RESEARCH/INVESTIGACIÓN

MORPHOMETRIC AND MOLECULAR IDENTIFICATION OF ROOT-LESION NEMATODES, *PRATYLENCHUS* SPP., FROM COSTA RICA

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ABSTRACT

Gamboa-Cortés, E., W. Peraza-Padilla, and P. Castillo. 2023. Morphometric and molecular identification of root-lesion nematodes, *Pratylenchus* spp., from Costa Rica. Nematropica 53:41-57.

Root-lesion nematodes, *Pratylenchus* spp., are among the top three most significant nematode parasites of a wide range of vascular plants including several economically important crops. There are more than 100 described species. Identification at the species level is difficult due to morphological similarities and overlapping morphometric characters. This research was carried out between 2017 and 2019 with the aim of identifying *Pratylenchus* species associated with four crops in Costa Rica. Species of *Pratylenchus* were identified by their morphological features and with molecular methods. For molecular analysis, rDNA 28S expansion segments D2-D3 were amplified by PCR with D2A and D3B primers and the ITS1 region with the 18S and rDNA1 primers, which were subsequently sent for sequencing. Phylogenetic relationships between *Pratylenchus* species were established by Bayesian Inference, based on the sequences analyzed. Three species of *Pratylenchus* were detected: *P. brachyurus* associated with pineapple (*Ananas comosus*), *P. coffeae* with plantain (*Musa paradisiaca*), and *P. zeae* associated with rice (*Oryza sativa*) and star grass (*Cynodon nlemfuensis*). Of the three *Pratylenchus* species identified in this research, this is the first report of *P. zeae* associated with star grass in Costa Rica, however, this species had already been reported in rice in the country.

Key words: 28S, ITS, morphology, phylogenetic analysis, plant-parasitic nematodes, sequencing, taxonomy

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RESUMEN

Gamboa-Cortés, E., W. Peraza-Padilla, y P. Castillo. 2023. Identificación morfométrica y molecular de nematodos lesionadores de la raíz de Costa Rica. Nematropica 53:41-57.

Los nematodos lesionadores de raíces *Pratylenchus* spp. se encuentran entre las tres plagas de nematodos más importantes que parasitan una amplia gama de plantas vasculares, incluidos varios cultivos de importancia económica. Hay más de 100 especies descritas. La identificación a nivel de especie es difícil debido a las similitudes morfológicas y los caracteres morfométricos superpuestos. La presente investigación se llevó a cabo entre el 2017 y el 2019 con el objetivo de identificar especies de *Pratylenchus* asociadas a cuatro cultivos en Costa Rica. Se identificaron especies de *Pratylenchus* por sus características morfológicas y con métodos moleculares. Para los análisis moleculares se amplificaron por medio de PCR los segmentos de expansión D2-D3 del gen 28S del ADNr utilizando los cebadores D2A y D3B, y la región ITS1 con los cebadores 18S y rDNA1 los cuales posteriormente se enviaron a secuenciar. Se establecieron relaciones filogenéticas entre las especies de *Pratylenchus* por medio de Inferencia Bayesiana, con base en las secuencias analizadas. Se detectaron tres especies de *Pratylenchus*: *P. brachyurus* asociada al cultivo de piña (*Ananas comosus* L.), *P. coffeae* asociada con el cultivo de plátano (*Musa paradisiaca*) y *P. zeae* asociada a arroz (*Oryza sativa*) y a un cultivo de pasto estrella. De las tres especies de *Pratylenchus* identificadas en esta investigación, *P. zeae* se reporta por primera vez asociada a pasto estrella en Costa Rica; sin embargo, en el cultivo del arroz ya había sido previamente reportada.

Palabras clave: 28S, ITS, morfología, análisis filogenético, nematodos fitoparásitos, secuenciación, taxonomía

INTRODUCTION

Costa Rica has an agricultural production area of approximately 2 million ha. This area represents 47% of the total national surface with 66% cultivated with perennial, annual, and short grass crops (Instituto Nacional de Estadísticas y Censos [INEC], 2015). All agricultural crops are attacked by many different pathogens that interfere with agricultural productivity (Sosa *et al.*, 1997). Within this biotic factor are plant-parasitic nematodes. Plant-parasitic nematodes are of economic importance since they have distribution patterns and population dynamics in the field that change depending upon the crop and management practices (Guzmán *et al.*, 2013). These organisms cause mechanical damage, affecting plant physiological processes, reducing vigor and the ability of the plant to withstand stress. Furthermore, they cause small wounds that favor the entry of other pathogens (Guzmán *et al.*, 2011).

Exact data and records of crop damage caused by plant-parasitic nematodes are not extensive in Costa Rica; however, the number of nematicides that are applied may be an indicator of the extensive prevalence of plant-parasitic nematodes in the country. Between 2005 and 2009, of the total tons of active ingredients of imported plant protection products, 4.5% were the nematicides terbufos, ethoprophos, and oxamyl (Bravo *et al.*, 2015). In 2008, 4 kg ai/ha of ethoprophos was applied to banana, representing 17% of the total application of nematicides in this crop and in tomato, 32% of active ingredient was applied. The use of these nematicides is common in crops such as melon, plantain, sugar cane, and rice (Bravo *et al.*, 2013).

Among the plant-parasitic nematodes are the migratory endoparasitic root-lesion nematodes (*Pratylenchus* spp.). A significant feature of these nematodes, unlike sedentary endoparasitic nematodes, is that all stages of development are infective (Chaves, 2014). *Pratylenchus* is considered the third most important nematode with significant economic impact on crops worldwide (Jones *et al.*, 2013). There are previous studies that have been important in the characterization of *Pratylenchus* species in Costa Rica. Zamora-Araya *et al.* (2016), taxonomically and molecularly identified the *Pratylenchus* species associated with rice, coffee, and an ornamental plantation, which are all crops of agricultural interest in Costa Rica. There are different methods to identify nematodes. Traditional (morphological) identification techniques continue to be a fundamental part of the physical characterization of nematodes (García, 2006). In addition, there are molecular tools used for the identification of nematode species and pathotypes such as the Polymerase Chain Reaction (PCR) technique with specific primers, Restriction Fragment Length Polymorphism (RFLP)-PCR, and sequencing (Vejl *et al.*, 2002). These tools allow for the separation of cryptic or morphologically indistinguishable species (Duncan *et al.*, 1999; De Luca *et al.*, 2012). The Nematology Laboratory of the School of Agricultural Sciences at the Universidad Nacional, Heredia, Costa Rica characterizes nematode species using taxonomic and molecular criteria; this identification process is called polyphasic. Using this approach, it has been possible to have an updated registry of the main nematode species found in Costa Rica as well as those of interest in this research.

