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LICENCIATURA EN INGENIERÍA EN AGRONOMÍA

First morphometric and molecular study of *Hemicriconemoides*  
(Nematoda: Criconematidae) in Costa Rica

Trabajo final de graduación, en modalidad de Artículo Científico, sometido a consideración del Tribunal Examinador de la Escuela de Ciencias Agrarias de la Universidad Nacional para optar al grado de Licenciatura en Ingeniería en agronomía

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Murillo-Valdés et al. 2024. 2024. First morphometric and molecular study of *Hemicriconemoides* in Costa Rica

**First morphometric and molecular study of *Hemicriconemoides* (Nematoda: Criconematidae) in Costa Rica**

Mariana Valdés Murillo

**Trabajo final de graduación, en modalidad de Artículo Científico, sometido a consideración del Tribunal Examinador de la Escuela de Ciencias Agrarias de la Universidad Nacional para optar al grado de Licenciatura en Ingeniería en agronomía**

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## INTRODUCCIÓN

Los nematodos son organismos con forma vermiforme, cilíndrica y simetría bilateral, que en su mayoría son microscópicos y se encuentran en una gran diversidad de ambientes y en casi todas partes del mundo. Se clasifican generalmente en nematodos de vida libre y fitoparásitos, y pueden ser endoparásitos o ectoparásitos en las plantas. Entre los síntomas que destacan de los nematodos fitoparásitos se encuentran las agallas a nivel de las raíces, lesiones necróticas, clorosis en las plantas, disminución del vigor, deficiencia de crecimiento y marchitez en la parte aérea (Castilla et al., 2017; Uribe et al., 2020).

Los nematodos fitoparásitos son un problema para las plantas cultivadas, ya que provocan pérdidas importantes en la producción agrícola debido a los daños que pueden provocar (Artavia y Peraza, 2020). En general, provocan diferentes tipos de daños que se relacionan según la ubicación donde se alimentan de la planta. Pueden afectar el crecimiento y el rendimiento de los cultivos, reducir las funciones fisiológicas de las raíces y la parte aérea, causar agallas radicales, necrosis en raíces, deformaciones en bulbos y tallos, entre otros. Estos daños pueden ser perjudiciales para los productores debido a los daños económicos que se producen (Artavia, 2018; Guzmán et al., 2020). Según Guzmán et al. (2012), existen aproximadamente 4105 especies diferentes de nematodos fitoparásitos. Estos microorganismos son muy abundantes y pueden reproducirse con facilidad, lo que conlleva a la dificultad de su control. Se estima que las pérdidas anuales en cultivos pueden llegar a los \$80 billones al año, lo que equivale a entre el 11 y el 14% de las pérdidas agrícolas en climas tropicales y subtropicales (Elling, 2013; Kumar et al., 2020).

La familia Criconematidae, comúnmente conocida como nematodos anillados, presenta como característica en su cuerpo anulaciones transversales de la cutícula. Además, según sus hábitos alimenticios, son ectoparásitos de raíz polífagos y migratorios, y se encuentran en plantas herbáceas

Murillo-Valdés et al. 2024. 2024. First morphometric and molecular study of *Hemicriconemoides* in Costa Rica y leñosas cultivadas y silvestres. Los nematodos anillados están ampliamente distribuidos y se pueden encontrar en diferentes tipos de suelos, como es el caso de los arenosos (Clavero et al., 2022; Peraza, 2014).

En Costa Rica, los primeros registros de especies pertenecientes a la familia Criconematidae datan de la década de los setenta (González, 1978); sin embargo, posterior a esa fecha solo se han realizado algunas investigaciones (Guzmán et al., 2011; López & Salazar, 1988; Peraza, 2014; Salazar & López, 1987; Sancho & Salazar, 1985; WingChing et al., 2008), pero ninguna de ellas incluye la identificación del género *Hemicriconemoides*.

La familia Criconematidae contiene 18 géneros y 5 subfamilias, entre los que se encuentra el género *Hemicriconemoides* sp. , el cual es un ectoparásito migratorio. Generalmente habitan en áreas tropicales en el mundo y se encuentran asociados con los cultivos agrícolas y variedad de plantas entre plantas aversas y ornamentales (Geraert, 2010; Munawar et al., 2018).

En el caso de Costa Rica, *Hemicriconemoides* se ha identificado a nivel de género; sin embargo, no existe a la fecha, un estudio que involucre la caracterización taxonómica, morfológica y molecular; únicamente se han realizado observaciones de caracteres morfológicos para su identificación. Es fundamental tener a disposición estrategias de identificación más confiables que involucren también los métodos moleculares.

En este sentido, los métodos basados en las secuencias de ácido desoxirribonucleico (ADN) para la caracterización de nematodos son comunes actualmente y se emplean para diferenciar especies, incluidas algunas pertenecientes a la familia Criconematidae a la que pertenece el género *Hemicriconemoides* (Powers et al., 2010, 2011, 2017; Powers et al., 2014, 2016). Por otra parte, la amplia variabilidad intraespecífica y la poca heterogeneidad interespecífica entre las especies ha sido una de las razones fundamentales para emplear los métodos de reconocimiento molecular (Barsalote et al., 2017; Karaca et al., 2021; Palomares et al., 2018).

La correcta identificación de nematodos fitoparásitos es sumamente importante, ya que en ocasiones la sintomatología es atribuida a otros fitopatógenos o a factores abióticos por lo que el nivel de daño que ocasionan realmente es subestimado (Gandarilla et al., 2014). En este sentido, es necesario un diagnóstico adecuado de nematodos el cual se refuerce con una caracterización precisa y confiable, ya que podría tener un impacto positivo en la reducción del uso de agroquímicos y las pérdidas económicas ocasionadas por estos fitoparásitos.

Sobre el uso de moléculas químicas, en Costa Rica no existen datos exactos ni registros de pérdidas agrícolas por causa de los nematodos, aunque sí existen cifras de la cantidad de nematicidas importados al año para el control de la población de nematodos (Bravo et al., 2013; Bravo et al., 2015). Entre 2017-2020 las importaciones de nematicidas fueron de 3,839,632 kg de ingrediente activo. En su gran mayoría la cantidad de estos productos químicos fueron 1,3-D con 252,197.3 kg, cadusafos con 292,567.6 kg, carbosulfan con 11,032.0 kg, etoprofos con 1,137,730.4, fenamifos con 329,843.8, forato con 307,86883, oxamil con 928,091 y terbufos con 928,091. Así mismo, existen otros ingredientes activos los cuales tienen prohibición de exportación como es el caso de triazofos, aldicarb, DBCP y carbofuran, entre otros (F. Ramírez, comunicación personal, 12 de abril de 2023).

Al realizar una identificación adecuada de un determinado nematodo se podría realizar una aplicación racional de los productos químicos ya que, al ser una práctica muy utilizada por los agricultores, que causa costos económicos y efectos adversos en la salud humana y el medio ambiente, en muchas ocasiones no son lo suficientemente efectivos para el control (Quirós &Pereza, 2023).En el laboratorio de Nematología de la Escuela de Ciencias Agrarias (ECA), se viene realizando una caracterización de especies de nematodos mediante criterios taxonómicos y moleculares, para identificación de nuevas especies presentes en Costa Rica. Por lo anterior, el objetivo de esta investigación es realizar por primera vez una correcta caracterización taxonómica y molecular de

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especies del género de *Hemicriconemoides* presentes en el país, con el propósito de aumentar el conocimiento de la nematofauna en Costa Rica y sentar las bases para su manejo racional.

## **OBJETIVOS**

### 2.1 General

- Realizar una correcta caracterización taxonómica y molecular de especies del nematodo fitoparásito *Hemicriconemoides* (Nematoda: Criconematidae) presentes en el país, con el propósito de aumentar el conocimiento de la nematofauna en Costa Rica.

### 2.2 Específicos

- Identificar morfológica y morfométricamente especies de *Hemicriconemoides*, con el uso de claves pictóricas y dicotómicas mediante la comparación de caracteres excluyentes como herramienta en la correcta caracterización de especies.
- Identificar molecularmente especies de *Hemicriconemoides* utilizando técnicas como PCR y secuenciación de ADN para posteriormente realizar una relación filogenética con especies de la misma familia reportadas en la base de datos de GenBank®.
- Elaborar una lista actualizada y precisa de las especies de *Hemicriconemoides* presentes en Costa Rica, junto con su distribución geográfica y sus hospedantes potenciales con el fin de contribuir al conocimiento de la nematofauna del país y facilitar su manejo en el futuro.

