



Integrative taxonomy of two known dagger nematodes of the genus *Xiphinema* (Nematoda: Longidoridae) from the Amazon basin in South America, including the first report of *X. brasiliense* Lordello, 1951 from Colombia and Ecuador

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Abstract Three populations of dagger nematodes (*Xiphinema* spp.), one recovered from a cassava field (*Manihot esculenta*) in northern Ecuador and two other from a maize field (*Zea mays*) in southern Colombia, were characterized through an integrative approach using morphological data and molecular phylogenetic analysis based on the D2–D3 expansion segments of the 28S and ITS1 region of the ribosomal RNA (rRNA) and the partial mitochondrial gene cytochrome c oxidase subunit 1 (COI mtDNA)

sequences. Integrating morphological hypotheses with genetic data, the pseudomonodelphic population from Colombia was identified as *X. krugi* and the monodelphic populations from Colombia and Ecuador were identified as *X. brasiliense*. Bayesian inference (BI) trees for these three molecular markers allowed establishing phylogenetic relationships highlighting the importance of using phylogenetic data for accurate dagger nematode identification at the species level. To our knowledge, this is the first report of *X. brasiliense* from Ecuador and Colombia, and the first record of *X. krugi* in a southerly Colombian region, extending the geographical distribution of these species within South America. In this work, morphological information has been updated to distinguish among *Xiphinema* species within morphospecies Group 1 and 2. New molecular markers were generated and new knowledge on the genetic diversity, molecular evolution and the geographic distribution of monodelphic *Xiphinema* species is revealed. This research is one of the first systematic studies for *Xiphinema* detection in Colombia and Ecuador by an integrative taxonomy.

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Introduction

Xiphinema Cobb, 1913, commonly known as dagger nematodes, is the most abundant and diverse genus within the family Longidoridae Thorne, 1935 (Dorylaimida Pearse, 1942; suborder Dorylaimina Pearse, 1936; phylum Nematoda Potts, 1932) (Potts, 1932; Pearse, 1936, 1942; Taylor & Brown, 1997; Gutiérrez-Gutiérrez et al., 2016; Ahmed & Holovachov, 2021).

The non-americanum-group of the genus *Xiphinema* comprises around 238 valid species (Ali et al., 2024; Cid del Prado-Vera et al., 2024). Due to this species richness and large morphological diversity, the *Xiphinema* non-americanum group was divided into eight morphospecies groups based on diagnostic morphological features (Loof & Luc, 1990, 1993; Loof et al., 1996; Brown & Halbrecht, 1997; Peraza-Padilla et al., 2017; Munawar et al., 2018). Within the proposed groups, members belonging to the morphospecies Groups 1 and 2 are characterized by females having a monodelphic reproductive system with only the posterior branch developed and pseudomonodelphic reproductive system with anterior branch incomplete, usually without ovary (monogenic), respectively (Loof & Luc, 1990, 1993; Loof et al., 1996; Brown & Halbrecht, 1997; Coomans et al., 2001; Peraza-Padilla et al., 2017, 2018; Munawar et al., 2018; Varela-Benavides et al., 2018).

Dagger nematodes of the genus *Xiphinema* are a remarkable group of micro-invertebrates of the phylum Nematoda comprising ectoparasitic enopleans of vascular plants including both pteridophytes and spermatophytes (Decraemer & Chaves, 2012; Taylor & Brown, 1997). Their ectoparasitic feeding causes destructive effects on the roots of their host plants (Gutiérrez-Gutiérrez et al., 2011a, 2016; Jones et al., 2013; Taylor & Brown, 1997). However, the main reason for its EU quarantine status on the European and Mediterranean Plant Protection Organization (EPPO) list (OEPP/EPPO, 2022) is due to its ability to retain/transmit economically important nepoviruses (genus Nepovirus, family Comoviridae) (Taylor & Brown, 1997; Trudgill et al., 1983).

Integrative taxonomy is the most efficient approach for the accurate diagnostic and identification over the wide range of families of plant parasitic nematodes that included the former Longidoridae family (Gutiérrez-Gutiérrez et al., 2010, 2011b, 2013, 2018, 2020;

Archidona-Yuste et al., 2016a, b, 2020; Munawar et al., 2018; Pour Ehtesham et al., 2023; Ali et al., 2024; Cid del Prado-Vera et al., 2024). Until recently, routine identification of *Xiphinema* populations was exclusively based on morphology (Coomans et al., 2001; Loof & Luc, 1990; Loof et al., 1996). Nowadays, DNA barcoding based on the sequencing RNA-based markers represents a powerful approach for species-level taxonomic identification and phylogenetic construction within the genus *Xiphinema* (He et al., 2005; Archidona-Yuste et al., 2016a, b, 2020; Peraza-Padilla et al., 2017, 2018; Munawar et al., 2018; Varela-Benavides et al., 2018; Cai et al., 2018, 2019; Mobasser et al., 2019; Ali et al., 2024; Cid del Prado-Vera et al., 2024). Likewise, the use of molecular tools has uncovered cryptic species within this genus (Archidona-Yuste et al., 2016b; Gutiérrez-Gutiérrez et al., 2010; Oliveira et al., 2006).

Biogeography and phylogeny based on selected morphological characters of the genus *Xiphinema* was analysed in great detail by Coomans et al. (2001). According to these biogeographic criteria, South America and the Caribbean islands are the less diverse continental region, followed in ascending order by Oceania, China, South Africa, North America, India and Europe (Coomans et al., 2001; Decraemer & Robbins, 2007). Previous studies have reported the occurrence and geographical distribution of dagger nematodes in South America and Caribbean islands (Coomans et al., 2001; Doucet et al., 1998; Peraza-Padilla et al., 2018; Varela-Benavides et al., 2018). To date, a total of ten *Xiphinema* species have been reported to be present in Colombia, although in fact only four species, namely *X. colombiense* Hunt, 1982, *X. krugi* Lorde-Ilo, 1955, *X. llanosum* Siddiqi & Lenné, 1990, and *X. riocaquetae* Hunt, 1982, were formally described (Barsalote et al., 2017; Doucet et al., 1998; Hunt, 1982; Siddiqi & Lenné, 1990; Volcy, 1990). In 1990, Volcy described morphologically and morphometrically several populations of *X. krugi* in northwest Colombia. On the other hand, in Ecuador only a single species of *Xiphinema* has been reported to date, namely *X. macrostylum* (Esser, 1966). To our knowledge, no previous studies published with detailed integrative descriptions including measurements, morphological analyses and DNA barcoding of Colombian and Ecuadorian populations of dagger nematodes have been published.

Nematological surveys carried out in 2014 and 2023, in agroforestry areas within the Amazon basin of Ecuador and Colombia, respectively, revealed low to moderate infestations (6–15 nematodes per 250 cm³ of soil) of three *Xiphinema* populations, two monodelphic and one pseudomonodelphic. Initial hypothesis based on a morphological evaluation under a microscope showed that three populations morpho-anatomically looked like Group 1 and 2 morphospecies (Coomans et al., 2001; Loof & Luc, 1990; Munawar et al., 2018; Peraza-Padilla et al., 2017). The goals of the present work were: 1) to characterize morphologically and morphometrically three populations of *Xiphinema*, two monodelphic and one pseudomonodelphic, and compare them with previous publications; 2) to characterize molecularly these three populations using the D2-D3 expansion segments of the 28S rRNA, ITS1, and partial COI gene sequences and 3) to establish the phylogenetic relationships of the identified *Xiphinema* species with available sequenced species. This study will bring new information to distinguish dagger nematode species, generate new molecular markers and reveal new knowledge on the genetic diversity, molecular evolution and the geographic distribution of monodelphic *Xiphinema* species.