The aim of the present study was to determine *Pratylenchus* species associated with plantain (*Musa paradisiaca*), pineapple (*Ananas comosus*), star grass (*Cynodon nlemfuensis*), and rice (*Oryza sativa*) in different localities than those sampled by Zamora Araya *et al.* (2016). The information generated in this study will help government institutions related to the agricultural sector, as well as producers and extension agents related to pest management.

MATERIALS AND METHODS

Nematode populations and extraction

Nematodes were collected from soil and root samples from plantain, pineapple, star grass, and rice from different locations (Table 1). Each soil sample was a composite of 20–25 soil cores collected to a depth of 5–40 cm with an Oakfield tube of 2.5-cm diameter. Samples were placed in labelled plastic bags, sealed, and brought back to the nematology laboratory where they were stored at 4°C until processed for nematode extraction. Adults (males and females) were extracted from 100 cm^3 of soil and 10 g of root by the centrifugal-flotation method described by Jenkins (1964). Extracted nematodes were placed in a 60 x 15 mm circular box, consisting of a squared plastic container for nematode enumeration.

Morphological studies

Using an inverted microscope (Olympus CK30-F100, Japan) and a nematode picking tool, 20 females and 10 males (when present) were picked and transferred to a drop of water placed on a rectangle of 3% water agar (WA) (Grewal, 1990). The WA rectangle was placed on a slide to observe each specimen. Micrographs of nematodes were taken with a Nikon DS-Fi1 camera (Nikon Eclipse 80, Japan) fitted to a microscope with differential interference contrast (DIC). Photographs were edited with Photoshop CS4 (Adobe, San Jose, CA). The specimens were then measured, and measurements were taken in micrometers (µm) with abbreviations as defined in Siddiqi (2000).

DNA extraction, PCR, and sequencing

DNA was extracted from 10 *Pratylenchus* adults from each sampled crop (Solano *et al.*, 2013). Polymerase Chain Reaction (PCR) amplification of the D2-D3 expansion segments of the 28S gene (28S) from the ribosomal DNA (rDNA) was performed with primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'TCGGAAGGAACCAGCTACTA-3') (Castillo *et al.*, 2003; He *et al.*, 2005; Palomares-Rius *et al.*, 2008). The internal transcribed spacer ITS1 (ITS1) region was amplified with primers rDNA2 (5'-TTGATTACGTCCCTGCCCTTT-3') and rDNA1.58S (5'-ACGAGCCGAGTGATCCACCG-3') (Cherry *et al.*, 1997).

Sequencing was performed on a multilayer DNA sequencer (Model 3130XL Genetic Analyzer; Applied Biosystems, Foster City, CA) using BigDye Terminator Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA). Sequences were edited and aligned with BioEdit v. 7.2.5 (Hall,

Table 1. Detailed description of the environmental characteristics of each of the collection sites.

Crop	Locality	Latitude	Longitude	Elevation (masl)	Temperature	Precipitation (mm/annual)
Plantain	Paraíso de Sixaola, Talamanca, Limón	9°28'41.85"N	82°37'11.99"W	8	Min: 22°C Max: 30°C Mean: 26°C	298
Rice	Laurel, Corredores, Puntarenas	8°28'28.6"N	82°55'27.0"W	16	Min: 22°C Max: 32°C Mean: 27°C	413
Star grass	San Vicente de Ciudad Quesada, San Carlos, Alajuela	10°17'13.85"N	84°23'5.47"W	1672	Min: 18°C Max:26°C Mean: 22°C	296
Pineapple	Pital, San Carlos, Alajuela	10°27'10.45"N	84°16'13.41"W	275	Min: 18°C Max: 26°C Mean: 22°C	296
Pineapple	Guápiles, Guápiles, Limón	10°20'47.97"N	83°80'83.83"W	50	Min: 22°C Max: 30°C Mean: 26°C	298

1999) and assembled with SeqmanPRO v.9.0. (DNA STAR Lasergene, Madison, WI). Each sequence was compared with *Pratylenchus* sequences downloaded from GenBank and sequences were submitted under the accession numbers: 28S *P. coffeae* (MH018684-MH018685), *P. zeae* (MH0108686-MH018688), *P. brachyurus* (MH018682-MH018683). ITS1 *P. coffeae* (MH020808-MH020809), *P. zeae* (MH020810-MH020811), *P. brachyurus* (MH020807).

Phylogenetic analysis

Sequences of the 28S and ITS1 genes of different species of *Pratylenchus* were retrieved from GenBank. The taxa and outgroups used to analyze the sequences of the 28S were the same as those used by Subbotin *et al.* (2008) and Palomares-Rius *et al.* (2014). *Nacobbus aberrans* was used as the outgroup for the ITS1 phylogeny as Palomares-Rius *et al.* (2014) and Zamora-Araya *et al.* (2016). Multiple alignments were made with MAFFT v.7.205. (Katoh and Standley, 2013). Phylogenetic analyses were performed based on Bayesian Inference (BI) criterion (Schwarz, 1978) with MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2003). The best-fitted model for nucleotide substitution was determined by jModelTest v. v2.1.10 (Darriba *et al.*, 2012). FigTree program v1.4.3 was used to visualize the phylogenetic trees (Rambaut, 2009).

RESULTS

Morphological, morphometric, and molecular analysis allowed for the identification of *Pratylenchus* species associated with four crops of agricultural importance in Costa Rica (pineapple, plantain, rice, and star grass). *Pratylenchus coffeae* was identified from a farm dedicated to the cultivation of plantain in Talamanca, Limón province. *Pratylenchus brachyurus* was found associated with two pineapple plantations in San Carlos, Alajuela, and Guápiles, Limón province. *Pratylenchus zeae* was found in association with rice in Laurel, Corredores, Puntarenas and star grass in San Vicente de Ciudad Quesada, San Carlos, Alajuela province.

Morphometric parameters established by other researchers for *P. coffeae*, *P. brachyurus,* and *P. zeae* agreed with the data obtained in this research (Tables 2, 3, and 4). However, some measurements had higher or lower values than those of the type of population, which is normal in taxonomic identifications.

Morphological characterization

Pratylenchus coffeae (Ryss, 1988) (Table 2; Fig. 1) was found in the roots and soil of plantain in Talamanca locality, Huetar Atlántica zone, in the province of Limón, Costa Rica. Females (n = 20) variable in body length of 421 to 700 µm and maximum body width (MBW) of 17.0 to 25.0 µm, as well as in the shape and thickness of the tail. Lip region with two annuli. Back edge of lip frame strongly sclerotized. Lateral field with four lines, with internal bands between them. Stylet size between 13.5 to 18.0 µm, with a dorsal gland opening (DGO) 1.5 to 3.5 µm from stylet base. Excretory pore between 74 to109 µm from anterior end. Pharynx between 113 to 151 µm long. Vulva located between 80 to 143 µm from anterior end, representing 77.5 to 83.1% with respect to total body length. Anterior gonad between 130 to 307 µm long. Post-vulval uterine sac between 17 to 32 µm long. Tail shape variable, truncated or hemispherical 20 to 30 annuli and 24 to 37 µm long. Spermatheca large broadly oval to almost round, with a ratio of 76-84% of the vulvar position regarding body length, and female tail tip truncate or hemispherical.