**FIRST MORPHOMETRIC AND MOLECULAR STUDY OF *HEMICRICONEMOIDES*  
(NEMATODA: CRICONEMATIDAE) IN COSTA RICA**

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ABSTRACT

*Hemicriconemoides* spp. are migratory root ectoparasites widespread in warmer areas of the tropics and subtropics. The aim of this study was to identify species of *Hemicriconemoides* using morphometric and molecular methods to contribute to the knowledge of these organisms. Soil samples were collected from cocoa, peppermint, ornamental plants, fig, aloe and grasses. Nematodes were isolated using the sieving and flotation-centrifugation method, and their morphology was assessed by light microscopy. Molecular characterization was performed using partial expansion domains D2-D3 sequences of the 28S ribosomal gene, partial sequences of the ITS1 and the cytochrome c oxidase subunit I (COI) mitochondrial gene, which allowed phylogenetic analysis between species identified in this study and others reported in GenBank. Two species of nematodes were identified: *H. strictathecatus* and *H. rosae*, marking the first record and

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Murillo-Valdés et al. 2024. 2024. First morphometric and molecular study of *Hemicriconemoides* in Costa Rica characterization of these species in Costa Rica. Phylogenetic analysis of COI, 28S D2-D3 expansion domains and ITS1 ribosomal DNA regions highlight the importance of using molecular data for accurate species identification. Furthermore, our findings will be useful in determining what other species exist in Costa Rica and to which crops they may be associated.

*Keywords:* plant-parasitic nematode, integrative taxonomy, morphology, DNA sequencing, phylogeny, new record.

## RESUMEN

*Hemicriconemoides* spp. son ectoparásitos migratorios de raíces y están ampliamente distribuidos en áreas cálidas de los trópicos y subtrópicos. El objetivo de este estudio fue identificar especies de *Hemicriconemoides* utilizando métodos morfométricos y moleculares para contribuir al conocimiento de estos organismos. Se recolectaron muestras de suelo de cacao, hierbabuena, plantas ornamentales, higuera, *Aloe vera* y pastos. Los nematodos se aislaron utilizando el método de tamizado y flotación-centrifugación, y su morfología se evaluó mediante microscopía óptica. El análisis molecular se realizó mediante la secuenciación de los dominios de expansión D2-D3 del gen ribosomal 28S, así como la región ITS1 y el gen mitocondrial de la subunidad I del citocromo c oxidasa (COI), lo que permitió el análisis filogenético entre las especies identificadas en este estudio y otras reportadas en el GenBank. Se identificaron dos especies de nematodos: *H. strictathecatus* y *H. rosae*, representado el primer registro y caracterización de estas especies en Costa Rica. El análisis filogenético de todas las regiones secuenciadas destaca la importancia de utilizar datos moleculares para la identificación precisa de especies. Además, nuestros hallazgos serán útiles para determinar qué otras especies existen en Costa Rica y a cuáles cultivos pueden estar asociadas.

*Palabras clave:* nematodo fitoparásito, taxonomía integradora, morfología, secuenciación de ADN, filogenia, nuevo registro.

## INTRODUCTION

The genus *Hemicriconemoides* (Chitwood & Birchfield, 1957) includes 55 nominal species, according to recent studies (Inserra et al., 2014; Munawar et al., 2019; Nguyen et al., 2020).

*Hemicriconemoides* spp. are migratory ectoparasitic nematodes associated with plant roots. They are commonly called sheathoid nematodes, because in the adult stages their body cuticle is covered by an external layer or sheath composed of prominent smooth rings. In addition, males have lateral and sublateral structures along their body (Sharma Chaubey, 2023). These morphological features give them a distinctive appearance reminiscent of delicate caudal wings (Chitwood and Birchfield, 1957; Khan et al., 2019; Sharma and Chaubey, 2023). These nematodes significantly affect crops such as millet, rice, tubers, citrus, banana, dates, pineapple and grapevine (Vovlas et al., 2000; Munawar et al., 2018; Khan et al., 2019; Azimi and Pedram, 2020; Budiman, 2020; Nguyen et al., 2020; Shokoohi, 2022). Their geographic distribution covers tropical regions of several continents such as Africa, America, Australia, South and Southeast Asia and Southern Europe.

In Costa Rica, this nematode has only been identified at the genus level using morphological criteria; however, to date, there is no integrated study that includes morphological, morphometric and molecular tools for a precise identification of the species present.

Correct identification of plant-parasitic nematodes is important, as sometimes plant symptoms are incorrectly attributed to other plant pathogens or abiotic factors, leading to an underestimation of the damage they cause (Gandarilla et al., 2014). Therefore, an accurate and

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reliable diagnosis of nematodes is essential to optimize integrated pest management strategies, which can contribute to a decrease in the use of agrochemicals and to the reduction of economic losses in agriculture.

DNA sequence-based methods are commonly used today for nematode identification. Along with morphological techniques, these methods allow the differentiation and classification of species, including those belonging to the genus *Hemicriconemoides* (Shama & Chaubey, 2023; Mirghasemi et al., 2024). In addition, wide intraspecific variability and low interspecific heterogeneity between species has been one of the fundamental reasons for using molecular recognition methods (Barsalote et al., 2017; Palomares et al., 2018; Karaca et al., 2021).

Correct identification of nematodes allows rational application of nematicides, which mitigates economic costs and adverse effects on human health and the environment associated with the indiscriminate use of these chemicals, which are often ineffective (Quirós & Peraza, 2023). In the Nematology Laboratory of the School of Agrarian Sciences at Universidad Nacional, characterization of nematode species is conducted using taxonomic and molecular criteria that help to accurately understand the nematode fauna present in Costa Rica. Therefore, the objective of this research was to carry out, for the first time, a morphometric and molecular characterization of species within the genus *Hemicriconemoides* in the country. This research aims to enhance the understanding of the nematode fauna in Costa Rica and establish a foundation for proper management.

## MATERIALS AND METHODS

## Collection and isolation of nematodes

Six soil samples were collected from different areas of Costa Rica in which the *Hemicriconemoides* genus was found. samples included agricultural crops, pastures and ornamentals from San José, Alajuela and Puntarenas provinces (Table 1). Between 10 and 15 random subsamples were taken from the first 30 cm of soil depth, until a sample consisting of approximately 1 kg of soil was obtained. Samples were stored in polyethylene plastic bags and transferred to the Nematology Laboratory at Universidad Nacional for processing.

Table 1. Geographic characteristics of the sample collection sites included in this study.

Province	Canton	District	Sample sites	Latitude	Longitude	Altitude (masl)
Alajuela	Upala	San Antonio	Cacao ( <i>Theobroma cacao</i> )	10°55'55"N	84°57'40"W	44
	Alajuela	Guácima	Hierbabuena ( <i>Mentha spicata</i> )	9°57'40"N	84°14'29"W	807
San José	San José	Carmen	Sheffera ( <i>Schefflera arboricola</i> ) y Cosmos ( <i>Cosmos bipinnatus</i> )	9°56'05"N	84°04'15"W	1161
	San Pedro	Montes de Oca	Higuera ( <i>Ficus benjamina</i> )	9°55'46"N	84°03'24"W	1183
	Curridabat	Curridabat	Sábila ( <i>Aloe vera</i> )	9°55'37"N	84°02'35"W	1212
Puntarenas	Parrita	Palo Seco	Pasto	9°57'20"N	85°08'48"W	58

## Extraction, mounting and fixation of nematodes

Female nematodes were extracted using the flotation-centrifugation method in sugar solution of Jenkins (1964). Each nematode population was fixed with 4% formaldehyde at 70°C using the

Murillo-Valdés et al. 2024. 2024. First morphometric and molecular study of *Hemicriconemoides* in Costa Rica modified rapid method of Seinhorst (1959). Fixed nematodes were transferred to counting dishes and placed on Cobb slides with paraffin according to the technique described by de Maeseneer and D'Herde (1963). The Cobb slides were labeled and added to the nematode collection of the Criconematidae family at the Nematology Laboratory of the School of Agrarian Sciences at Universidad Nacional de Costa Rica.

### Morphological and morphometric characterization

Taxonomic identification at the genus level was carried out using morphological characters, original descriptions and identification keys according to Geraert (2010). Females were measured in  $\mu\text{m}$  with a Nikon eclipse 80i high-resolution microscope connected to a Nikon DS-Fi1 camera where micrographs of the populations were taken. From each nematode population, between 10 and 13 females were measured. The parameters measured were: L (body length), St (stylet length), DGO (distance from the base of the stylet to the dorsal esophageal gland), stylet knob height, stylet knob width, diameter at mid-body, first lip annulus diameter, second lip annulus diameter, third body annulus diameter, tail length, R (ring number), Rex (number of rings from the excretory pore), RVan (number of rings from the vulva to the posterior part),  $V\% \left( \frac{(L-VL)}{L} * 100 \right)$ ,  $St\%L \left( \frac{St}{L} * 100 \right)$  and VL/VBD. Adobe Photoshop® CS6 was used to edit and assemble the micrographs of the identified populations.

