

## RESEARCH REPORT

### MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF POPULATIONS OF *HEMICRICONEMOIDES STRICTATHECATUS* ESSER, 1960 AND *H. ROSAE* RATHOUR, SHARMA, SINGH & GANGULY, 2003 (NEMATODA: CRICONEMATIDAE) FROM COSTA RICA

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#### ABSTRACT

Valdés-Murillo, M., W. Peraza-Padilla, R. Artavia-Carmona, J. Aráuz-Badilla and I. Hilje-Rodríguez. 2026. Morphological and molecular characterization of populations of *Hemicriconemoides strictathecatus* Esser, 1960 and *H. Rosae* Rathour, Sharma, Singh & Ganguly, 2003 (Nematoda: Criconematidae) from Costa Rica. *Nematropica* 56: 1-19.

During nematological studies conducted in Costa Rica, five populations of sheathoid nematodes (*Hemicriconemoides* spp.) were detected in samples collected in Alajuela, Puntarenas, and San José provinces. Morphological and molecular analyses showed that five sheathoid nematode populations from Alajuela and San José provinces match with *Hemicriconemoides strictathecatus*, a species described from Florida, USA and reported in many other countries. A population from Puntarenas province fit morphologically and molecularly with *H. rosae*, a species described in Uttar Pradesh, India and also reported in China. This is the first record of the occurrence of these two species in Costa Rica. Phylogenetic analysis of *Hemicriconemoides* species were performed based on partial sequences of the D2-D3 domains of the 28S ribosomal DNA gene, the ITS1 region and subunit I of the cytochrome c oxidase (*COI*) mitochondrial gene. This integrative approach also supported the identification of *H. strictathecatus* and *H. rosae* as valid species newly recorded in Costa Rica.

*Key words:* DNA sequencing, integrative taxonomy, morphology, phylogeny, sheathoid nematode.

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#### RESUMEN

Valdés-Murillo, M., W. Peraza-Padilla, R. Artavia-Carmona, J. Aráuz-Badilla e I. Hilje-Rodríguez. 2026. Caracterización morfológica y molecular de poblaciones de *Hemicriconemoides strictathecatus* Esser, 1960 y *H. Rosae* Rathour, Sharma, Singh & Ganguly, 2003 (Nematoda: Criconematidae) de Costa Rica. *Nematropica* 56: 1-19.

Durante estudios nematológicos llevados a cabo en Costa Rica, se detectaron cinco poblaciones del nematodo vaina (*Hemicriconemoides* spp.) en muestras recolectadas en las provincias de Alajuela, Puntarenas y San José. Los análisis morfológicos y moleculares mostraron que cinco poblaciones de

nematodos vaina de las provincias de Alajuela y San José eran congruentes con *Hemicriconemoides strictathecatus*, una especie descrita en Florida, EUA y reportada en muchos otros países. Una población de la provincia de Puntarenas coincidió morfológica y molecularmente con *H. rosae*, una especie descrita en Uttar Pradesh, India y reportada también en China. Este es el primer registro de la ocurrencia de estas dos especies en Costa Rica. Se hicieron análisis filogenéticos de especies del género *Hemicriconemoides* a partir de secuencias parciales de los dominios D2-D3 del gen 28S del ADN ribosomal, la región ITS1 y la subunidad I del gen mitocondrial del citocromo c oxidasa (*COI*). Este enfoque integrador también respaldó la identificación de *H. strictathecatus* y *H. rosae* como especies válidas recién registradas en Costa Rica.

*Palabras clave:* Filogenia, morfología, nematodo vaina, secuenciación de ADN, taxonomía integrativa.

## INTRODUCTION

The genus *Hemicriconemoides* (Chitwood and Birchfield, 1957), later redescribed by Dasgupta et al., (1969), currently includes 55 nominal species, according to recent studies (Inserra et al., 2014; Munawar et al., 2019; Nguyen et al., 2020; Van den Berg et al., 2015). *Hemicriconemoides* spp. are migratory ectoparasitic nematodes associated with plant roots. They are commonly called sheathoid nematodes because in adult stages, their body cuticle is covered by an external layer or sheath composed of prominent smooth annuli. 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 & Birchfield, 1957; Khan et al., 2019; Sharma & Chaubey, 2023). Infestations of these nematodes may suppress the yield in crops such as millet, rice, tubers, citrus, banana, dates, pineapple, and grapevine (Azimi & Pedram, 2020; Budiman, 2020; Khan et al., 2019; Munawar et al., 2018; Nguyen et al., 2020; Shokoohi, 2022; Vovlas et al., 2000). Their geographic distribution covers tropical regions of several continents such as Africa, America, Australia, South and Southeast Asia, and Southern Europe.

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, accurate and reliable diagnosis of nematodes such as *Hemicriconemoides* is essential for optimizing integrated pest management (IPM) strategies,

particularly in high-value tropical crops where this genus has been reported. For example, *Hemicriconemoides mangiferae* has been associated with root damage in mango (*Mangifera indica*) in Asia and parts of Latin America (Khan et al., 2019); *H. cocophillus* has been reported affecting coconut (*Cocos nucifera*) in tropical regions, including the Caribbean (Munawar et al., 2018); and *H. kanayaensis* has been linked to growth reduction in grapevines (*Vitis vinifera*) (Vovlas et al., 2000). These cases underscore the importance of precise identification for effective management, particularly in tropical agroecosystems where these nematodes may be underdiagnosed.

Although *Hemicriconemoides* is not typically the direct target of nematicide trials, some broad-spectrum nematicides such as fluopyram and oxamyl have shown incidental suppression of ring nematodes in field and greenhouse settings (Schumacher et al., 2022). Additionally, soil amendments and biofumigation with Brassicaceae plants (e.g., mustard, radish) have demonstrated general nematicidal effects that may impact populations of *Hemicriconemoides* (Matthiessen & Kirkegaard, 2006; Zasada & Ferris, 2004). In perennial cropping systems, integrated management practices, such as pre-plant fumigation followed by nematicide applications, can indirectly reduce *Hemicriconemoides* densities as part of a broader impact on soil nematode communities (Chitwood, 2003).