Males (n = 10) were found in 50% of the sample. Body length ranged from 469 to 562 µm, with MBW of 17.0 to 20.0 µm. Stylet between 14.0 to 16.0 µm and a DGO 3.0 to 4.0 µm from stylet base. Four lines at lateral field. Excretory pore 82 to 97 µm from anterior end. Pharynx 113 to 137 µm long. Spicule between 16 to 24 µm long and the gubernaculum between 3.0 to 6.0 µm long. Tail 26 to 34 µm long.

Pratylenchus zeae (Graham, 1951) (Table 3; Fig. 2) was found in roots and soil of star grass in the town of San Vicente de Ciudad Quesada, San Carlos, in the province of Alajuela, Costa Rica. Also, it was found associated with roots and soil of rice in the town of Laurel, Corredores, in the province of Puntarenas, Costa Rica. To the best of our knowledge, this is the first report of *P. zeae* in star grass in Costa Rica. Females (n = 20) had a body length ranging from 307 to 534 µm, MBW of 16.0 to 21.0 µm. Lip region showing three annuli. Stylet between 13.0 to 16.0 µm long with a DGO of 2.0 to 3.0 µm. Lateral fields with four lines

Table 2. Morphometric measurements of females and males of the *Pratylenchus coffeae.*

Host	Plantain (*Musa* sp.)		Ryss, 1988	
	Female (*µm*)	Male (*µm*)	Female (*µm*)	Male (*µm*)
n	20	10	10	10
L	554.2±78.9(420.9-700.0)	515.3±32.2(468.6-562.3)	620.0 (490.0-650.0)	520.0 (440.0-600.0)
A	26.6±1.9 (23.2-30.9)	28.7±1.3 (26.9-30.7)	28.0 (25.0-31.0)	28.0 (25.0-32.0)
B	4.1±0.5 (3.2-4.9)	17.4±1.6 (14.3-19.9)	5.9 (5.1-6.6)	6.2 (5.6-6.9)
C	18.8±2.0 (14.4-21.7)	34.6±3.9 (26.3-40.7)	21.0 (19.0-22.0)	20 (18.0-23.0)
c´	2.3±0.2 (2.0-2.7)	2.0±0.3 (1.6-2.5)	3.2	2.0
G1	32.7±5.7 (24.0-43.9)	-	41.5	-
O	18.3±3.6 (10.5-22.5)	21.8±2.6 (18.5-25.5)	18.7	20.0
M	43.4±3.3 (38.6-49.4)	44.4±2.7 (41.1-50.4)	50.0	40.0
MB	43.7±4.8 (34.1-52.8)	3.7±5.6 (27.1-42.4)	41.9	41.0
MBW	20.9±2.5 (17.3-25.0)	18.8±.9 (17.0-2.1)	20.0	14.0
ABW	12.7±1.5 (10.8-16.8)	15.0±1.7 (12.0-17.8)	10.0	12.0
Lip annulments	1.6±0.5 (1.0-2.0)	1.9±0.3 (1.0-2.0)	2.0	2.0
Stylet length	16.7±1.0 (13.6-18.0)	14.9±0.7 (13.7-15.7)	17 (15-18)	16.0 (15.0-17.0)
Conus	6.9±0.5 (6.1-7.8)	6.6±0.4 (6.0-7.3)	8.0	6.0
DGO from stylet base	2.9±0.6 (1.6-3.6)	3.2±0.3 (2.8-3.7)	3.0	3.0
Excretory pore length	93.7±11.4 (73.8-109.4)	89.2±5.4 (81.5-96.5)	65.0	70.0
Esophagus length	135.5±13.5 (113.1-151.4)	126.0±8.4 (113.0-136.5)	106.0	101.0
Post-corpus length	59.4±10.0 (39.0-79.9)	47.1±5.1 (36.8-53.5)	42.5	41.5
N° of side lines	4.1±0.2 (4.0-5.0)	4±0.0 (4.0-4.0)	4.0	4.0
V	109.4±16.9 (80.0-142.9)	-	100.0	-
Anterior gonad length	181.0±40.8 (129.8-307.3)	-	200.0	-
Post vulvar uterine sac	24.2±4.7 (16.7-31.7)	-	21.5	-
V%	80.2±1.7 (77.5-8.1)	-	79 (76.0-82.0)	-
Tail length	29.5±2.7 (24.4-37.0)	29.8±2.8 (25.9-33.9)	26.3	25.0

Table 3. Morphometric measurements of females of the *Pratylenchus zeae.*

	(*Cynodon nlemfuensis*)	(*Oryza sativa*)	Graham, 1951
Host	Female (*μm*)	Female (*μm*)	Female (*μm*)
n	20	20	
L	354.3±28.4 (306.5-440.9)	430.2±38.6 (341.3-533.9)	540.0 (360.0-580.0)
A	19.6±1.7 (15.8-23.1)	23.7±2.6 (19.4-30.9)	27.0 (25.0-30.0)
B	3.7±0.3 (3.2-4.6)	3.8±0.3 (3.1-4.4)	6.5 (5.4-8.0)
C	15.9±2.2 (12.7-21.0)	17.0±1.5 (14.6-19.2)	15.2 (17.0-21.0)
c´	2.1±0.1 (1.9-2.4)	2.5±0.3 (1.8-2.9)	2.5
G1	24.6±3.6 (19.6-31.5)	28.9±3.8 (23.5-38.4)	21.3
O	17.6±24 (13.0-22.7)	16.6±2.4 (11.9-20.4)	-
M	46.7±3.7 (37.3-54.9)	46.7±3.3 (37.3-50.7)	40.6
MB	39.2±3.5 (30.9-46.9)	42.0±3.2 (37.0-49.7)	41.7
MBW	18.1±0.8 (16.4-19.8)	18.2±1.4 (16.3-21.6)	23.7
ABW	10.7±0.7 (9.4-11.9)	10.4±0.6 (9.0-12.2)	10.0
Lip annulments	2.0±0.2 (1.0-2.0)	2.1±0.2 (2.0-3.0)	3.0
Stylet length	14.1±0.6 (13.2-161)	14.4±0.8 (13.1-15.7)	15.5 (15.0-17.0)
Conus	6.6±0.4 (5.9-7.4)	6.7±0.7 (5.0-7.6)	6.3
DGO from stylet base	2.5±0.3 (2.0-3.0)	2.4±0.4 (1.6-2.9)	-
Excretory pore length	67.0±6.0 (54.7-78.1)	72.2±9.9 (50.6-86.1)	70.0
Esophagus length	95.0±7.9 (77.3-109.5)	114.2±8.4 (99.2-132.2)	113.8
Post-corpus length	37.7±5.3 (26.8-49.2)	48.0±5.0 (40.4-58.0)	47.5
N° of side lines	3.8±0.6 (2.0-4.0)	4.0±0.0 (4.0-4.0)	4.0
V	105.1±9.8 (87.5-123.0)	122.9±12.2 (101.4-158.1)	86.8
Anterior gonad length	87.1±13.2 (66.9-198.2)	123.9±15.4 (102.8-155.9)	115.0
Post vulvar uterine sac	17.2±4.2 (10.1-26.4)	24.6±3.6 (19.0-34.2)	25.0
V%	70±2.2 (66-75)	71±1.7 (69-75)	71.0 (68.0-76.0)
Tail length	22.6±2.3 (18.4-26.3)	25.6±3.5 (17.8-30.7)	25.0
N°. of tail annuli	20.4±2.0 (16.0-24.0)	23.3±1.9 (20.0-27.0)	19.0