### Molecular characterization

#### DNA extraction, PCR and sequencing

DNA was extracted from individual females per population according to the protocol of Solano et al. (2013), with some modifications. Nematodes were then transferred to sterile 0.2 ml Eppendorf tubes containing 47 µl of Tris-HCL, pH 8.0 (0.2 M). Each tube was added with 3 µl of proteinase K (20 mg/ml) and then subjected to an ultrasonic bath at 60 °C for 10 minutes. Then they were incubated in a thermocycler at 60 °C for 30 minutes, followed by vortexing. A 15-minute cold incubation at -20 °C was then carried out, followed by a 10-minute hot incubation at 90 °C. Tubes were vortexed again and a second incubation was carried out, with cold and warm temperature cycles. Samples were then vortexed again before being centrifuged for 2 minutes at 2000 rpm. Finally, the DNA samples were stored at -20 °C for later polymerase chain reaction (PCR).

Three genomic regions were amplified by PCR. D2-D3 region of the 28S rDNA gene was amplified with primers D2A (5'-ACAAGTACCGTGAGGGAAAGT TG-3 ')/D3B (5'-TCGGAAGGAACCAGCTACTA-3') (De Ley et al.,1999) and ITS1 region with the primer set rDNA2 (5'-TTGATTACGTCGCTGCCCTTT-3') (Vrain et al., 1992)/rDNA1.58S (3'-ACGAGCCGAGTGATCCACCG-5') (Cherry et al., 1997). The cytochrome c oxidase subunit I (COI) gene was amplified with the primer set COI-F5 (5'AATWTWGGTGTGGAAC-3')/COI-R9 (5'CTTAAACATAATGRAAATGWGCWACWACATAATAAGTATC-3) (Powers et al., 2014). Polymerase chain reaction (PCR) conditions were adjusted according to the protocol described by Cordero et al. (2012), using a 25 µl reaction mix containing 5.5 µl nuclease-free water, 12.5 µl DreamTaq™ PCR Master Mix (2X) (ThermoScientific™) (including buffer, dNTPs, MgCl<sub>2</sub> and DreamTaq polymerase), 1 µl of each primer at a concentration of 10 µM and 5 µl of extracted DNA. The temperature profile for the 28S and ITS1 regions consisted of an initial cycle at 94 °C for 4 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. The reaction concluded with a final extension cycle at 72°C for 10 minutes.

For amplification of the COI region of mitochondrial DNA, a 30 µL reaction mixture was used. This consisted of 9 µL of DNA, 1.2 µL of nuclease-free water, 15 µL of DreamTaq™ PCR Master Mix (2X) (ThermoScientific™), and 2.4 µL of each primer at 20 µM. The PCR included an initial cycle at 94°C for 5 minutes, followed by 50 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was then performed at 72°C for 5 minutes.

PCR products were analyzed by gel electrophoresis on 1% (w/v) TopVision™ agarose (100 V/1 hour). 5 µl of each PCR product with 2 µl of 6X DNA loading buffer or dye (ThermoScientific™) were loaded with 1X GelRed®. DNA bands were observed under UV light and GeneRuler™100 bp Plus (ThermoScientific™) was used as a molecular weight marker.

The resulting PCR products were sent to Macrogen Inc. (South Korea) for purification and bi-directional Sanger sequencing. The resulting sequences were edited and assembled with BioEdit v.7.2.5 (Hall, 1999). Finally, each sequence was compared with the GenBank® database using the BLAST (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information).

### Phylogenetic analysis

The 28S, COI and ITS1 sequences obtained in this study were used in separate phylogenetic analysis. Sequences from other species of *Hemicriconemoides* from the GenBank database were also included. The selection of outgroups for phylogenetic analysis was based on previously published studies (Subbotin et al., 2005).

Multiple sequence alignments were performed using MAFFT version 7.450 (Kato et al., 2019). jModelTest v.2.1.10 (Darriba et al., 2012) was used to determine the best-fitting nucleotide

Murillo-Valdés et al. 2024. 2024. First morphometric and molecular study of *Hemicriconemoides* in Costa Rica substitution model to generate the trees, based on the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Decision Theory (DT). For phylogenetic reconstruction, MrBayes v.3.2.6 (Ronquist et al., 2012) was used to generate the trees according to the most probable substitution model for each alignment.

## RESULTS

The integrated analysis (morphological, morphometric, and molecular) of six populations of *Hemicriconemoides* confirmed the presence of *H. strictathecatus* and *H. rosae* in Costa Rica.

### Description and morphometry

*Hemicriconemoides strictathecatus* (Esser, 1960) (Figure 1, Table 2), was found in soil samples collected from cacao (*Theobroma cacao*), fig (*Ficus benjamina*), peppermint (*Mentha spicata*), aloe (*Aloe vera*) and ornamental plants Schefflera (*Schefflera arboricola*) and Cosmos (*Cosmos bipinnatus*) at Alajuela (n = 11) and San José (n = 43) provinces. Females showed variable body size (412.7 - 668.6  $\mu\text{m}$  long) with body diameter between 27.6 and 37.4  $\mu\text{m}$ . The number of rings along the body varied from 137 to 159. Females had a long, thin stylet, often slightly curved dorsally (69.5 - 86.1  $\mu\text{m}$ ), with anchor-shaped basal nodules, anteriorly serrated and posteriorly rounded 5.1 - 7.5  $\mu\text{m}$  wide and 2.6 - 5.0  $\mu\text{m}$  long

In the population from San José National Park associated with the ornamentals Shefflera and Cosmos, specimens showed pointed, conical tail ends, unlike the other populations where specimens with pointed and slightly rounded tail ends were observed.

The Labial region is flattened and narrow, with two rings, the first with a slightly smaller diameter than the second one. Dorsal gland orifice (DGO) between 2.9 and 6.6  $\mu\text{m}$ , posterior to the base of the stylet. The pharynx is 103.5 - 131.8  $\mu\text{m}$  long with a fused procorpus and metacorpus. The excretory pore was found posterior to the base of the pharynx, between annulations 32 and 45 from the anterior end. The vulva is distinguished by its cleft structure and the lack of prominent lips, with 11 - 15 annulations to the tail.

The spermatheca is round, with a length of 12.5 - 29.9  $\mu\text{m}$  and a diameter of 10.9 - 18.6  $\mu\text{m}$ . The tail is conical with the last rings frequently irregular and sometimes curved dorsally or ventrally. The anus is located posterior to the vulva with three or four annulations. Neither juveniles nor males were found in this population.

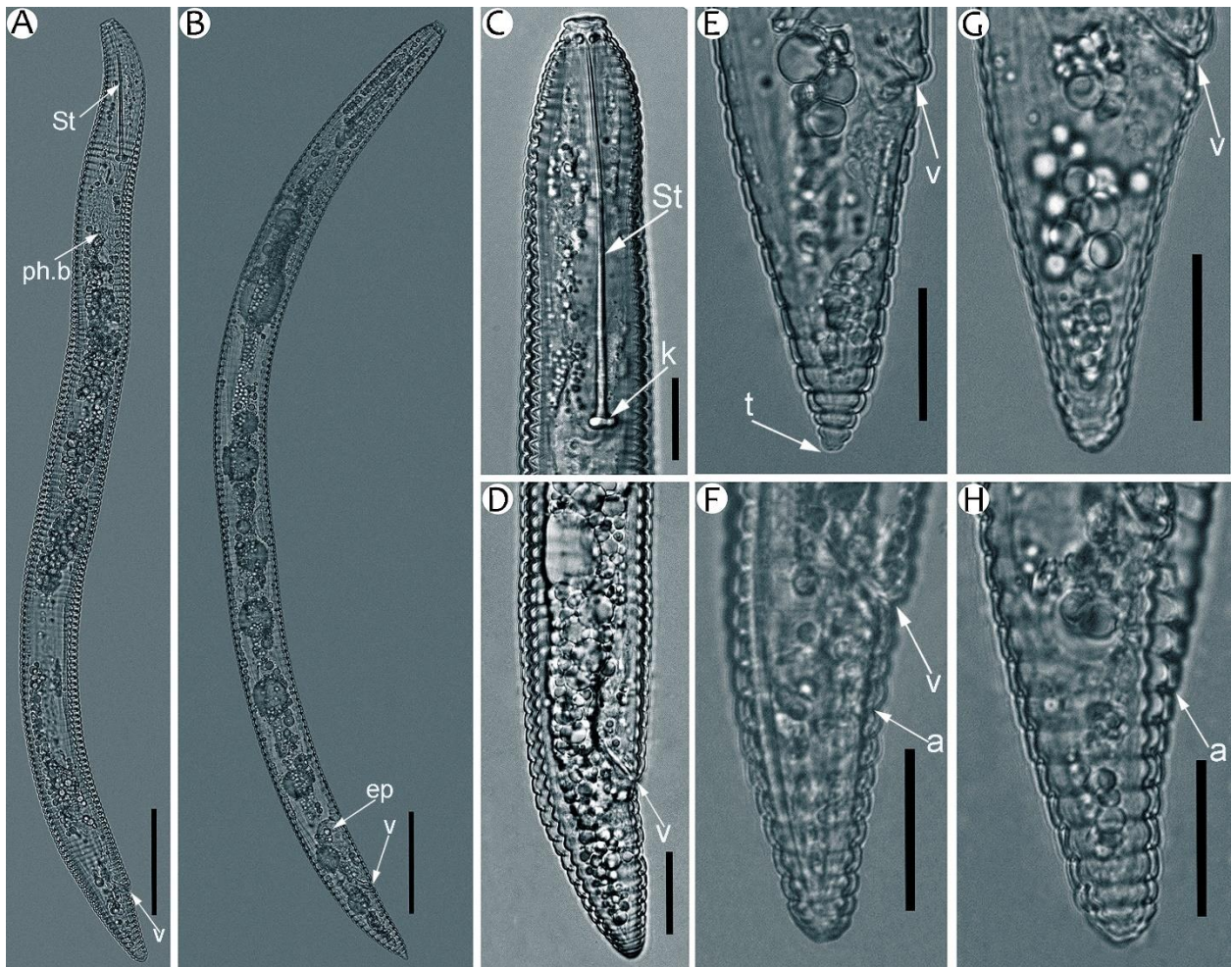


Figure 1. Light micrographs of *H. strictathecatus* females. A and B. Whole body showing stylet (St), base of pharyngeal bulb (ph.b), vulva (v) and excretory pore (ep). B. Anterior region showing stylet (St) and knobs (k). D-H. Posterior region showing vulva (v), anus (a) Scales: A and B = 60  $\mu\text{m}$ . C-H = 20  $\mu\text{m}$ .