In Costa Rica, sheathoid nematodes have 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 precise

identification of the species occurring in the country.

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* (Mirghasemi et al., 2024; Sharma & Chaubey, 2023). 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; Karaca et al., 2020; Palomares et al., 2018).

Therefore, in order to obtain new information on some of the species of *Hemicriconemoides* occurring in Costa Rica, a study was conducted to determine: i) the distribution of sheathoid nematode species in three provinces of Costa Rica and ii) the morphological and molecular characterization of these sheathoid nematode populations based on sequences of the 28S and the ITS nuclear ribosomal DNA and the mitochondrial COI gene.

## MATERIALS AND METHODS

### *Collection and isolation of nematodes*

One hundred and fifty soil samples were collected from different areas of Costa Rica, six of which were from agricultural crops, ornamental plants, and grasses, and tested positive for the genus *Hemicriconemoides*. Samples were obtained from the following host plants: cocoa (*Theobroma cacao*) and peppermint (*Mentha spicata*) from Alajuela province; Schefflera (*Schefflera arboricola*), Cosmos (*Cosmos bipinnatus*), fig (*Ficus benjamina*), and Aloe (*Aloe vera*) from San José and one additional sample from an unspecified grass from Puntarenas province (Table 1).

Between 10 and 15 random subsamples were taken from the first 30 cm of soil depth, until a composite sample consisting of approximately 1 kg of soil per site was obtained. Samples were stored in plastic bags and transferred to the Laboratorio de Nematología of the Universidad Nacional for further processing.

### *Extraction, mounting, and fixation of nematodes*

Female nematodes were extracted using the flotation-centrifugation method in sugar solution of Jenkins (1964). Nematodes were fixed in 4% formaldehyde at 70°C using the modified rapid Seinhorst method (Seinhorst, 1959) and preserved on Cobb slides using the paraffin method (Eisenback & Hunt 2020). Labeled Cobb slides were added to the Criconematidae family nematode collection of the Nematology Laboratory at Escuela de Ciencias Agrarias, Universidad Nacional de Costa Rica.

### *Morphological and morphometric characterization*

Taxonomic identification at genus level was carried out by using morphological characters, original descriptions, redescrptions, and identification keys according to Geraert (2010). For each sample, eleven adult females were selected, and measured in micrometers ( $\mu\text{m}$ ) using a high-resolution Nikon Eclipse 80i optical microscope (Nikon Corporation, Tokyo, Japan) equipped with a Nikon DS-Fi1 digital camera. This system enabled the acquisition of high-quality micrographs of the studied populations. The resulting images were subsequently edited and assembled using Adobe Photoshop® CS6 to enhance visualization and facilitate comparison of key morphological traits.

### *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 transferred to sterile 0.2 ml Eppendorf tubes containing 47  $\mu\text{l}$  of 0.2 M Tris-HCl, pH 8.0, 3  $\mu\text{l}$  of proteinase K (20 mg/ml) was added to each tube, and they were placed in an ultrasonic bath at 60°C for 10 min. Then they were incubated in a thermocycler at 60°C for 30 min, followed by vortexing. A 15-min cold incubation at -20°C was then carried out, followed by a 10-min hot incubation at 90°C. Tubes were vortexed again, and a second incubation was carried out with cold/hot temperature cycles. Samples were finally vortexed before being centrifuged for 2 min at 2000 rpm. DNA samples were stored at -20°C for posterior polymerase chain reactions (PCR).

Three genomic regions were amplified by PCR. D2-D3 region of the 28S rDNA gene was

amplified with primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') /D3B (5'-TCGGAAGGAACCAGCTACTA-3') (De Ley et al., 1999), ITS1 region with the primer set rDNA2 (5'-TTGATTACGTCGCTGCCCTTT-3') (Vrain et al., 1992) /rDNA1.58s (3'-ACGAGCCGAGTGATCCACCG-5') (Cherry et al., 1997), and subunit I of the cytochrome c oxidase (*COI*) gene was amplified with the primer set COI-F5 (5'-AATWTWGGTGTGGAACCTTCTTGAAC-3')/COI-R9 (5'-CTTAAACATAATGRAAAATGWGCWACWACATAATAAGTATC-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  $\mu$ l reaction mix containing 5.5  $\mu$ l nuclease-free water, 12.5  $\mu$ l DreamTaq™ PCR Master Mix (2X) (Thermo Scientific™) (including buffer, dNTPs, MgCl<sub>2</sub> and DreamTaq polymerase), 1  $\mu$ l of each primer (10  $\mu$ M), and 5  $\mu$ l of the extracted DNA. For amplification of the mtDNA COI region, a 30  $\mu$ l reaction mixture was prepared consisting of 9  $\mu$ l of DNA, 1.2  $\mu$ l of nuclease-free water, 15  $\mu$ l of DreamTaq™ PCR Master Mix (2X) (Thermo Scientific™), and 2.4  $\mu$ l of each primer (20  $\mu$ M). The temperature profile 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 min.

PCR products were analyzed by electrophoresis on 1% (w/v) TopVision™ agarose gels (100 V/1 hr) stained with 1X GelRed® (Biotium, Fremont, California, USA). DNA bands were observed under UV light and amplicons sizes were determined against GeneRuler™100 bp Plus DNA ladder (Thermo Scientific™, Waltham, Massachusetts, USA).