extending to the end of the tail. Excretory pore between 51 to 86 µm from anterior end. Pharynx 77 to 132 µm long. Anterior gonad between 66.2 to 189.2 µm long. Post-vulval uterine sac between 10.0 to 34.0 µm long. Round and small spermatheca, without sperm. Vulva 87.5 to 158.0 µm from anterior end, representing 66 to 75% with respect to body length. Tail shape variable, usually pointed, narrowly rounded and smooth, with 18.0 to 31.0 µm long and between 16 to 31 annuli. No male specimens were found in this population.

Pratylenchus brachyurus (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941) (Table 4; Fig. 3) was found in the roots and soil of pineapple in San Carlos, in the province of Alajuela, and in Guápiles, province of Limón, Costa Rica. Females (n = 20) with a body length ranging from 446 to 756 µm and MBW of 19.0 to 32.0 µm. Lip region with two annuli. Stylet 15.0 to 20.0 µm long with a DGO of 2.0 to 9.0 µm from stylet base. Lateral fields with four lines extending to the end of the tail. Excretory pore between 76 to 118 µm from anterior end. Pharynx between 113 to 170 µm long. Anterior gonad between 103 to 280 µm long. Post-vulval uterine sac between 15.0 to 34.0 µm in length. Spermatheca round and small. Vulva located at 69 to 102 µm from anterior end and representing 81-87% with respect to body length. Tail tip with variable shape, generally closely rounded, and smooth, with a length between 23 to

Table 4. Morphometric measurements of females of the *Pratylenchus brachyurus.*

Host	(*Ananas comosus*)[y] Female (*μm*)	(*Ananas comosus*)[z] Female (*μm*)	Van der Berg, 1971 Female (*μm*)
n	20	20	12
L	555.4±54.7 (464.3-639.0)	575.6±73.3 (446.1-756.1)	560.0 (520.0-600.0)
A	23.3±2.0 (18.2-25.9)	23.1±2.0 (18.3-26.5)	20.0 (18.0-23.0)
B	4.0±0.5 (2.9-4.8)	4.0±0.5 (3.3-5.5)	3.9 (3.4-5.2)
C	20.6±2.9 (14.4-25.6)	19.5±2.0 (15.7-24.1)	19.0 (16.0-23.0)
c′	2.0±0.2 (1.7-2.3)	2.0±0.2 (1.5-2.6)	2.4
G1	28.8±5.0 (18.3-37.8)	30.5±5.6 (20.3-38.3)	18.3
O	14.6±2.2 (11.8-21.1)	15.0±2.5 (11.1-20.2)	12.5
M	43.7±2.9 (38.0-48.3)	44.4±3.1 (39.3-51.7)	50.0
MB	45.1±5.7 (33.8-55.3)	43.3±5.4 (26.3-50.7)	40.6
MBW	23.9±2.2 (19.1-27.7)	25.0±3.5 (18.7-32.0)	21.0
ABW	13.9±1.1 (12.0-15.7)	14.9±1.7 (11.9-17.9)	14.0
Lip annulments	2.0±0.0 (2.0-2.0)	2.0±0.0 (2.0-2.0)	2.0
Stylet length	17.6±1.0 (15.2-19.1)	18.4±1.0 (16.9-20.2)	19.0 (18.0-21.0)
Conus	7.7±0.6 (6.5-8.7)	8.1±0.6 (7.0-9.2)	6.5
DGO from stylet base	2.6±0.4 (2.1-3.8)	2.8±0.5 (2.1-3.9)	3.0
Excretory pore length	102.5±8.4 (81.3-117.3)	98.7±11.0 (76.1-118.4)	97.4
Esophagus length	139.1±17.2 (112.6-162.5)	143.2±11.5 (121.8-169.8)	155.3
Post-corpus length	62.3±7.7 (49.1-76.2)	62.1±9.6 (40.9-82.1)	63.1
N° of side lines	4.0±0.0 (4.0-4.0)	4.0±2.1 (4.0-4.0)	4.0
V	85.4±9.2 (72.3-100.4)	86.7±9.0 (68.5-102.0)	94.7
Anterior gonad length	159.1±28.2 (117.1-209.9)	176.1±42.3 (102.6-279.9)	102.7
Post vulvar uterine sac	23.6±4.4 (15.6-34.0)	18.6±2.1 (15.0-24.4)	30.3
V%	85±1.5 (81-87)	85±1.3 (82-87)	86.0 (84.0-87.0)
Tail length	27.2±3.1 (22.5-32.7)	29.7±3.5 (23.3-36.4)	33.0
N°. of tail annuli	16.2±2.4 (13.0-21.0)	17.9±1.8 (14.0-21.0)	19.0

[y] San Carlos, Alajuela province

[z] Guápiles, Limón province

36 µm long and between 13 to 21 annuli. Males are rarely present; no specimens were found in this study.

Molecular characterization

Sequences of the 28S D2-D3 of *P. coffeae* (MH018684-MH018685) from plantain showed 99.7% and 99.7% similarity with sequences of *P. coffeae* from Vietnam (KC857658) and China (MG906758), respectively. Sequences of the 28S D2-D3 region of *P. zeae* (MH018686-MH018688) showed 98% similarity with *P. zeae* (KT971361) from San Carlos, Alajuela, Costa Rica in rice and reported by Zamora Araya *et al.* (2016), and with *P. zeae* (JN020932 and JN020931) associated with *Cymbopogon* sp. in China (Zhao, 2011). *Pratylenchus brachyurus* sequences (MH018682-MH018683) from this study showed 97%, 98%, and 99% similarity with *P. brachyurus* associated with Cerasus sp. in Japan (EU130842) (Subbotin *et al.*, 2008), *Alocasia macrorrhizos* in China

