Scientific Note

First report of *Quinisulcius capitatus* (Allen, 1955) Siddiqi, 1971 (Telotylenchidae) in Costa Rica: morphological and molecular characterization[1]

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ABSTRACT

During a survey of plant-parasitic nematodes in Costa Rica, *Quinisulcius capitatus* was isolated and identified for the first time in three locations: Barva and Santa Bárbara in the Heredia province, and Vázquez de Coronado in the San José province. The nematodes were extracted from soil samples by the sugar solution centrifugation-flotation method, and the specimens described and characterized based on integrative taxonomy. The adult nematodes of *Q. capitatus* are in accordance with the type population, but displayed slight morphometric variations in relation to those originally described: body length of 649.5-875.7 *vs*. 630-850, style length of 13.7-17 *vs*. 16-18 and percentage of vulva of 52.7-59.9 *vs*. 51-58. The D2-D3 expansion segments of the 28S rDNA from the recovered populations were sequenced and used in the phylogenetic analysis. The Costa Rican populations showed a high similarity and formed a separate clade with the Pakistani and Ethiopian populations deposited in the GenBank® database. This study expands the species geographic distribution and provides the first morphometric and molecular characterization of *Q. capitatus* from Costa Rica.

KEYWORDS: Plant-parasitic nematode, stunt nematode, integrative taxonomy.

RESUMO

Primeiro relato de *Quinisulcius capitatus* (Allen, 1955) Siddiqi, 1971 (Telotylenchidae) na Costa Rica: caracterização morfológica e molecular

Durante uma pesquisa de nematoides parasitas de plantas na Costa Rica, *Quinisulcius capitatus* foi isolado e identificado pela primeira vez em três locais: Barva e Santa Bárbara na província de Heredia, e Vázquez de Coronado na província de San José. Os nematoides foram extraídos de amostras de solo pelo método de centrifugação-flotação em solução de açúcar, e os espécimes descritos e caracterizados com base na taxonomia integrativa. Os nematoides adultos de *Q. capitatus* estão de acordo com a população tipo, mas apresentaram leves variações morfométricas com relação àqueles originalmente descritos: comprimento do corpo de 649,5-875,7 *vs*. 630-850, comprimento do estilete de 13,7-17 *vs*. 16-18 e porcentagem de vulva de 52,7-59,9 *vs*. 51-58. Os segmentos de expansão D2-D3 do rDNA 28S das populações recuperadas foram sequenciados e usados na análise filogenética. As populações da Costa Rica mostraram alta similaridade e formaram um clado separado com as populações do Paquistão e da Etiópia depositadas no banco de dados GenBank®. Este estudo amplia a distribuição geográfica da espécie e fornece a primeira caracterização morfométrica e molecular de *Q. capitatus* da Costa Rica.

PALAVRAS-CHAVE: Nematoide parasita de plantas, nematoide de atrofia, taxonomia integrativa.

The *Quinisulcius* genus includes more than 17 species (Geraert 2011) commonly known as stunt nematodes. *Quinisulcius capitatus* was originally described in the USA (Allen 1955) and is widely distributed throughout the Americas, Europe and Africa (Doucet 1986, Manuwar et al. 2021). It is a polyphagous ectoparasite of plant roots with a wide host range that includes horticultural plants, grasses and forest trees (Greco et al. 1992).

This species is widely distributed and found in tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), cabbage (*Brassica oleracea* var. *capitata*) and potato (*Solanum tuberosum* L.) crops in many countries worldwide (Bafokuzara 1996, Baimey et al. 2009, Geraert 2011, Hussain et al. 2019). Plant damage caused by this nematode is sometimes difficult to detect. Therefore, assessing its impact on crop yield is a challenge. Furthermore, by feeding directly on the plant root system, the nematode enhances the entry of other plant pathogens (Singh et al. 2013, Kefelegn et al. 2023).