Table 2. Comparative morphometric characters of *Hemicriconemoides strictathecatus* females from this study with the original description.

	<i>Hemicriconemoides strictathecatus</i> (Esser, 1960)					
Origen	San José, San José	Upala, San José	Montes de Oca, San José	Alajuela, Alajuela	Curridabat, San José	Esser (1960) **USA
Host	Sheffera ( <i>Schefflera arboricola</i> ) y Cosmos ( <i>Cosmos bipinnatus</i> )	<i>Theobroma cacao</i>	<i>Ficus benjamina</i>	<i>Mentha spicata</i>	<i>Aloe vera</i>	<i>Cocos nucifera</i>
Nematodes	12 ♀♀	10 ♀♀	11 ♀♀	11 ♀♀	10 ♀♀	X ♀♀
L	595.4 ± 53.6 (512.3-667.3)	635.5 ± 23.1 (611.7-667.3)	532.0 ± 56.1 (434.6-611.3)	597.7 ± 40.4 (538.3-668.6)	558.7 ± 61.2 (412.7-630.4)	490.0 - 590.0
DGO	5.4 ± 0.6 (4.5- 6.2)	5.2 ± 0.4 (4.3- 5.5)	4.9 ± 0.7 (2.9- 5.3)	5.5 ± 0.2 (5.1- 5.8)	5.7 ± 1.1 (3.1- 6.6)	-
Lip height	4.5 ± 0.8 (3.0 - 5.7)	5.3 ± 0.3 (4.7 - 5.6)	4.7 ± 0.3 (4.5 - 5.2)	5.0 ± 0.5 (4.3- 5.7)	4.5 ± 0.8 (3.3- 5.9)	-
Lip width	9.5 ± 0.5 (8.7 - 10.5)	9.7 ± 0.2 (9.3 - 10.0)	8.9 ± 0.5 (8.3 - 9.9)	9.3 ± 0.3 (9.0 - 10.1)	8.9 ± 1.2 (7.4 - 11.3)	-
Second lip annulus diam.	11.2 ± 0.6 (10.1 - 12.3)	11.4 ± 0.5 (10.3 - 11.9)	10.6 ± 0.4 (9.9 - 11.3)	11.7 ± 0.5 (11.0 - 12.4)	11.2 ± 1.1 (9.3 - 12.8)	-
First body annulus diam.	13.8 ± 0.8 (12.9 - 15.5)	14.1 ± 0.6 (13.5 - 15.0)	12.8 ± 0.7 (11.4 - 13.6)	13.7 ± 0.8 (12.6 - 15.4)	13.7 ± 0.8 (12.6 - 15.4)	-
Second body annulus diam.	15.9 ± 0.7 (15.2 - 17.1)	16.1 ± 0.6 (15.1 - 16.8)	15.1 ± 0.4 (14.1 - 15.8)	17.4 ± 1.6 (15.3 - 19.9)	15.4 ± 1.0 (13.7 - 16.7)	-
Third body annulus diam.	18.1 ± 1.0 (16.4 - 19.7)	18.2 ± 0.3 (17.6 - 18.7)	16.6 ± 0.6 (15.4 - 17.8)	17.5 ± 0.9 (15.6 - 18.3)	16.6 ± 1.0 (15.0 - 18.1)	-
Stylet length	79.3 ± 2.9 (74.6 - 83.4)	80.3 ± 3.1 (76.2 - 85.1)	77.0 ± 4.0 (69.5 - 82.7)	82.4 ± 1.7 (79.8 - 85.1)	80.1 ± 4.9 (72.3 - 86.1)	73 - 83
Stylet knob width	6.7 ± 0.6(5.1. - 7.5)	6.7 ± 0.4 (6.1 - 7.3)	6.2 ± 0.1 (6.1. - 6.6)	6.5 ± 0.5 (5.7. - 7.1)	6.7 ± 0.4 (6.1. - 7.5)	-
Stylet knob length	3.1 ± 0.4 (2.6 - 3.8)	4.4 ± 0.2 (4.1 - 4.8)	3.6 ± 0.3 (3.1 - 4.3)	3.4 ± 0.2 (3.1 - 3.7)	3.7 ± 0.7 (2.9 - 5.0)	-
Anterior end to nerve ring	106.0 ± 8.8 (81.3 - 112.7)	107.5 ± 3.5 (102.4 - 112.9)	107.1 ± 3.6 (101.5 - 112.2)	105.2 ± 2.9 (101.0 - 110.5)	104.1 ± 5.8 (95.3 - 112.5)	-
Anterior to excretory pore	144.2 ± 8.5 (130.6 - 155.4)	154.5 ± 5.2 (146.6 - 164.8)	144.4 ± 2.5 (140.3 - 149.8)	144.9 ± 6.1 (134.0 - 154.5)	122.8 ± 12.9 (97.7 - 141.6)	-
Pharynx Length	128.7 ± 9.4 (103.5 - 142.4)	129.7 ± 7.2 (122.1 - 140.8)	122.2 ± 5.2 (112.0 - 128.5)	121.4 ± 2.0 (118.0 - 124.3)	122.8 ± 6.1 (110.0 - 131.8)	-
Width at mid-body	33.3 ± 1.2 (31.3 - 35.2)	33.1 ± 1.5 (29.3 - 34.8)	29.0 ± 1.5 (27.6 - 32.7)	32.8 ± 2.2 (29.1 - 37.4)	32.2 ± 3.0 (25.3 - 36.4)	-
Width at anus	22.9 ± 1.4 (20.0 - 25.2)	21.4 ± 2.3 (17.0 - 24.7)	19.1 ± 1.0 (17.2 - 20.5)	22.1 ± 0.9 (20.4 - 23.3)	32.2 ± 3.0 (25.3 - 36.4)	-
Vulva body diam. (VD)	26.4 ± 1.3 (24.9 - 29.9)	25.8 ± 1.3 (23.4 - 27.8)	22.3 ± 1.7 (20.4 - 26.5)	23.3 ± 1.0 (22.4 - 25.6)	21.8 ± 3.4 (17.3 - 28.6)	-
R	154.1 ± 3.0 (150.0 - 159.0)	142.4 ± 4.0 (137.0 - 149.0)	142.7 ± 3.3 (138.0 - 148.0)	145.5 ± 5.8 (137.0 - 156.0)	149.6 ± 3.8 (144.0 - 156.0)	127-152
Rst	24.1 ± 1.8 (21.0 - 28.0)	22.7 ± 1.4 (20.0 - 25.0)	24.7 ± 1.0 (24.0 - 27.0)	24.3 ± 1.3 (23.0 - 26.0)	26.2 ± 2.4 (22.0 - 31.0)	-
Rph	36.2 ± 2.9 (32.0 - 40.0)	34.3 ± 3.6 (30.0 - 42.0)	34.7 ± 2.9 (28.0 - 38.0)	29.4 ± 1.2 (28.0 - 31.0)	32.2 ± 4.7 (22.0 - 40.0)	-
Rex	38.8 ± 1.6 (37.0 - 41.0)	40.7 ± 2.7 (35.0 - 45.0)	42.3 ± 1.8 (40.0 - 45.0)	35.0 ± 2.9 (32.0 - 41.0)	38.4 ± 1.5 (36.0 - 41.0)	38
Rv	13.3 ± 0.9 (12.0 - 15.0)	13.4 ± 1.1 (11.0 - 15.0)	13.2 ± 1.2 (11.0 - 15.0)	13.8 ± 0.8 (13.0 - 15.0)	13.3 ± 0.7 (12.0 - 14.0)	11-14
RVan	3.3. ± 0.5 (3.0 - 4.0)	3.2 ± 0.6 (3.0 - 5.0)	3.6 ± 0.5 (3.0 - 4.0)	3.3 ± 0.5 (3.0 - 4.0)	3.0 ± 0.0 (3.0 - 3.0)	3
Ran	9.8 ± 1.0 (8.0 - 11.0)	10.1 ± 1.6 (6.0 - 12.0)	9.4 ± 1.5 (7.0 - 12.0)	7.1 ± 1.0 (6.0 - 9.0)	10.2 ± 0.6 (9.0 - 11.0)	9-11
a	17.9 ± 1.3 (16.0- 19.9)	19.2 ± 0.8 (17.7 - 20.9)	18.5 ± 1.7 (15.7 - 22.2)	18.3 ± 1.1 (15.9 - 19.9)	17.4 ± 1.0 (15.3 - 18.7)	
b	4.6 ± 0.5 (3.8- 5.5)	4.9 ± 0.4 (4.3 - 5.5)	22.7 ± 0.4 (3.6 - 5.1)	4.9 ± 0.3 (4.5 - 5.6)	4.6 ± 0.6 (3.5 - 5.5)	

