RESEARCH NOTE/NOTA DE INVESTIGACIÓN

FIRST REPORT OF PRATYLENCHUS PANAMAENSIS IN THE SOUTHERN REGION OF COSTA RICA

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ABSTRACT


Coffee (Coffea arabica L.) is one of the most valuable tropical crops in Costa Rica. The plant-parasitic nematodes Meloidogyne exigua and Pratylenchus spp. are a major problem on coffee in the country. A population of Pratylenchus sp. was collected from a coffee plantation in the locality of Daniel Flores, Pérez Zeledón in the southern region of Costa Rica. Morphological and morphometric analyses were performed on 20 females and 20 males. Two nuclear markers, the internal transcribed spacer (ITS) and the expansion segment D2-D3 of the 28S gene (28S), and one mitochondrial marker, the partial cox1 gene, were amplified and sequenced. Phylogenetic relationships between the Pratylenchus sp. from Costa Rica and other Pratylenchus spp. were estimated with the Bayesian Inference method. For females, the following measurements (mean ± standard deviation) were: body length: 548.25 μm ± 20.79, stylet length: 15.43 μm ± 0.55, and %V: 79.39 ± 0.96. Males had the following measurements: body length: 473.78 μm ± 18.17, stylet length: 14.84 μm ± 0.39, and spicule length: 15.06 μm ± 1.06. Additionally, the Pratylenchus sp. population had lateral fields with four equidistant incisures. The phylogenetic analyses based on the ITS and the 28S placed the Pratylenchus sp. sequences under study in a clade with sequences of P. panamaensis, supported with high posterior probability values (99% and 100%, respectively). This is the first report of P. panamaensis in the southern region of Costa Rica.

Key words: Bayesian Inference, coffee, cox1, ITS, 28S, P. panamaensis, morphometrics, root-lesion nematode sequencing

RESUMEN


El café es uno de los cultivos tropicales más valiosos en Costa Rica. Los nematodos parásitos de plantas Meloidogyne y Pratylenchus son un problema mayor en café en este país. Una población de Pratylenchus sp., fue colectada de una plantación de café en la localidad de Daniel Flores, Pérez Zeledón en la región sur de Costa Rica. Se llevó a cabo un análisis morfométrico y morfológico en veinte hembras y veinte machos. Dos marcadores nucleares, el espaciador transcripto interno (ITS por sus siglas en inglés) y los segmentos de expansión D2-D3 del gen 28S (28S), y un marcador mitocondrial el gen parcial cox1 se amplificaron y secuenciaron. Las relaciones filogenéticas entre las especies de Pratylenchus de Costa Rica y otros Pratylenchus spp. fueron estimadas con el método de Inferencia Bayesiana. Para las hembras, se obtuvieron las siguientes medidas promedio, longitud del cuerpo: 548.25 μm ± 20.79, estilete: 15.43 μm ±
result indicates that collected during a field survey of plant-parasitic A population of coffee in the southern region of Costa Rica. The objective of our study was to describe one Costa Rica, but misidentified, for a while. The population densities over time (Arita, 2016). Coffee is a perennial crop, making it a suitable host for nematodes to take advantage of by affecting coffee (Siddiqi, 2000; Zamora-Araya, 2014; Zamora-Araya et al., 2016). The root-lesion nematodes Pratylenchus spp. are migratory endoparasites that move through root tissues while feeding, leaving behind necrotic tissue (Handoo et al., 2008). Ten species, P. coffeae, P. loosi, P. brachyurus, P. panamaensis, P. gutierrezi, P. panamaensis, P. pratensis, P. goodeyi, P. vulnus, and P. zeae, have been reported affecting coffee (Siddiqi, 2000; Zamora-Araya et al., 2016). Coffee is a perennial crop, making it a suitable host for nematodes to take advantage of by producing more offspring and increasing their population densities over time (Arita et al., 2020). In Costa Rica, morphological and molecular tools have allowed the identification of P. coffeae and P. gutierrezi associated with coffee (Golden et al., 1992; Sandoval, 2015; Zamora-Araya et al., 2016). Three populations of P. gutierrezi associated with coffee in Costa Rica (coded as K1 and K3) and Guatemala (K2) were characterized with the D3 region from the 28S gene. Later, it was suggested that sequences from K1 (AF170440) and K2 (AF170441) should be considered as conspecific of P. panamaensis (Zamora-Araya et al., 2016). This result indicates that P. panamaensis has been in Costa Rica, but misidentified, for a while. The objective of our study was to describe one population of Pratylenchus sp. associated with coffee in the southern region of Costa Rica.

