restricted to a small number of sites (SILVA et al., 2017). The main species reported in Brazil are *P. brachyurus*, *P. zeae* and *P. coffeae*, although more than 70 species have been reported worldwide. Few studies were carried out in Brazil to characterize *Pratylenchus* species, particularly populations in sugarcane fields. Thus, the aim of this study was to morphometrically characterize *Pratylenchus* populations in several farms cultivated with sugarcane.

MATERIAL AND METHODS

Thirty farms continuously cultivated with sugarcane were sampled at three sugarcane mills of Pernambuco state, Brazil: a) Santa Teresa Mill, located in Goiana; b) Cruangi II Mill, located in Timbaúba, and c) Salgado Mill, located in Barreiros. Five composite samples were collected from each farm. Each composite sample consisted of 2 kg of soil and 500 g of roots from three subsamples collected at the central point of sugarcane plantation of the farm. Aliquots of 250 cm^3 of soil were used for extracting the nematodes according to Jenkins (1964), and 10 g of roots for extracting the nematodes according to blender centrifugal flotation method (BEZOOIJEN, 2006). Extracts were brought up to 40 mL and kept refrigerated (4 °C), before semi-permanent slides were prepared for the specific identification. After the processing of soil and roots, 40 adult females obtained from each sample were completely measured, totalizing 1200 specimens in all study. At sampling time plants or ratoon were older than 10 months.

The following structures were measured: stylet length, diameter of the stylet bulb, overall body length (L), body diameter, % distance of vulva from anterior end (V), tail size, body diameter at anus height, anterior end to the end of the esophagus, anterior end to the esophagus-intestine junction, and body diameter at stylet height. In addition, the following De Man indices were calculated: a (relationship between body length and largest body width), b (relationship between body length and distance from anterior end to pharyngo-intestinal junction), b' (relationship between body length and distance from anterior end to esophagus end); c (relationship between body length and tail length), and c' (relationship between tail length and tail diameter at anus height). *Pratylenchus* genus was set apart from the others using keys and descriptions of Mai et al. (1996) and then identified to species level using identification keys of Castillo and Vovlas (2007) and Gonzaga and Santos (2010), under a compound microscope (400-1,000x).

Data analysis was performed by means of

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classical statistics by using descriptive statistics (mean, standard deviation, standard error of the mean, minimum, maximum, and coefficient of variation) using the software SAS 9.4 (SAS, 2017).

RESULTS AND DISCUSSION

Among the collected samples in the sugarcane crop-growing areas, 30 *Pratylenchus* populations from the different areas were identified; 26 of them (86.7%) containing exclusively *P. zeae* and four of them (13.3%) with atypical *Pratylenchus* population. The identification of *P. zeae* was initially based on the absence of males (extremely rare), a typical characteristic of this species, and vulva position (V = 71 ± 3%) since it is in the most anterior body position.