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c	17.6 ± 1.8 (15.6- 21.2)	20.4 ± 6.0 (14.4 - 33.9)	22.7 ± 3.9 (15.9 - 28.2)	23.0 ± 3.5 (18.8 - 29.6)	13.3 ± 4.5 (9.4 - 22.5)	
c'	1.5 ± 0.2 (1.3- 1.8)	1.6 ± 0.4 (1.1 - 2.4)	1.3 ± 0.2 (1.0 - 1.5)	1.2 ± 0.1 (0.9 - 1.3)	1.4 ± 0.3 (1.4 - 2.3)	
VL/VB	1.8 ± 0.2 (1.5- 2.0)	1.9 ± 0.2 (1.6 - 2.2)	1.8 ± 0.2 (1.5 - 2.0)	2.1 ± 0.3 (1.4 - 2.6)	1.7 ± 0.3 (1.4 - 2.3)	1.5-2.0
T/ABD	1.5 ± 0.2 (1.3- 1.8)	1.6 ± 0.4 (1.1 - 2.4)	4.5 ± 10.8 (1.0 - 37.1)	1.3 ± 0.1 (1.3 - 1.5)	1.4 ± 0.3 (0.9 - 1.8)	-
VA/T%	26.7 ± 4.8 (18.9 - 34.6)	31.0 ± 10.8 (17.7 - 56.3)	32.6 ± 7.0 (20.0 - 43.3)	32.3 ± 8.9 (19.9 - 45.0)	44.8 ± 9.7 (27.1 - 55.4)	-
Tail length	34.0 ± 4.1 (28.5- 41.7)	33.2 ± 7.8 (18.2 - 43.8)	24.4 ± 5.2 (18.3 - 37.1)	26.4 ± 3.1 (20.9 - 29.8)	30.0 ± 5.0 (21.1 - 35.9)	-
St%L	13.4 ± 1.5 (11.2 - 16.3)	12.7 ± 0.8 (11.6 - 13.7)	14.4 ± 0.9 (13.3 - 16.1)	13.8 ± 0.9 (12.6 - 15.2)	14.5 ± 1.5 (12.9 - 17.8)	-
Vulva to anus distance	12.6 ± 2.2 (8.2 - 17.1)	15.1 ± 4.0 (10.4 - 23.4)	12.8 ± 2.2 (7.9 - 16.6)	15.7 ± 3.2 (11.6 - 21.8)	15.7 ± 3.2 (11.6 - 21.8)	-
Vulva to tail tip	47.4 ± 3.9 (41.7 - 53.5)	49.6 ± 4.5 (41.5 - 58.8)	39.9 ± 4.4 (33.9 - 48.6)	50.1 ± 7.8 (32.1 - 58.4)	36.2 ± 8.2 (22.7 - 50.5)	-
Anterior end of vulva	550.7 ± 49.0 (468.6 - 621.3)	597.6 ± 25.7 (568.3 - 641.3)	496.6 ± 48.2 (401.8 - 562.7)	540.0 ± 46.1 (471.3 - 618.3)	509.3 ± 64.7 (375.3 - 577.5)	-
Spermatheca length	21.2 ± 4.0 (15.6 - 29.9)	21.0 ± 1.6 (17.7 - 23.2)	21.2 ± 2.6 (17.2 - 25.5)	21.0 ± 4.3 (13.9 - 27.1)	16.2 ± 3.9 (12.5 - 22.5)	-
Spermatheca width	14.6 ± 2.2 (11.2 - 18.6)	15.5 ± 1.3 (13.7 - 17.1)	13.4 ± 2.2 (10.9 - 16.7)	14.8 ± 2.9 (10.9 - 17.3)	14.1 ± 2.5 (10.9 - 17.2)	-
Vulva-Spermatheca distance	47.3 ± 10.5 (32.1 - 63.6)	48.9 ± 4.9 (40.9 - 58.7)	47.8 ± 9.2 (32.4 - 58.5)	54.3 ± 9.0 (41.8 - 66.4)	42.6 ± 7.8 (27.5 - 49.7)	-

Abbreviations: n (number of specimens measured), L (body length), St (stylet length), DGO (distance from base of stylet to dorsal esophageal gland), R (body ring number), Rex (number of rings from anterior end to excretory pore), RVan (number of rings from vulva to posterior end), St%L (stylet length/body length x 100), and VL/VBD (distance from vulva to posterior end/vulval body diameter).

*Hemicriconemoides rosae* (Rathour, Sharma, Singh & Ganguly, 2003) (Figure 2, Table 3). This species was found in a soil sample collected from a pasture at Puntarenas province (n = 11). Females have a small body size, 422.4 - 509.8  $\mu\text{m}$  long with distinct rounded body rings, a body diameter between 23.1 and 33.8  $\mu\text{m}$ . The number of rings along the body varied between 112 and 126. The labial region is round with a prominent labial disk with two rings, the first ring is smaller (7.4 - 9.6  $\mu\text{m}$ ) than the second one (10.7 - 14.9  $\mu\text{m}$ ).

The cephalic structure is well developed and moderately sclerotized. The stylet is long (41.6 - 59.9  $\mu\text{m}$ ), with anchor-shaped nodules anteriorly and rounded posteriorly. They are 6.3 - 8.1  $\mu\text{m}$  wide and 3.1 - 4.1  $\mu\text{m}$  long. The dorsal esophageal gland orifice (DGO) is located 3.8 - 5.9  $\mu\text{m}$  posterior to the base of the stylet. It has a widened procorpus fused with the metacarpus. The pharynx is 90.1-108.5  $\mu\text{m}$  long. The excretory pore is located 32 - 35 rings posterior to the base of the pharynx (21 - 27 rings).

The vulva is straight, located between rings 9-12, slit-shaped with a prominent membranous sheath. Females have a well-developed oval spermatheca, 10.2-21.4  $\mu\text{m}$  long and 17.3-24.5  $\mu\text{m}$  in diameter. The tail is conical, with a dorsal convexity and a sheath that encloses the tip and gradually narrows. The anus is located 2 or 3 rings posterior to the vulva. Juveniles and males were not found in this population.

