RESEARCH NOTE/NOTA DE INVESTIGACIÓN

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

J. Abarca-Durán, L. A. Núñez-Rodríguez, L. Flores-Chaves, and D. A. Humphreys-Pereira*

Laboratory of Nematology, Crop Protection Research Center (CIPROC), Agronomy School, University of Costa Rica, San José, Costa Rica 2060; *Corresponding author: danny.humphreys@ucr.ac.cr

ABSTRACT

Abarca-Durán, J., L.A. Núñez-Rodríguez, L. Flores-Chaves, and D.A. Humphreys Pereira. 2022. Presence of *Pratylenchus panamaensis* in the Southern region of Costa Rica. Nematropica 52:85-93.

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 *cox*1 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, *cox*1, *ITS*, *28S*, *P. panamaensis*, morphometrics, root-lesion nematode sequencing

RESUMEN

Abarca-Durán, J., L. A. Núñez-Rodríguez, L. Flores-Chaves, and D. Humphreys Pereira. 2022. Presencia de *Pratylenchus panamaensis* en la región Sur de Costa Rica. Nematropica 52:85-93.

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 *cox*1 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 ±

0.55, y %V: 79.39 ± 0.96. Los machos obtuvieron las siguientes mediciones promedio, longitud de cuerpo: 473.78 µm ± 18.17, estilete: 14.84 µm ± 0.39, y longitud de espículas: 15.06 µm ± 1.06. Adicionalmente, la población de *Pratylenchus* sp. presentó líneas laterales con cuatro equidistantes incisuras. El análisis filogenético basado en la región *ITS* y el gen *28S* ubican las secuencias obtenidas en este estudio en el clado con las secuencias de *P. panamaensis*, sustentado con valores de probabilidad posterior (99% and 100%, respectivamente). Este es el primer reporte de *P. panamaensis* en la región sur de Costa Rica.

Palabras clave: Inferencia Bayesiana, café, *P. panamaensis*, *cox*1, *ITS*, *28S*, morfometría, secuenciación, nemátodo lesionador de la raíz

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 cm^3 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 (*28S*), and the partial mitochondrial gene, *cox*1. 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 *cox*1 gene. The *ITS* region was analyzed using DNA from single individuals (eight samples total) (Williamson *et al.*, 1997; Sandoval-Ruiz *et al.*, 2020). Briefly, one nematode was placed in a 1.5

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'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Subbotin *et al.*, 2000), whereas the *28S* was amplified with primers D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (De Ley *et al.*, 1999). The partial *cox*1 gene was obtained with primers F7bP (5GGDTGRACWTTHTAYCCNCC-3′) (Ozbayrak *et al.*, 2019) and JB4 (5′ CATTTTCATTATGTTCTTTCTTTA3′) (Derycke *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 *cox*1, 58 ºC for the *ITS*), 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 *cox*1 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

