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A new dagger nematode, *Xiphinema poasense* n. sp. (Nematoda: Longidoridae), from Costa Rica

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Summary – A new dagger nematode, *Xiphinema poasense* n. sp., is described and illustrated from three populations extracted from soil associated with a combined plantation of *Eucalyptus* sp., *Cupressus* sp. and *Pennisetum* sp. and wild plants from a tropical pre-montane forest in Costa Rica. The new dagger nematode is characterised by a moderate body size 2612 (2416-3042) μm long, a rounded lip region 15.0 (13.5-16.5) μm broad, separated from the body contour by a shallow depression, amphidial fovea large, stirrup-shaped, a very long odontostyle (175 (164-188) μm), stylet guiding ring located 167 (136-181) μm from anterior end, vulva situated anterior to mid-body (36-40%), anterior genital branch complete but strongly reduced, without uterine differentiation, female tail short, hemispherical to convex-conoid with a c′ ratio = 0.7 (0.6-0.8) and bearing two pairs of caudal pores, and male absent. Integrative diagnosis was completed with molecular data using D2-D3 expansion segments of 28S rRNA, ITS1 region, partial 18S-rRNA and the partial mitochondrial gene cytochrome c oxidase subunit 1 (*coxI*). The phylogenetic relationships based on D2-D3 segments of this species with other *Xiphinema* spp. of the *X.* non-*americanum* group indicated that *X. poasense* n. sp. clustered with other species with a reduced anterior genital branch from the morphospecies Group 2, *viz.*, *X. costaricense* and *X. krugi*. However, the phylogeny of *coxI* and partial 18S rRNA gene revealed that the new species did not cluster with *Xiphinema* species having the anterior genital branch absent or reduced (*i.e.*, morphospecies Groups 1 and 2, respectively).

Keywords – 18S, Bayesian inference, *coxI*, *Cupressus* sp. description, D2-D3 segments, *Eucalyptus* sp., ITS1, longidorids, molecular, morphology, morphometrics, new species, *Pennisetum* sp., phylogeny, plant-parasitic nematode, pre-montane forest, rRNA, taxonomy.

Among dorylaimid nematodes, dagger nematodes of the genus *Xiphinema* Cobb, 1913 comprise an abundant number of species with a variety of morphological characteristics that turn them into a difficult group of plant-parasitic nematodes in a diagnostic context. Furthermore, dagger nematodes are of economic interest because they can cause significant losses to an important number of crops and some are vectors of plant viruses (Brown & Trudgill, 1998). Due to their large morphological diversity, *Xiphinema* was categorised into the *X. americanum*- and *X.* non-*americanum* groups (Coomans *et al.*, 2001). The *X.* non-*americanum* group comprises a complex of about 220 species characterised by having a large body and long odontostyle (Loof & Luc, 1990; Coomans *et al.*, 2001). These species can be recognised by the degree of the anterior female genital branch development, uterine differentiation and tail shape. These characteristics were included in a polytomous key containing eight morphospecies groups (Loof & Luc, 1990). However, recent taxonomic studies have revealed the existence of complex cryptic species within *Xiphinema* (Palomares-Rius *et al.*, 2014; Archidona-Yuste *et al.*, 2016a, b, c), which makes it necessary to apply integrative taxonomy based on morphology and DNA sequences. These approaches have been efficiently applied to the accurate identification of *Xiphinema* spp. (Barsi & De Luca, 2008; Gutiérrez-

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Gutiérrez *et al.*, 2010, 2012; Palomares-Rius *et al.*, 2014; Archidona-Yuste *et al.*, 2016a, b, c; Peraza-Padilla *et al.*, 2017a, b). Sequencing rRNA markers (18S, ITS regions, the D2-D3 segments, and mitochondrial markers, such as cytochrome c oxidase subunit 1 gene (*coxI*)) constitute a useful tool for species identification, revealing phylogenetic relationships within Longidoridae (De Luca *et al.*, 2004; He *et al.*, 2005; Subbotin *et al.*, 2014; Zasada *et al.*, 2014; Archidona-Yuste *et al.*, 2016a, b, c).

To complete the study on the species diversity of the genus *Xiphinema* in Costa Rica, nematological surveys of several regions were conducted in 2015 and 2016, revealing a moderate soil infestation (15-57 nematodes (500 cm^3 soil)$^{-1}$) of three unidentified populations of *Xiphinema* fitting the overall characteristics of the *X.* non-*americanum* group species. Initial morphometric studies showed that these populations morphologically match morphospecies Group 3 (with anterior female genital branch complete but strongly reduced and a uterine region without differentiation) (Loof & Luc, 1990). That prompted us to carry out detailed studies, using light microscopy and molecular characterisation, which indicated that these populations were conspecific and should be assigned to a new species. In the present study, we describe this new species as *Xiphinema poasense* n. sp. using integrative taxonomy. Costa Rica has been shown to have a rich nematode diversity and many nematode species have not yet been characterised (Esquivel, 2003; Powers *et al.*, 2009; Andrássy & Esquivel, 2012; Peraza-Padilla *et al.*, 2017a, b).