In Costa Rica, there are no previous studies on the occurrence of stunt nematodes, although there are recent records from the Universidad Nacional de

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Costa Rica (data not published) that identifies this nematode at the genus level.

In the present study, three stunt nematode populations of the *Q. capitatus* species were isolated and studied morphologically and molecularly. Since this species is plant-parasitic and occurs in other countries (Smiley et al. 2006, Thompson et al. 2008), the present study aimed to provide a detailed molecular and morphometric characterization of the species and conduct a phylogenetic analysis of this and other stunt nematodes from the Telotylenchidae family.

Soil samples were collected from the rhizosphere of agronomic crops during 2022 and 2023. Each soil sample consisted of approximately 1 kg of soil, from 15-20 subsamples, which were collected systematically from the top 5-30 cm soil layer. The samples were transported in thermally insulated containers to the laboratory for processing.

The nematodes were extracted from the soil samples by the sugar solution centrifugation and flotation method (Jenkins 1964). Following extraction, they were killed and fixed in 4 % formaldehyde at 70 ºC. Then, they were gradually transferred to glycerin using a modified version of the Seinhorst's slow method (Seinhorst 1959) and mounted on slides for observation and preservation. All specimens from this study were deposited in the nematode collection at the Laboratorio de Nematología of the Universidad Nacional de Costa Rica. The sampling sites and host plants of the isolated nematode populations are detailed in Table 1.

Fixed female nematodes were measured and photographed using a Nikon Eclipse 80*i* microscope connected to a Nikon DS-Fi1 DS camera. Morphometric measurements and ratios typically used for morphological identification of *Quinisulcius* spp. were recorded. To establish correspondence between morphological/morphometric measurements and molecular data, those measured nematodes were used for genomic DNA extraction.

For molecular phylogenetic studies, genomic DNA was extracted from individual nematodes following the protocol outlined by Solano et al. (2013). The D2-D3 region of the 28S rDNA was amplified with primers D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) (De Ley et al. 1999). The PCR amplification conditions were adapted based on Cordero et al. (2012). The PCR reaction mix was adjusted to 25 µL, with 5.5 µL of nuclease-free water, 12.5 µL of 2X DreamTaq™ PCR Master Mix (Thermo Scientific™), 1 µL of each primer (10 µM) and 5 µL of DNA solution. Temperature cycling conditions were as follows: an initial cycle of 94 ºC for 2 min, followed by 45 cycles of 94 ºC for 45 sec, 55 ºC for 45 sec (annealing), 72 ºC for 1 min and a final cycle of 72 ºC for 5 min. The PCR products were subjected to electrophoresis (100 V/1 hour) on 1 % (w/v) TopVison agarose gels (Thermo Scientific™) prepared with 1X TBE and visualized under UV light using Gene Ruler (Thermo Scientific™) 100bp Plus DNA ladder to estimate amplicon sizes.

The PCR products were sent to Macrogen, Inc. (South Korea) for purification and bi-directional Sanger sequencing. Subsequently, the obtained sequences were manually edited and assembled using BioEdit v.7.2.5 (Hall 1999) and compared with existing sequences in the GenBank® database through the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information (NCBI). All sequences newly obtained from this study were deposited in the GenBank® database with their corresponding accession numbers (PP194270, PP194271 and PP194272).

The phylogenetic reconstruction was performed using partial 28S D2-D3 expansion domains sequences obtained in this study and from other stunt nematodes from the GenBank® database. Multiple sequence alignment was performed with MAFFT v.7.490 (Katoh et al. 2019). jModelTest v.2.1.10 was used to select the best-fit nucleotide substitution model based on Akaike Information Criterion (AIC) (Darriba et al. 2012). Bayesian inference (BI) was

Table 1. Sampling sites and host information from which the *Quinisulcius capitatus* populations were covered in this study in Costa Rica.