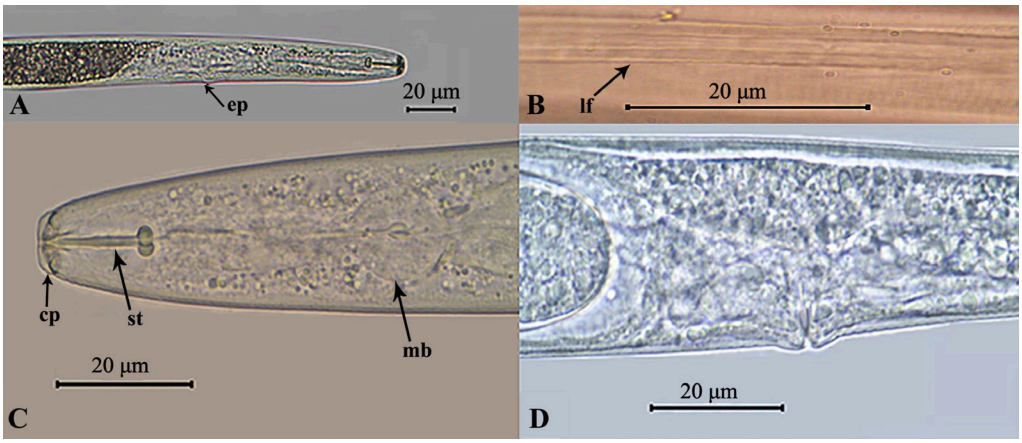

Figure 1. Light micrographs of a *Pratylenchus panamaensis* female from the southern region of Costa Rica. A) esophageal region, B) lateral fields, C) anterior region, and D) vulva. Abbreviations: ep = excretory pore, st = stylet, mb = median bulb, lf = lateral fields at midbody; cp = cephalic region, v = vulva.

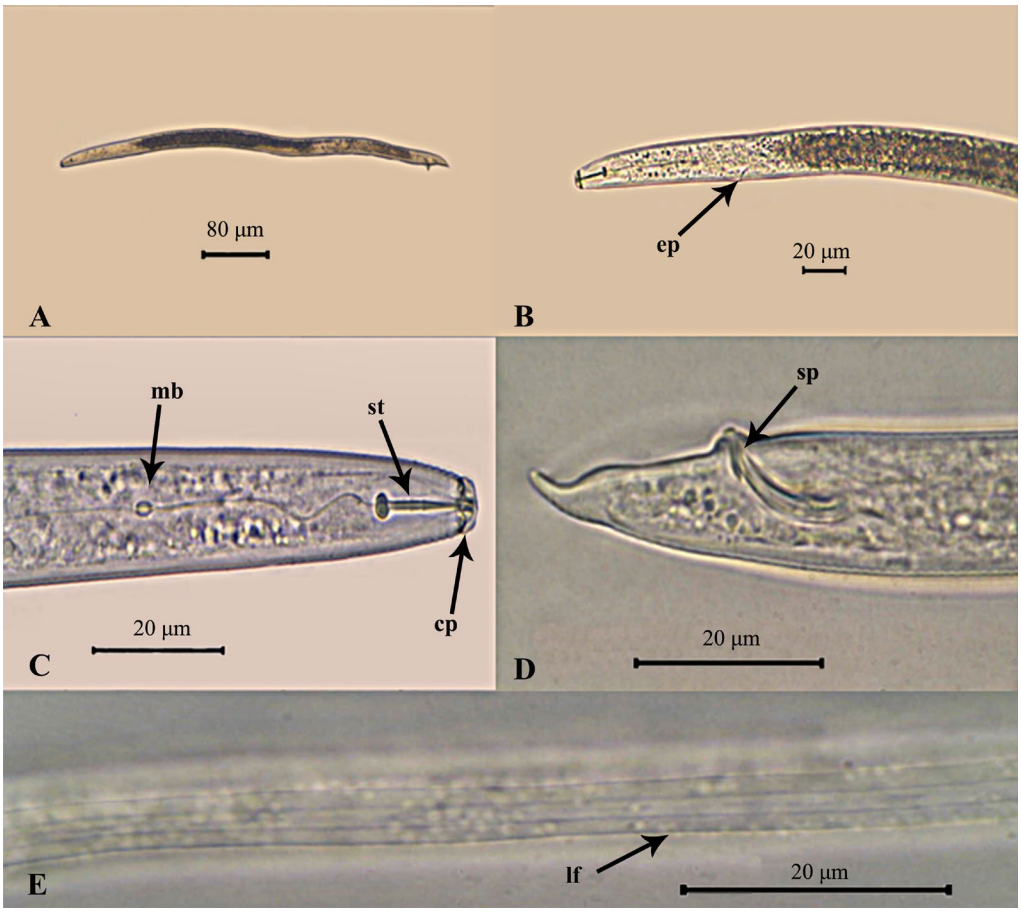

Figure 2. Light micrographs of a *Pratylenchus panamaensis* male from the southern region of Costa Rica. A) whole body, B-C) anterior region, D) tail region, and E) lateral field at midbody. Abbreviations: st = stylet, mb = median bulbe, lf = lateral fields; cp = cephalic region, sp = spicules.