Females of *P. zeae* had a thin body (Figure 1A) with a low lip region, but not angular and not separated from the body. Although this parameter is variable in *Pratylenchus*, it is more characteristic in certain species (OLOWE; CORBETT, 1984). The specimens showed a normal stylet (Figure 1B) with flattened bulbs and strong anterior projections, as shown in (Figure 1C), which is considered an important morphological character of this species (CASTILLO; VOVLAS, 2007). In addition, a clearly excretory pore, a dorsal esophageal gland overlapping the intestine (Figure 1D), and a quite developed median bulb (Figure 1E) were observed. However, the most anterior vulva position (Figure 2A) is the most outstanding diagnostic character in this species, when compared to other species from Brazil (GONZAGA; SANTOS, 2010).
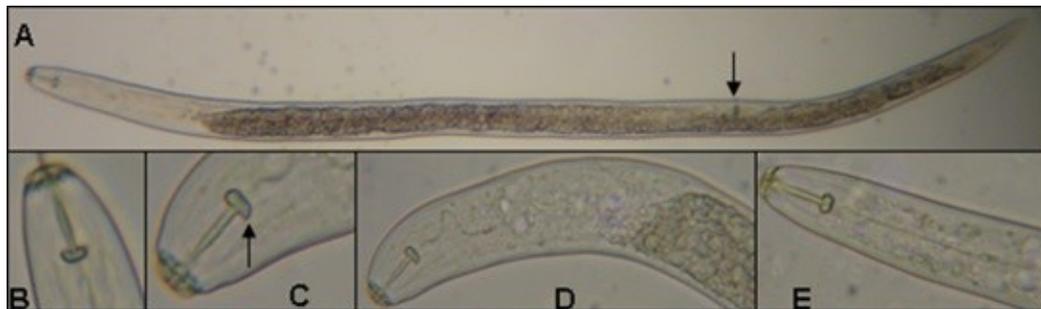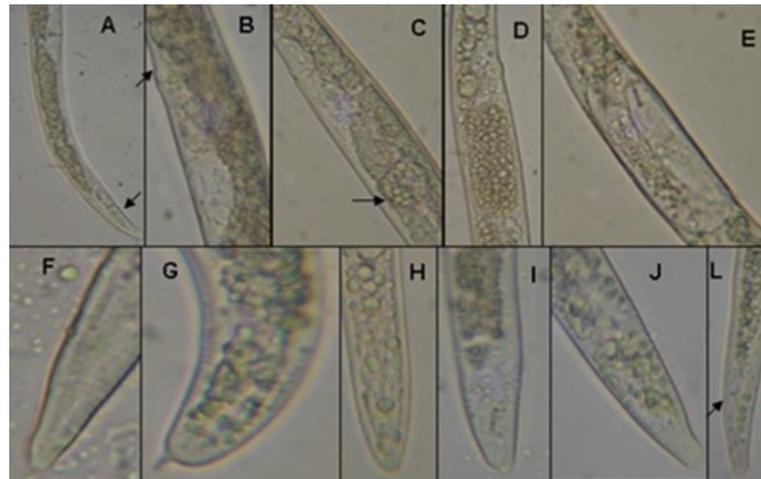

Figure 1. A) Photomicrograph of females of *Pratylenchus zeae* showing the thin body and vulva position (arrow). B) Lip region with a normal stylet. C) Stylet showing a bulb with an anterior projection (arrow) and typical low lip region of three lip rings as *P. zeae*. D) Visible excretory pore (arrow) and dorsal esophageal gland overlapping the intestine. E) Anterior region showing the median bulb (arrow).

To identify species of this genus, one of the main parameters analyzed is the number of lip rings present in the lip region. Some species have two rings, others four, and most commonly three lip rings, as observed in our study, which represent the majority of the species of the genus, such as *P. zeae*. Although most *Pratylenchus* species identified in our study had a constant number of rings in the lip region (three lip rings), few of them exhibited incomplete transverse annulus. On account of this variation we measured 40 adult females per sample, in line with Castillo and Vovlas (2007), who recommend to make measurements of at least 10 or 20 females of the species under study before it can be safely classified.

A narrow, subacute, rounded tail with predominant smooth terminus was observed in the species analyzed in this study (Figure 2A). Pointed and smooth tail, typical of *P. zeae*, were also observed, sometimes with a dorsally curved terminus

and pointed with annuli at the end (Figure 2F, 2G, 2H, 2I, 2J and 2L). The great variation in tail shape of the genus *Pratylenchus* make it a good taxonomic characteristic, however it should be the last parameter to be considered because of environmental influence and variation within species (DOUCET et al., 2001).

The post-uterine sac in *P. zeae* is short (Figure 2B) and females do not exhibit functional spermatheca, which has a round shape and most of the time is empty, without sperm (Figure 2C), a characteristic of species without males, being considered as small or absent for some authors (OLOWE; CORBETT, 1984; CASTILLO; VOVLAS, 2007). Variations in the shape of this structure may result from artifacts of preparation, such as when pressing the coverslip during preparation. The specimens were always developed showing adult females with eggs (Figure 2D) and juveniles of the second stage (Figure 2E).

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Figure 2. A) Most anterior vulva position with an arrow showing the anus. B) Photomicrograph of a female showing a short post-uterine sac (arrow). C) Short rounded spermatheca (arrow) typical of *Pratylenchus zeae*. D) Female of *P. zeae* with eggs E) and juveniles of the second stage. F-L) Variations in tail shape (L-arrow indicates the anus).