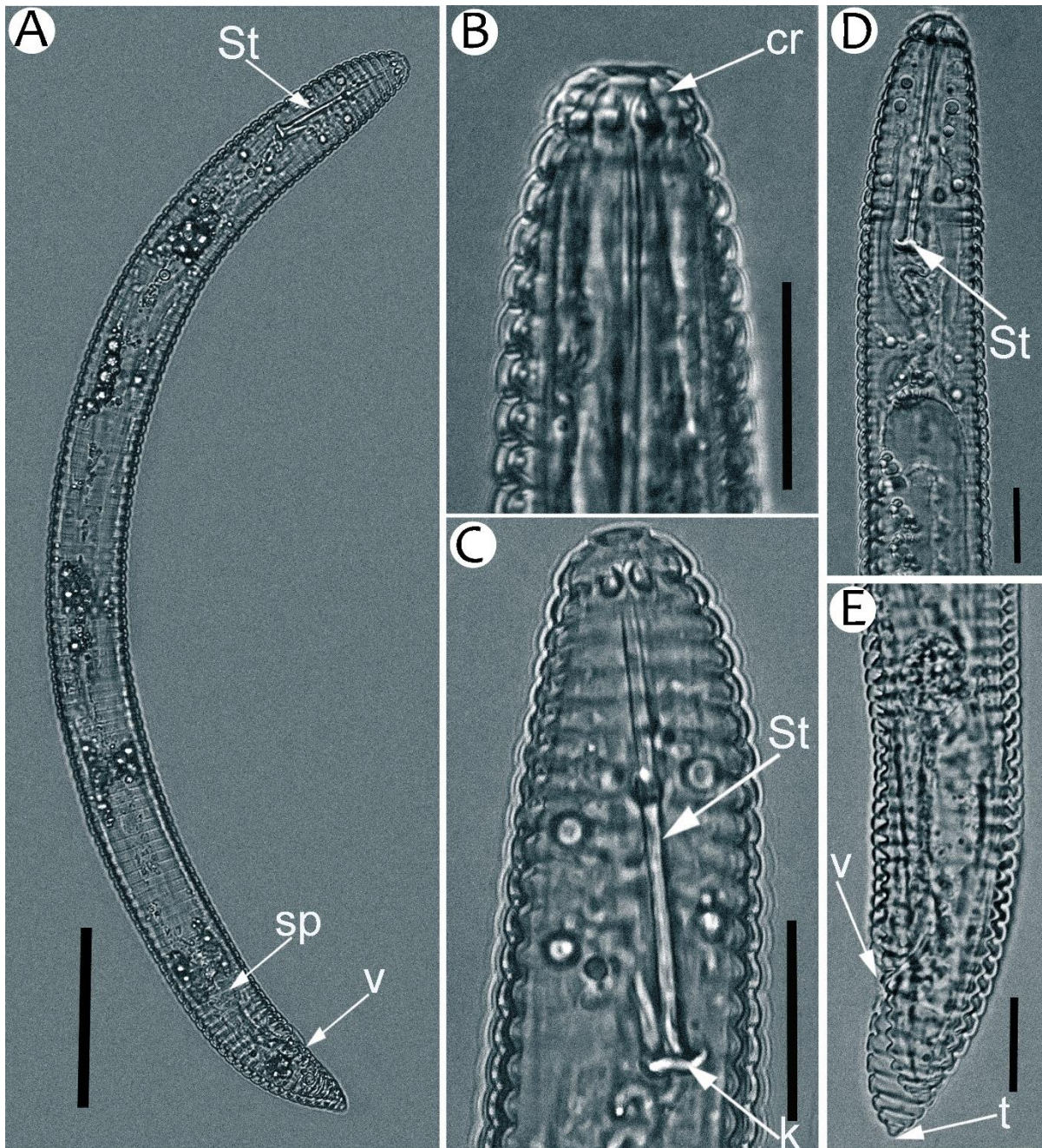


Figure 2. Light micrographs of *H. rosae* females. A. whole body showing stylet (St), spermatheca (sp) and vulva (v). B, C and D. Anterior region showing cephalic region (cr), stylet (St) and knobs (k). E. Posterior region showing vulva (v) and tail (t). Scales: A= 50  $\mu$ m, B, C, D and E = 20  $\mu$ m.

Table 3. Comparative morphometric characters of *Hemicriconemoides rosae* females from this study with the original description.

<i>Hemicriconemoides rosae</i> (Rathour, Sharma, Singh & Ganguly, 2003)		
		Rathour, Sharma, Singh & Ganguly (2003) **
Origin	San José	India
Host	Grass	Sugar cane
n	11 ♀♀	13 ♀♀
L	454.1 ± 33.6 (422.4-509.8)	470-510
DGO	4.6± 0.9 (3.8- 5.9)	-
Lip height	4.9± 0.3 (4.4- 5.6)	-
Lip width	8.7 ± 0.7 (7.4 - 9.6)	-
Second lip annulus diam.	12.4± 1.2 (10.7 - 14.9)	-
First body annulus diam.	14.5 ± 1.3 (12.3 - 17.6)	-
Second body annulus diam.	17.1 ± 1.4 (15.8 - 20.8)	-
Third body annulus diam.	18.7 ± 1.7 (17.2 - 23.4)	-
Stylet length	48.9± 5.5 (41.6 - 59.9)	50-55
Stylet knob width	6.9 ± 0.5 (6.3 - 8.1)	-
Stylet knob length	3.4 ± 0.3 (3.1 - 4.1)	-
Anterior end to nerve ring	81.7 ± 7.2 (72.6 - 90.3)	-
Anterior to excretory pore	97.9 ± 16.4 (82.9 - 115.4)	-
Pharynx Length	97.9 ± 5.8 (90.1 - 108.5)	-
Width at mid-body	28.0 ± 3.0 (23.1 - 33.8)	-
Width at anus	18.7 ± 1.9 (15.1 - 22.2)	-
Vulva body diam. (VD)	21.9 ± 1.6 (20.1 - 25.3)	-
R	119.4 ± 4.8 (112.0 - 126.0)	-
Rst	17.7 ± 1.8 (15.0 - 21.0)	-
Rph	24.0 ± 1.7 (21.0 - 27.0)	-
Rex	33.5 ± 1.3 (32.0 - 35.0)	-
Rv	10.5 ± 1.0 (9.0 - 12.0)	6-9
RVan	2.9 ± 0.3 (2.0 - 3.0)	-
Ran	7.5 ± 1.1 (6.0 - 9.0)	-
a	16.4 ± 2.2 (13.9 - 22.0)	
b	4.7 ± 0.3 (4.3 - 5.3)	
c	22.7 ± 2.7 (17.1 - 27.5)	
c'	1.1 ± 0.1 (0.9 - 1.3)	
VL/VB	0.7 ± 0.1 (0.5 - 1.0)	-
T/ABD	1.1 ± 0.1 (0.9 - 1.3)	-
VA/T%	33.9± 8.0 (24.9 - 47.8)	-
Tail length	20.3 ± 2.7 (17.2 - 24.7)	-
St%L	10.8 ± 1.0 (9.0 - 12.2)	-
Vulva to anus distance	10.6 ± 2.1 (8.5 - 14.4)	-
Vulva to tail tip	423.1 ± 29.8 (390.0 -477.6)	-
Anterior end of vulva	31.7± 4.8 (23.3 - 39.6)	-
Spermatheca length	14.0 ± 3.3 (10.2 - 21.4)	-
Spermatheca width	20.7 ± 2.4 (17.3 - 24.5)	-
Vulva-Spermatheca distance	46.7 ± 6.7 (38.2 - 54.3)	-

Abbreviations: n (number of specimens measured), L (body length), St (stylet length), DGO (distance from base of stylet to dorsal esophageal gland), R (body ring number), Rex (number of rings from anterior end to excretory pore), RVan (number of rings from vulva to posterior end), St%L (stylet length/body length x 100), and VL/VBD (distance from vulva to posterior end/vulval body diameter).

## Molecular identification

Partial sequences of the three analyzed genomic regions were obtained: D2-D3 domains of the 28S gene, ITS1 region of nuclear ribosomal DNA, and the mitochondrial COI gene (Cytochrome Oxidase I). All sequences of *H. strictathecatus* from Costa Rica (Table 4) showed high similarity with previously identified *H. strictathecatus* from the United States (Van den Berg et al., 2015). D2-D3 (28S) sequences showed between 99.7% and 100% similarity with KM516172, KM516173, and KM516176, while COI region exhibited similarity between 99.3% and 99.4% with KM516196, KM516197, and KM516198. ITS1 region showed similarity between 99.7% and 100% with accessions KM516186, KM516187, KM516188, and KM516191 (Van den Berg et al., 2015). D2-D3 sequences.

*H. rosae* (Table 4) showed 100% similarity with sequences of *H. rosae* from India (MK371811 and MK371813) (Khan et al., 2019) and China (MW938525 and MW938526) (Zhao & Zeng, 2021). ITS sequences exhibited 99.4% similarity with accessions from India (MK371814-MK371816) (Khan et al., 2019). COI region of *H. rosae* was not evaluated because primers COI-F5/COI-R9 do not amplify this region in this species.

Table 4. Collection data and GenBank accessions of *Hemicriconemoides* species from this study.

Species	Location	Host	Sample code	GenBank accession number		
				28S rDNA	ITS 1 rDNA	COI mtDNA
<i>H. strictathecatus</i>	San Antonio, Upala, Alajuela	<i>Theobroma cacao</i>	200C	PV069073	PV069097	PV237142
	La Guácima, Alajuela	<i>Mentha spicata</i>	254C	PV069075	PV069095	PV237140
	El Carmen, San José	<i>Schefflera arboricola</i>	219C	PV069074	PV069094	PV237138
		<i>Cosmos bipinnatus</i>				
	San Pedro, Montes de Oca, San José	<i>Ficus benjamina</i>	172C	PV069076	PV069098	PV237141
Curridabat, San José	<i>Aloe vera</i>	276C	PV069077	PV069096	PV237139	
<i>H. rosae</i>	Palo Seco, Parrita, Puntarenas	Pasto	145C	PV069078	PV069100	-

Phylogenetic trees for all analyzed regions (Figs. 4, 5, and 6) were generated using Bayesian inference (MCMC ngen = 1,100,000, subsamplefreq = 200) under the Akaike Information Criterion (AIC). jModelTest determined that the best nucleotide substitution model for generating the phylogenetic trees was the GTR+G model for the D2D3 and ITS1 regions, and GTR+I+G for the COI region