Methods

Nematode sampling, extraction, and morphological studies

Nematode surveys were carried out in 2014 and 2023, in cassava (*Manihot esculenta* Crantz) and maize fields (*Zea mays* L.) localized in agroforestry areas within the Amazon basin from Ecuador and Colombia, respectively (Table 1). *Xiphinema* specimens

were extracted from 250 cm³ soil samples using a modified Cobb washing, sieving and decantation technique and a modified Baermann method (Flegg, 1967). As result of these surveys, three populations of *Xiphinema* were isolated from these two soil samples from the northern area of the Ecuadorian Amazon, Sucumbios province, Cuyabeno (Ecuador) and the southwest area of the Colombian Amazon, Sibundoy, Putumayo state (Colombia), respectively (Table 1). Additional soil samples were collected to guarantee the necessary specimens for morphological and/or molecular analyses.

Xiphinema specimens were hand-picked, and kept in both TAF fixative solution (triethanolamine) and ethanol 70% (v/v) for morphological and molecular analyses, respectively. Measurements were made on specimens fixed in TAF and subsequently processed to glycerol using Seinhorst's method (Seinhorst, 1959) as modified by De Grisse (1969). The light micrographs and measurements were performed using an Olympus BX50 light microscope equipped with an Olympus DP70 digital camera (Olympus BX50, Hamburg, Germany) and Cell software (Olympus Software Imaging for Life Sciences). All measurements were expressed in micrometers (µm). All abbreviations are as defined in Jairajpuri and Ahmad (1992). Etymology used to describe the female genital branches is in accordance with Coomans et al. (2001).

DNA extraction, PCR and sequencing

For DNA extraction, a single individual nematode was placed in a 3 µL drop of sterile water on the slide and crushed with a sterilized and pointed pin. Subsequently, the crushed nematode was pipetted into a PCR tube containing 20 µL of lysis buffer solution consisting of 10 µL ddH₂O, 6 µL 10×PCR buffer, 2

Table 1 Two *Xiphinema* species recovered from the northern Ecuador and southern Colombia, their species names, locality, host plants, sequenced loci and GenBank accession numbers

Species	Sample code	Locality	Host	Genbank accessions		
				28S	ITS1	COI
<i>X. brasiliense</i>	COL-P-01	Sibundoy, Putumayo	maize	PQ009926	PP999470	PP991512
<i>X. brasiliense</i>	ECU-S-01	Cuyabeno, Sucumbios	cassava	PQ009927	-	-
<i>X. krugi</i>	COL-P-01	Sibundoy, Putumayo	maize	PQ009925	-	PP991511

(-) Not obtained or not performed

$\mu\text{L Mg}^{2+}$ and 2 μL of proteinase K (20 mg/mL) (Nal-gene®). Later, tubes were centrifuged at 13,000 rpm for 45 s to 1 min and frozen at $-80\text{ }^{\circ}\text{C}$ (for 1 h 30 min). Samples were mixed for 12 s, centrifuged at 13,000 rpm for 45 s to 1 min, and incubated at $57\text{ }^{\circ}\text{C}$ (1 h 30 min), $65\text{ }^{\circ}\text{C}$ (2 h) and $95\text{ }^{\circ}\text{C}$ (15 min). Finally, the DNA suspension was cooled to $4\text{ }^{\circ}\text{C}$ and used for conducting PCR (Ali et al., 2024). Three sets of primers (synthesized by STABVIDA, Caparica, Portugal) were used in the PCR analyses to amplify the D2-D3 fragments of the 28S and the ITS region of the rDNA, and the COI gene region of the mtDNA. Primers for amplification of 28S were forward primer 391F (5′-AGCGGAGGAAAAGAACT-3′) or D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) (De Ley et al., 1999; Nunn, 1992) and reverse primer D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Nunn, 1992). Primers for amplification of ITS region were forward primer TW81 (5′-GTTTCCGTA GGTGAACCTGC-3′) and the reverse primer AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) (Curran et al., 1994). Primers for amplification of the partial COI gene region were forward primer COIF (5′-GAT TTTTGGKCATCCWGARG-3′) and reverse primer COIR (5′-CWACATAATAAGTATCATG-3′) (Lazarova et al., 2006). The 50 μL PCR was performed using Master Mix DNA polymerase (NZYTech, Lisbon, Portugal) according to the manufacturer's protocol in a Bio-Rad Thermal Cycler (Bio-Rad Laboratories, CA, USA). All PCR assays were conducted as described by Gutiérrez-Gutiérrez et al., (2018, 2020). These PCR assays were conducted using DNA positive controls and a negative control containing sterile water. The PCR cycle conditions were followed as described by Ali et al. (2024). Each PCR product was purified and sequenced by STABVIDA (Caparica, Portugal). All new sequences of the Colombian and Ecuadorian populations of dagger nematode species were deposited into the GenBank database under the accession numbers indicated on the Table 1.

Phylogenetic analysis

DNA sequences were analysed, trimmed, and assembled into contigs using DNA Baser Sequence Assembler (version 4.0), Heracle BioSoft, from <https://www.dnabaser.com/download/download.html>. The newly obtained rDNA sequences (D2–D3 expansion segments of 28S rRNA gene and partial ITS1) and

mtDNA sequences (partial COI mtDNA gene) were compared with those of the other nematode species available at the GenBank sequence database using the BLAST homology search program. Likewise, these newly obtained rDNA and mtDNA sequences from all known dagger nematode species found in this survey (Table 1), together with other available selected sequences from the National Center for Biotechnology Information (NCBI) were used for phylogenetic analyses. Outgroup taxa for each studied gene were chosen according to previously published data by Peraza-Padilla et al. (2018), Varela-Benavides et al. (2018) and Ali et al. (2024). MAFFT v. 7 (Katoh et al., 2019) and ClustalX2 (Larkin et al., 2007) with default parameters were used for sequence alignment. Later, ambiguous regions were removed with Gblocks v. 0.91b (Castresana, 2000) using less stringent parameters (www.phylogeny.fr). Homogeneities of base frequencies and optional substitution models for 28S rRNA, ITS1 and COI mtRNA datasets were tested with jModelTest2 (Tanabe, 2011). Bayesian inference (BI) was performed using the software MrBayes v. 3.2.1 (Ronquist et al., 2012). For BI, the substitution model was selected by the Bayesian information criterion (BIC) and three different models were selected for this study. Convergence of the MCMC chains and burn-in length were assessed and checked in Tracer 1.7.1 (Rambaut et al., 2018). Between 2,000,000 to 5,000,000 generations was performed for each BI analysis, sampling every 100th tree and discarding 25% of samples as 'burn-in'. Finally, the final consensus trees were visualized using FigTree v. 1.4.3 (Rambaut, 2009).

Results

Morphology and morphometrics of dagger nematode species of the genus *Xiphinema* recovered from Colombia and Ecuador

Xiphinema brasiliense Lordello, 1951 (Fig. 1; Table 2).