(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 delimitated by four lines without areolation along the body, differing from *P. gutierrezi* that has lateral fields consisting of three bands delimitated 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 *cox*1, respectively.

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 (*cox*1). 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 *cox*1 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

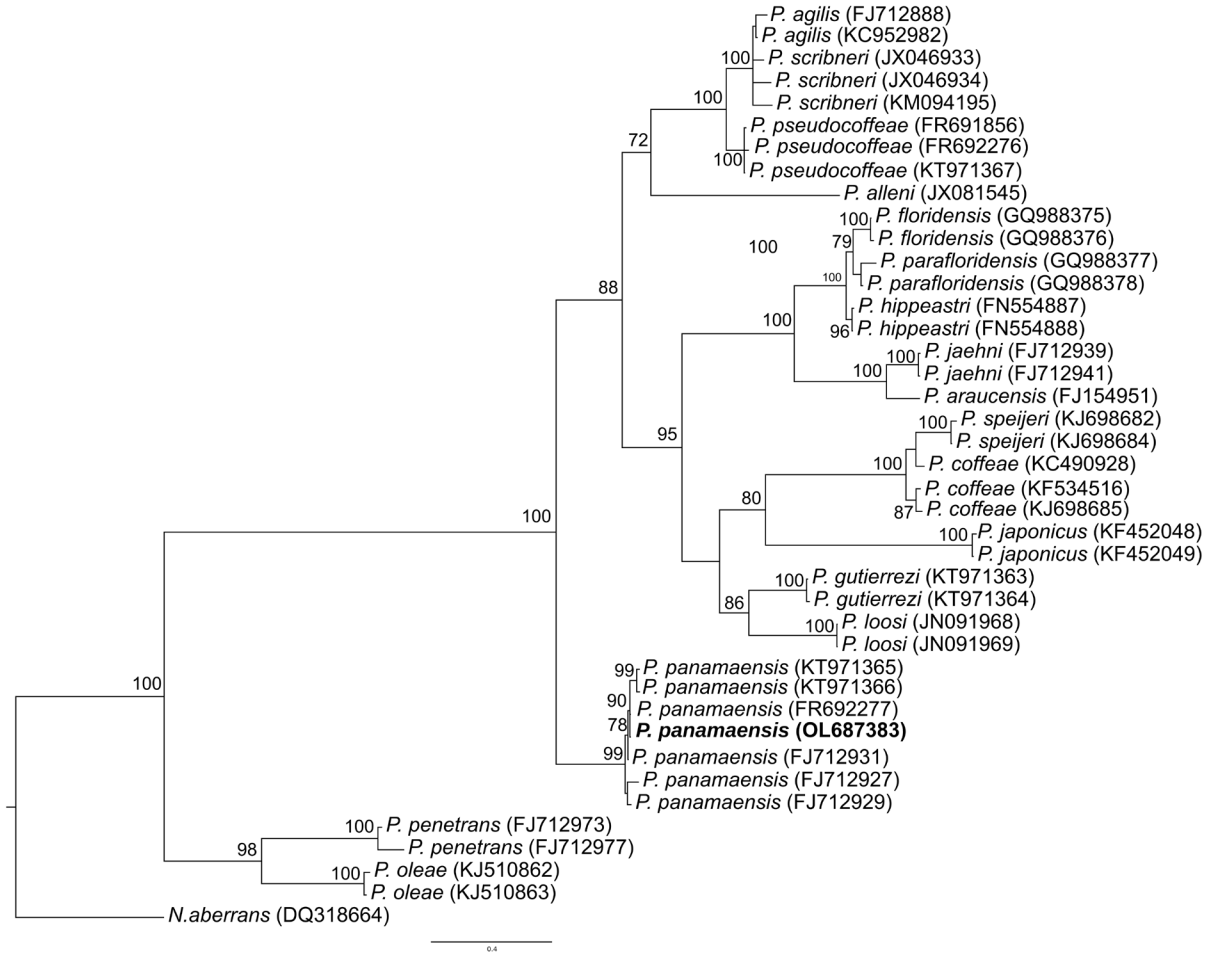

Figure 3. Phylogenetic analysis based on the *ITS* region of *Pratylenchus* species using the Bayesian Inference method under the HKY+I+G model. Posterior probabilities above 70% are given for appropriate clades. Newly obtained sequences are in bold. Scale bar = expected changes per site.