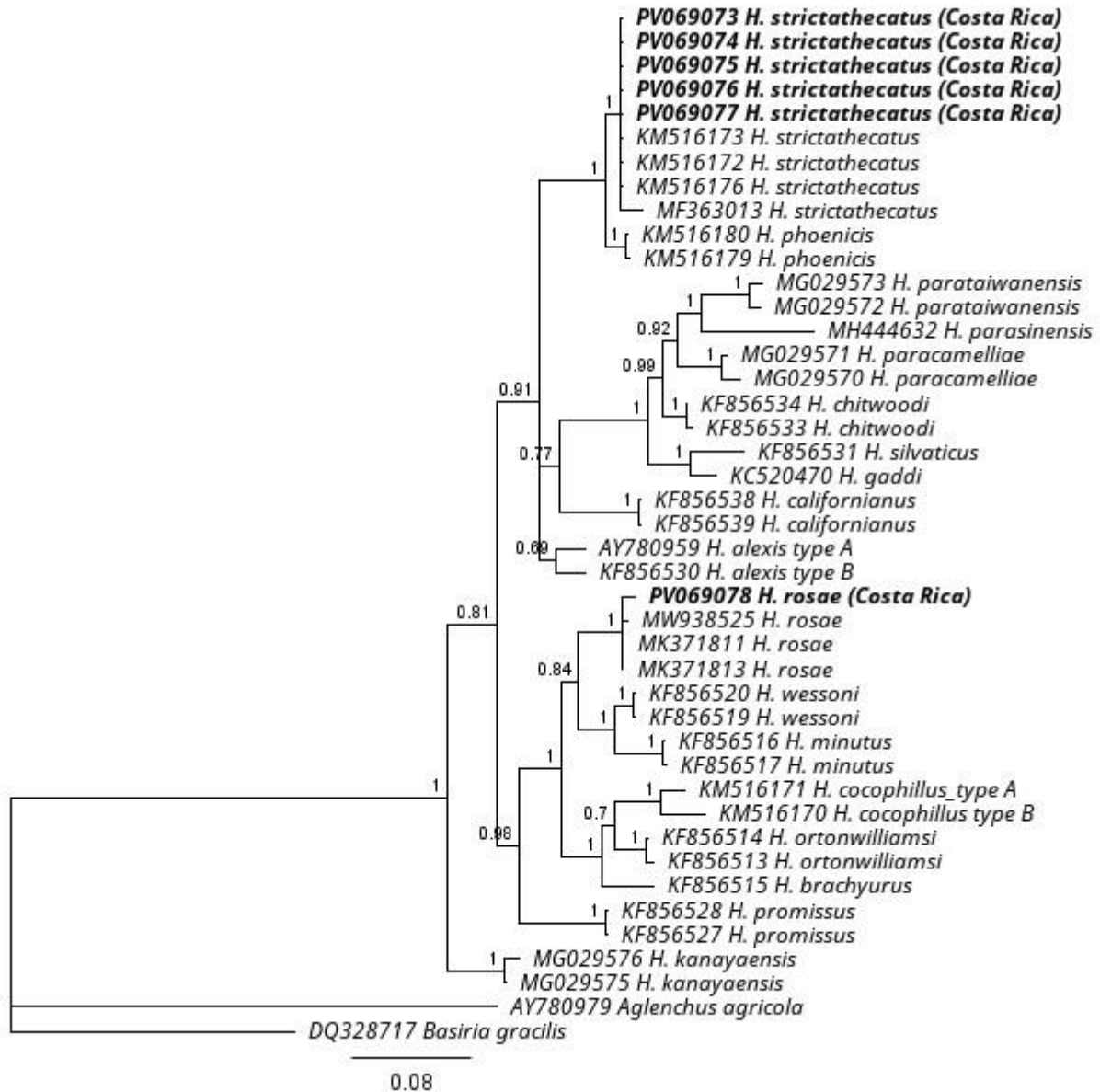


Figure 3. Phylogenetic tree constructed by Bayesian inference (BI) under the GTR+G model from partial sequences of the 28S D2-D3 domain. Relevant clades presented a probability greater than 70%. Sequences from this study are highlighted in bold.

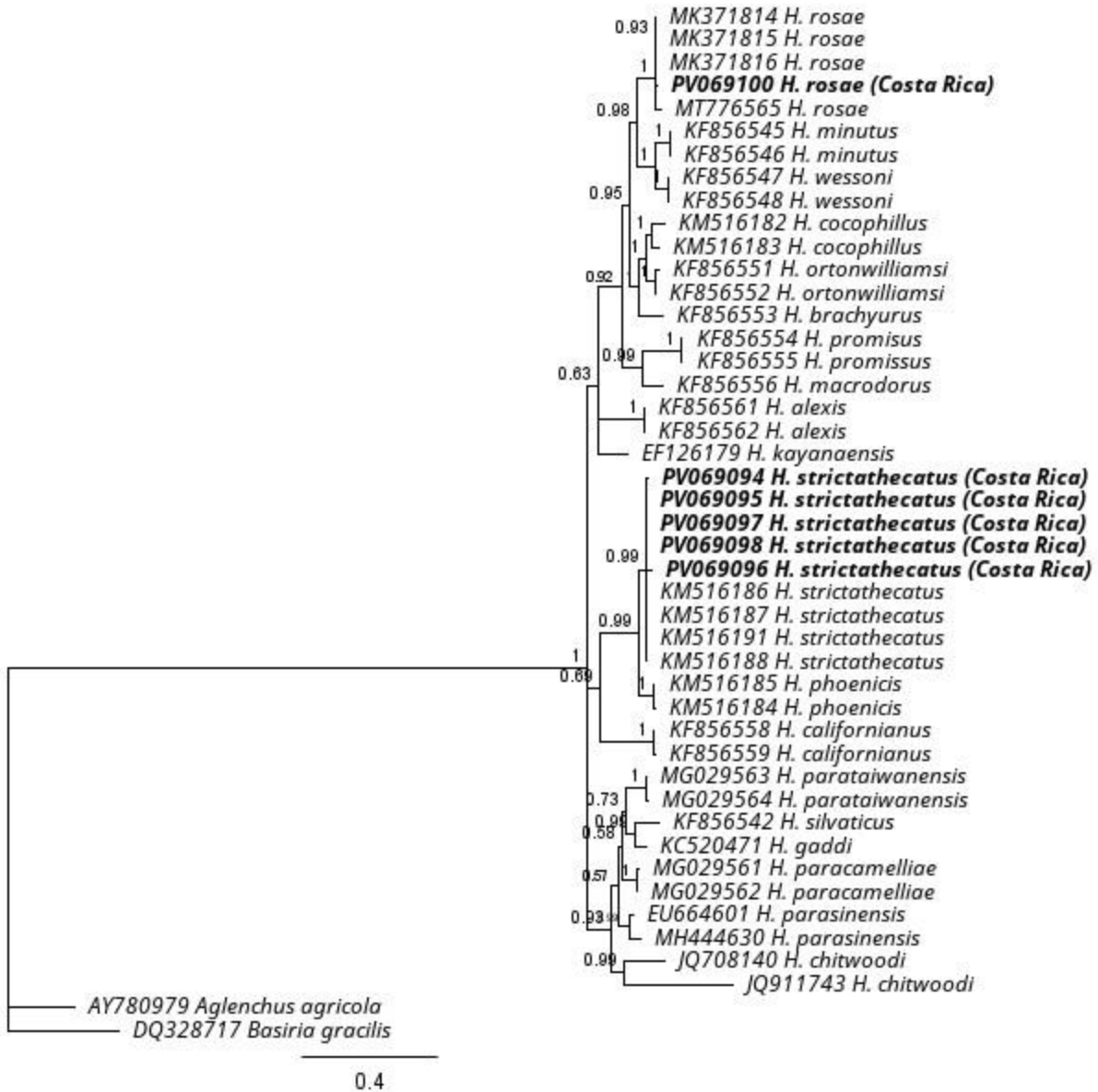


Figure 4. Phylogenetic tree constructed by Bayesian inference (BI) under the GTR+G model, from partial sequences of the ITS region (with primers rDNA1.58s/rDNA2). Relevant clades presented a probability greater than 50 %. Sequences from this study are highlighted in bold.