Atypical *Pratylenchus* population (10a, 12a, and 14a, all of them from Goiana) regarding vulva position (Figure 3A), presented a lip region slightly different from the typical body of nematodes with three lip rings, similar to *P. penetrans*. Another similar characteristic was the predominance of a median bulb, highly developed stylet, and evident stylet muscle (Figure 3B). Tail (Figure 3C) resembles those of both *P. penetrans* and *P. coffeae* despite the predominance of a truncated tail terminus in *P. coffeae* and conical and smooth in *P. penetrans*. In addition, rectum size between species seems to have more or less the same distance (Figure 3C and 3D). Males found in the studied populations exhibited a bursa involving the entire tail (Figure 3D), a characteristic that does not differentiate the main species found in Brazil, although males do not have relevant diagnostic characters for identifying the species.

Figure 3. A) Photomicrograph of the body of *Pratylenchus* sp. (population 10a) showing the vulva position (arrow). B) Anterior end showing the stylet bulb and medium bulb of *Pratylenchus* sp. C) Photomicrograph of the tail of *Pratylenchus* sp. D) Posterior region of a male of *Pratylenchus* sp. with the bursa involving the entire tail (arrow).

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Vulva morphology of population 12a (Figure 4A) resembles those of *P. penetrans* and *P. coffeae*. Vulva position of *P. penetrans* is not a relevant diagnostic character for identifying the nematode. No marked difference of this character was observed under the light microscope in relation to *P. coffeae*. The post-uterine sac of *P. penetrans* is short and similar to that of the studied nematode. The rounded and slightly oval spermatheca (Figure 4B) resembled that of *P. penetrans*, in contrast to the oval and functional structure in *P. coffeae*. Females of all atypical *Pratylenchus* populations showed a well-developed ovary under the light microscope (Figure 4C).
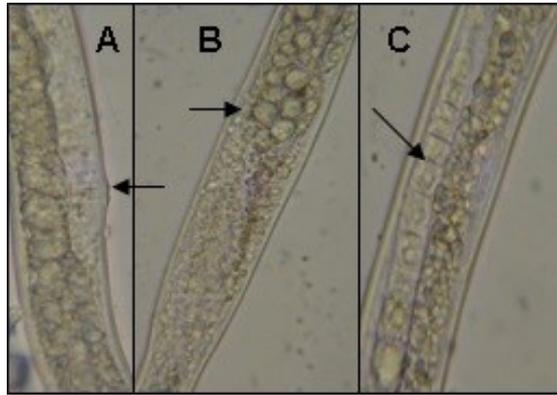
Figure 4. A) Photomicrograph of the female of *Pratylenchus* sp. (population 12a) showing the vulva position (arrow). B) Functional rounded spermatheca (arrow). C) Ovary showing germinative region (arrow).

The first population studied (population 1a from Goiana) also had atypical specimens. Tail size was small in relation to the body and the vulva was located in the posterior portion compared to that observed in other common species found in Brazil (Figure 5A). Well-developed females without male presence were observed, exhibiting a large and massive stylet similar to that of *P. brachyurus* (Figure 5A; Figure 5B). It was not possible to observe spermatheca and post-uterine sac as shown in (Figure 5C).
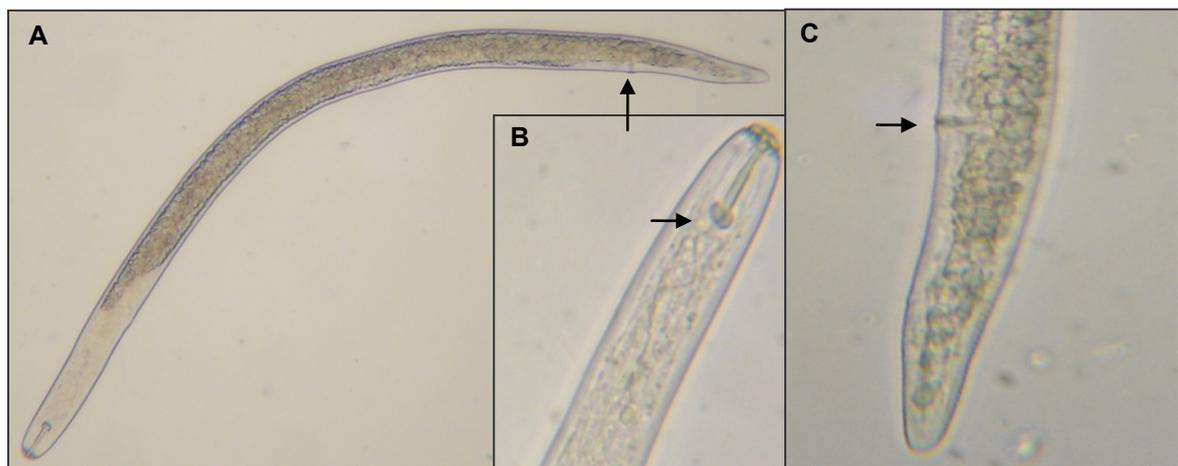
Figure 5. A) Photomicrograph of a female of *Pratylenchus* sp. (population 1a) with the indication of the vulva position in C; B) Anterior region showing the stylet (arrow) with spherical basal nodules; C) Hemispheric tail as a typical smooth terminus in P. *brachyurus*.