Location	Host	Latitude	Longitude	Altitude (m)
Barva, Heredia	Tomato (*Solanum lycopersicum* L.)	10º01'23"N	84º06'41"W	1,250
Santa Bárbara, Heredia	Tomato (*Solanum lycopersicum* L.)	10º02'00"N	84º09'46"W	1,106
Vázquez de Coronado, San José	Bean (*Phaseolus vulgaris* L.)	09º59'03"N	83º59'54"W	1,431

performed under the transition model with invariable sites and a gamma-shaped distribution (GTR + I + G) using Mr. Bayes v.3.2.6 (mcmc ngen = 1100000, subsamplefreq = 200) (Huelsenbeck & Ronquist 2001) in Geneious Prime® 2024.0.5. The outgroup was chosen based on previous publications.

No males were found, and females presented a straight to slightly ventrally arcuate body after heat fixation (Figure 1), with maximum body diameter between 22.1 and 28.4 µm; cuticle marked with fine transverse striation 1-1.2 µm apart; lateral fields originated below the cephalic region and expanded to form five lines that extended to the end of the tail; labial region with 2.9-5.6 µm high and 7.1-9.4 µm wide, with framework slightly sclerotized; small stylet with 13.7-17 µm long and conus of 8.8-9.0 µm with three round knobs with posteriorly inclined margins; dorsal gland orifice of 2.1-3.6 µm from knobs; elongated and cylindrical procorpus, gradually expanding into the postcorpus or the median pharyngeal bulb, which had an oval shape and featured a prominent valvular apparatus at its center; isthmus elongated and surrounded by the nerve ring, located at 80-100 µm from the anterior end of the body; excretory pore positioned from the anterior margin to the middle of the basal pharyngeal bulb, approximately 95-115 µm from the anterior extremity; small pharyngeal-intestinal valve; secretory-excretory pore at 97.8-133.2 µm from the anterior end; tail of 42.4-60.1 µm long, with a distinctly annulated and cylindrical conoid terminus; didelphic-amphidelphic reproductive system, with branches outstretched in opposite directions; phasmids anterior to middle of the tail; body diameter at the vulva of 21.5- 27.1 µm and at the anal region of 14.6-19.4 µm (Table 2).