Locality and habitat

Specimens collected from the rhizosphere of cassava (*M. esculenta*) and maize (*Z. mays*) fields at Cuyabeno, Sucumbíos, Ecuador and Sibundoy, Putumayo, Colombia respectively (Table 1).

Brief description

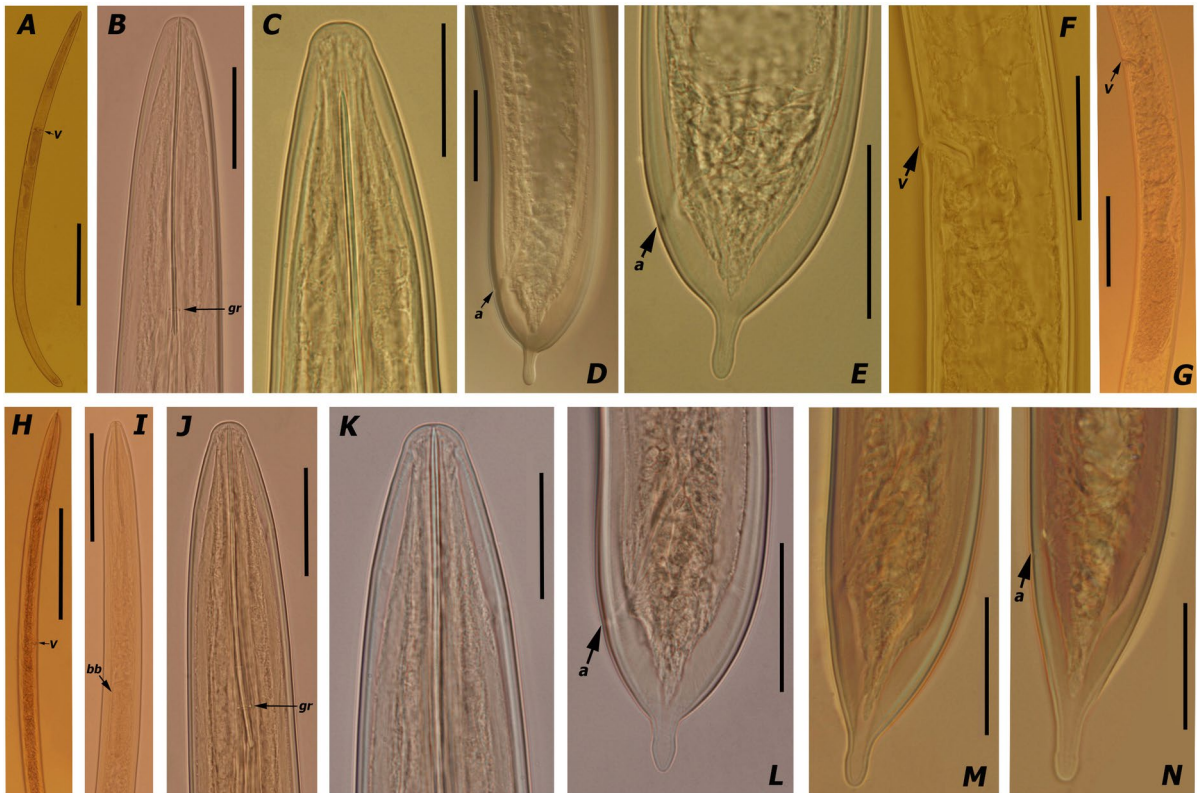


Fig. 1 Light micrographs of *Xiphinema brasiliense* Lordello, 1951 females from the rhizosphere of maize (*Zea mays* L) and cassava (*Manihot esculenta* Crantz) fields from Sibundoy, Putumayo, Colombia (A–G) and Sucumbios, Ecuador (H–N) respectively. A: Entire female specimen; B–C, J–K: Lip region; D–E, L: Female tail region; F: Vulva region; G: Pos-

terior reproductive branch; H–I: Female anterior body region; M: tail region of the fourth stage juveniles (J4); N: tail region of the third stage juveniles (J3). Abbreviations: a=anus; bb=basal bulb; gr=guiding ring; v=vulva. (Scale bars: A=375 µm; B, J=40 µm; C, D, E, K, N=25 µm; F=50 µm; G=100 µm; H=400 µm; I=120 µm; L=33 µm; M=30 µm)

Colombian and Ecuadorian specimens of this species were characterized by having a body with *post mortem* habitus weakly curved. Cuticle smooth under light microscope, 2.8 (2.1–3.7) µm (Colombian population) and 2.5 (2.2–2.8) µm (Ecuadorian population) thick at mid-body, but thicker at the head and tail level. Labial region rounded, almost continuous, separated from the rest of the body by a weak shallow constriction. Odontostyle 1.8 and 1.6 times longer than the odontophore in both Colombian and Ecuadorian populations, respectively. Guide ring localized at 112 µm and 104 µm distances from anterior end in Colombian and Ecuadorian populations, respectively. Basal bulb cylindrical, 3.6 and 4.4 times longer than wide in Colombian and Ecuadorian populations, respectively. Female reproductive system monodelphic with anterior branch absent and posterior branch

complete with 288.7 (190.0–411.9) µm (Colombian population) and 245.9 (147.4–471.0) µm (Ecuadorian population), uterus unipartite, without uterine differentiation and ovary reflexed. Vulva anteriorly located at 31% and 28% of total body length for Colombian and Ecuadorian populations, respectively; vagina posteriorly obliquely bent, occupying about one half of the body width. Tail short and conoid ending with a well-developed axial peg, 10 and 13 µm long for both Colombian and Ecuadorian populations, respectively. Males were not found for this species. Only fourth (J4) and third (J3) juvenile stages were found and they were distinguished from other stages according to Robbins et al. (1996) (Table 2). J4 and J3 juvenile stages were similar to adults except for their smaller size, smaller odontostyle length, higher index c' , and absence of sexual characteristics. J4 tail

Table 2 Morphometrics of *Xiphinema brasiliense* Lordello, 1951 and *Xiphinema krugi* Lordello, 1955, from the rhizosphere of cassava (*Manihot esculenta* Crantz) and maize (*Zea*mays L.) from the northern Ecuador and southern Colombia, respectively. All measurements in μm and in the format: mean $\pm$ s.d. (range)*