In general, the analyzed variables showed small variation among the studied populations (Table 1). The variables size and width of stylet were more stable when compared to the other variables, with averages of 18.20 and 4.55, respectively, in accordance with Castillo and Vovlas (2007) regarding *P. zeae*, which may enable the identification of this parthenogenetic species since males are very rare. In fact, body length of females ranged from 0.57 to 0.70 mm, as shown in Table 1.

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Table 1. Stylet length (SL), stylet bulb diameter (SBD), overall body length (L), body diameter (BD), anterior end to vulva (AEV), tail size (T), body diameter at anus height (AD), and anterior end to the end of esophagus (LEE), measurements of anterior end to the esophagus-intestine junction (LEIJ) and body diameter at stylet (BDS),**b**ody length/largest body width (A), body length/LEE (B), body length/tail length (C), AEV /L × 100 (V), L/ LEIJ (BL), tail length/DA (CL) of *Pratylenchus* females collected from 30 farms growing sugarcane (*Saccharum* sp.).

Population					Parameter: mean/ standard deviation					
	SL (μm)	SBD (μm)	L (mm)	BD (μm)	AEV (μm)	T (μm)	AD (μm)	LEE (μm)	LEIJ	BDS
1	18.09 ± 1.02	4.55 ± 0.01	0.59 ± 0.08	25.43 ± 4.63	423.85 ± 59.27	168.93 ± 35.50	14.70 ± 2.20	131.1 ± 22.11	105.46 ± 19.38	-
1a	22.75	4.55	0.67	22.75	577.85	95.55	18.20	154.70	131.95	-
2	18.09 ± 0.01	4.55 ± 0.001	0.59 ± 0.07	25.59 ± 3.92	407.11 ± 61.70	163.23 ± 23.50	15.24 ± 2.41	134.79 ± 1.64	105.22 ± 21.09	-
3	18.20 ± 0.01	4. 55 0.01	0.59 ± 0.06	24.68 ± 3.55	426.90 ± 48.14	168.23 ± 19.83	14.56 ± 1.84	133.54 ± 16.14	104.19 ± 10.69	-
4	18.20 ± 0.01	4.55 ± 0.01	0.63 ± 0.07	25.93 ± 5.17	453.97 ± 60.56	177.56 ± 19.88	14.44 ± 1.75	137.97 ± 15.87	108.17 ± 10.83	-
5	18.08 ± 0.71	4.55 ± 0.01	0.59 ± 0.07	23.88 ± 3.04	429.17 ± 52.19	169.14 ± 30.75	14.10 ± 1.72	14.10 ± 1.72	104.19 ± 14.30	-
6	18.08 ± 0.71	4.55 ± 0.01	0.63 ±0.51	25.13 ± 3.85	429.91 ± 38.16	180.97 ± 22.65	14.44 ±1.75	136.72 ± 14.16	105.78 ± 9.97	-
7	18.08 ± 0.71	4.55 ± 0.01	0.58 ±0.06	22.75 ± 2.30	422.69 ± 41.76	166.53 ± 22.78	13.87 ±1.43	137.63 ± 17.89	101.92 ± 13.81	-
8	18.08 ± 0.71	4.55 ± 0.01	0.59 ± 0.05	23.88 ± 2.47	426.79 ± 40.30	167.55 ± 17.90	13.76 ± 1.25	141.61 ± 13.55	100.66 ± 9.11	-
9	18.20 ± 0.01	4.55 ± 0.01	0.63 ± 0.06	23.66 ± 3.88	454.09 ± 50.77	176.42 ± 21.84	14.21 ± 2.10	148.89 ± 15.79	109.88 ± 11.35	-