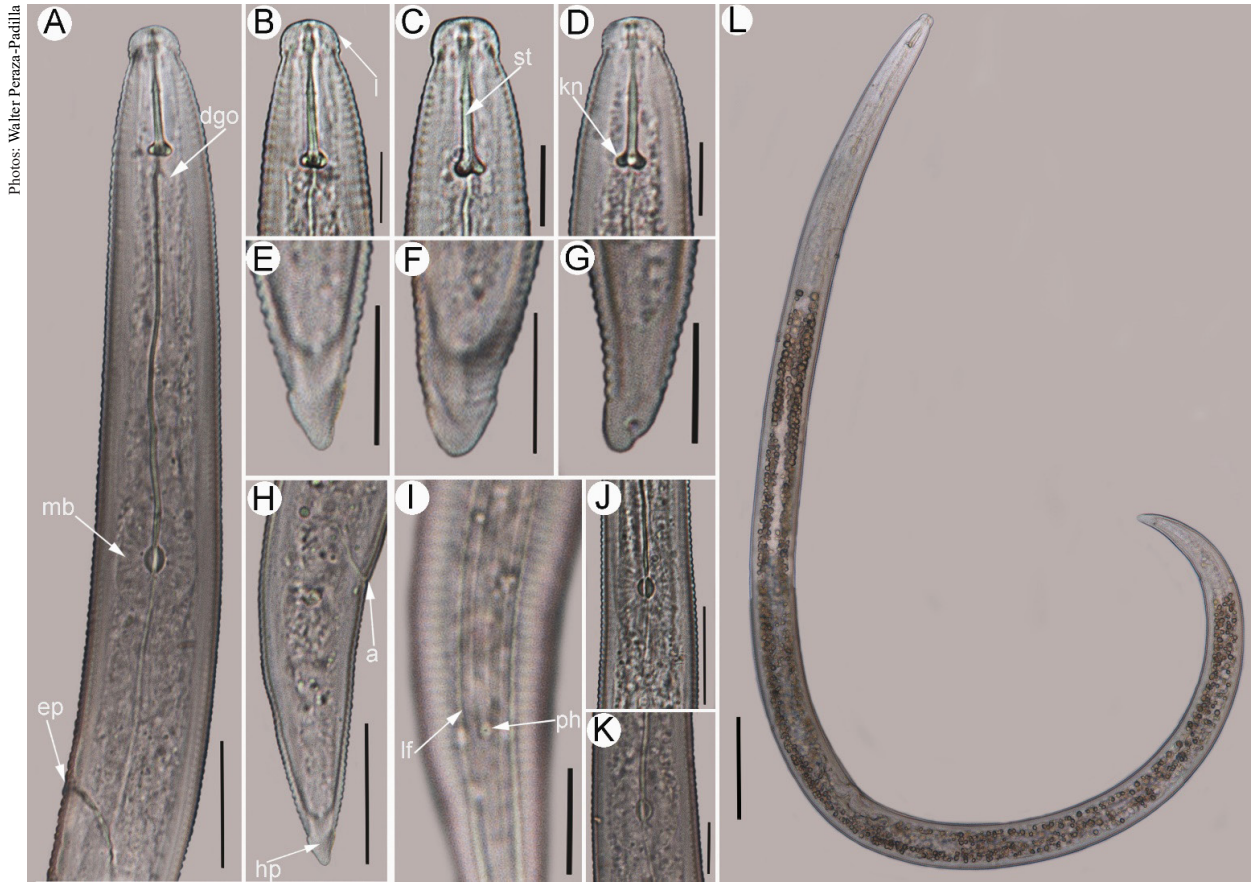

Figure 1. Photomicrographs of the *Quinisulcius capitatus* females: A) anterior region showing the dorsal gland orifice, median bulb and excretory pore position; B-D) anterior region showing the lip (B), stylet (C) and stylet knobs (D); E, F, G) posterior region showing the tail shape; H) posterior region with the anus position and hyaline portion; I) lateral fields and phasmid; J, K) median bulb; L) entire body. dgo: dorsal gland orifice; mb: median bulb; ep: excretory pore; l: lip; st: stylet; kn: knobs; a: anus; hp: hyaline portion; lf: lateral fields; ph: phasmid. Scales: A-J: 20 µm; K: 10 µm; L: 40 µm.

Table 2. Comparative morphometric characters of *Quinisulcius capitatus* females from the present and other studies.

