RESEARCH NOTE/NOTA DE INVESTIGACIÓN

NEW RECORD OF THE AMARYLLIS LESION NEMATODE, *PRATYLENCHUS HIPPEASTRI*, ASSOCIATED WITH STRAWBERRY IN THE CENTRAL REGION OF COSTA RICA

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ABSTRACT

Brenes-Campos, R., L. A. Núñez-Rodríguez, L. Flores-Chaves, and D. A. Humphreys-Pereira. 2021. New record of the amaryllis lesion nematode, *Pratylenchus hippeastri* associated with strawberry in the central region of Costa Rica. Nematropica 51:131-136.

Pratylenchus species are among the most important groups of plant-parasitic nematodes worldwide due to their wide distribution and host range, and negative impacts on crop yields. In strawberries, they are considered a major problem because of their direct damage to plants and interaction with other plant pathogens. As part of a nematode survey on strawberries in the central region of Costa Rica, one population of *Pratylenchus* collected from the province of Heredia was characterized morphologically and with three molecular markers. The analysis of morphological and morphometric characters, as well as DNA sequencing of the internal transcribed spacer (ITS), the 28S large subunit ribosomal DNA, and the mitochondrial cytochrome oxidase (COI) genes confirmed the identity of this population as *P. hippeastri*. This study is the first report of *P. hippeastri* associated with strawberry in the country and worldwide. Further studies should be focused on pathogenicity essays in different varieties to assess its potential damage on strawberry production and to develop strategies for its integrated management.

Key words: COI, DNA, identification, morphology, *Pratylenchus hippeastri*, strawberry

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RESUMEN

Brenes-Campos, R., L. A. Núñez-Rodríguez, L. Flores-Chaves, and D. A. Humphreys-Pereira. 2021. Nuevo hallazgo del nematodo lesionador de amarilis, *Pratylenchus hippeastri* asociado a fresa en la Región Central de Costa Rica. Nematropica 51:131-136.

Pratylenchus se encuentra entre los nematodos fitoparásitos más importantes a nivel mundial, debido a su larga distribución, amplio rango de hospederos y el impacto negativo sobre el rendimiento de los cultivos. En fresa, se considera un problema importante por el daño directo o por su interacción con otro patógenos. Como parte de un muestreo de nematodos en plantaciones de fresa en la Región Central de Costa Rica, una población de *Pratylenchus* recolectada en la provincia de Heredia fue caracterizada morfológica y molecularmente con tres marcadores moleculares. El análisis de caracteres morfológicos y morfométricos, así como la secuenciación de ADN ribosomal (regiones ITS y 28S) y mitocondrial (COI) confirmaron la identidad de esta población como *P. hippeastri*. Este estudio es el primer hallazgo de *P. hippeastri* asociado a fresa en el país y en el mundo. Estudios posteriores sobre patogenicidad en diferentes variedades deberían realizarse para determinar el posible daño en la producción de fresa y desarrollar estrategias para su manejo integrado.

Palabras clave: ADN, COI, fresa, identificación, morfológica, *Pratylenchus hippeastri*

Strawberry (*Fragaria* x *ananassa* Duch.) production in Costa Rica is concentrated in the central region of the country due to its temperate climate (INEC, 2015). This region includes the provinces of Cartago, Alajuela, San Jose and Heredia with 151 ha of strawberry fields (SEPSA, 2018). One of the most devastating phytosanitary problems on strawberries are the root-lesion nematodes (*Pratylenchus* spp.). The genus *Pratylenchus* is included in the list of the top ten most important plant-parasitic nematodes worldwide ranked in third position (Jones *et al.*, 2013). The genus contains more than 103 valid species (Nguyen et al., 2019; Handoo *et al.*, 2021). In Costa Rica, root-knot nematodes, *Meloidogyne* spp., and *Pratylenchus* are the two most predominant groups of plant-parasitic nematodes found in roots and soil samples from strawberry (unpublished data). Species of *Pratylenchus* that have been identified in the country from several plant species include: *P. coffeae*, *P. pseudocoffeae*, *P. zeae*, *P. brachyurus*, *P. penetrans*, *P. crenatus*, *P. bolivianus*, *P. gutierrezi*, *P. teres*, and *P. scribneri* (López and Salazar, 1990; Sandoval, 2015; Zamora *et al.*, 2016). Only *P. penetrans* is reported to be associated with strawberry in Costa Rica (Sandoval, 2015). The objective of this study was to identify at the species level a new *Pratylenchus* population isolated from strawberry in Costa Rica.