10	18.20 ± 0.01	4.55 ± 0.01	0.68 ± 0.07	24.79 ± 3.56	489.01 ± 52.21	193.26 ± 18.22	1.67 ± 1.92	150.71 ± 16.29	112.27 ± 14.10	-
10a	18.20	4.55	0.57 ± 0.77	27.30 ±31.85	445.90 ±655.20	100.10 ±122.85	13.65 ±18.20	127.40 ±195.65	91.00 ±159.25	18.20 ±22.75
11	18.20 ± 0.01	4.55 ± 0.01	0.65 ± 0.64	25.48 ± 4.22	467.51 ± 46.59	183.13 ± 22.77	14.90 ± 2.05	147.87 ± 13.19	112.38 ± 989	-
12	18.20 ± 0.01	4.55 ± 0.001	0.63 ± 0.07	23.89 ± 3.82	455.34 ± 58.08	174.83 ± 23.80	14.33 ± 1.64	147.53 ± 15.13	110.22 ± 9.74	-
12a	18.20 ± 0.01	4.55 ± 0.001	0.53-0.57	22.75-27.30	386.75-445.90	122.55-141.05	13.65	141.05-150.15	113.75 ±122.85	18.20
13	18.20	4.55	0.68 ± 0.05	24.80 ± 3.25	486.74 ± 42.73	193.03 ± 17.57	15.24 ± 2.19	152.20 ± 13.27	114.66 ± 10.10	-
14	18.20 ± 0.01	4.55 ± 0.001	0.63 ± 0.11	24.68 ± 3.55	467.17 ± 46.41	184.73 ± 23.64	14.22 ± 1.52	145.71 ± 16.24	109.77 ± 13.48	-
14a	18.20	4.55	0.61	22.75	486.85	131.85	13.65	163.80	109.30	18.20
15	18.20 ± 0.71	4.55 ± 0.001	0.65 ± 0.06	24.46 ± 3.52	468.99 ± 49.07	187.23 ± 22.72	15.02 ± 2.11	143.44 ± 15.07	110.79 ± 12.04	-
16	18.20 ± 0.71	4.55 ± 0.001	0.66 ± 0.07	26.96 ± 4.04	468.42 ± 54.06	187.12 ± 21.29	15.02 ± 2.11	144.80 ± 14.23	112.27 ± 10.87	-
17	18.20 ± 0.71	4.55 ± 0.001	0.69 ± 0.05	25.14 ± 2.91	487.08 ± 37.30	200.66 ± 21.13	14.22 ± 1.52	145.73 ± 24.76	117.85 ± 11.69	-
18	18.20 ± 0.71	4.55 ± 0.001	0.67 ± 0.05	26.96 ± 4.29	479.00 ± 39.61	187.80 ± 15.65	14.56 ± 1.84	154.81 ± 15.43	124.33 ± 15.12	17.63 ± 1.52
19	18.20 ± 0.01	4.55 ± 0.01	0.63 ± 0.05	24.00 ± 3.85	456.82 ± 38.02	173.92 ± 21.07	14.90 ± 2.30	140.14 ± 14.79	114.32 ± 17.70	17.52 ± 1.64
20	18.20 ± 0.01	4.55 ± 0.001	0.68 ± 0.06	26.05 ± 3.26	495.50 ± 46.09	185.41 ± 23.11	15.13 ± 2.06	151.97 ± 16.51	118.07 ± 12.15	18.20 ± 0.75
21	18.20 ± 0.01	4.55 ± 0.001	0.69 ± 0.05	24.91 ± 3.98	490.15 ± 40.52	198.04 ± 15.75	14.79 ± 2.24	149.01 ± 8.29	117.39 ± 18.60	17.52 ± 1.64
22	18.20 ± 0.01	4.55 ± 0.001	0.67 ± 0.06	25.94 ± 3.84	486.05 ± 50.80	189.17 ± 24.06	14.56 ± 1.89	149.47 ± 11.26	121.03 ± 12.14	17.86 ± 1.25
23	18.20 ± 0.01	4.55 ± 0.001	0.65 ± 0.05	24.12 ± 2.56	464.78 ± 38.25	184.84 ± 17.59	14.67 ± 1.92	152.77 ± 12.44	117.96 ± 9.90	17.86 ± 1.21
24	18.20 ± 0.71	4.55 ± 0.001	0.63 ± 0.07	22.86 ± 1.27	455.11 ± 55.12	175.86 ± 24.76	13.88 ± 1.01	149.01 ± 9.75	115.68 ± 8.66	17.75 ± 1.39
25	18.20 ± 0.01	4.55 ± 0.001	0.63 ± 0.06	23.77 ± 4.05	449.77 ± 56.61	178.59 ± 21.18	14.33 ± 1.94	149.47 ± 17.83	113.07 ± 14.33	17.52 ± 2.19