Coffee (Coffea arabica L. and C. canephora Pierre ex A. Froehner) is the most valuable tropical export crop in the world, with exports around 129.5 million bags (60-kg bags) worldwide between 2020 and 2021 (International Coffee Organization, 2022). Among the phytosanitary problems on coffee in Costa Rica are the plant-parasitic nematodes, Meloidogyne and Pratylenchus species (Humphreys-Pereira et al., 2014; Zamora-Araya et al., 2016). The root-lesion nematodes Pratylenchus spp. are migratory endoparasites that move through root tissues while feeding, leaving behind necrotic tissue (Handoo et al., 2008). Ten species, P. coffeae, P. loosi, P. brachyurus, P. panamaensis, P. gutierrezi, P. panamaensis, P. pratensis, P. goodeyi, P. vulnus, and P. zeae, have been reported affecting coffee (Siddiqi, 2000; Zamora-Araya et al., 2016). Coffee is a perennial crop, making it a suitable host for nematodes to take advantage of by producing more offspring and increasing their population densities over time (Arita et al., 2020). In Costa Rica, morphological and molecular tools have allowed the identification of P. coffeae and P. gutierrezi associated with coffee (Golden et al., 1992; Sandoval, 2015; Zamora-Araya et al., 2016). Three populations of P. gutierrezi associated with coffee in Costa Rica (coded as K1 and K3) and Guatemala (K2) were characterized with the D3 region from the 28S gene. Later, it was suggested that sequences from K1 (AF170440) and K2 (AF170441) should be considered as conspecific of P. panamaensis (Zamora-Araya et al., 2016). This result indicates that P. panamaensis has been in Costa Rica, but misidentified, for a while. The objective of our study was to describe one population of Pratylenchus sp. associated with coffee in the southern region of Costa Rica.

A population of Pratylenchus sp. was collected during a field survey of plant-parasitic nematodes associated with coffee in the locality of Daniel Flores, Perez Zeledón, in the Southern region of Costa Rica. Three root and soil composite samples (each consisted of roots from 15 healthy coffee plants and the surrounding soil) were collected randomly (zig zag pattern) and transported to the Laboratory of Nematology, Crop Protection Research Center (CIPROC), Agronomy School, University of Costa Rica, San Jose, Costa Rica. Ten grams of roots and 100 cm³ of soil from each composite sample were processed using the flotation-centrifugation method (Caveness and Jensen, 1955; Alvarado and López, 1985). Population density ranged from 120 to 2,400 Pratylenchus sp./100 g of roots; no individuals were found in the soil. Twenty-five Pratylenchus sp. females and males were hand-picked and placed on carrot discs (four carrot discs in total) for in vitro reproduction at 28°C for 45 days (Coyne et al., 2014). Nematodes were extracted from the carrot disc with the flotation-centrifugation method and temporarily mounted on slides sealed with nail-polish and killed by gentle heat with a lighter. The specimens were photographed and measured with a digital camera EUROMEX (DC5000 Wi-Fi; Arnhem, Netherlands) attached to an Olympus BH-2 microscope (Tokyo, Japan).

Two DNA extraction methods were used to analyze three molecular markers, two ribosomal DNA markers, the intergenic transcript spacer (ITS) and the expansion segment D2-D3 of the 28S gene, and the partial mitochondrial gene, cox1. Eight nematode samples (1,000 nematodes per sample) were used for genomic DNA extraction according to the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Massachusetts, USA) to study the markers D2-D3 of the 28S gene (28S), and the partial cox1 gene. The ITS region was analyzed using DNA from single individuals (eight samples total) (Williamson et al., 1997; Sandoval-Ruíz et al., 2020). Briefly, one nematode was placed in a 1.5

Palabras clave: Inferencia Bayesiana, café, P. panamaensis, cox1, ITS, 28S, morfometría, secuenciación, nemátodo lesionador de la raíz
ml tube with 10 μl of water, 10 μl of DreamTaq (Thermo Fisher Scientific) PCR buffer and 1.5 μl of Proteinase K (20 mg/ml; Thermo Fisher Scientific), and the solution was incubated for 16 hr at 56°C and 95°C for 20 min.