PCR products were sent to Macrogen Inc. (South Korea) for purification and bi-directional Sanger sequencing. Resulting sequences were edited and assembled with BioEdit v.7.7.1 (Hall, 1999). Each sequence was compared with sequences deposited in GenBank® using BLAST® (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information, Bethesda, Maryland, USA). The new sequences were submitted to the GenBank® database under the accession numbers PV069073-PV069078 for the 28S rDNA region, PV069094-PV069100 for the ITS1 rDNA region, and PV237138-PV237142 for the COI mtDNA region, as indicated in Table 2.

#### Phylogenetic analysis

Separate phylogenetic analyses were performed using the D2-D3 28S, ITS and COI partial sequences obtained from the sheathoid nematode populations collected and from other Criconematidae accessions from the GenBank database. Outgroups selection was based on previously published studies (Subbotin et al., 2005).

Multiple sequence alignments were performed using the FFT-NS-2 algorithm in MAFFT v.7.490 (Katoh & Standley 2013). jModelTest v.2.1.10 (Darriba et al., 2012) was used to determine the best-fitting nucleotide substitution model to generate the trees, based on the Akaike Information Criterion (AIC) (Akaike, 1973), Bayesian Information Criterion (BIC) (Schwarz, 1978), and Decision Theory (DT) (Minin et al. 2003). For phylogenetic reconstruction, MrBayes v.3.2.6 (Huelsenbeck & Ronquist, 2001) was used to generate the trees in Geneious Prime® v.2025.2.2 (Biomatters Ltd.) according to the most probable substitution model for each alignment.

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

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

## RESULTS

The analysis of the six *Hemicriconemoides* populations, each corresponding to a composite soil sample collected from different geographic locations, revealed the presence of *H. strictathecatus* and *H. rosae* in Costa Rica.

*Hemicriconemoides strictathecatus* Esser, 1960 (Fig. 1 in this study, 2 in Esser (1960), and 1-4 in Van den Berg et al. (2015)).

Measurements (See Table 3).

### Female

Females in the studied populations showed variable body size and number of body annuli. Labial region was flattened and narrow, with two annuli, the first having a slightly smaller diameter than the second, features not explicitly detailed in the original description by Esser (1960). Dorsal esophageal gland orifice (DGO), between 2.9 and 6.6  $\mu\text{m}$ , posterior to stylet knobs. Stylet was long and thin, ranging from 69.5 to 86.1  $\mu\text{m}$  in length, which aligns with the original measurement of 73-83  $\mu\text{m}$ . It was often slightly curved dorsally and ended in anchor-shaped basal knobs which were anteriorly serrated and posteriorly rounded. Stylet knobs were anchor-shaped, anteriorly serrated, and posteriorly rounded measuring 5.1-7.5  $\mu\text{m}$  wide and 2.6-5.0  $\mu\text{m}$  long. Pharynx was 103.5-131.8  $\mu\text{m}$  long with a fused procorpus and metacarpus. Excretory pore posterior to the pharyngeal basal bulb between annuli 32 and 45 from the anterior end. Vulva cleft and lacking prominent lips, usually located 11-15 annuli from tail terminus. Spermatheca was round, 12.5-29.9  $\mu\text{m}$  long, and a diameter of 10.9-18.6  $\mu\text{m}$ . Conical tail with the last annuli frequently irregular and sometimes dorsally or ventrally curved. Anus three or four annuli posterior to the vulva.

### Male and juveniles

Not found

### Remarks

Specimens of the population from San José province (associated with *Schefflera arboricola*

and *Cosmos bipinnatus*) showed pointed or conical tail terminus unlike those from other localities that exhibited rounded tail terminus.

*Hemicriconemoides rosae* Rathour et al., 2003 (Fig. 2 in this study and 1 in Rathour et al. (2003)). Measurements (See Table 4).

### Female

Females have a small body size, 422.4-509.8  $\mu\text{m}$  long, falling within the original range of 470-510  $\mu\text{m}$ , with distinct rounded 112-126 body annuli. Round labial region with a prominent labial disk and two lip annuli, the first smaller (7.4-9.6  $\mu\text{m}$ ) than the second one (10.7-14.9  $\mu\text{m}$ ). Distinct cephalic framework well-developed and moderately sclerotized. Stylet 41.6-59.9  $\mu\text{m}$  long, which is slightly broader but still consistent with the original range of 50-55  $\mu\text{m}$ . Knobs anteriorly anchor-shaped and posteriorly rounded. Dorsal esophageal gland orifice (DGO) located 3.8-5.9  $\mu\text{m}$  posterior to stylet knobs. Pharynx with a widened procorpus fused with the metacarpus. Excretory pore with 32-35 annuli below the pharyngeal basal bulb. Straight slit-like vulva with prominent membranous sheath and located 9-12 annuli from the tail terminus and slightly exceeding the 6-9 annuli reported in the original description. Oval spermatheca 10.2-21.4  $\mu\text{m}$  long and 17.3-24.5  $\mu\text{m}$  in diameter. Conical tail with a dorsal convexity and a pointed tail terminus ranging from 17.2 to 24.7  $\mu\text{m}$ , a measurement not reported in the original data but typical for the genus. Anus two or three annuli posterior to the vulva.

### Male and juveniles

Not found

### Remarks

Morphological characteristics of Costa Rican population fit those reported for the type of population in Uttar Pradesh, India by Rathour et al. (2003).

Table 2. Species and populations of *Hemicriconeimoides* from Costa Rica characterized in the present study.