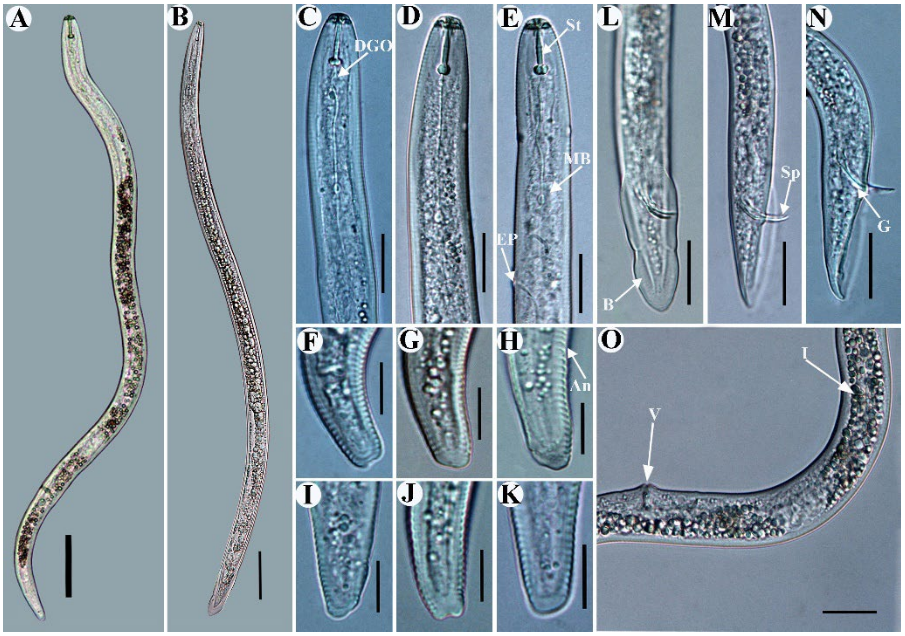

Figure 1. Photomicrographs of *Pratylenchus coffeae* in plantain. A. Female. B. Male. C, D and E. Anterior region of the female, showing: Dorsal gland opening (DGO), Median Bulb (MB), excretory pore (EP) and stylet (St). F, G, H, I, J, and K. Posterior region of the female: Annules (An). L, M, N. View of the male's tail: Bursa (B), Spicule (Sp), and gubernaculum (G). O. Posterior region of female, vulva (V) and Intestine (I). Scales: A: 50 µm, B: 30 µm, C, D, E, F, G, H, I, J, K, L, M, N, O: 20 µm).

(MH142615) (Hou, 2018), and in Brazil (KT948338), respectively.

The best nucleotide substitution models to generate the phylogenetic trees were GTR + I + G and TVM + I + G with the BI criterion for 28S and ITS1, respectively. Phylogenetic relationships between *Pratylenchus* species inferred from the analysis of the ITS1 (Fig. 4) and 28S (Fig. 5) showed similar clusters in their topology. The dendrogram generated with the ITS1 showed that *P. coffeae* (MH020808-MH020809) clustered with the *P. coffeae* (KR106212) from China (Huang and Jiang, 2015), and with *P. coffeae* (KY424204) also from China (Wang *et al.*, 2017) (Fig. 4). *Pratylenchus* zeae (MH020810-MH020811) grouped with *P. zeae* accession KY424186 from China (Wang *et al.*, 2017), and accession JN020935 from Saipan, United States (Zhao, 2011). *Pratylenchus brachyurus* (MH020807) grouped with *P. brachyurus* accession KY828251 from Belgium (Janssen *et al.*, 2017) and *P. brachyurus* (MG738356) from China (Wang and Liao, 2018).

The dendrogram generated with the 28S showed that *P. coffeae* (MH018684-MH018685) grouped with *P. coffeae* (LT965041) from South Africa (Fanelli *et al.*, 2018) (Fig. 5). *Pratylenchus zeae* (MH018686-MH018688) grouped with *P. zeae* accessions KY424263 from China (Wang *et al.*, 2017) and JN020932 and JN020931 from Saipan, United States (Zhao, 2011). Also, *P. brachyurus* (MH018682-MH018) grouped with *P. brachyurus* accession KY424286 from China (Wang *et al.* 2017) and *P. brachyurus* accession KT948328 from Brazil (Ribeiro *et al.*, 2016).

DISCUSSION

The presence of *Pratylenchus* spp. in Costa Rica indicates potential for damage to the sampled crops and the need for appropriate management strategies. Previous studies in Costa Rica revealed the presence of some species associated with different crops. For example, *P. zeae* was identified in rice (López and Salazar, 1988; Sandoval, 2015; Zamora-Araya *et al.*, 2016) and sugarcane (López and Salazar, 1990; Sandoval, 2015); *P. brachyurus* in pineapple (López and Salazar, 1990), pepper (Sandoval, 2015), greater yam and yampee (Humphreys-Pereira *et al.*, 2017); *P. coffeae* in

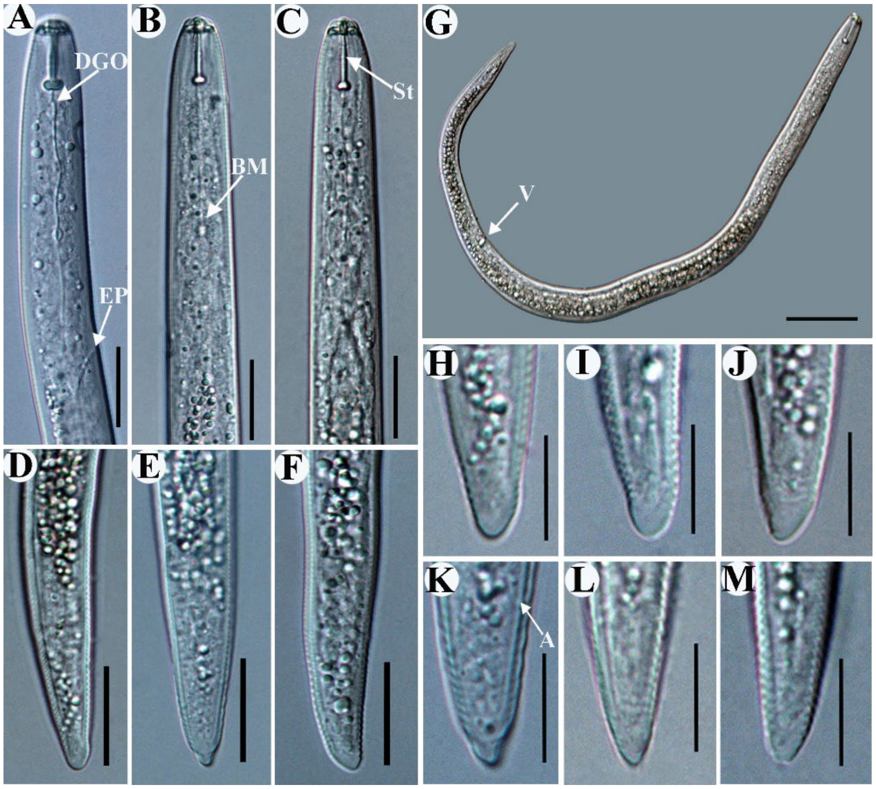

Figure 2. Photomicrographs of *Pratylenchus zeae* in rice. A, B, C. Lateral view of the female's front part showing the dorsal gland opening (DGO), Excretory pore (EP), Median bulb (MB), Stylet (St). D, E and F. Side view of the tail of the female. G. Female showing the vulva (V). H, I, J, K, L and M. Terminal part of the tail of females: Anus (A). Scales: A, B, C, D, E, F, H, I, J, K, L, M: 20 µm, G: 50 µm.).