The ITS was amplified with primers TW81 (5'-GTTTCGCTAGGTGAACTGC-3') and AB28 (5'-ATATGCTTAAGTTACGCGGT-3') (Subbotin et al., 2000), whereas the 28S was amplified with primers D2A (5'-ACAAGTACGGTAAAGGTTG-3') and D3B (5'-TCGGAAGGAACTCTATA-3') (De Ley et al., 1999). The partial cox1 gene was obtained with primers F7bP (5GGDTGRACWTTHTAYCCNCC-3') (Ozbayrak et al., 2019) and JB4 (5'CATTTCATATTGTTACTCTTTA-3') (Deruycke et al., 2010). PCR reactions were performed in a final volume of 25 μl as described by Brenes-Campos et al. (2021). The PCR amplification conditions were as follows: 95ºC for 30 s, followed by 35 cycles at 95ºC for 5 s, 30 s (55ºC for the 28S and cox1), 72ºC for 40 s, and a final extension step at 72ºC for 3 min. PCR products were bidirectionally sequenced at Macrogen Inc. (Seoul, South Korea).

A total of 40, 83, and 51 Pratylenchus spp. sequences of the ITS, 28S, and the partial cox1 gene were downloaded from GenBank, respectively. Sequences generated in this study and those retrieved from GenBank were edited and analyzed with the software BioEdit v.7.0.5.3 (Hall, 1999). The software jModelTest v. 2.1.10 (Darriba et al., 2012) was used to select the best substitution model for each gene. The software MrBayes v.3.2.6 was used to perform the phylogenetic analysis (Ronquist et al., 2012), and trees were visualised using the program FigTree v.1.4.3 (Rambaut and Drummond, 2012).

The Pratylenchus population from coffee was characterized as amphimictic, a flat encephalic region, and the presence of a strong stylet and knobs. Morphometric values are presented in μm in the format of mean ± standard deviation (range). For females (n = 20), body length: 548.25 ± 20.79 (515.34-587.4), a: 21.44 ± 0.82 (20.51-23.70), b': 4.29 ± 0.04 (4.23-4.35), c: 18.83 ± 2.90 (14.41-24.78), c': 2.35 ± 0.14 (1.98-2.61), V: 79.39 ± 0.96 (77.36-80.51), stylet length: 15.43 ± 0.55 (14.74-16.56), m: 44.55 ± 1.20 (43.08-46.63), O: 17.55 (14.31-19.35), MB: 43.54 ± 1.13 (41-45), and tail length: 29.65 ± 23.13-37.92 (Fig. 1). For males (n = 20), body length: 473.78 ± 18.17 (448.24-504.87), a: 26.43 ± 1.02 (25.15-28.66), b': 4.82 ± 0.06 (4.67-4.90), c: 20.81 ± 0.49 (20.32-22.09), c': 1.90 ± 0.04 (1.82-1.96), stylet length: 14.84 ± 0.39 (14.27-15.54), spicules: 15.06 ± 1.06 (13-16.64), and gubernaculum: 3.36 ± 0.34 (2.56-3.96) (Fig. 2).

Measurements for females from the Costa Rican P. panamaensis population differ from P. coffeae by being smaller: body length (548.2 μm vs. 561-790 μm), stylet length (15.4 μm vs 16-19.5 μm) (Lira et al., 2014; Wang et al., 2020), ratio c

Figure 1. Light micrographs of a Pratylenchus panamaensis female from the southern region of Costa Rica. A) esophageal region, B) lateral fields, C) anterior region, and D) vulva. Abbreviations: ep = excretory pore, st = stylet, mb = median bulb, lf = lateral fields at midbody; cp = cephalic region, v = vulva.
(18.8 μm vs. 18.7 μm) (Wang et al., 2020), and V (79.4 μm vs. 80.8-83.2 μm). It differed from P. thornei for its longer tail length (29.6 μm vs. 20.8-27 μm) (Divsalar et al., 2018) and from P. brachyurus for being smaller: body length (548.2 μm vs. 567.8 μm), stylet (15.4 μm vs. 18.4 μm), and V: (79.7 μm vs. 84.8 μm) (Roman and Hirschmann, 1969). The body length from the P. panamaensis population from Costa Rica did not differ with P. gutierrezi except for stylet length (15.5 μm vs. 16.8-17 μm) (Golden et al., 1992; Zamora-Araya et al., 2016). Moreover, the P. panamaensis population from Costa Rica had lateral fields consisting of three bands delimited by four lines without areolation along the body, differing from P. gutierrezi that has lateral fields consisting of three bands delimited by four lines with areolation along the body (Zamora et al., 2016). Additionally, this population was different from another populations of P. panamaensis in body length (547.4 μm vs. 417.7 –491.8 μm), however, similar in stylet length (15.4 vs. 15.3-16.2) and V (79.8 μm vs. 76.1-84.1 μm) (Zamora-Araya et al., 2016; Duncan et al., 1999).