Sample ID	Species	Location	Host	Sample code	Collectors	GenBank accession number			
						28S rDNA	ITS1 rDNA	COI mtDNA	COI mtDNA
1	<i>H. strictathecatus</i>	Bijagua, Alajuela	<i>Theobroma cacao</i>	200C	Valdés, Aráuz, Peraza	PV069073	PV069097	PV237142	PV237142
2	<i>H. strictathecatus</i>	Guácima, Alajuela	<i>Mentha spicata</i>	254C	Valdés, Aráuz, Peraza	PV069075	PV069095	PV237140	PV237140
3	<i>H. strictathecatus</i>	Carmen, San José	<i>Schefflera arboricola</i> and <i>Cosmos bipinnatus</i>	219C	Valdés, Aráuz, Peraza	PV069074	PV069094	PV237138	PV237138
4	<i>H. strictathecatus</i>	San Pedro, San José	<i>Ficus benjamina</i>	172C	Valdés, Aráuz, Peraza	PV069076	PV069098	PV237141	PV237141
5	<i>H. strictathecatus</i>	Curridabat, San José	<i>Aloe vera</i>	276C	Valdés, Aráuz, Peraza	PV069077	PV069096	PV237139	PV237139
6	<i>H. rosae</i>	Parrita, Puntarenas	Grass	145C	Valdés, Aráuz, Peraza	PV069078	PV069100	–	–

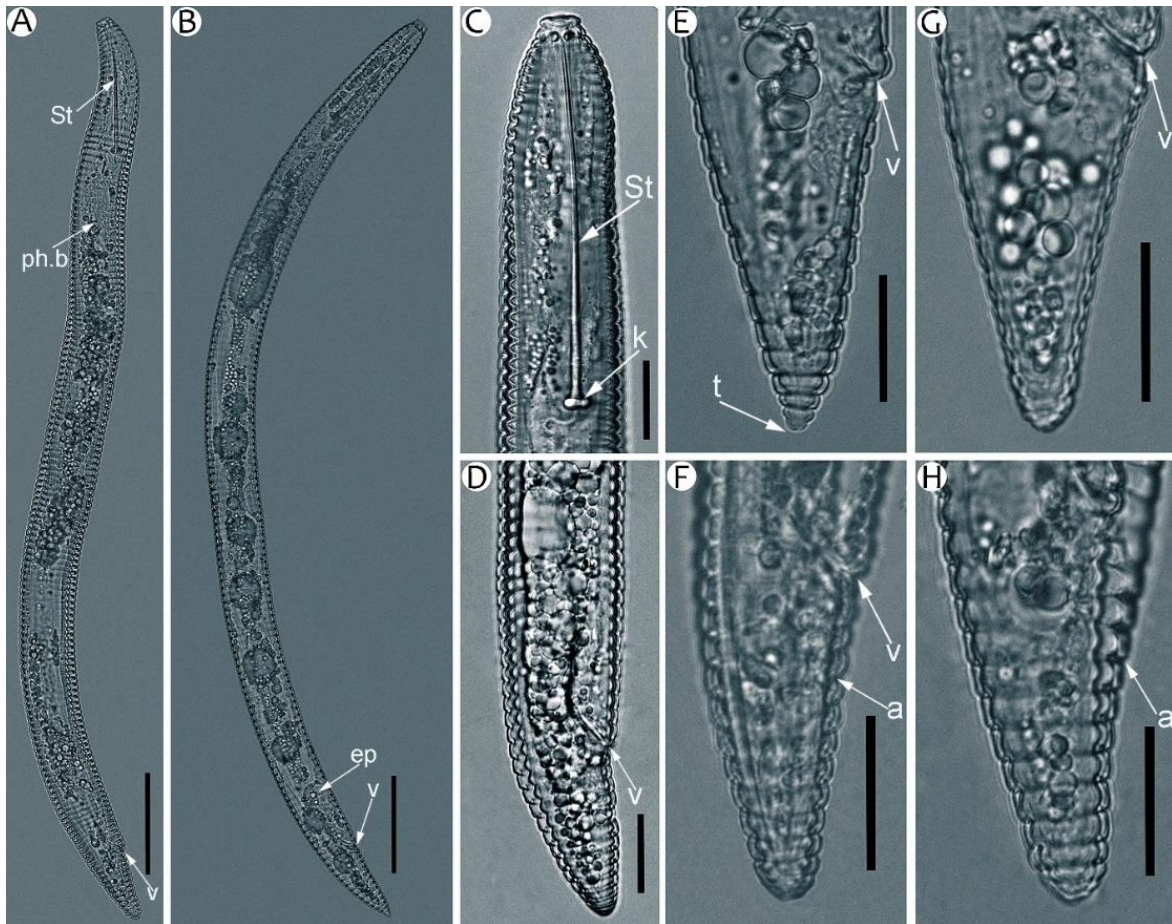


Figure 1. Light micrographs of *H. strictathecatus* females. A and B. Whole body showing stylet (St), base of pharyngeal bulb (ph.b), vulva (v) and excretory pore (ep). C. Anterior region showing stylet (St) and knobs (k). D-H. Posterior region showing vulva (v), anus (a) Scales: A and B = 60  $\mu\text{m}$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