Species	<i>X. brasiliense</i>	<i>X. brasiliense</i>			<i>X. krugi</i>
Sample code	COL-P-01	ECU-S-01			COL-P-01
Character	Females	Females	J4	J3	Females
n	10	7	3	2	12
L	1724.3 $\pm$ 97.0 (1597.0–1870.4)	1965.3 $\pm$ 122.0 (1841.0–2151.1)	1416.3 $\pm$ 71.5 (1346.0–1489.0)	1127.0–1148.0	2068.5 $\pm$ 148.4 (1878.9–2411.7)
a	35.6 $\pm$ 3.5 (27.7–40.3)	39.3 $\pm$ 5.4 (29.4–45.0)	36.8 $\pm$ 0.4 (36.4–37.0)	34.0–35.4	41.2 $\pm$ 2.2 (38.2–45.2)
b	4.6 $\pm$ 0.4 (4.3–5.4)	5.1 $\pm$ 1.1 (3.7–6.8)	4.0 $\pm$ 0.2 (3.9–4.2)	4.1–4.2	5.1 $\pm$ 0.3 (4.4–5.6)
c	57.0 $\pm$ 8.5 (49.8–79.9)	54.6 $\pm$ 3.9 (50.6–61.9)	34.4 $\pm$ 1.6 (33.2–36.2)	23.6–25.7	71.5 $\pm$ 6.8 (62.5–83.9)
c'	0.9 $\pm$ 0.1 (0.6–1.1)	1.1 $\pm$ 0.0 (1.0–1.1)	1.4 $\pm$ 0.0 (1.4–1.5)	1.9–1.9	0.8 $\pm$ 0.1 (0.7–0.9)
V (%)	30.7 $\pm$ 1.3 (29.0–32.8)	28.0 $\pm$ 1.1 (27.0–29.7)	-	-	35.4 $\pm$ 1.5 (33.8–38.4)
Gonad posterior	288.7 $\pm$ 81.0 (190.0–411.9)	245.9 $\pm$ 122.7 (147.0–471.9)	-	-	293.6 $\pm$ 60.3 (218.4–398.0)
Odontostyle length	127.6 $\pm$ 10.8 (117.7–146.0)	119.8 $\pm$ 1.9 (117.7–122.8)	101.2 $\pm$ 1.4 (100.4–102.8)	86.5–87.5	116.4 $\pm$ 3.8 (112.1–124.3)
Replacement odontostyle length	-	-	118.9 $\pm$ 1.5 (117.2–119.8)	99.9–101.9	-
Odontophore length	71.9 $\pm$ 5.9 (67.2–84.4)	73.5 $\pm$ 1.4 (72.0–76.2)	61.6 $\pm$ 0.9 (60.7–62.6)	55.0–55.6	68.7 $\pm$ 3.6 (59.7–72.3)
Lip region width	12.8 $\pm$ 0.7 (11.8–13.5)	13.0 $\pm$ 0.4 (12.2–13.5)	11.6 $\pm$ 0.7 (11.2–12.4)	10.5–10.9	13.4 $\pm$ 0.6 (12.8–14.9)
Lip region height	4.2 $\pm$ 0.8 (3.2–5.5)	3.8 $\pm$ 0.5 (2.8–4.3)	3.4 $\pm$ 0.5 (2.9–3.9)	3.4–3.5	5.2 $\pm$ 0.6 (4.3–5.9)
Oral aperture-guiding ring	112.2 $\pm$ 10.0 (95.1–130.0)	104.3 $\pm$ 5.9 (94.5–112.4)	89.6 $\pm$ 0.6 (84.4–96.1)	73.3–74.0	97.7 $\pm$ 8.7 (83.2–109.9)
Pharynx length	375.6 $\pm$ 41.5 (301.2–425.6)	400.8 $\pm$ 82.1 (302.0–518.6)	354.8 $\pm$ 25.2 (336.0–383.5)	268.0–283.3	405.4 $\pm$ 23.4 (360.0–452.1)
Pharyngeal basal bulb length	91.6 $\pm$ 2.8 (88.5–97.2)	103.7 $\pm$ 13.0 (85.0–120.0)	81.8 $\pm$ 2.7 (79.0–84.5)	65.4–75.9	97.7 $\pm$ 4.3 (89.6–101.8)
Pharyngeal basal bulb width	26.0 $\pm$ 4.9 (19.3–37.4)	23.9 $\pm$ 3.5 (19.0–28.7)	19.5 $\pm$ 1.4 (18.0–20.8)	17.4–17.5	26.9 $\pm$ 2.9 (23.5–33.0)
Gladularium length	78.9 $\pm$ 2.5 (74.6–82.0)	74.5 $\pm$ 7.3 (66.2–79.9)	-	-	84.0 $\pm$ 1.1 (83.1–85.2)
Cardia length	12.1 $\pm$ 5.1 (7.3–19.2)	9.9 $\pm$ 2.4 (7.6–12.4)	-	-	10.8 $\pm$ 3.1 (6.5–14.0)
Max body width	48.8 $\pm$ 5.9 (43.4–64.4)	50.7 $\pm$ 6.9 (44.6–64.9)	38.5 $\pm$ 1.6 (37.0–40.2)	32.4–33.2	50.8 $\pm$ 4.0 (44.4–60.5)
Body width at anus	32.6 $\pm$ 2.6 (29.7–36.3)	33.2 $\pm$ 1.0 (32.2–35.2)	29.1 $\pm$ 0.9 (28.1–29.9)	23.5–25.2	34.4 $\pm$ 1.2 (32.4–36.8)
Body width at guiding ring	38.4 $\pm$ 3.3 (34.2–45.5)	41.1 $\pm$ 4.5 (38.1–54.3)	33.3 $\pm$ 1.1 (32.5–34.5)	26.8–27.7	37.2 $\pm$ 1.6 (35.5–41.6)
Tail length	30.6 $\pm$ 3.4 (22.3–35.2)	36.0 $\pm$ 2.2 (33.9–40.4)	41.1 $\pm$ 0.5 (40.6–41.6)	30.3–33.0	29.1 $\pm$ 2.8 (23.9–34.8)
Vagina total	26.9 $\pm$ 3.0 (21.3–31.0)	33.9 $\pm$ 5.5 (29.4–40.6)	-	-	33.9 $\pm$ 2.8 (28.7–39.6)
Intrauterine egg length	172.8 $\pm$ 38.1 (130.0–203.0)	-	-	-	194.7 $\pm$ 22.0 (180.0–220.0)

Table 2 (continued)

Species	<i>X. brasiliense</i>	<i>X. brasiliense</i>			<i>X. krugi</i>
Sample code	COL-P-01	ECU-S-01			COL-P-01
Character	Females	Females	J4	J3	Females
Intrauterine egg width	41.2 ± 7.2 (36.0–49.5)	-	-	-	42.3 ± 4.1 (39.9–47.0)
Cuticle wide at mid-body	2.8 ± 0.5 (2.1–3.7)	2.5 ± 0.2 (2.2–2.8)	1.9 ± 0.1 (1.8–2.1)	1.5–1.8	2.6 ± 0.4 (2.2–3.3)
Rectum length	29.9 ± 4.9 (21.3–33.8)	34.1 ± 2.9 (30.1–37.9)	29.9 ± 4.8 (24.4–33.6)	18.0–25.8	35.9 ± 4.1 (29.2–42.6)
J	5.8 ± 1.7 (3.4–9.7)	4.0 ± 1.3 (2.7–6.2)	4.3 ± 0.8 (3.7–5.2)	2.7–4.1	11.5 ± 1.9 (8.62–14.3)
Peg	10.1 ± 2.2 (4.3–11.9)	13.0 ± 1.5 (11.5–15.9)	13.3 ± 2.5 (10.4–15.1)	-	-

*Abbreviations are defined in [Jairajpuri and Ahmad \(1992\)](#)

shape showed a similar pattern to females; while J3 tail shape was more slender than females, with a peg much less sharply offset (Fig. 1M–N).