The objectives of this study were: *i*) to characterise the new *X.* non-*americanum*-species morphometrically and molecularly using the D2-D3 segments, ITS1, partial 18S rRNA, and partial *coxI* gene sequences; and *ii*) to determine the phylogenetic relationships of these dagger nematode populations within the *X.* non-*americanum* group species.

Materials and methods

Nematode population sampling and extraction

Soil samples were taken during the rainy season from 2015 to 2016 in eucalyptus trees and forests (Table 1). Five samples were taken from each place in an area of 50 m^2 where each sample was composed of ten cores 1 m away one from another, taken to a depth of 10-40 cm with a Dutch auger 5 cm wide. The samples were transported in plastic labelled bags, sealed and brought to the nematology laboratory where they were processed or stored at 4°C until processed. For each sample, nematodes were extracted from 500 cm^3 of soil using the protocol described by the California Department of Food and Agriculture (CDFA, 2015) and the centrifugal flotation method (Coolen, 1979).

Nematode morphological identification

Extracted nematodes were heat-killed, fixed in 4% formaldehyde and processed to pure glycerin using Seinhorst's (1966) method. Finally, they were mounted on permanent glass slides to allow handling, observation and measurement using a light microscope and an ocular micrometer. Specimens were examined using a Zeiss III compound microscope with Nomarski differential interference contrast up to a magnification of 1000×. Morphometric study of each nematode population included classical diagnostic features of the Longidoridae (*i.e.*, de Man

Table 1. *Xiphinema poasense* n. sp. sampled and sequenced from Costa Rica in this study.

Locality, province	Host-plant	Sample	GenBank accession no.			
			D2-D3 expansion segments of 28S rRNA	ITS1	18S	*coxI*
Toro Amarillo, Valverde Vega, San Carlos Alajuela	*Eucalyptus*, cypress and fountain grass	CTF01	MF461347, MF461348	MF461339	MF461351	MF461334
Aguas Zarcas, San Carlos, Alajuela	Forest	CTF02	MF461349	–	–	MF461335
Cerro Dantas, San Rafael de Heredia	Forest	ACC28	MF461350	MF461340	MF461352	MF461336

–: Not obtained.

body ratios, lip region and amphid shape, oral aperture-guiding ring distance, odontostyle and odontophore shape and length; Jairajpuri & Ahmad (1992)). All measurements were expressed in micrometres (μm), unless otherwise indicated in the text. For line drawings of the new species, light micrographs were imported to CorelDraw Graphics Suite software version X8 and redrawn. All other abbreviations used are as defined in Jairajpuri & Ahmad (1992). In order to clarify and confirm the detected developmental stage of juveniles of the new *Xiphinema* species, a scatter plot of the functional and replacement odontostyle in relation to body length of juvenile and female developmental stages was done.

Nematode molecular identification

For molecular analyses, and in order to avoid mistakes in case of mixed populations in the same sample, two live specimens from each sample were temporarily mounted in a drop of 1 M NaCl containing glass beads (to avoid nematode crushing/damaging specimens) to ensure specimens conformed with the unidentified populations. Following morphological confirmation, nematode DNA was extracted from single individuals and PCR assays were conducted as described by Archidona-Yuste *et al.* (2016a, b, c). The D2-D3 segments were amplified using the D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) primers (De Ley *et al.*, 1999). The ITS1 region was amplified using forward primer 18S (5′-TTG ATT ACG TCC CTG CCC TTT-3′) (Vrain *et al.*, 1992) and reverse primer rDNA1 5.8S (5′-ACG AGC CGA GTG ATC CAC CG-3′) (Cherry *et al.*, 1997). The portion of 18S rRNA was amplified using primers 988F (5′-CTC AAA GAT TAA GCC ATG C-3′), 1912R (5′-TTT ACG GTC AGA ACT AGG G-3′), 1813F (5′-CTG CGT GAG AGG TGA AAT-3′) and 2646R (5′-GCT ACC TTG TTA CGA CTT TT-3′) (Holterman *et al.*, 2006). Finally, the portion of the *coxI* gene was amplified as described by Lazarova *et al.* (2006) using the primers COIF (5′-GATTTTTTGGKCATCCWGARG-3′) and COIR (5′-CWACATAATAAGTATCATG-3′).