Data of each population are means of 40 adult females.
Populations 1 to 14 from Goiana; populations 15 to 22 from Timbaúba; populations 23 to 30 from Barreiros.

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Table 1. Continuation.

Population	Parameter: mean/ standard deviation
	SL (μm)	SBD (μm)	L (mm)	BD (μm)	AEV (μm)	T (μm)	AD (μm)	LEE (μm)	LEIJ	BDS
26	18.09 ± 0.01	4.55 ± 0.001	0.65 ± 0.05	25.14 ± 3.41	461.48 ± 46.02	186.10 ± 22.40	14.45 ± 2.27	147.88 ± 18.08	113.75 ± 13.97	17.63 ± 1.83
27	18.20 ± 0.71	4.55 ± 0.001	064 ± 0.06	25.37 ± 4.10	459.10 ± 33.16	182.80 ± 28.58	14.79 ± 2.24	149.01 ± 11.32	113.98 ± 8.17	17.52 ± 1.64
28	18.20 ± 0.01	4.55 ± 0.001	064 ± 0.05	23.89 ± 3.37	460.12 ± 46.73	178.93 ± 22.39	14.45 ± 1.75	143.21 ± 18.71	109.43 ± 11.33	17.63 ± 1.52
29	18.09 ± 0.01	4.55 ± 0.001	0.70 ± 0.06	25.48 ± 4.58	501.41 ± 52.31	198.84 ± 23.68	14.79 ± 2.24	157.77 ± 18.81	122.96 ±14.66	18.09 ± 1.25
30	18.20 ± 0.71	4.55 ± 0.001	0.63 ± 0.05	24.23 ± 2.98	454.09 ± 44.73	177.68 ± 18.43	14.79 ± 1.99	142.64 ± 14.51	105.67 ±10.06	17.97 ± 1.00