In 2020, during a plant-parasitic nematode survey of strawberry fields in the central region of Costa Rica, one *Pratylenchus* population was collected from the province of Heredia (locality Vara Blanca). The strawberry plants were in a greenhouse on raised-beds covered with black plastic mulch, and plants were asymptomatic aboveground. Five plants were collected in a zig-zag pattern. The entire root system and soil surrounding the roots were sampled from the five plants and combined into a composite sample. Nematodes were extracted from roots using the flotation-centrifugation method (Caveness *et al.*, 1955; Alvarado and López, 1985). The population density was 21,800 nematodes/100 g of roots and 17 nematodes/100 cm^3 soil. The nematodes from the roots were cultured on carrot discs at 28°C (Coyne *et al.*, 2014) for 3 months and used for further morphological and molecular analysis. For light microcopy studies, temporary mounts were established in water, and nematodes were relaxed by heating gently. Nematodes were measured and photographed with an Olympus BH-2 microscope (Olympus, Toyko, Japan) equipped with an Euromex digital camera (model DC.5000-Wifi; Arnhem, The Netherlands).

DNA was extracted from ~1,000 nematodes using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Pittsburgh, PA) following the manufacturer's instructions (eight DNA samples were prepared in this study). Three molecular markers were used: the internal transcribed spacer (ITS) region and the D2-D3 expansion segment of the 28S gene (28S) from ribosomal DNA, and the partial COI gene (COI) from the mitochondrial DNA genome. The primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') were used to amplify the ITS region (Subbotin *et al.*, 2001). The primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') (De Ley *et al.*, 1999) were used to amplify the D2-D3 region of 28S rDNA. The partial COI gene was amplified with primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB5 (5'-AGCACCTAAACTTAAAACATAATGAAAATG-3') (Derycke *et al.*, 2005). The amplification reaction included 1× PCR reaction buffer (DreamTaq Buffer, Thermo Fisher Scientific), 0.8 µM of each primer, 1 µl of 20 mg/ml BSA (Thermo Fisher Scientific), 0.1 mM DNTP mix (Thermo Fisher Scientific), 1.5 units of DreamTaq polymerase (Thermo Fisher Scientific), 3 µl of the DNA preparation in a final volume of 25 µl. Amplification conditions were as described by Humphreys-Pereira *et al.* (2017). The PCR products were sequenced bidirectionally at Macrogen (Seoul, Korea). Eight sequences were obtained for each molecular marker.

Sequences generated in this study were aligned using ClustalW (Thompson *et al.,* 1994) in Bioedit (Hall, 1999) with sequences retrieved from

GenBank. The phylogenetic relationships within the genus *Pratylenchus* were estimated with the Bayesian Inference method in Mrbayes 3.2 (Ronquist *et al.*, 2012). The substitution model GTR + I + G was used as the best fit nucleotide model for the three molecular markers. The Bayesian analysis was conducted with four MCMC chains (three heated, one cold) and 106 generations (sampled every 1,000 generations). The first 2,500,000 generations (2,500 trees) were discarded as burn-in. The tree topologies were visualized using the program FigTree 1.4.3 (Rambaut, 2012).