yam (*Dioscorea* spp.), greater yam and yampee (Humphreys-Pereira *et al.*, 2017), and *Aglaonema commutatum* (López and Salazar, 1990); *P. gutierrezi* in coffee (Golden *et al.*, 1992; Sandoval, 2015; Zamora-Araya *et al.*, 2016); *P. penetrans* in *Rumohra adiantiformis* (López and Salazar, 1990), onion, strawberry. and lily (Sandoval, 2015); *P. crenatus* in onion and potato; *P. pseudocoffeae* on *Aster* sp. (Sandoval, 2015) and *Chrysanthemum* sp. (Zamora-Araya *et al.*, 2016); *P. speijeri* in plantain (Sandoval, 2015); and, *P. bolivianus* in Fer (Zamora-Araya *et al.*, 2016).

According to Silva and Inomoto (2002), *P. coffeae* is closely related to coffee cultivation in Costa Rica. However, Fernández and Quesada (2013) and Esquivel and Peraza (2010) found *P. coffeae* also associated with banana cultivation in the Huetar Norte region where the most important plant-parasitic nematodes in Musaceae were *Radopholus*, *Pratylenchus,* and *Meloidogyne* (Guzmán *et al.*, 2013). According to Gowen *et al.* (2005), *P. coffeae* is among the most common nematodes in *Musa* plantations in the world, causing the destruction of primary roots in plants and up to 30% losses if not controlled.

Pratylenchus coffeae can be distinguished from the closely related species (*P. loosi*) by body annulation, the position of the vulva and the shape of the female tail, truncated or hemispherical in *P. coffeae*, and without cut or finely pointed in *P. loosi*. The matrix codes for *P. coffeae* are: A1, B2, C2, D3, E3, F6, G2, H1, I2, J1-2-3, K1 (Castillo and Vovlas, 2007). This species is widespread in both Central and South America. It has been reported in yam and plantain in Belize (Bridge *et al.*, 1996), and in banana in Colombia (Grisales-Lopez and Lescot, 1999), and Puerto Rico (Oramas and Román, 1982). The morphometric analysis, together with the descriptions made by Bridge *et al.* (1996) in Belize, Moura *et al.* (2001) in Brazil, Bridge (1976) in Ecuador, Marbán-Mendoza and Flores (1993), and Ryss (1988) in Costa Rica allowed for the identification of the *P. coffeae* population associated with banana cultivation.

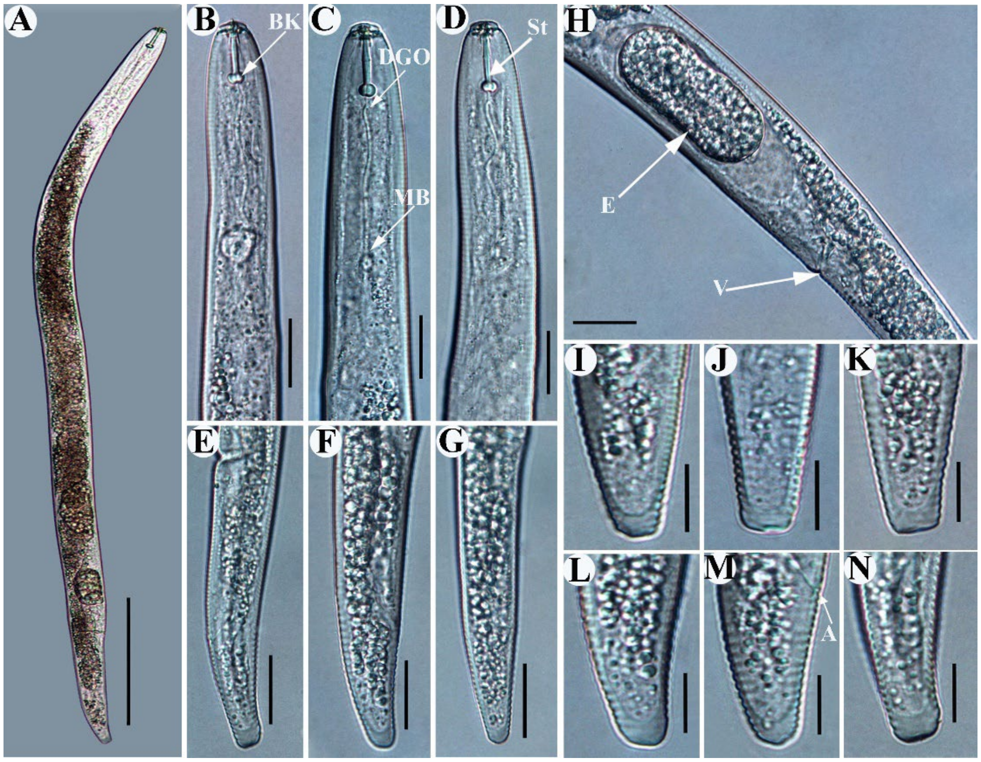

Figure 3. Photomicrographs of *Pratylenchus brachyurus* in pineapple. A. Female. B, C, D. Lateral view of the female's front body, showing Basal knobs (BK), Dorsal gland opening (DGO), Median bulb (MB), Stylet (St). E, F and G. Side view of the tail of the female. H. Egg inside the female's body, Vulva (V). I, J, K, L, M and N. Terminal part of the tail of females: Anus (A). Scales. A: 100 µm. B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q: 20 µm).

According to Ryss (1988), *P. coffeae* differs from other *Pratylenchus* species by the number of lip annuli (2), V ratio 79% (76-82%), post-vulval uterine sac 21.5 µm long, the shape of the smooth subspherical tail and the presence of males with a spicule length 17.0 µm and gubernaculum of 6.0 µm. When comparing the measurement of this investigation with the original description of the species, it was observed that the specimens had two labial annuli, V ratio 80.2% (77.5-83.1%), post-vulval uterine sac 24.0 µm, and a spherical tail tip shape with irregular termination, and the presence of males with a spicule 18.0 µm and a gubernaculum of 4.0 µm long. In addition, other measurements such as stylet length, number of lateral lines, anal body width (ABW), c and c´ ratio, length and number of tail annuli were coincident.