All different PCR assays were carried out according to the conditions described by Archidona-Yuste *et al.* (2016a, b, c). Then the amplified PCR products were purified using ExoSAP-IT (Affmetrix, USB Products) and used for direct sequencing on a DNA multicapillary sequencer (Model 3130XL genetic analyser, Applied Biosystems), using the BigDye Terminator Sequencing Kit V.3.1 (Applied Biosystems), at the Stab Vida sequencing facilities (Caparica, Portugal). The newly obtained sequences were submitted to the GenBank database under accession numbers indicated on the phylogenetic trees and in Table 1.

Phylogenetic analyses

D2-D3 segments, partial 18S rRNA, and partial *coxI* sequences of different *Xiphinema* species belonging to *X.* non-*americanum* group from GenBank were used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen following previous published studies (He *et al.*, 2005; Holterman *et al.*, 2006; Gutiérrez-Gutiérrez *et al.*, 2013; Tzortzakakis *et al.*, 2015; Archidona-Yuste *et al.*, 2016a, b, c). Multiple sequence alignments of the different genes were made using the Q-INS-i algorithm of MAFFT V.7.205 (Katoh & Standley, 2013), which accounts for secondary RNA structure. Sequence alignments were manually visualised using BioEdit (Hall, 1999) and edited by Gblocks ver. 0.91b (Castresana, 2000) in Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). Percentage similarity between sequences was calculated using the sequence identity matrix in BioEdit. For that, the score for each pair of sequences was compared directly and all gap or place-holding characters were treated as a gap. When the same position for both sequences had a gap it was not treated as a difference. Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The best-fit model of DNA evolution was obtained using JModelTest V.2.1.7 (Darriba *et al.*, 2012) with the Akaike Information Criterion (AIC). The best-fit model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC were then given to MrBayes for the phylogenetic analyses. General time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for the 18S rRNA and transversion model with invariable sites and a gamma-shaped distribution (TVM + I + G) for the D2-D3 segments and the partial *coxI* gene. These BI analyses were run separately per dataset using four chains for 1×10^6 generations for all molecular markers. A combined analysis of the three genes was not undertaken due to some sequences not being avail-

able for all species. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees from all analyses were visualised using TreeView (Page, 1996) and FigTree software V.1.42 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Xiphinema poasense[*] n. sp.
(Figs 1-6)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body cylindrical, slender, tapering towards anterior end, nearly straight upon fixation. Cuticle 2.5-4.0 μm at mid-body, and 8.0-10.0 μm at tail tip, marked by very fine superficial transverse striae mainly in tail region. Lip region continuous, rounded, differentiated from body by a slight depression; 2.0-2.8 times as broad as high. Amphidial fovea cup-like with aperture 8.0-10.5 μm wide or occupying *ca* 50-70% of lip region diam., located slightly anterior to depression marking lip region. Two pairs of body pores present between anterior end and guiding ring. Odontostyle very long and slender, 164-188 μm long, 9.0 (7.1-9.6) times lip region diam. Guiding ring double with guide sheath 12-14 μm long. Odontophore half or more of odontostyle length long, with moderately developed flanges. Pharynx 580-620 μm long, consisting of a slender and weakly muscular anterior portion separated from pharyngeal bulb by a constriction. Pharyngeal bulb from 2-4 times as long as wide, occupying about 20% of total pharynx length and comprising three nuclei as typical for genus. Nucleus of dorsal gland (DN) large, located at 14.3% (11.3-19.8%) of pharyngeal bulb length, being larger than the two ventrosublateral nuclei (SVN), which are located at 46.6% (36.3-55.3%) of terminal bulb length (location of gland nuclei according to Loof & Coomans, 1972). Cardia conical, 14-30 μm long and 10-23 μm wide. Vestigium visible in some specimens, *ca* 4.0 μm in length. Intestine simple, prerectum long, 8.6-13.2 anal body diam. long and rectum 0.6-1.0 anal body diam. long. Female reproductive system didelphic-amphidelphic, anterior genital branch shorter than posterior one at nearly half its length, probably not functional. Anterior genital branch variable in length (103-230 μm) and bearing a small ovary also varying in length (30-50 μm), uterus tripartite, 80-140 μm long, oviduct 70-90 μm long, both well developed and separated by a distinguished sphincter. Posterior genital branch well developed, consisting of a tripartite uterus 100-140 μm long, an oviduct 80-130 μm long, and a developed ovary 60-155 μm long. No uterine differentiation. Sperm not found in genital tract. Two mature eggs observed in posterior genital branch, 253 and 275 μm long, both four times as long as wide. Eggs or developed oocytes not observed in any female specimens within anterior genital branch, which is therefore non-functional. Vulva in form of a transverse slit, situated anterior to mid-body (36-40%), vagina extending inwards for 30-40 μm, occupying 40-70% of body diam. Ovejector well developed, 50-56 μm wide. Caudal region hemispherical, tail short convex conoid, *ca* 0.6-0.8 anal body diam. long, bearing one pair of caudal pores.