Population	Parameter: mean/ standard deviation	
	A	B	C	V	BL	CL	
1	23.64 ± 3.24	4.58 ± 0.67	3.55 ± 0.31	71.59 ± 2.89	71.59 ± 2.98	11.68 ± 2.86	*P. zeae*
1a	29.60	4.35	5.25	85.81	5.10	7.05	*Pratylenchus* sp.
2	22.44 ± 3.51	4.29 ± 0.66	10.90 ± 1.85	71.13 ± 4.07	5.58 ± 1.11	3.57 ± 0.46	*P. zeae*
3	24.39 ± 2.93	4.50 ± 0.63	3.55 ± 0.28	71.77 ± 4.20	5.75 ± 0.67	11.65 ± 1.47	*P. zeae*
4	24.90 ± 3.92	4.61 ± 0.62	3.55 ± 0.23	71.78 ± 1.93	5.87 ± 0.74	12.42 ± 1.81	*P. zeae*
5	25.27 ± 3.43	4.38 ± 0.63	3.56 ± 0.29	71.78 ± 2.50	5.82 ± 0.96	12.04 ± 1.78	*P. zeae*
6	25.64 ± 3.36	4.68 ± 0.61	3.53 ± 0.30	71.90 ± 3.08	6.04 ± 0.72	12.67 ± 1.97	*P. zeae*
7	26.08 ± 2.70	4.34 ± 0.61	3.56 ± 0.21	71.68 ± 1.89	0.86 ± 5.87	12.13 ± 2.23	*P. zeae*
8	25.18 ± 2.49	4.25 ± 0.47	3.58 ± 0.25	71.38 ± 3.39	5.88 ± 0,86	12.26 ± 1.79	*P. zeae*
9	27.11 ± 3.90	4.27 ± 0.60	3.59 ± 0.30	71.99 ± 2.05	5.77 ± 0.71	12.59 ± 2.07	*P. zeae*
10	27.75 ± 3,32	4.55 ± 0.61	3.51 ± 0.13	73.51 ± 0.13	6.13 ± 0.91	13.35 ±1.95	*P. zeae*
10a	20.85 ±28.33	3.49 ± 4.46	5.50 ± 8.67	78.40 ±85.33	4.55 ± 6.40	4.63 ± 6.83	*Pratylenchus* sp.
11	25.92 ± 3.23	4.42 ± 0.49	3.56 ± 0.21	71.87 ± 1.64	5.80 ± 0.49	12.42 ±1.80	*P. zeae*
12	26.85 ± 4.44	4.29 ± 0.44	12.32 ± 2.04	72.22 ± 2.05	5.73 ± 0.56	3.66 ± 0.26	*P. zeae*
12a	20.00 ±23.20	3.74 ± 3.79	9.00 ±10.33	73.28 ±78.40	4.62 ± 4.64	3.74 ± 4.63	*Pratylenchus* sp.
13	27.72 ± 3.25	4.50 ± 0.56	12.85 ± 1.74	71.58 ± 1.69	5.97 ± 0.70	3.53 ± 0.21	*P. zeae*
14	26.30 ± 5.60	4.38 ± 0.88	13.09 ± 1.86	72.10 ± 2.19	5.84 ± 1.18	3.46 ± 0.54	*P. zeae*
14a	26.60	3.69	9.67	80.45	5.54	4.59	*Pratylenchus* sp.
15	27.02 ± 3.09	4.59 ± 0.51	12.60 ± 1.70	71.72 ± 2.51	5.94 ± 0.66	3.51 ± 0.23	*P. zeae*
16	24.64 ± 3.11	4.55 ± 0.48	12.65 ± 1.91	71.41 ± 1.90	5.86 ± 0.55	3.51 ± 0.23	*P. zeae*
17	27.61 ± 2.98	4.64 ± 0.48	14.23 ± 1.88	70.84 ± 1.84	5.89 ± 0.71	3.44 ± 0.20	*P. zeae*
18	25.19 ± 3.59	4.34 ± 0.55	13.03 ± 1.51	71.86 ± 1.26	5.42 ± 0.65	3.55 ± 0.15	*P. zeae*
19	26.76 ± 3.70	4.54 ± 0.57	11.93 ± 2.36	72.46 ± 1.93	5.62 ± 0.85	3.65 ± 0.27	*P. zeae*
20	26.41 ± 3.22	4.52 ± 0.45	12.49 ± 1.98	72.72 ± 2.40	5.82 ± 0.51	3.80 ± 0.32	*P. zeae*
21	28.08 ± 3.40	4.63 ± 0.45	13.55 ± 1.33	71.18 ± 1.63	5.72 ± 0.59	3.48 ± 0.19	*P. zeae*
22	26.22 ± 3.70	4.50 ± 0.48	13.11 ± 2.02	72.57 ± 3.50	5.58 ± 0.69	3.59 ± 0.31	*P. zeae*

Data of each population are means of 40 adult females.
Populations 1 to 14 from Goiana; populations 15 to 22 from Timbaúba; populations 23 to 30 from Barreiros.

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Table 1. Continuation.