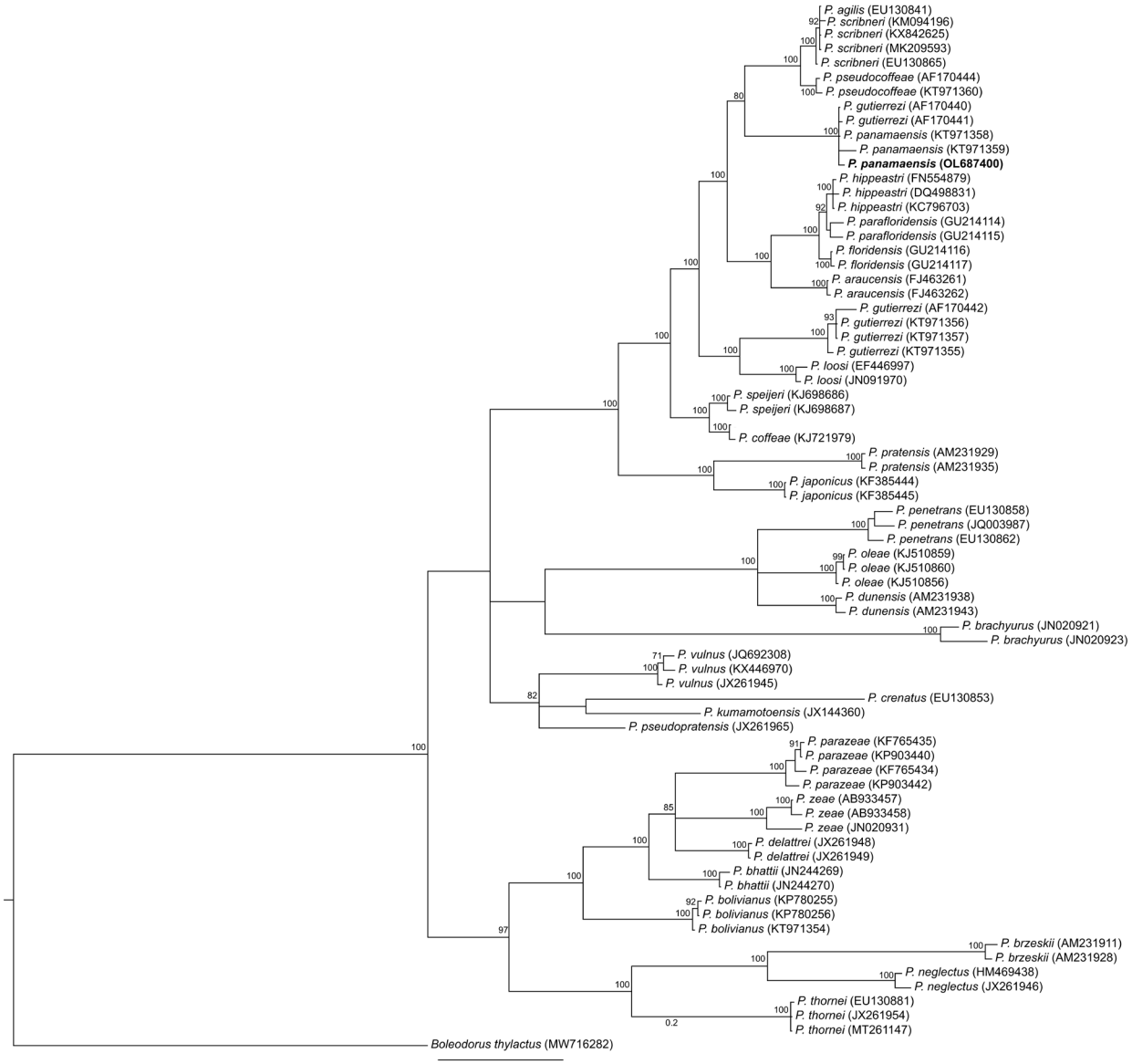

Figure 4. Phylogenetic analysis based on the D2-D3 fragment (28S) of *Pratylenchus* species using the Bayesian Inference method under the GTR+I+G model. Posterior probabilities above 70% are given for appropriate clades. Newly obtained sequences are in bold. Scale bar = expected changes per site.