Present study			
Origin	Santa Bárbara, Heredia	Barva, Heredia	Vázquez de Coronado, San José
Host	Tomato	Tomato	Bean
n	11 ♀♀	11 ♀♀	11 ♀♀
L	722.0 ± 54.7 (664.6-818.5)	768.6 ± 55.8 (649.5-875.7)	733.2 ± 55.6 (661.7-808.5)
a	28.9 ± 1.4 (26.5-31.3)	30.5 ± 1.6 (27.6-33.0)	28.8 ± 0.9 (27.7-30.1)
b	5.1 ± 0.3 (4.8-5.7)	5.0 ± 0.2 (4.6-5.3)	4.9 ± 0.2 (4.7-5.4)
c	15.0 ± 1.0 (13.9-16.7)	14.6 ± 1.3 (13.2-17.3)	15.5 ± 0.6 (14.6-16.6)
c’	2.8 ± 0.1 (2.6-2.9)	3.0 ± 0.3 (2.4-3.3)	2.8 ± 0.1 (2.5-3.0)
V%	56.4 ± 0.8 (55.0-57.6)	56.0 ± 1.7 (52.7-58.6)	56.4 ± 1.5 (54.6-59.9)
Lip height	4.7 ± 0.5 (3.9-5.6)	4.4 ± 0.3 (3.8-5.1)	3.4 ± 0.3 (2.9-4.1)
Lip width	8.4 ± 0.5 (7.3-9.3)	8.2 ± 0.5 (7.1-8.8)	8.5 ± 0.4 (8.0-9.4)
Stylet length	15.8 ± 0.8 (14.8-17.0)	15.8 ± 0.9 (14.2-16.9)	15.0 ± 0.8 (13.7-16.2)
DGO	3.0 ± 0.3 (2.6-3.6)	2.8 ± 0.3 (2.2-3.3)	2.9 ± 0.4 (2.1-3.5)
MB length	15.9 ± 1.2 (14.4-18.1)	16.7 ± 1.1 (15.0-18.5)	16.3 ± 1.0 (14.5-18.3)
MB width	12.7 ± 1.0 (11.3-14.1)	12.8 ± 0.9 (11.0-13.9)	12.2 ± 0.9 (11.4-14.2)
Excretory pore	116.4 ± 10.7 (97.8-131.1)	122.8 ± 6.9 (107.9-133.2)	120.6 ± 5.7 (112.6-129.9)
Pharynx	143.3 ± 15.3 (120.2-169.4)	155.0 ± 10.1 (134.3-165.7)	149.2 ± 7.2 (141.1-163.0)
MBD	25.0 ± 1.9 (22.1-27.4)	25.2 ± 1.3 (23.5-27.8)	25.4 ± 2.0 (22.5-28.4)
VBD	24.2 ± 1.8 (21.5-26.8)	25.1 ± 1.4 (22.4-27.1)	23.9 ± 1.3 (21.7-25.7)
ABD	17.1 ± 1.1 (15.9-19.4)	17.4 ± 0.8 (16.4-18.8)	16.9 ± 1.2 (14.6-18.9)
Tail	48.3 ± 3.0 (45.7-56.5)	53.1 ± 6.0 (42.4-60.1)	47.3 ± 2.9 (42.9-51.3)
Phasmid position	Anterior to middle of tail	Anterior to middle of tail	Anterior to middle of tail

Other studies				
Origin	Allen 1955** (USA)	Iqbal et al. 2021 (Pakistan)	Kefelegn et al. 2023 (Ethiopia)	Munawar et al. 2021 (Canada)
Host	Pear	Several crops	Chickpea	Grass
n	13 ♀♀	50 ♀♀	10 ♀♀	20 ♀♀
L	630-850	605-856	699 ± 11.6 (667-707)	810.3 ± 44.6 (744-911)
a	30-38	28.3-37.7	31.2 ± 1.3 (29.7-34.4)	41.4 ± 1.8 (38.6-43.7)
b	5.0-5.8	4.5-6.9	5.4 ± 0.2 (5.1-5.7)	5.5 ± 0.3 (5.0-6.3)
c	12-17	13.4-19.0	14.7 ± 0.7 (14.1-16.3)	22.4 ± 1.1 (19.9-23.8)
c’	-	2.4-3.5	1.9 ± 0.2 (1.5-2.1)	2.6 ± 0.2 (2.2-3.2)
V%	51-58	53.3-59.6	56.6 ± 2.2 (52.7-59.0)	57.4 ± 1.5 (53.4-59.8)
Lip height	-		4.0 ± 0.5 (3.2-4.5)	4.0 ± 0.2 (3.7-4.4)
Lip width	-		7.6 ± 0.5 (7.0-8.3)	7.6 ± 0.4 (6.9-8.3)
Stylet length	16-18	13-19	18.8 ± 0.6 (17.8-19.7)	18.3 ± 1.0 (15.5-20.4)
DGO	-		-	-
MB length	-		16.1 ± 0.3 (15.7-16.6)	14.0 ± 1.6 (11.3-16.9)
MB width	-		13.1 ± 0.6 (12.2-13.9)	10.4 ± 1.4 (8.4-14.2)
Excretory pore	-	96-144	121 ± 3.3 (113-123)	128.6 ± 5.3 (121-139)
Pharynx	-	114-174	129 ± 5.1 (123-138)	147.8 ± 5.8 (140.2-159.0)
MBD	-	20-26	22.4 ± 1.0 (20.5-23.5)	19.0 ± 1.5 (16.9-21.3)
VBD	-		-	18.4 ± 1.3 (15.7-20.8)
ABD	-	14-19	25.3 ± 3.2 (23.0-34.2)	13.8 ± 1.1 (11.2-15.2)
Tail	-	40-56	47.8 ± 3.0 (41.0-50.0)	35.8 ± 2.4 (31.3-40.4)
Phasmid position	Middle of tail	Anterior to middle of tail	Middle of tail	Middle of tail