Population	Parameter: mean/ standard deviation						
	A	**B**	**C**	**V**	**BL**	**CL**	
23	27.10 ± 2.54	4.27 ± 0.41	12.75 ± 1.69	71.57 ± 1.72	5.53 ± 0.52	3.52 ± 0.21	*P. zeae*
24	27.69 ± 3.15	4.25 ± 0.50	12.68 ± 1.58	71.97 ± 2.42	5.47 ± 0.56	3.63 ± 0.32	*P. zeae*
25	26.83 ± 3.61	4.24 ± 0.60	12.60 ± 1.81	71.57 ± 1.20	5.61 ± 0.76	3.52 ± 0.16	*P. zeae*
26	26.05 ± 3.11	4.42 ± 0.56	13.16 ± 2.56	71.27 ± 2.21	5.76 ± 0.77	3.50 ± 0.26	*P. zeae*
27	25.71 ± 3.21	4.32 ± 0.41	12.51 ± 1.93	71.66 ± 2.78	5.66 ± 0.59	3.56 ± 0.33	*P. zeae*
28	27.12 ± 3.29	4.54 ± 0.72	12.50 ± 1.80	71.98 ± 4.46	5.90 ± 0.75	3.60 ± 0.32	*P. zeae*
29	27.94 ± 3.16	4.49 ± 0.53	13.61 ± 1.82	71.56 ± 1.56	5.76 ± 0.73	3.54 ± 0.18	*P. zeae*
30	26.31 ± 3.08	4.46 ± 0.50	12.14 ± 1.47	71.86 ± 1.56	6.01 ± 0.67	3.56 ± 0.19	*P. zeae*

Data of each population are means of 40 adult females.
Populations 1 to 14 from Goiana; populations 15 to 22 from Timbaúba; populations 23 to 30 from Barreiros.

Females showed continuous lip region (Figure 1A), average stylet length from 18.09 to 18.31 μm, rounded and nonfunctional spermatheca (Figure 2C), and subacute and smooth tail terminus with variations (Figures 2F-L), classifying the subpopulation as P. zeae (OLOWE; CORBETT, 1984; CASTILLO; VOVLAS, 2007). In addition, the average value of V% varied from 70 to 72 in this species, indicating an important character for identifying P. zeae. This is a parthenogenetic species (rare males), and V% value is the lowest among the studied species. Morphometric data related to amphimictic subpopulations (populations 10a, 12a, and 14a) showed a similarity with P. penetrans.

Among the most important morphometric variables for identifying this species is V% value with an average of 73 to 80 (Table 1), which are in accordance with previous reports (CASTILLO; VOVLAS, 2007). The variables V% and stylet length from the first studied population had the highest values found in this study. Morphometric data related to this parthenogenetic subpopulation evidenced that the species is a P. brachyurus since it had a stylet length of 22.75 μm and V% value of 85.81, as shown in (Table 1).

Morphological analysis evidenced an angular lip region with continuous lip plate, spherical, strong, and well developed stylet basal nodules, nonfunctional spermatheca, and hemispheric and smooth tail terminus, in addition to the absence of males (Figure 5), fitting in the description of P. brachyurus population (CASTILLO; VOVLAS, 2007). The other morphometric variables included in (Table 1) were less important to identify the species when compared to those mentioned.

Some populations of Pratylenchus were atypical in relation to P. zeae; for example, population 1a, which exhibited morphometric characters similar to those of P. brachyurus. Other populations (10a, 12a, and 14a) showed males similar to P. penetrans. Although some morphometric characteristics can be modified by biotic and abiotic factors (DOUCET et al., 2001), the presence of other Pratylenchus species is expected, as reported by Bellé et al. (2014) in sugarcane plantations in south Brazil.

The identification of Pratylenchus species is based on morphological and/or morphometric differences of each species. However, their general morphology is similar, making it even more difficult to identify them. This low similarity level is applicable only when populations come from different geographical regions, which suggests that Pratylenchus genome is highly variable, in addition to inter- and intraspecific variability for Pratylenchus species. This problem tends to worsen when a mixed population occurs in the same area. The taxonomy of this genus is often questioned and special attention must be paid in species determination.

Fast recognition and identification are premise and benefit for making effective control plan. Although studies performed for identifying Pratylenchus species must be used in the molecular analysis (YU et al., 2012; MOKRINI et al., 2013; NORONHA et al., 2017), morphological characterization of the root lesion nematode is indispensable and provide base data for nematode identification.

CONCLUSIONS

Pratylenchus zeae is ordinarily present in the sugarcane crop-growing areas of Goiana, Timbaúba and Barreiros of Pernambuco state, although atypical root lesion nematode species may occur in a lower amount and frequency. Further studies to clarify the identity and distribution of these other species are

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encouraged.

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