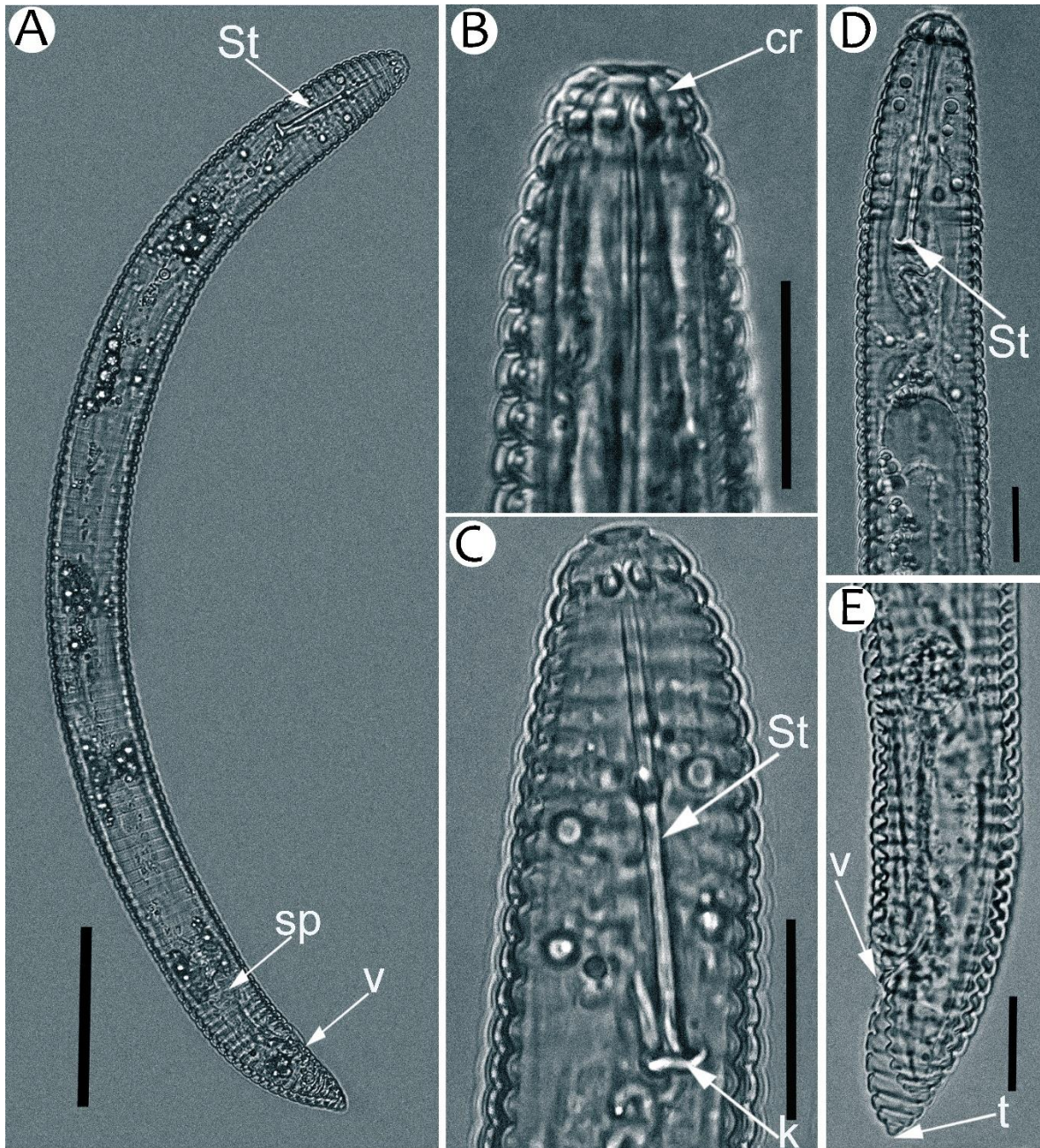


Figure 2. Light micrographs of *H. rosae* females. A. whole body showing stylet (St), spermatheca (sp) and vulva (v). B, C, and D. Anterior region showing cephalic region (cr), stylet (St) and knobs (k). E. Posterior region showing vulva (v) and tail (t). Scales: A = 50  $\mu\text{m}$ , B, C, D and E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

Table 3. Comparative morphometric characters of *Hemicriconemoides strictathecatus* females from this study and those in the original description (Esser, 1960)\* and topotype specimens (Van den Berg et al., 2015).

Location	This study				Van den Berg et al. 2015		Esser, 1960*	
	Carmen, San José <i>Schefflera arboreola</i> and <i>Cosmos bipinnatus</i>	Bijagua, Alajuela <i>Theobroma cacao</i>	San Pedro, San José <i>Ficus benjamina</i>	Guácima, Alajuela <i>Mentha spicata</i>	Curridabat, San José <i>Aloe vera</i>	Key West, Florida <i>Cocos nucifera</i>	Key West, Florida <i>Cocos nucifera</i>	
Host	12 ♀♀	10 ♀♀	11 ♀♀	11 ♀♀	10 ♀♀	4 ♀♀	10 ♀♀	
n								
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)	596 ± 4.2 (592-603)	560 (510-590)	
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)	79.9 ± 2.1 (77.5-82.5)	79 (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)	6.9 ± 0.7 (6.3-7.5)	-	
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)	3.6 ± 0.1 (3.5-3.8)	-	
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 end 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)	142 ± 2.5 (140-145)	-	
Pharynx (ph) 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)	-	-	
Midium body diam. (MBD)	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)	32.2 ± 0.6 (31.3-32.5)	-	
Anus body diam. (ABD)	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)	21.8 ± 3.4 (17.3-28.6)	19.7 ± 0.6 (18.8-20.0)	-	
Vulval body diam. (VBD)	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 ± 3.2 (13.3-24.4)	25 ± 3 (25.0-26.3)	-	
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)	136 ± 4 (132-140)	138 (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)	20 ± 1.4 (19-22)	-	
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)	35 ± 1.7 (34-37)	38	

Table 3 (Cont'd). Comparative morphometric characters of *Hemicriconemoides strictathecatius* females from this study and those in the original description (Esser, 1960)\* and topotype specimens (Van den Berg et al., 2015).