Remarks

A Colombian population of this species was found in a rizospheric soil sample of a maize field at the Sibundoy municipality, Putumayo department, southern Colombia. Additionally, an Ecuadorian population of this same species was also recovered from a soil sample collected around roots of a cassava crop at Cuyabeno municipality, Sucumbíos province, northern Ecuador. These *Xiphinema* populations presented low to moderate nematode densities in both soil samples (6 to 12 individuals/250 cm³ of soil). The morphological and morphometrical characters of both populations closely agree with the original description of this species ([Lordello, 1951](#)). It matches well with other worldwide populations of this same species, except for minor intraspecific differences in the body length, a and c index, odontostyle length and pharyngeal basal bulb length (i.e. [Alkemade & Loof, 1990](#); [Cohn & Sher, 1972](#); [Cordero, 2003](#); [Ferraz, 1980](#); [Lamberti et al., 1987](#); [Oliveira et al., 2003](#); [Zeng et al., 2016](#)), which may be due to geographical intraspecific variability. This species was originally described parasitizing potato plants in Brazil ([Lordello, 1951](#)). Since then, it has been reported in association with a wide range of cultivated and uncultivated plants in several countries belonging South and Central America (i.e. Colombia, Costa Rica, Cuba, Guatemala, French Guyana, Peru, Venezuela,) ([Cohn & Sher, 1972](#); [Ferraz, 1980](#);

[Hunt, 1982](#); [Lamberti et al., 1987](#); [Alkemade & Loof, 1990](#); [Luc & Coomans, 1992](#); [Costa Manso et al., 1994](#); [Dias-Silveira & Herrera, 1995](#); [Doucet et al., 1998](#); [Coomans et al., 2001](#); [Cordero, 2003](#); [Esquivel, 2003](#)). Out of South America, this species has been only reported from Nigeria ([Cohn & Sher, 1972](#)) and China ([Zeng et al., 2016](#)). Although this species has already been cited in Colombia by [Hunt \(1982\)](#), no molecular, morphological and morphometric data have previously been provided for those populations. Thus, to our knowledge this is the first record of this species in Ecuador and Colombia, extending its geographical distribution in South America.

Xiphinema krugi [Lordello, 1955](#) (Fig. 2; Table 2).

Locality and habitat

Specimens collected from the rhizosphere of a maize (*Z. mays*) field at Sibundoy, Putumayo, Colombia (Table 1).

Brief description

The body of the specimens presented a *post mortem* habitus in hook or J shaped. Cuticle smooth along of body with 2.6 (2.2–3.3) µm thick in the vulval region, but thicker at the tail portion. Labial region hemispherical or rounded, separated from the rest of the body by a weak depression. Odontostyle 1.7 times longer than the odontophore. Guide ring localized at 97.7 µm distance from anterior end. Basal bulb elongated, 3.6 times longer than wide. Female reproductive system pseudomonodelphic with an anterior genital branch reduced and incomplete (partially atrophied) and posterior genital branch developed (ovary, oviduct, sphincter and uterus presents) with 296.3

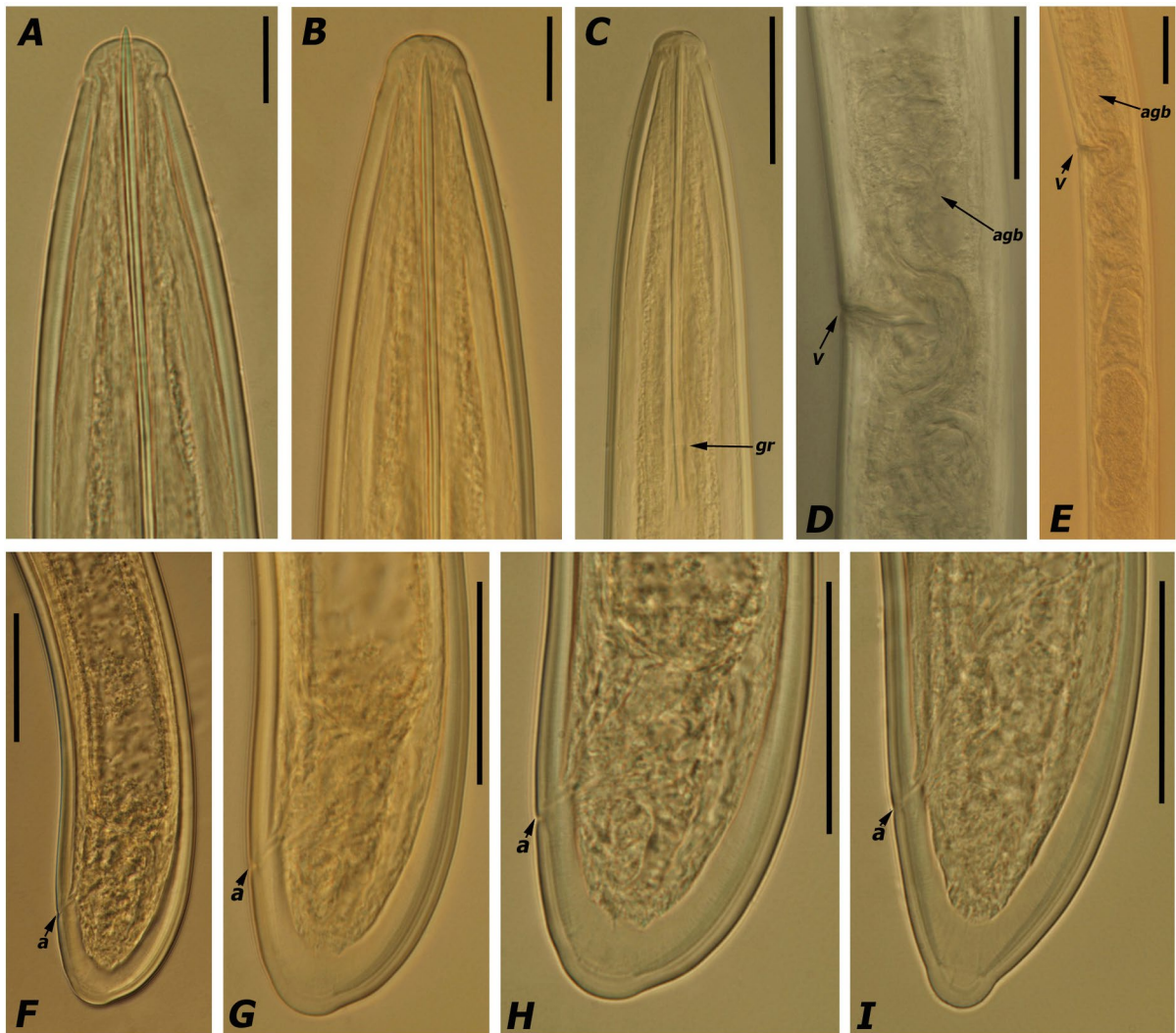


Fig. 2 Light micrographs of *Xiphinema krugi* Lordello, 1955 females from the rhizosphere of a maize (*Zea mays* L) field from Sibundoy, Putumayo, Colombia (A–J). A–C: Lip region; D: Vulval region including details of anterior genital branch; G: Details of anterior and posterior genital branches;

F–I: Female tail region. Abbreviations: a=anus; agb=anterior genital branch; gr=guiding ring; v=vulva. (Scale bars: A–B=13 μ m; C=37 μ m; D–E=50 μ m; F–H=35 μ m; I=32 μ m)

(218.4–398.0) μ m length, ovary reflexed. Vulva anterior; vagina occupying more than a half of the body width (around 68% of the body diameter). Tail short, subconoid, with a slight bulge more or less developed but never distinct peg. Males and juveniles were not found for this specie Fig. 2.