All measurements are in µm and in the form mean ± standard deviation (range). ** Original description. L: total body length; a: L/maximum body diameter; b: L/MB (median bulb); b’: L/pharyngeal length; c: L/anal body diameter; c’: anal body diameter/tail length; V%: vulva percentage (distance from anterior end/L x 100); stylet length: from the base of the knobs to the anterior part of the stylet; DGO (dorsal gland orifice): length from the base of the knobs to the opening of the dorsal gland; MB length: length of the median bulb; MB width: width of the median bulb; MBD (maximum body diameter): maximum width of the body; VBD (vulva body diameter): width of the body at the level of the vulva; ABD (anal body diameter): width of the body at the level of the anus.

Adult *Q. capitatus* nematodes from the populations examined in the present study were slightly longer and wider than those described in the original report and in other reports. The nematodes had a longer stylet and tail and maintained morphological characteristics such as lip and tail morphology, consistent indistinct hemizonid, and weakly developed spermatheca and vulva appearance, as those described in the study of Kefelegn et al. (2023). The morphometric characteristics of individuals identified in the present study did not exhibit significant differences from North American populations and other geographic regions (Allen 1955, Hooper 1959, Loof 1959, Knobloch & Laughlin 1973, Saltukoglu & Coomans 1975).

Quinisulcius capitatus males were not found in any of the samples examined in this study. Although males of the species were described by Allen (1955), no males were found or reported in later studies: Hooper (1959), Knobloch & Laughlin (1973), Maqbool (1982), Mekete et al. (2008) and Siddiqi (1961). This coincides with Costa Rican populations, where, to date, no males have been found or identified. However, Iqbal et al. (2021) reported the presence of males in *Q. capitatus* Pakistani populations. It is important to note that there is no molecular evidence confirming that these males were indeed *Q. capitatus*. Since Iqbal et al. (2021) did not conduct molecular characterization exclusively on males, it is not possible to definitively ascertain that the males belonged to the same species as the females. This highlights the need for further molecular studies to confirm the identity of male nematodes within these populations.

Quinisulcius capitatus populations were found in soil samples from tomato (*Solanum lycopersicum* L.) rhizosphere from Barva and Santa Bárbara at the Heredia province, and bean (*Phaseolus vulgaris* L.) rhizosphere from Vázquez de Coronado at the San José province. Although there are no previous official records of stunt nematodes in Costa Rica, the data collected by the Laboratorio de Nematología at the Universidad Nacional de Costa Rica indicates the presence of some *Tylenchorhynchus* sp. populations in pitahaya, grasses and other crops, indicating that we could be dealing with other species of the Telotylenchidae family to which the *Q. capitatus* described in this study belongs.

We found a low number of *Q. capitatus* individuals, less than 25 per 100 g of soil, in samples from crops cultivated in Costa Rica. The low population density in these areas could be attributed to soil disturbance, as sampled sites are subject to intensive tillage, crop rotation and other land use practices for vegetable cultivation.