Location	<i>Hemicriconemoides strictathecatius</i>									
	This study					Van den Berg et al., 2015				
Host	Carmen, San José <i>Schefflera arboricola</i> and <i>Cosmos bipinnatus</i>	Bijagua, Alajuela <i>Theobroma cacao</i>	San Pedro, San José <i>Ficus benjamina</i>	Guácima, Alajuela <i>Mentha spicata</i>	Curridabat, San José <i>Aloe vera</i>	Key West, Florida <i>Cocos nucifera</i>	Key West, Florida <i>Cocos nucifera</i>	Key West, Florida <i>Cocos nucifera</i>		
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)	12 ± 1.0 (12-14)	12 ± 1.0 (12-14)	12 ± 1.0 (12-14)	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)	3.3 ± 0.5 (3.0-4.0)	3.3 ± 0.5 (3.0-4.0)	3.3 ± 0.5 (3.0-4.0)	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 ± 0.5 (9-10)	9 ± 0.5 (9-10)	9 ± 0.5 (9-10)	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)	18.5 ± 0.3 (18.3-19.0)	18.5 ± 0.3 (18.3-19.0)	18.5 ± 0.3 (18.3-19.0)	17.8 (15.6-19.0)	
b	4.6 ± 0.5 (3.8-5.5)	4.9 ± 0.4 (4.3-5.5)	4.4 ± 0.4 (3.6-5.1)	4.9 ± 0.3 (4.5-5.6)	4.6 ± 0.6 (3.5-5.5)	-	-	4.4 (4.1-4.7)	4.4 (4.1-4.7)	
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)	20.8 ± 1.1 (18.9-22.5)	18.6 ± 0.9 (17.9-19.8)	18.6 ± 0.9 (17.9-19.8)	18.6 ± 0.9 (17.9-19.8)	17.5 (16.0-19.3)	
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)	1.6 ± 0.04 (1.6-1.7)	1.6 ± 0.04 (1.6-1.7)	1.6 ± 0.04 (1.6-1.7)	-	
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)	1.9 ± 0.1 (1.8-2.0)	1.9 ± 0.1 (1.8-2.0)	1.9 ± 0.1 (1.8-2.0)	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%	27.4 ± 5.3 (18.9-34.6)	37.6 ± 15. (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)	35.9 ± 6 (31.0-42.0)	35.9 ± 6 (31.0-42.0)	35.9 ± 6 (31.0-42.0)	-	
Tail length (T)	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)	32.2 ± 1.6 (30.3-33.7)	32.2 ± 1.6 (30.3-33.7)	32.2 ± 1.6 (30.3-33.7)	-	
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)	13.4 ± 0.4 (13.0-14.0)	13.4 ± 0.4 (13.0-14.0)	13.4 ± 0.4 (13.0-14.0)	-	
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)	11.5 ± 1.8 (10.0-13.8)	11.5 ± 1.8 (10.0-13.8)	11.5 ± 1.8 (10.0-13.8)	-	
Vulva to tail end	47.4 ± 3.9 (41.7-53.5)	49.6 ± 4.5 (41.5-58.8)	41.8 ± 1.4 (39.9-44.6)	50.1 ± 7.8 (32.1-58.4)	42.7 ± 1.7 (40.7-46.7)	45.0 ± 2.0 (42.5-47.5)	45.0 ± 2.0 (42.5-47.5)	45.0 ± 2.0 (42.5-47.5)	-	
Anterior end to 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)	548 ± 4.3 (543-553)	548 ± 4.3 (543-553)	548 ± 4.3 (543-553)	-	
V%	92.5 ± 1.7 (90.6-97.3)	94.0 ± 1.9 (91.0-97.0)	92.0 ± 3.6 (81.7-95.3)	90.3 ± 2.1 (85.4-92.5)	91.1 ± 5.0 (77.6-94.9)	91.9 ± 0.4 (91.6-92.4)	91.9 ± 0.4 (91.6-92.4)	91.9 ± 0.4 (91.6-92.4)	92.7 (91.3-93.2)	
Spermatheca length	21.2 ± 4.0 (15.6-29.9)	21.0 ± 1.6 (17.7-23.2)	21.2 ± 2.6 (10.9-25.5)	21.0 ± 4.3 (13.9-27.1)	21.6 ± 1.6 (19.0-23.9)	-	-	-	-	
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)	50.2 ± 1.5 (47.9-52.3)	-	-	-	-	

Measurements in µm and in the form: mean ± s.d. (range). Abbreviations: n (number of specimens measured), L (body length), DGO (distance from base of stylet to dorsal esophageal gland), St (stylet length), R (number of body annulus), Rst (number of annulus from anterior end to stylet base), Rph (number of annulus from anterior end to pharynx base), Rex (number of annulus from anterior end to excretory pore), RV (number of annulus from posterior end to vulva), RVan (number of annulus from vulva to anus), Ran (number of annulus from posterior end to anus), a (L/MBD), b (L/ph), c (L/T), c' (T/ABD), VL/VBD (distance from vulva to posterior end / vulval body diameter), T/ABD (Tail length / anal body diam.), VA/T% ((distance from anus to vulva / total tail length) × 100), St/L ((stylet length / body length) × 100), and V% ((distance from anterior end to vulva / body length) × 100).

Table 4. Comparative morphometric characters of *Hemicriconemoides rosae* females from this study and those in the original description (Rathour et al., 2003)\*.

<i>Hemicriconemoides rosae</i>		
Origin	Parrita, Puntarenas	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)	-

*Molecular analysis*

Phylogenetic trees for all analyzed regions (Figs. 3, 4, and 5) were generated by 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 28S D2D3 and ITS1 regions, and GTR+I+G for the COI region. 28S D2-D3 analysis included 41 sequences of *Hemicriconemoides* while ITS and COI alignments

included 43 and 18 sequences, respectively.

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 which were obtained from populations collected in Orlando, Palm Beach, and Gainesville, Florida, from *Zoysia* grass (*Zoysia japonica*) and ornamental turfgrass (Van den Berg et al., 2015). Additionally, they showed phylogenetic proximity and grouped with *H. phoenicis* (Van den Berg et al., 2015).