Morphometric data were obtained from 15 females, and measurements are provided in the following format: mean (range). The female body is slender with length = 594 (565-625) µm (Fig. 1A), maximum body diameter = 23 (21-24) µm. Lateral field with four lines (Fig. 1E). The stylet is short and thick with length = 15 (14-16) µm, basal knobs usually rounded with a height = 2.2 (2.0-2.4) µm and width = 4.4 (3.3-5.6) µm (Fig. 1B), dorsal esophageal gland opening (DGO) = 3.0 (2.5-3.5) µm. Anterior end to center of the metacorpus = 61.8 (60.2-62.9) µm, body width = 20.2 (16.1-22.0) µm, vulval body diameter = 21.2 (19.0-23.3) µm with protruded lips (Fig. 1D) and distance between vulva and anus = 99.7 (89.7-111.0) µm, anal body width = 13.3 (10.8-16.2) µm. Tail is conoid with bluntly pointed or subhemispherical, smooth terminus with length = 37.5 (30.8-43.3) µm (Fig. 1C, D). De Man ratios are a = 27.9 (32.1-31.5) µm, b = 5.1 (4.6-6.1), c = 16.1 (4.6-6.1), c' = 2.6 (2.4-2.9), V = 77.3 (76.4-79.1). No males were found. The morphometric details of female were consistent with *Pratylenchus hippeastri* (Inserra *et al.*, 2007; Gu *et al.*, 2014; Wang *et al.*, 2016; Knoetze *et al.*, 2019).

The eight sequences per marker were identical. The ITS rDNA sequence (929 bp length) showed more than 99% identity (differing at 0 -1

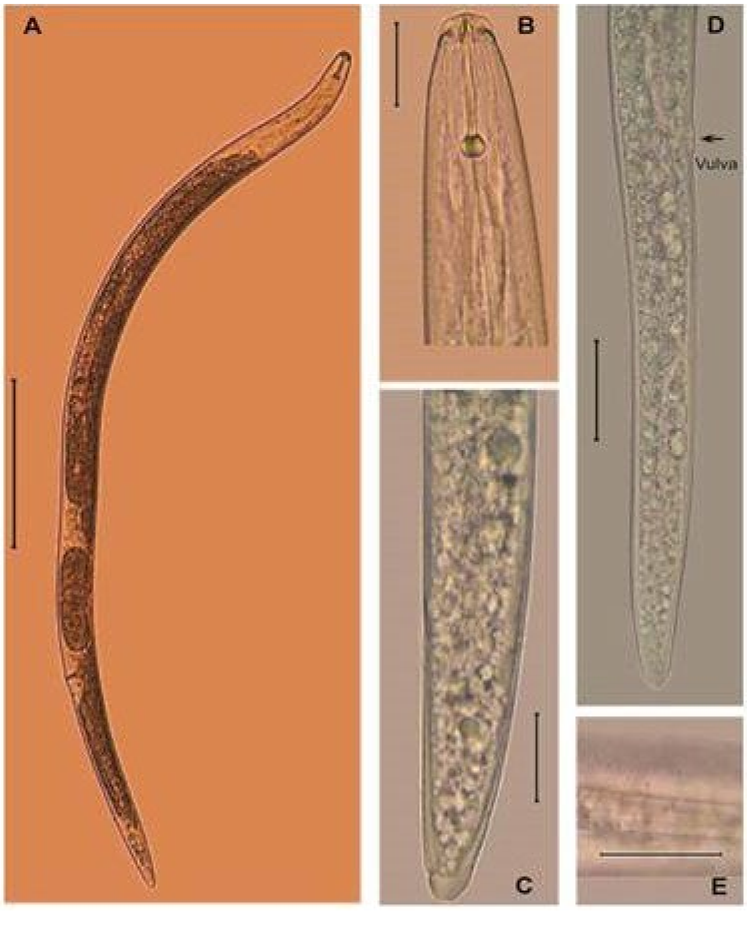

Figure 1. Light micrographs of *Pratylenchus hippeastri* female from Costa Rica. A: Entire body; B: Anterior region; C-D: Posterior end; E: Lateral lines near mid-body. (Scale bar: A: 100 µm, B: 10 µm, C: 10 µm, D: 25 µm and E: 20 µm).

bp) with sequences of *P. hippeastri* from South Africa (MW019435; MW015781), Japan (KC796699), and USA (FN554887, FJ712932, MT090056, FN554888). The 28S rDNA (744 bp) sequence was more than 99% identical (differing at 0-2 bp) with populations of *P. hippeastri* from Israel (KJ001715), Japan (KP161608), South Africa (MK749422), and USA (DQ498829, GU214113, MT090065). The COI mtDNA (393 bp) sequence showed more than 97% identity (differing at 9-12 bp) with sequences of *P. hippeastri* from China (KY424098, KY424099), South Africa (MW042870), and USA (MT093835, MT093836).