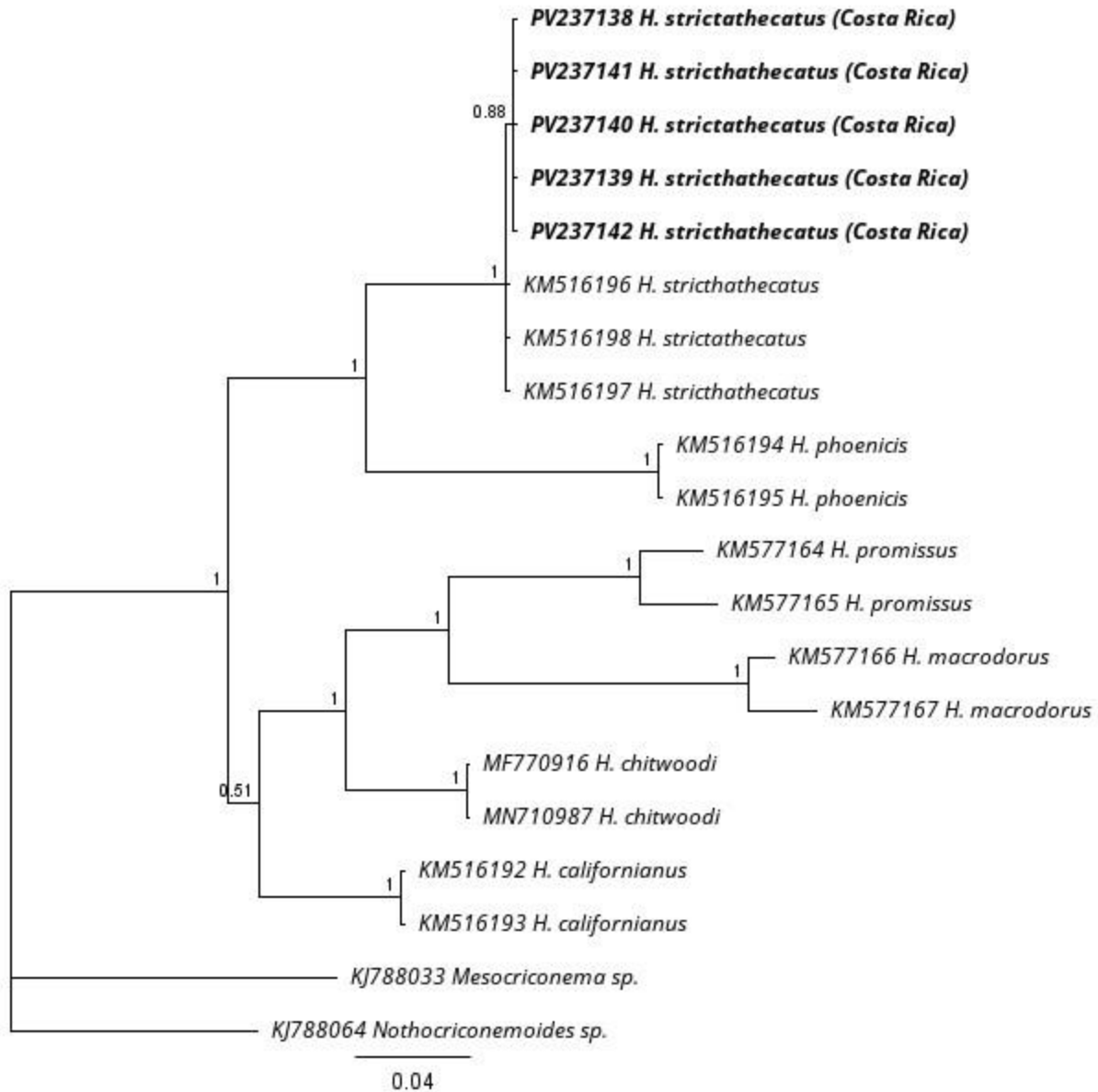


Figure 5. Phylogenetic tree constructed by Bayesian inference (BI) under the GTR+I+G model, from partial sequences of the COI region. Relevant clades presented a probability greater than 50%. Sequences from this study are highlighted in bold.

The D2-D3 (28S), ITS1, and COI sequences of *H. strictathecatus* from Costa Rica formed well-defined clades supported by high bootstrap values. They clustered in a single clade along with sequences of *H. strictathecatus* from the United States (Van den Berg et al., 2015).

Additionally, they showed phylogenetic proximity and grouped with *H. phoenicis* (Van den Berg et al., 2015).

Sequences of *H. rosae* from Costa Rica (D2-D3, ITS) also formed a well-defined single clade with high posterior probability value (100%) alongside sequences of the same species reported in India (Khan et al., 2019). These sequences showed a close relationship with *H. wessoni* and *H. minutus* (Van den Berg et al., 2014).

## DISCUSSION

The genus *Hemicriconemoides* was originally described by Chitwood and Birchfeld in 1957 and later redescribed by Dasgupta et al., (1969). According to the results obtained in this study, this genus is not widely distributed in Costa Rica, since it was detected in few soil samples and in low population densities. *Hemicriconemoides* is characteristic of warm climate regions and is usually found in the rhizosphere of ornamental, medicinal, aromatic plants and other hosts (Edward & Misra, 1964; Edward et al., 1965; Rathour et al., 2003, Van den Berg et al., 2014; María et al., 2018; Khan et al., 2019).

*H. strictathecatus* has been reported in Key West, Florida, United States, where it has been mainly associated with coconut palms (*Cocos nucifera*) (Van den Berg et al., 2014). This nematode has also caused damage to fruit trees such as mango (*Mangifera indica*) and various species of ornamental grasses (McSorley et al., 1980; Crow, 2013). In addition to its presence in the United States, it has also been reported in South Africa, China, Taiwan, and Venezuela (Van den Berg et al., 2014).

Populations of *H. strictathecatus* described in Costa Rica showed morphological variations in the shape of the tails, identifying two morphotypes: dotted and rounded tails. In addition, the stylet size of Costa Rican populations varied between 77.0 and 82.4  $\mu\text{m}$  long. These characteristics coincided with the findings reported by Van den Berg et al. (2014), where they mention morphological variation present among individuals of the same species.

In the isolated populations from San José (ornamentals Sheffera and Cosmos) as well as in Alajuela (cacao), females of *H. strictathecatus* showed predominantly conical and pointed tails, while in populations associated with mint from Alajuela, fig and *Aloe vera* from San José, females showed rounded conical tails were (Van den Berg et al., 2014). These differences can be attributed to intraspecific variations due to phenotypic plasticity, influenced by environmental conditions in which these populations inhabit (Munawar et al., 2019).

Morphometric results of *H. strictathecatus* were consistent with the original description of Esser (1960). However, some measurements showed differences in some parameters such as total body length (434.6 - 668.6 vs 490 - 590  $\mu\text{m}$ ), stylet length (69.5 - 86.1  $\mu\text{m}$  vs 73 - 83  $\mu\text{m}$ ), R (137 - 159  $\mu\text{m}$  vs 127 - 152  $\mu\text{m}$ ), Rex (32 - 45  $\mu\text{m}$  vs 38  $\mu\text{m}$ ), Rv (11 - 15  $\mu\text{m}$  vs 11 - 14  $\mu\text{m}$ ), Ran (6 - 12  $\mu\text{m}$  vs 9 - 11  $\mu\text{m}$ ), RVan (3 - 5  $\mu\text{m}$  vs 3  $\mu\text{m}$ ) and VL/VB (1.4 -2.6 vs 1.5 - 2.0  $\mu\text{m}$ ).

Molecular characterization, including ITS1, 28S, and COI sequences, revealed that our results of *H. strictathecatus* from Costa Rica were consistent with findings previously reported by Van den Berg et al. (2014) from Florida, USA. Furthermore, phylogenetic analysis showed a branching pattern that aligned with results from previous studies (Van den Berg et al., 2014; Munawar et al., 2018). *H. strictathecatus* consistently groups in a clade closely related to *H. phoenicis*, supporting Van den Berg et al. (2015) findings regarding the relationship between these species.

*H. rosae* was first isolated and described from the rhizosphere of rose plants from Bareilly district (Rathour et al., 2003). Subsequently, it was identified in sugarcane in Meerut district (Khan et al., 2019) and in mustard in Bulandshahr (Sharma and Kumar, 2023), all localities belonging to Uttar Pradesh, India.

Morphological and morphometric results show greater similarity with *H. rosae*, originally described by Rathour et al. (2003) and Khan et al. (2019). In this species, differences were also found between the studied population and the one described by Rathour et al. in 2003, in the average body length (422 - 509.8  $\mu\text{m}$  vs 470 - 510  $\mu\text{m}$ ) and stylet length (41.6 - 59.9  $\mu\text{m}$  vs 50 - 55  $\mu\text{m}$ ), R (112.0 - 126.0 vs 116), RV (9.0 - 12.0 vs 6 - 9), Rex (32.0 - 35.0 vs 22 - 30), St% L (9.0 - 12.2 vs 11) and VL/VB (0.5 - 1.0 vs 2.0).

Phylogenetic analyses indicated that *H. rosae* is closely related to *H. wessoni* and *H. minutus*, which is consistent with previous observations by Khan et al. (2019).

This study identified *H. strictathecatus* and *H. rosae* for the first time in Costa Rica, highlighting the importance of taxonomic and molecular research to enhance the understanding of these nematodes, which have been previously studied at the genus level in the region.

Morphological variability observed in the tails of *H. strictathecatus* indicates significant intraspecific diversity, influenced by environmental and genetic factors, which could complicate its identification and is thus a factor to consider for future studies.

The integration of morphological and molecular tools, including the amplification and sequencing of key genomic regions such as 28S, ITS1, and COI genes, allows broader genomic coverage and provides a comprehensive and accurate approach for the proper characterization of species.

This study contributes to the understanding of the nematode community in Costa Rica and lays the groundwork for future studies on the distribution and impact of these nematodes in various crops.

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