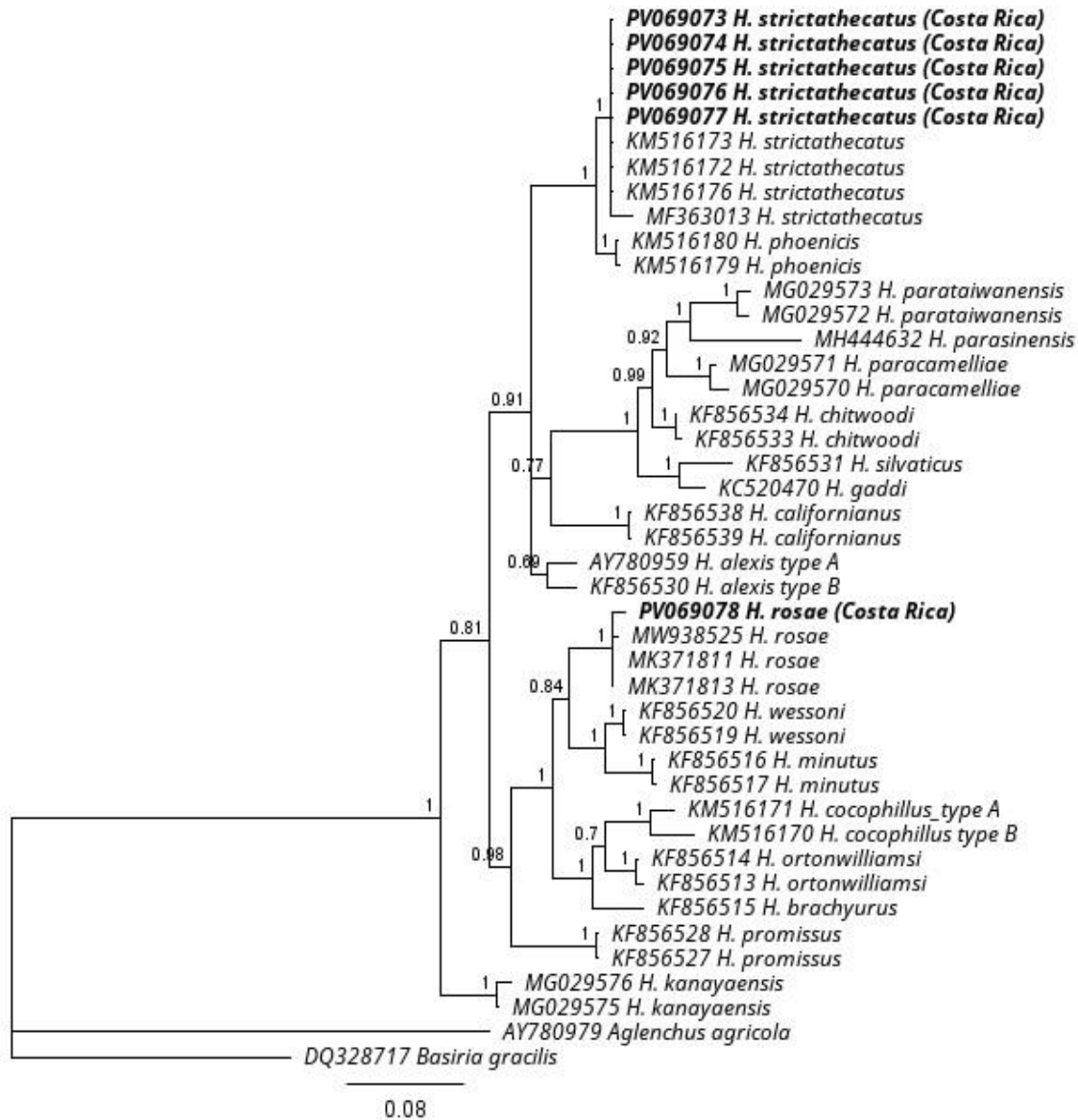


Figure 3. Phylogenetic tree constructed by Bayesian inference (BI) under the GTR+G model from partial sequences of the 28S D2-D3 domain. Relevant clades presented a probability greater than 70%. Sequences from this study are highlighted in bold.

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 and China obtained from *Rosa indica* in

Lucknow, Uttar, Pradesh, India (Khan et al., 2019) and from *Ruellia simplex* in Foshan, Guangdong, China (Zeng et al., 2022). These sequences showed a close relationship with *H. wessoni* and *H. minutus* (Van den Berg et al., 2014).