Males from the Costa Rican P. panamaensis population differed from P. coffeae by being smaller: body length (473.8 μm vs 511 μm), stylet length (14.8 μm vs. 15 μm), spicule length (15 μm vs. 17 μm), tail length (22.8 μm vs. 26 μm), and a (26.4 μm vs. 30.8 μm) (Wang et al., 2020); from P. gutierrezi for its longer body length (473.8 μm vs. 425.2-463.8 μm), a (26.4 μm vs. 24.5-24-7 μm), stylet length (14.8 μm vs. 15.3 μm) and spicule length (15 μm vs. 16.8-7.8 μm) (Zamora-Araya et al., 2016).

PCR amplification products were approximately 940 bp, 750 bp, and 730 bp for the ITS, the 28S, and the partial cox1, respectively.

Figure 2. Light micrographs of a Pratylenchus panamaensis male from the southern region of Costa Rica. A) whole body, B-C) anterior region, D) tail region, and E) lateral field at midbody. Abbreviations: st = stylet, mb = median bulbe, lf = lateral fields; cp = cephalic region, sp = spicules.
Eight sequences were generated from each marker. A unique haplotype was found within each DNA marker and uploaded to GenBank with accession numbers: OL687383 (ITS), OL687400 (28S) and OL687387 (cox1). The Blast analysis of the ITS had an identity percentage that ranged from 97.17% to 99.68% with *P. panamaensis* accessions (KT971365, KT971366, FR692277, FJ712931, FJ712927, FJ712929) whereas the 28S Blast analysis resulted in identity values from 99.45% to 99.74% with *P. panamaensis* accessions (AF170440, AF170441, EU130897, KT971358, KT971359 and EU130899). The phylogenetic analyses based on the ITS and the 28S grouped the *Pratylenchus* sp. sequence from coffee collected in the southern region of Costa Rica together with sequences of *P. panamaensis* (PP = 99% and 100%, respectively) (Fig. 3 and 4, respectively).

Our study provides the first *cox1* sequence from *P. panamaensis*. Therefore, the closest *Pratylenchus* sp. to the new *P. panamaensis* sequence was *P. alleni* (Fig. 5).

The use of molecular methods has increased the opportunity of clarifying the identity of closely related species or considered synonyms, such as *P. panamaensis* and *P. gutierrezi* (Siddiqi, 2000; Handoo et al., 2008). Our results agree with Zamora-Araya et al. (2016), who demonstrated that *P. panamaensis* and *P. gutierrezi* are valid species based on molecular information obtained from the 28S and the ITS regions. This is the first report of *P. panamaensis* in the southern region of Costa Rica. The identification of population K1 as *P. gutierrezi* (Duncan et al., 1999) and its reclassification as conspecific of *P. panamaensis* in

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**Figure 3.** Phylogenetic analysis based on the ITS region of *Pratylenchus* species using the Bayesian Inference method under the HKY+I+G model. Posterior probabilities above 70% are given for appropriate clades. Newly obtained sequences are in bold. Scale bar = expected changes per site.
Figure 4. Phylogenetic analysis based on the D2-D3 fragment (28S) of *Pratylenchus* species using the Bayesian Inference method under the GTR+I+G model. Posterior probabilities above 70% are given for appropriate clades. Newly obtained sequences are in bold. Scale bar = expected changes per site.
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2016 (Zamora-Araya et al., 2016) and the current study indicated that *P. panamaensis* has been in the country for more than 23 years. A more extended study should be performed to determine the distribution of this species in Costa Rica.

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