The ITS (data not shown), COI (Fig. 2A), and the 28S phylogenetic trees (Fig. 2B) revealed a highly supported clade that includes the *Pratylenchus* population from Costa Rica and other *P. hippeastri* sequences (Posterior Probability, PP =100, 100 and 98 %, respectively). The ITS and 28S topologies showed that the *P. hippeastri* clade was close to *P. floridensis* and *P. parafloridensis*. These results are in concordance with previous studies (Subbotin *et al.*, 2008; De Luca *et al.,* 2010; Handoo *et al.*, 2020). The 28S tree also showed that the population from Costa Rica is more closely related to the Florida (USA) (differing at 0-2 bp) and Israel (identical) populations than the Chinese (differing at 5 bp) and South Africa (differing at 2-4 bp) populations.

Pratylenchus hippeastri was given the common name of the amaryllis lesion nematode and has a narrow host range with only four hosts reported previously including amaryllis, bromeliads, and grapevine in North America (Inserra *et al.*, 2007; De Luca *et al.*, 2010; Handoo *et al.*, 2020), from apples in China and Africa (Wang *et al.*, 2016; Knoetze *et al.*, 2019), and *Acer palmatum* plant in Japan (Chen *et al.,* 2014). It was also collected from the rhizosphere of *Salix mucronata* in Africa (Shokoohi, 2019). This study is the first report of *P. hippeastri* associated with strawberry, and the first report of the species in Costa Rica specifically in the locality of Varablanca, Heredia. Studies on the life cycle of this nematode, pathogenicity assays on strawberry cultivars, and the distribution of the nematode in the country would generate new knowledge on the potential damage in the crop and to design a program for the nematode integrated management.

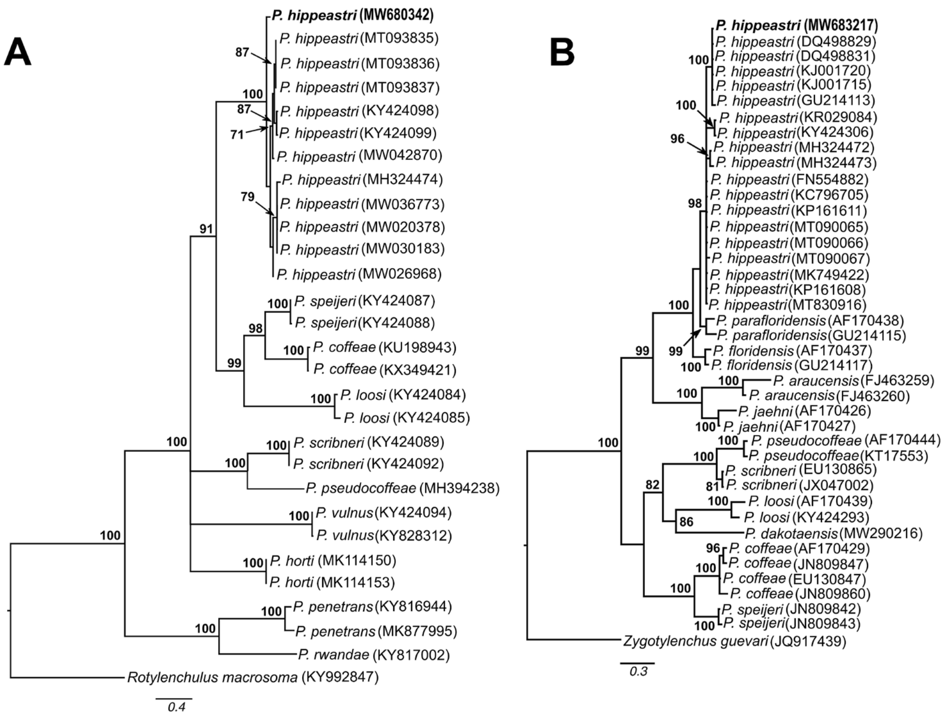

Figure 2. Phylogenetic relationships between *Pratylenchus hippeastri* and other *Pratylenchus* species as inferred from Bayesian analysis of the COI (A) and the 28S (B) gene sequences using the GTR + I + G model of nucleotide substitution. Posterior probabilities of over 70% are given for appropriate clades. New sequences are highlighted in bold.

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