Remarks

The population identified in the present study as *X. krugi* was extracted from a rizospheric soil sample collected from a maize field in the Sibundoy

municipality, Putumayo, Colombia. The morphology and measurements agree well with original and other descriptions of this species (Lordello, 1955; Oliveira et al., 2003; Peraza-Padilla et al., 2017). *Xiphinema krugi* was reported for the first time in a soil sample collected from several native forest trees at Piracicaba, Sao Paulo state, Brazil (Lordello, 1955). Since then, this species has been reported in several countries of South and Central America included Argentina, Brazil, Costa Rica, Colombia, Panamá, Salvador

and Uruguay (Loof & Maas, 1972; Luc & Hunt, 1978; Bala, 1984; Chaves & Coomans, 1984; Luc & Doucet, 1990; Luc & Coomans, 1992; Decramer et al., 1998; Doucet et al., 1998; Coomans et al., 2001; Oliveira et al., 2003, 2006; Ye & Robbins, 2010; Peraza-Padilla et al., 2017; Chaves et al., 2019) and also is widespread in the USA (Frederick & Tarjan, 1974; Robbins & Brown, 1991). This species has already been reported from Colombia by Volcy (1990), but no molecular data was provided for those populations. To our knowledge, these findings represent the first record of *X. krugi* associated with maize plants in a southerly Colombian region, extending its geographical distribution in this country and South America.

Molecular results and phylogenetic relationship of the two dagger nematode species of the genus *Xiphinema* recovered from Colombia and Ecuador and the remaining *Xiphinema* spp.

Sequence analysis and phylogenetic relationships of 28S rRNA gene

Two almost identical 28S sequences (more than 99% of similarity) of Colombian and Ecuadorian isolates of *X. brasiliense* (PQ009926 and PQ009927) matched closely (98–99% of similarity) to a Brazilian and a Chinese populations of the same species (AY601616 and KP793050), varying in 11–10 nucleotides and 5–4 indels. Likewise, the 28S sequence of *X. krugi* (PQ009925) showed a high homology (99–100%) with Costa Rican sequences of the same species, namely *X. krugi* (KX931060 – KX931063). For these species, the respective Bayesian phylogenetic tree (Fig. 3) showed that *X. krugi* was grouped together within the well-supported clade of the same species and is clearly separated from other morphologically similar species belonging to morphospecies Group 1 and 2. Furthermore, the Colombian and Ecuadorian isolates of *X. brasiliense* were closest to isolates of the same species from different world populations, but not within the same subclade (Fig. 3), mostly due to the intraspecific variations of the 28S sequences from isolates that distinguished Brazilian and Chinese populations from the Colombian and Ecuadorian, yielding intraspecies cladogenesis with one subclade inside this species clade.

Sequence analysis and phylogenetic relationships of ITS rRNA

The ITS sequence from the Colombian population of *X. brasiliense* (PP999470) showed a high sequence similarity with two Brazilian isolates of this same species (AY430182 and AY604184) (93 and 89% of similarity, respectively; query cover 93 and 49%, respectively) and several Thai isolates of *Xiphinema* sp. t XM-2022 (e.g. OP910319 to OP910324) (91–92% of similarity); presenting an intraspecific variation for *X. brasiliense* of 51–63 nucleotide mismatches and 38–42 indels. The corresponding Bayesian phylogenetic tree is represented in Fig. 4. ITS sequences from two Brazilian isolates of *X. brasiliense* (AY430182 and AY604184) and a Thai isolate of *Xiphinema* sp. t XM-2022 (OP910324) clustered together in a well-supported subclade within the clade of the Colombian isolate of *X. brasiliense* (PP999470), probably due to the high intraspecific variation exhibited by *X. brasiliense* for this marker.

Sequence analysis and phylogenetic relationships of COI mtDNA

The COI sequence from the Colombian population of *X. brasiliense* (PP991512) presented 77–78% similarity to two USA isolates of *X. setariae* Tarjan, 1964 (KX263044 and KX263048). The phylogenetic inference (Fig. 5) showed that our *X. brasiliense* was phylogenetically related with *X. tica* Peraza-Padilla, Cantalapiedra-Navarrete, Zamora-Araya, Palomares-Rius, Castillo, & Archidona-Yuste, 2017 and clearly separated from other *Xiphinema* spp.; however, our *X. brasiliense* COI sequence clustered, with good support, together Costa Rican isolates of *X. tica* (KY623500–KY623501). In relation to *X. krugi*, the Colombian (PP991511) and Costa Rican (KY816625–KY816626) populations clustered in a well-supported sub-clade.

Discussion

In this study, three populations of dagger nematodes of the genus *Xiphinema* collected from two agroforestry areas within the Amazon basin in northern Ecuador and southern Colombia, were characterized through an integrative approach using