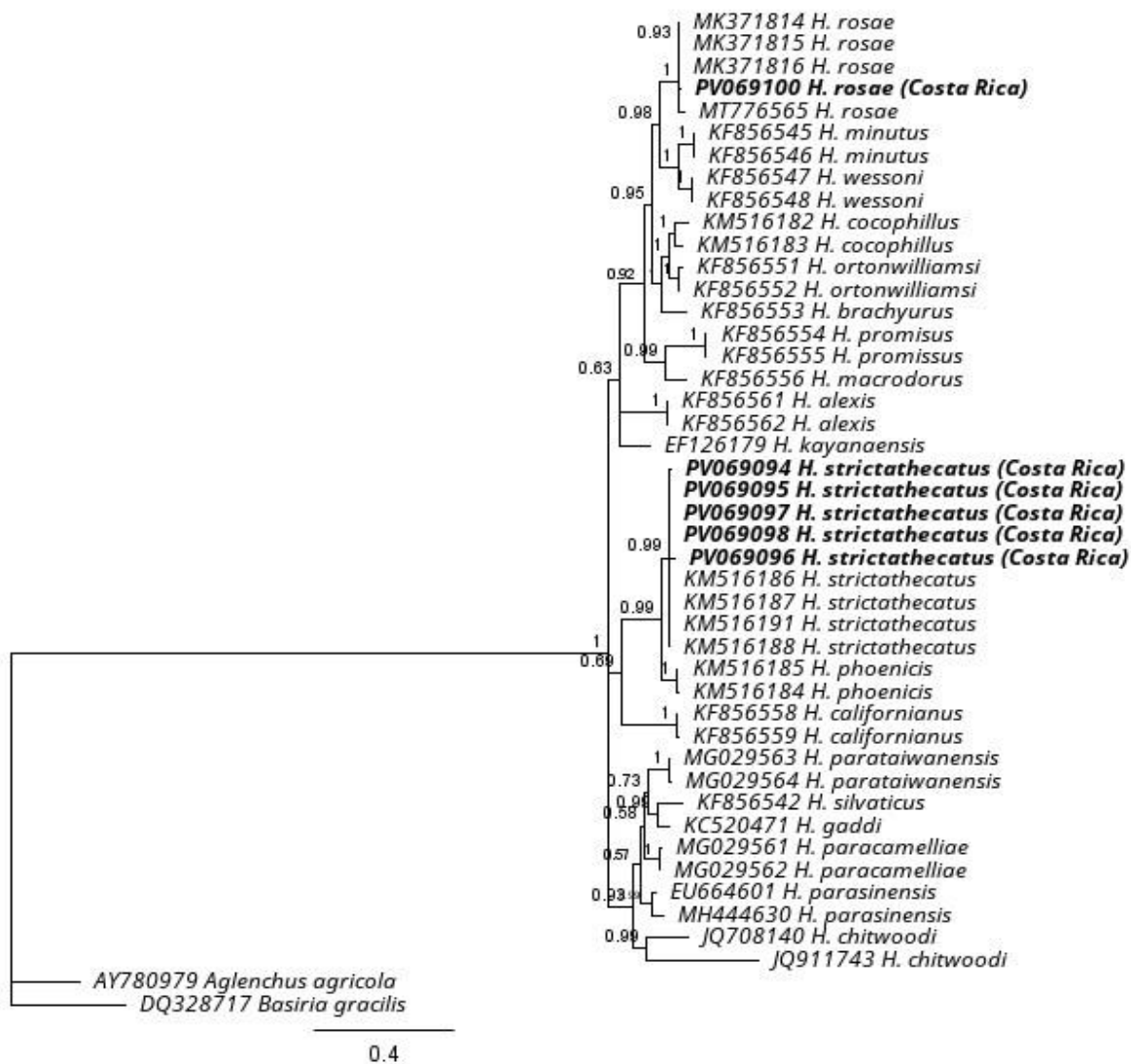


Figure 4. Phylogenetic tree constructed by Bayesian inference (BI) under the GTR+G model, from partial sequences of the ITS1 region (with primers rDNA2/rDNA1.58s). Relevant clades presented a probability greater than 70%. Sequences from this study are highlighted in bold.



## DISCUSSION

The results obtained in this study indicate that *Hemicriconemoides* has a limited distribution in Costa Rica, as it was detected in few soil samples and in low population densities. This genus is characteristic of warm climate regions and is commonly found in the rhizosphere of ornamental, medicinal, aromatic plants, and other hosts (López & Salazar, 1987; Ashokkumar & Vadivelu, 1991; Rathour et al., 2003; Chen & Liu, 2011; Gandarilla & Rivas, 2012; Van den Berg et al., 2014; Heydari et al., 2016; Munawar et al., 2018; Khan et al., 2019).

*Hemicriconemoides 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 (Phani et al., 2021). 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 Shefflera and Cosmos) and Alajuela (cocoa), females of *H. strictathecatus* showed predominantly conical and pointed tails, while in populations associated with mint from Alajuela, fig and Aloe from San José, females showed rounded conical tails (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 vs. 127–152), Rex (32–45 vs. 38), Rv (11–15 vs. 11–14), Ran (6–12 vs. 9–11), RVan (3–5 vs. 3), and VL/VB (1.4–2.6 vs. 1.5–2.0).

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. (2015) from Florida, USA. Furthermore, phylogenetic analysis showed a branching pattern that aligned with results from previous studies (Munawar et al., 2018; Van den Berg et al., 2014). *Hemicriconemoides 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.

*Hemicriconemoides 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 Chaubey, 2023), all localities belonging to Uttar Pradesh, India.

Mitochondrial COI region of *H. rosae* was not evaluated due to the lack of amplification with universal primers COI-F5/COI-R9. This amplification failure is likely attributable to low conservation and high variability in the primer binding sites within the mitochondrial COI gene of this species. Such sequence divergence hinders efficient annealing of universal primers, limiting their applicability across certain taxa. Therefore, designing species-specific primers for *H. rosae* is essential to enable reliable amplification and analysis of this genetic region, which is crucial for phylogenetic and genetic diversity studies.

Morphological and morphometric results showed overall similarity with *Hemicriconemoides rosae*, as originally described by Rathour et al. (2003) and later by Khan et al. (2019). However, some differences were observed between the studied population and the original description by Rathour et al. (2003), particularly in the following measurements: average body length (422–509.8  $\mu\text{m}$  vs. 470–510  $\mu\text{m}$ ), 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 ratio (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). Morphologically, these species share several general traits, including relatively short body length, moderately developed stylets, and comparable annuli counts. However, *H. rosae* can be differentiated from *H. wessoni* and *H. minutus* by subtle but consistent features such as lip region dimensions, stylet knob morphology, and tail annulation patterns. These distinctions support its separation as a valid species despite phylogenetic proximity.

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.

Integration of morphological and molecular tools, such as the amplification and sequencing of key genes (28S, ITS1, and COI), allows more precise species identification and population delimitation. This combination facilitates distinguishing morphologically similar species and detecting genetic variability among populations, which was fundamental for correctly identifying the two species and the six populations analyzed in the present study. This study provides relevant information on the identification and distribution of two nematode species of the genus *Hemicriconemoides* in Costa Rica, representing a significant advancement in the specific knowledge of these taxa. Furthermore, it lays the groundwork for future research on the distribution and impact of these nematodes on various crops.

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