With respect to *P. zeae*, it is common to find this nematode associated with rice plantations in Costa Rica (López *et al.*, 1987; López and Salazar 1988; Araya 2008; Guzmán *et al.*, 2011). The nematodes of greatest importance for rice cultivation in Costa Rica were: *Hirschmanniella oryzae*, *A. besseyi*, *Criconemella* sp., *Helicotylenchus* sp., *Longidorus* sp., *Meloidogyne arenaria*, *M. exigua*, *M. incognita*, *M. salasi*, *Paratrichodorus* sp., *P. zeae*, *Rotylenchulus* sp., *Trichodorus* sp., *Tylenchorhynchus* sp. and *Xiphinema* sp. (Fernández and Quesada, 2013). Furthermore, Duncan and Moens (2006) reported that *P. zeae* parasitizes cereal plantations, forage crops, sugar cane and tobacco in subtropical regions.

Pratylenchus zeae is characterized by a lip region with three annuli. Round and small spermatheca with a V ratio of 68-76%. Tail with variable tip shape, usually pointed. It is distinguished from closely related species such as *P. bolivianus* and *P. curvicauda* by the length of the stylet, position of the vulva, shape of the spermathecal, and tail shape. Males are rarely

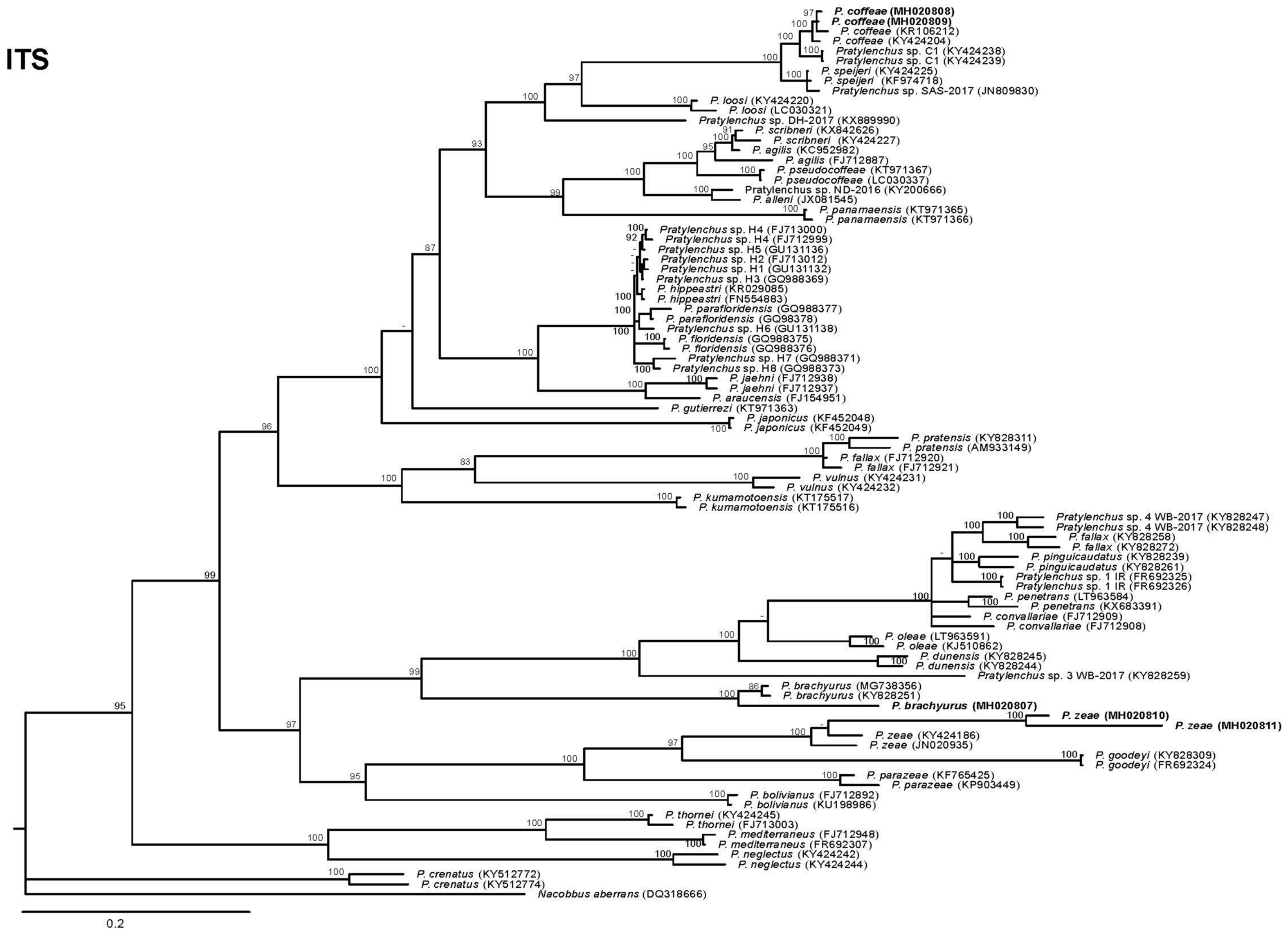

Figure 4. 50% consensus rule of a Bayesian Inference (IB) and Maximum Likelihood (MV) analysis tree generated from the ITS sequence data set for *Pratylenchus coffeae* species; *P. brachyurus*, and *P. zeae*. Codes generated for identification with the plantain (PL001, Pl002), Pineapple (PÑ003), Rice (Ar001), Star Grass (PE001).