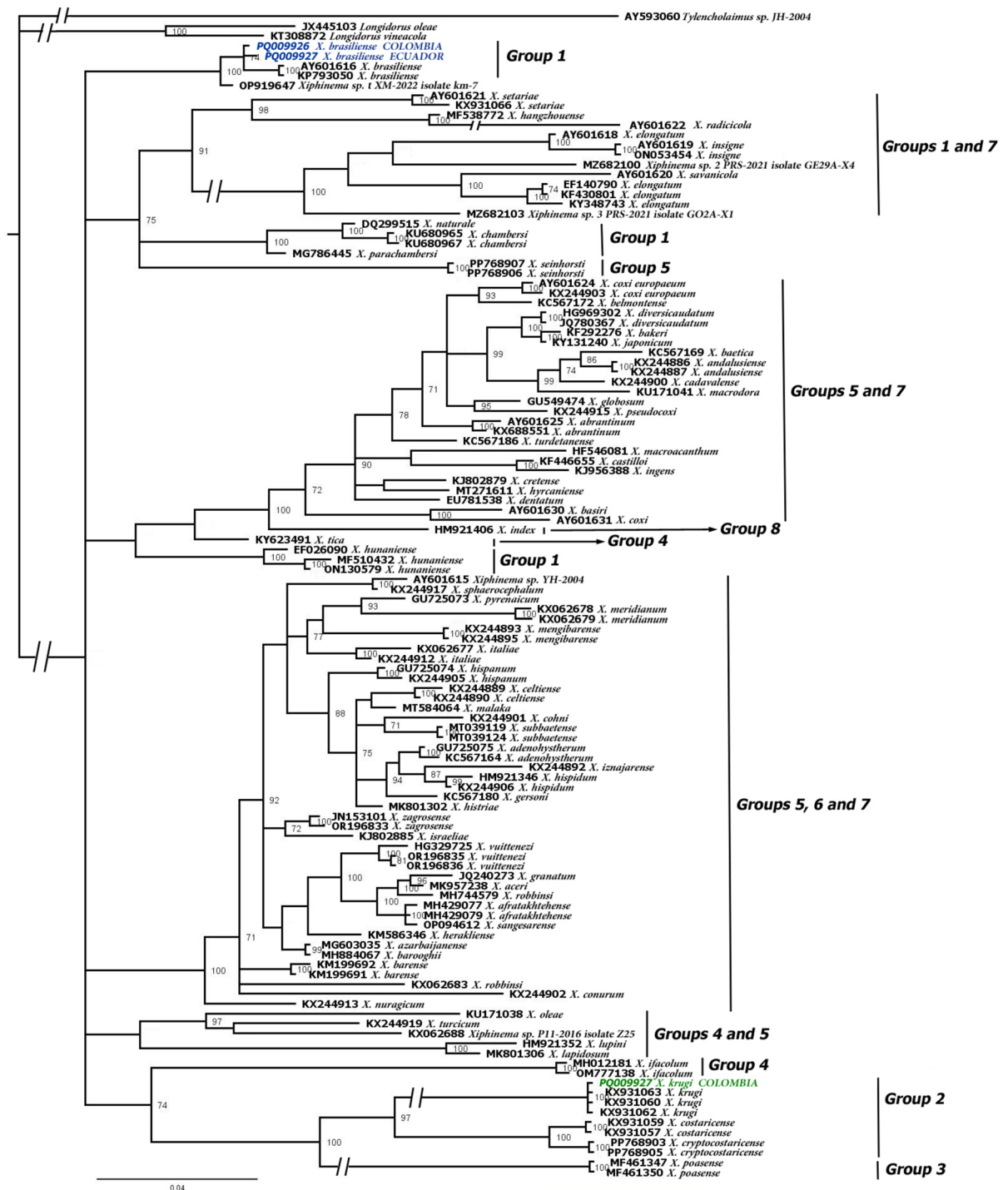


Fig. 3 Phylogenetic relationships of *Xiphinema brasiliense* Lordello, 1951 and *Xiphinema krugi* Lordello, 1955 with other *Xiphinema* species. Bayesian 50% majority rule consensus trees was inferred from 118 sequences of D2-D3 expansion segments of 28S rRNA sequences analysed under the

SYM+Gmodel. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are coloured in red and navy blue. Scale bar=expected changes per site

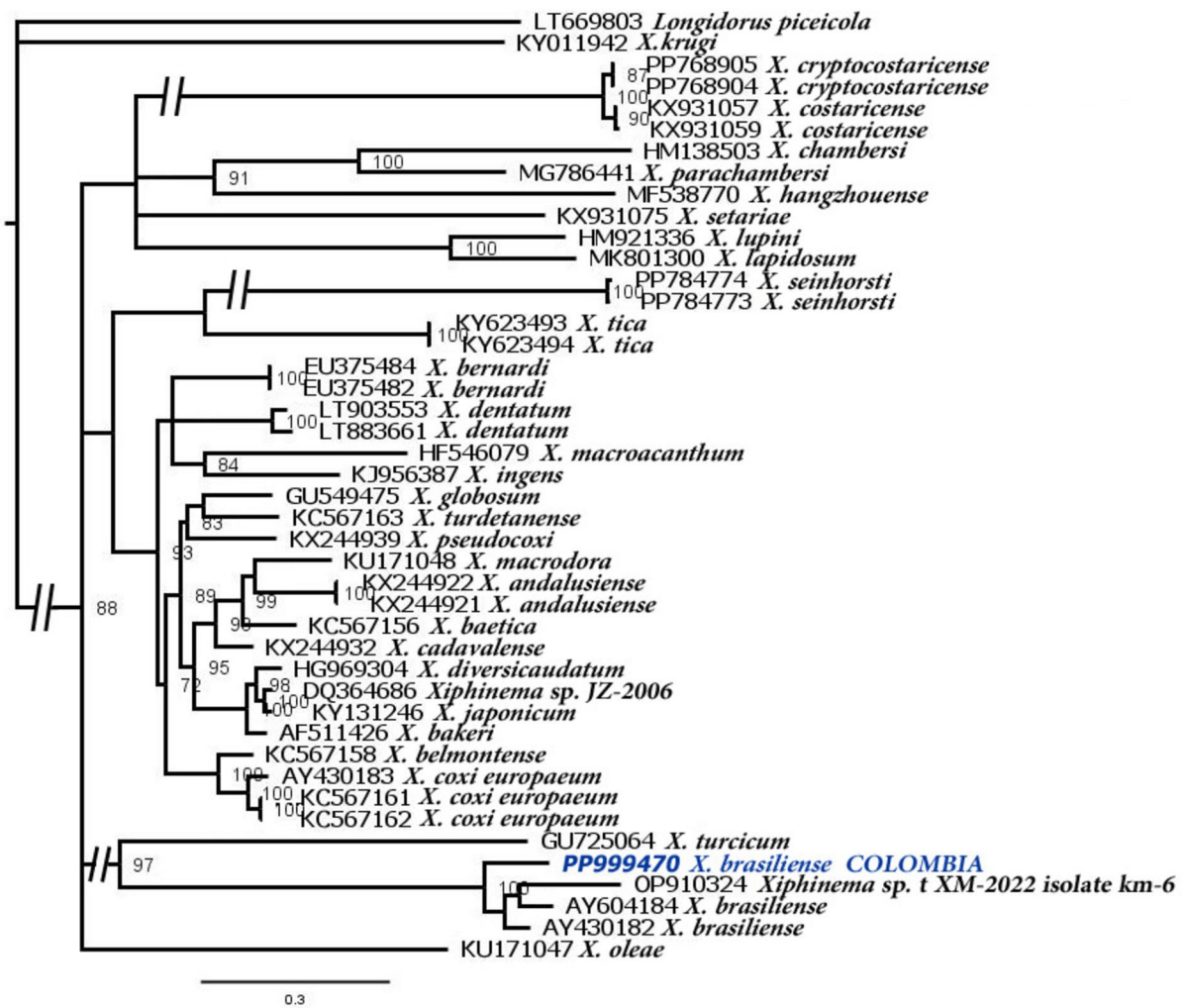


Fig. 4 Phylogenetic relationships of *Xiphinema brasiliense* Lordello, 1951 and *Xiphinema krugi* Lordello, 1955 with other *Xiphinema* species. Bayesian 50% majority rule consensus trees was inferred from 44 sequences of ITS1 rRNA ana-

lysed under the GTR+G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are coloured in pink and sky blue. Scale bar=expected changes per site

morpho-anatomical analysis and molecular phylogenetic data based on the rRNA and mtDNA genes. Using integrative taxonomy to identify dagger nematode species has already well documented (Ali et al., 2024; Archidona-Yuste et al., 2016b, 2020; Cid del Prado-Vera et al., 2024; Gu et al., 2022; Lazarova et al., 2019; Mobasser et al., 2019; Naghavi et al., 2022; Peraza-Padilla et al., 2018; Varela-Benavides et al., 2018; Vazifeh et al., 2019), however it is one of the first systematic studies using an integrative approach to identify monodelphic and pseudomonodelphic *Xiphinema* spp. belonging to the

morphospecies Group 1 and 2 in Ecuador and Colombia. The findings in this study expand the knowledge on the biodiversity of *Xiphinema* species in Colombia and Ecuador where several dagger nematode species have already been described (Barsalote et al., 2017; Doucet et al., 1998; Esser, 1966; Hunt, 1982; Siddiqi & Lenné, 1990; Volcy, 1990). Thus, *X. brasiliense* is a new record from Colombia and Ecuador, increasing the geographical distribution of this species.