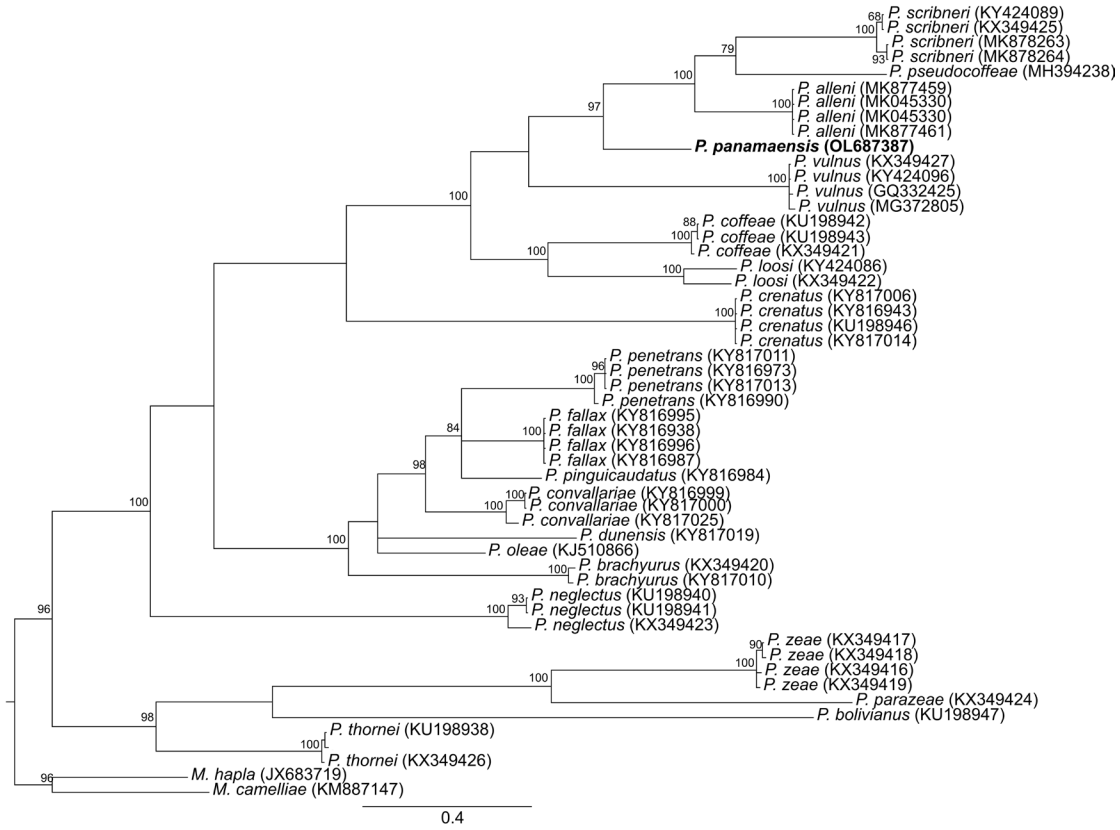

Figure 5. Phylogenetic analysis based on the partial cox1 gene of *Pratylenchus* species using the Bayesian Inference method under the GTR+I+G model. Posterior probabilities above 70% are given for appropriate clades. Newly obtained sequences in this study are in bold. Scale bar = expected changes per site.

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.

ACKNOWLEDGEMENTS

First author was also supported by the "Sistema de estudios de posgrado de la Universidad de Costa Rica".

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Received: *Accepted for publication:*

30/V/2022 *4/X//2022*

Recibido: *Aceptado para publicación:*