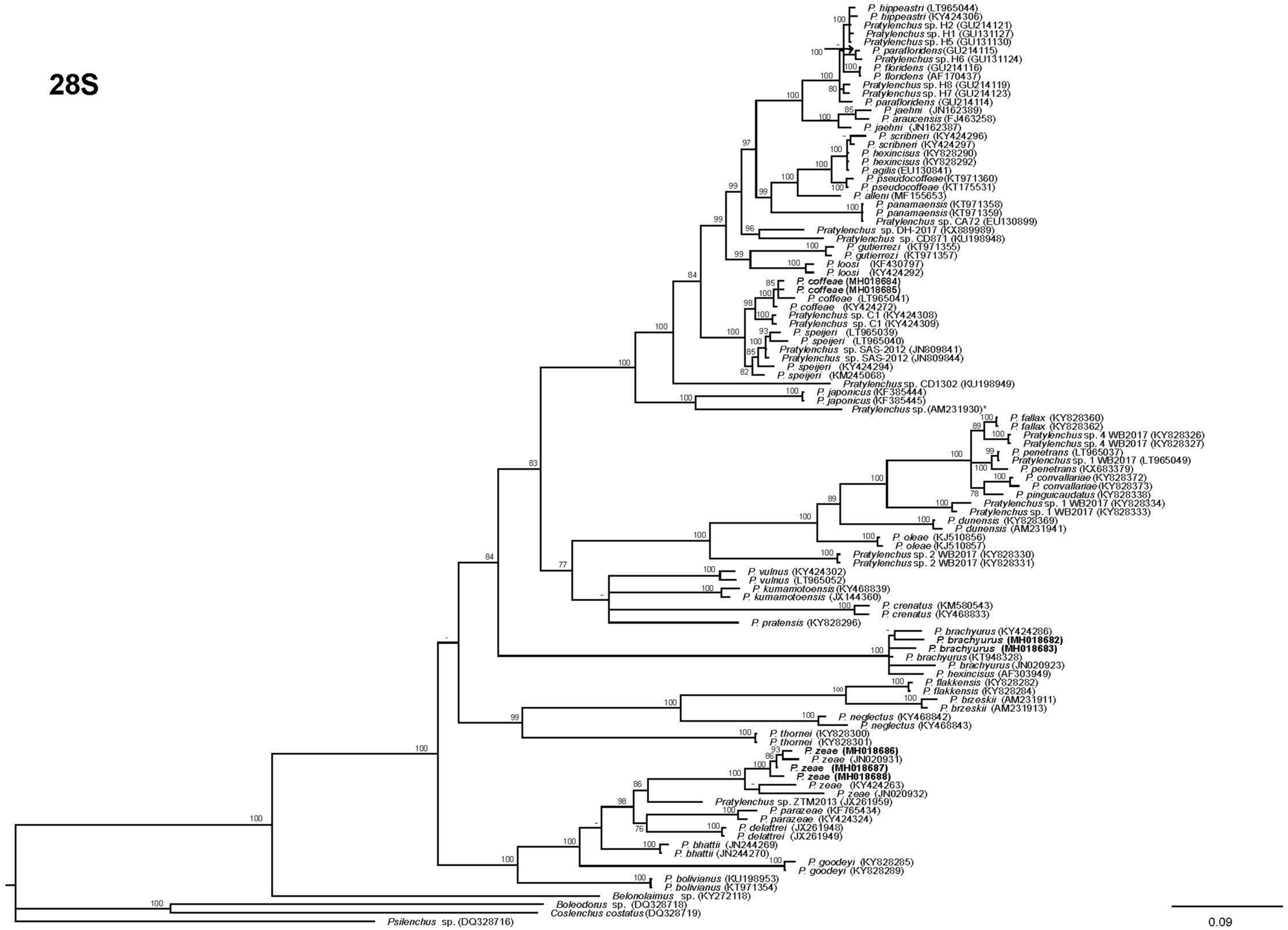

Figure 5. 50% consensus rule of a Bayesian Inference and Maximum Likelihood analysis tree generated from the D2-D3 region of the 28s rDNA of the sequence gene dataset for the *Pratylenchus coffeae* species; *P. brachyurus* and *P. zeae*. Codes generated for identification with the plantain (PL001, Pl002), Pineapple (PÑ002, PÑ003), Rice (Ar001, Ar002), Star Grass (PE001).

present; however, they are not essential for reproduction. Nonetheless, on the occasions when a male was found, it presented a thin arc-shaped spicule 14 to 15 µm long, with a gubernaculum 4 to 5 µm long and slightly indented bursa margins according to Castillo and Vovlas (2007). Studies by Inserra *et al.* (2005), showed similarity between *P. jordanensis* and *P. zeae*, suggesting that the former may be a synonym for the latter, however, molecular markers from the former species are lacking and may be also cryptic species. The matrix codes for *P. zeae* are: A2, B2, C3, D1, E1, F5, G3, H3, I2, J1, K1 (Castillo and Vovlas, 2007).

The morphometric analysis, together with the descriptions made by Doucet and Cagnolo (1998) in Argentina, López and Salazar (1990) in Costa Rica, Fernández and Ortega, 1998 in Cuba, Yu (2008) in Canada, and Graham (1951) in USA allowed the identification of the *P. zeae* populations associated with star grass and rice. According to Graham (1951), *P. zeae* is distinguished from other *Pratylenchus* species by the number of lip annuli (3), female stylet length (15.5 µm), V ratio 71.0% (68 to 76 µm), and pointed shape of the tail. Measurements of these populations are within the range with the original description of the species (Castillo and Vovlas, 2007).

In Costa Rica *P. brachyurus* has been found associated with pepper in Muelle de San Carlos, Alajuela (Sandoval, 2015) and pineapple (López and Salazar, 1990). In studies carried out by Guzmán *et al.* (2013), *Pratylenchus* and *Helicotylenchus* had the highest population densities in the Huetar Norte and Huetar Atlántica regions in pineapple cultivation.

The morphometric analysis agreed with other published descriptions of *P. brachyurus* populations associated with pineapple cultivations (Pinochet *et al.*, 1978; Stanton *et al.*, 1989; López and Salazar, 1990; Fourie *et al.*, 2001). According to Van der Berg (1989), *P. brachyurus* is distinguished from other *Pratylenchus* species by the number of lip annuli (2), stylet length (19 µm), V ratio 86% (84 to 87%), a pointed tail shape, discrete nonfunctional spermatheca, and a uterine sac less than body diameter. The tail is widely cone-shaped, smooth, and rounded, truncate or spatulate at the tip. Male presence is rare (Castillo and Vovlas 2007). This species is related to *P. japonicus* and *P. neobrachyurus*; however, it differs by the body and length of the stylet, the shape of the lip region, shape of the stylet knobs, the position of the vulva, and the shape of the female's tail (Corbett and Clark, 1983). The matrix code is: A1, B2, C4, D1, E4, F3, G3, H1, I4, J2-3, K1 (Castillo and Vovlas 2007).

Primers rDNA2-rDNA1.58S and the ones for the ITS1 region have been used for the identification of *Pratylenchus* species. Machado *et al.* (2007) and Machado *et al.* (2015) identified *P. brachyurus*, *P. zeae*, *P. coffeae*, *P. penetrans*, *P. jaehni* in Brazil using these universal primers. Zamora-Araya *et al.* (2016) used these primers for the first time to discriminate between *P. gutierrezi*, *P. bolivianus* and *P. pseudocoffeae* in Costa Rica. *Pratylenchus* coffeae and *P. brachyurus* data add to the few available records for Costa Rica of species identification with rDNA2 primers.

Pratylenchus are difficult to identify mainly due to interspecific characters overlapping and intraspecific variation in conventional morphological characters, which are very similar between one species and another (Castillo and Vovlas, 2007). Consequently, application of integrative taxonomical approaches (morphometrical and molecular) is essential for precise and accurate identification. It is important to carry out further research on the pathogenicity and host-parasite relationships of each identified species as well as new nematode species not reported for Costa Rica and their association with other host plants. In conclusion, combining the morphological aspects and molecular data and observations with the known ones allows for the correct identification and the existence of new records or species that have not yet been reported. It is extremely important to carry out this type of studies to have clarity regarding the species that may be damaging with a certain crop.

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