Morphospecies Group 1 is paraphyletic as well as most of the morphospecies groups studied until now. However, morphospecies Group 2 appears to

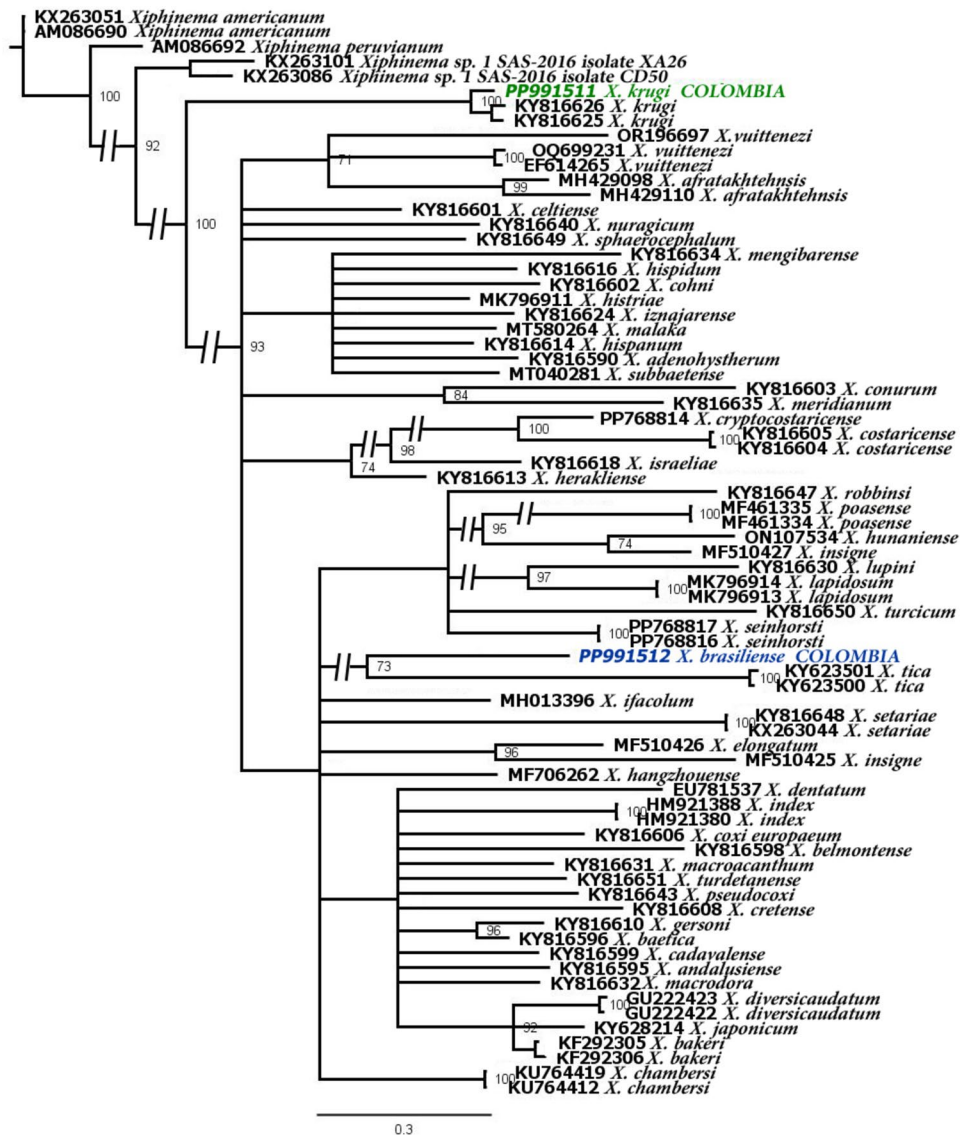


Fig. 5 Phylogenetic relationships of *Xiphinema brasiliense* Lordello, 1951 and *Xiphinema krugi* Lordello, 1955 with other *Xiphinema* species. Bayesian 50% majority rule consensus trees was inferred from 73 sequences of *COI* mtDNA analysed

under the HKY85 + G + I model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are coloured in green and purple. Scale bar = expected changes per site

be monophyletic, although to date there is a lack of sequences deposited in the GenBank database on this morphospecies group (Figs. 3, 4 and 5). Additionally, the phylogenetic analysis, particularly the phylogeny based on the 28S rRNA gene, showed that *X. krugi* is phylogenetically related to pseudomonodelphic species, such as *X. costaricense* Lamberti & Tarjan, 1974, and these are successively related to *X. poasense* Varela-Benavides, Peraza-Padilla,

Cantalapiedra-Navarrete, Palomares-Rius, Castillo, & Archidona-Yuste, 2018 belonging to Group 3 (dagger nematodes with the anterior genital branch complete and reduced) and *X. ifacolum* Luc, 1961 belonging to Group 4 (dagger nematodes with the female genital branches equal and presence of Z-differentiation). This supports the hypothesis proposed by Coomans et al. (2001), that the reduction of the anterior genital branch may represent a first step towards the origin

of pseudomonodelphism and that the monodelphic and pseudomonodelphic forms originated from didelphic ancestors. Likewise, according to Coomans et al. (2001) this hypothesis of the reducing complexity of the female reproductive system results in uterine reduction, the anterior position of the vulva, any uterine differentiations absent as Z- differentiations and/or crystalloids/spines, and huge uterine eggs, since the uterine elongation and differentiations assist the egg production in the two uterine branches at the same time. In any case, more studies with other molecular markers for these morphospecies Group 1 and 2 are necessary in order to determine the biology and phylogenetic relationships within the genus *Xiphinema*.

To date, the morphospecies Group 1 and 2 (Loof & Luc, 1990, 1993; Loof et al., 1996; Munawar et al., 2018) comprises around 9 and 7 valid species, which are mostly distributed in tropical and subtropical regions around the latitude of the Tropic of Cancer, including Central America and the top half of South American, several countries of the Middle East and Africa, excluding some countries in south and north, South Asia and the most of the counties belonging to Oceania (Brown & Halbrecht, 1997; Coomans et al., 2001; Doucet et al., 1998; Loof & Luc, 1990, 1993; Loof et al., 1996). This distribution suggests that morphospecies Group 1 and 2, such as morphospecies Group 3 and 4, are associated with high temperatures and humid conditions (Coomans et al., 2001). These findings are in accordance with Tarjan (1969), who described *Xiphinema* spp. with an anterior vulva and a short body length, that are usually associated with an equatorial climate characterized by high temperatures and abundant rainfall throughout the year. Spite of the Amazon basin, the largest drainage basin in the world localized in the tropical region, has been proposed as a major centre of diversity of *X. non-americanum* group species, particularly characterized by the presence of a remarkable diversity of monodelphic and pseudomonodelphic *Xiphinema* spp., it has not been extensively studied for many years (Coomans & Heyns, 1985; Coomans et al., 2001; Doucet et al., 1998).

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Declarations

Research involving human participants and/or animals No specific permits were required for the described fieldwork studies. Permission for sampling the cassava and maize fields was granted by the landowner. However, the plots are not protected in any way.

Informed consent All the authors certify that this work carried out in this research followed the principles of ethical and professional conduct have been followed. The funding source had no role the design of this study and not had any role during its execution, data collection and analyses, interpretation of the data, decision to publish, or preparation of the manuscript.

Conflict of interest All authors declare no conflict of interest. We also certify that all authors have reviewed the manuscript and approved the final version of manuscript before submission.

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