The results of the present study agree with those of Knight et al. (1997) and Iqbal et al. (2021), who also reported *Q. capitatus* associated with tomato crops. Similarly, other authors mentioned the presence of this species in a wide range of solanaceous plants, such as potato (Krishna Prasad & Sharma 1985, Hafez et al. 2010, Marais et al. 2015, Munawar et al. 2021) and tobacco (Mountain & McKeen 1962, Uludamar et al. 2018), and in legume crops, such as soybean (Mbatyoti et al. 2020) and chickpea (Kefelegn et al. 2023).

In the American continent, this species has also been reported in potato (Hafez et al. 2010, Munawar et al. 2021), rye and tobacco (Mountain & McKeen 1962), corn and sunflower (Doucet 1987, Wyse-Pester et al. 2002), sugarcane (Perichi et al. 2002), grass, wild poppy, barrel cactus, short thorn and cotton (Knobloch & Laughlin 1973), red clover and Kentucky bluegrass (Malek 1980) and switchgrass (Cassida et al. 2005). In Asia, *Q. capitatus* has been found in various fruits and crops such as pomegranate (Bekmurodov & Raxmatova 2020), apple (Sattorovich et al. 2021), peanut (Mirghasemi et al. 2014), smooth wild bean (Yan et al. 2019), potato, cherry, walnut, apple, maize, tomato, cabbage, carrot, cucumber and onion (Krishna Prasad & Sharma 1985, Iqbal et al. 2021), grape (Bobokeldieva & Khurramov 2022), grass and lily (Siddiqi 1961) and other cultivated crops (Kheiri et al. 2002). In Africa, host reports of *Q. capitatus* include soybean (Mbatyoti et al. 2020), coffee (Mekete et al. 2008), chickpea (Kefelegn et al. 2023), potato (Marais et al. 2015) and various weeds (Ntidi et al. 2012). In Europe, this species has been found in crops such as corn (Vovlas 1983), apple (Braasch 1978), tomato and tobacco (Uludamar et al. 2018), and grape (Antoniou 1981), whereas, in Oceania, it was found in cucumber, squash and tomato (Knight et al. 1997).

While this nematode demonstrates to have a broad host plant range, in Costa Rica it was only found in two tomato crops and in one bean field. This observation is consistent with Greco et al. (1992), pointing towards its common occurrence in legume crops.

Studies conducted by Malek (1980) revealed that *Q. capitatus* needs soil under low temperatures to thrive and reproduce. Similarly, Maqbool & Hashmi (1986) discovered that this nematode can exhibit a substantial infestation level in greenhouse-grown potato, when sandy clay soil is employed. According to Krishna Prasad & Sharma (1985), the occurrence of *Q. capitatus* and *Helicotylenchus dihystera* (Cobb 1983) Sher 1961 resulted in a reduced potato tuber yield ranging from 14 to 29 %. In the present study, all *Q. capitatus* populations were found in fields where there is intensive land use and permanent crop rotation.

The amplification of the 28S D2-D3 rDNA region yielded fragments of approximately 700 bp. The three newly obtained sequences of *Q. capitatus* from Costa Rica (PP194270-712 pb, PP194271-749 bp and PP194272-676 pb) exhibited high identity values (between 98.4 and 100 %) with sequences of *Q. capitatus* from Pakistan (MT703017-MT703025) (Iqbal et al. 2021) and *Q. capitatus* from Ethiopia (OP626319-OP626321) (Kefelegn et al. 2023) (between 99.3 and 100 %).

In the Bayesian phylogenetic tree presented in Figure 2, Costa Rican accessions grouped inside the major clade with other *Q. capitatus* and the clade received the maximum Bayesian posterior probability support, whereas the Canadian accession MW023387 occupied a position outside this clade, and its identity needs further studies.

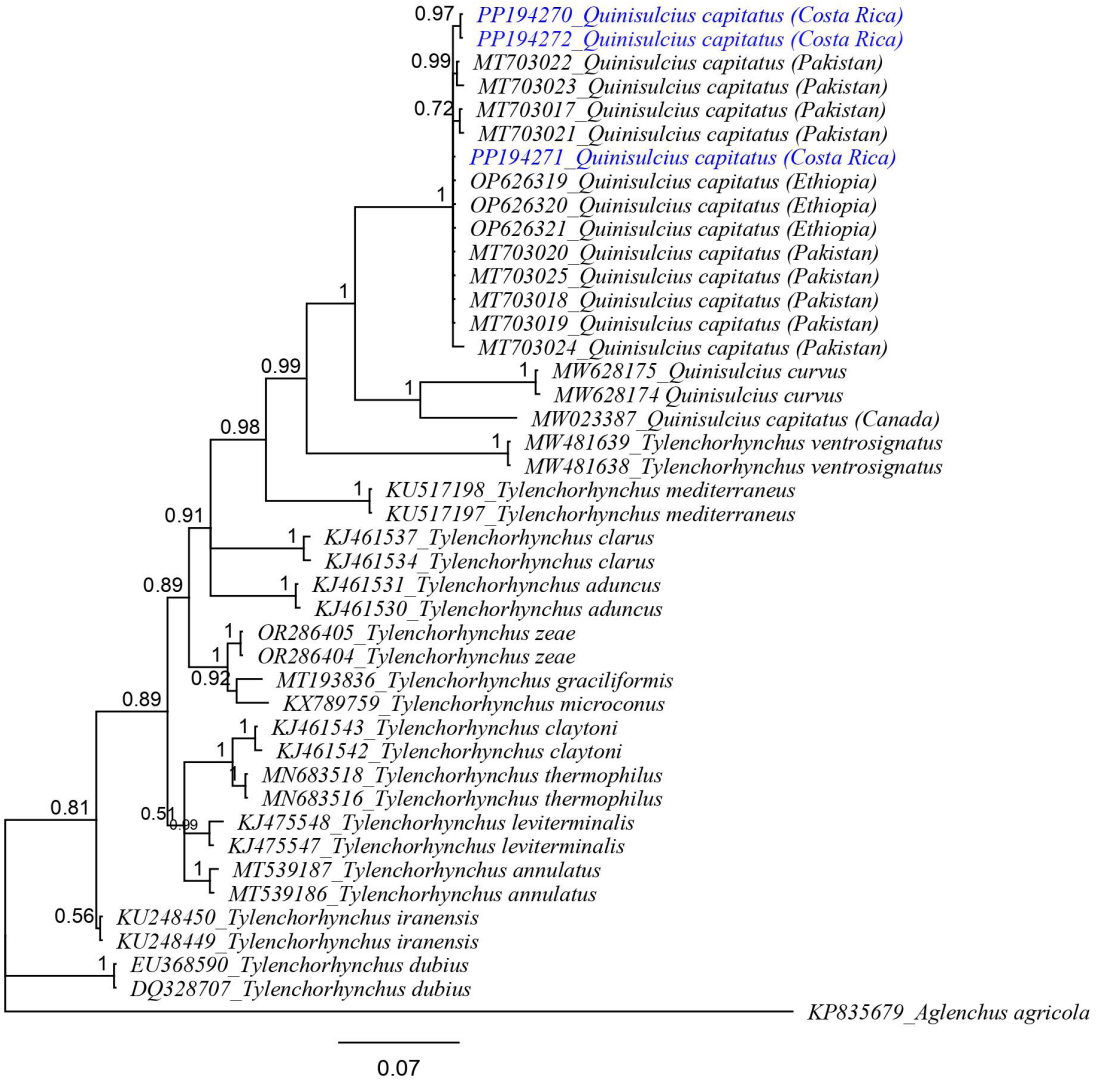

Figure 2. Phylogenetic tree generated by Bayesian inference from partial 28S rDNA D2-D3 expansion segments sequences of *Quinisulcius capitatus* from Costa Rica under the GTR + I + G model.

This study expands the species geographic distribution and provides the first morphometric and molecular characterization of *Q. capitatus* from Costa Rica.

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