Male

Male not found and no sperm seen in female genital tract.

Juveniles

All four juvenile stages (first-, second-, third- and fourth-stage) were identified using morphological characters such as body length, length of replacement and functional odontostyle (Robbins *et al.*, 1996). Juveniles similar to adults apart from developed reproductive system, shorter body length, slightly longer tail and presence of replacement odontostyle. Tail becoming progressively shorter in each moult but maintaining same tail shape as adults, tail of juveniles and adult female very similar. First juvenile stage characterised by replacement odontostyle tip located close to base of functional odontostyle and at level of odontophore, and almost hemispherical tail. In J2-J4, replacement odontostyle located at some distance from odontophore.

[*] The species epithet refers to the Poás volcano in Costa Rica, the geographic origin of the nematode.

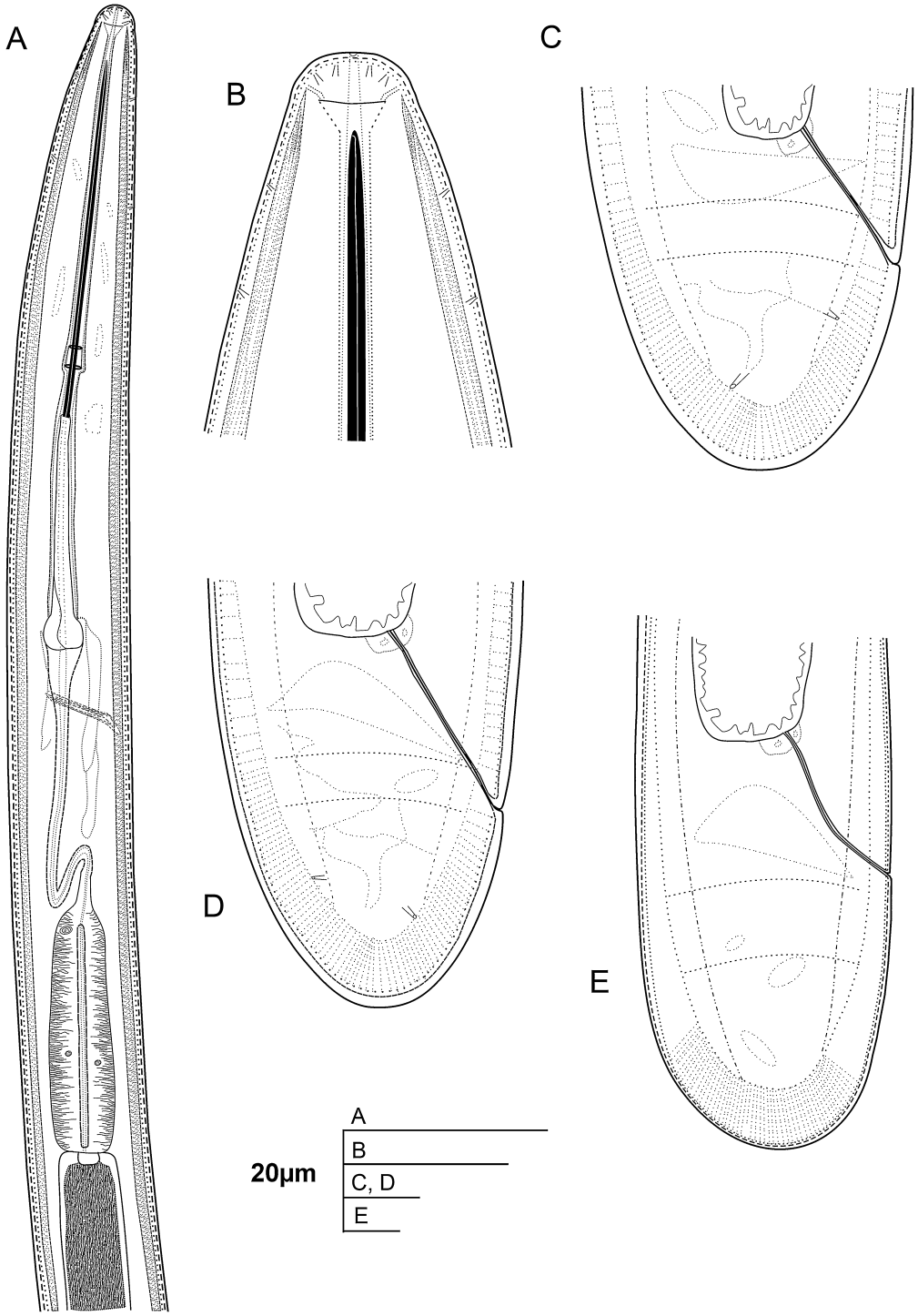

Fig. 1. Line drawings of *Xiphinema poasense* n. sp. A: Female pharyngeal region; B: Female lip region; C, D: Female tail region; E: First-stage juvenile tail region.

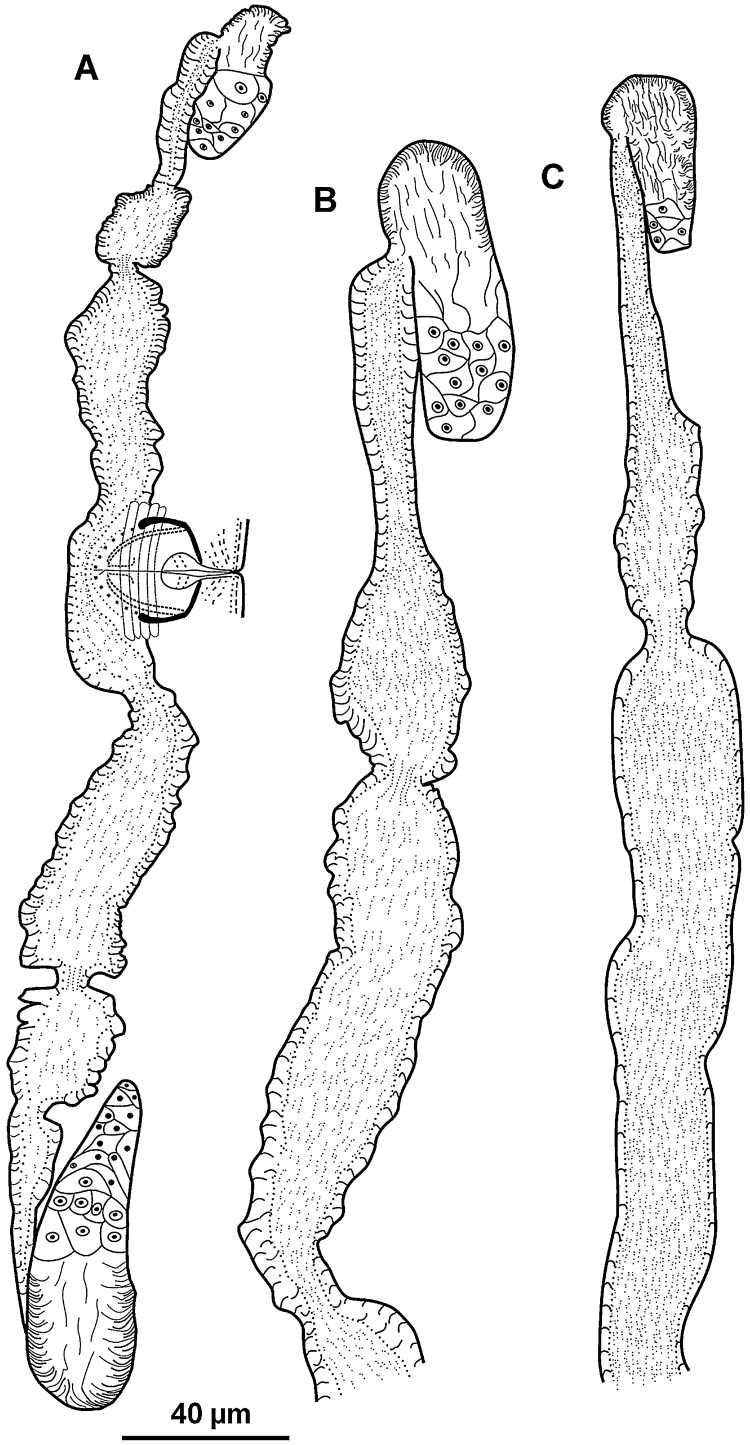

Fig. 2. Line drawings of *Xiphinema poasense* n. sp. A: Detail of anterior and posterior female gonads; B, C: Detail of anterior gonad. Abbreviations: a = anus; af = amphidial fovea; b = basal bulb; c = cardia; fl = flanges; gr = guiding ring; tro = tip of replacement odontostyle; V = vulva.

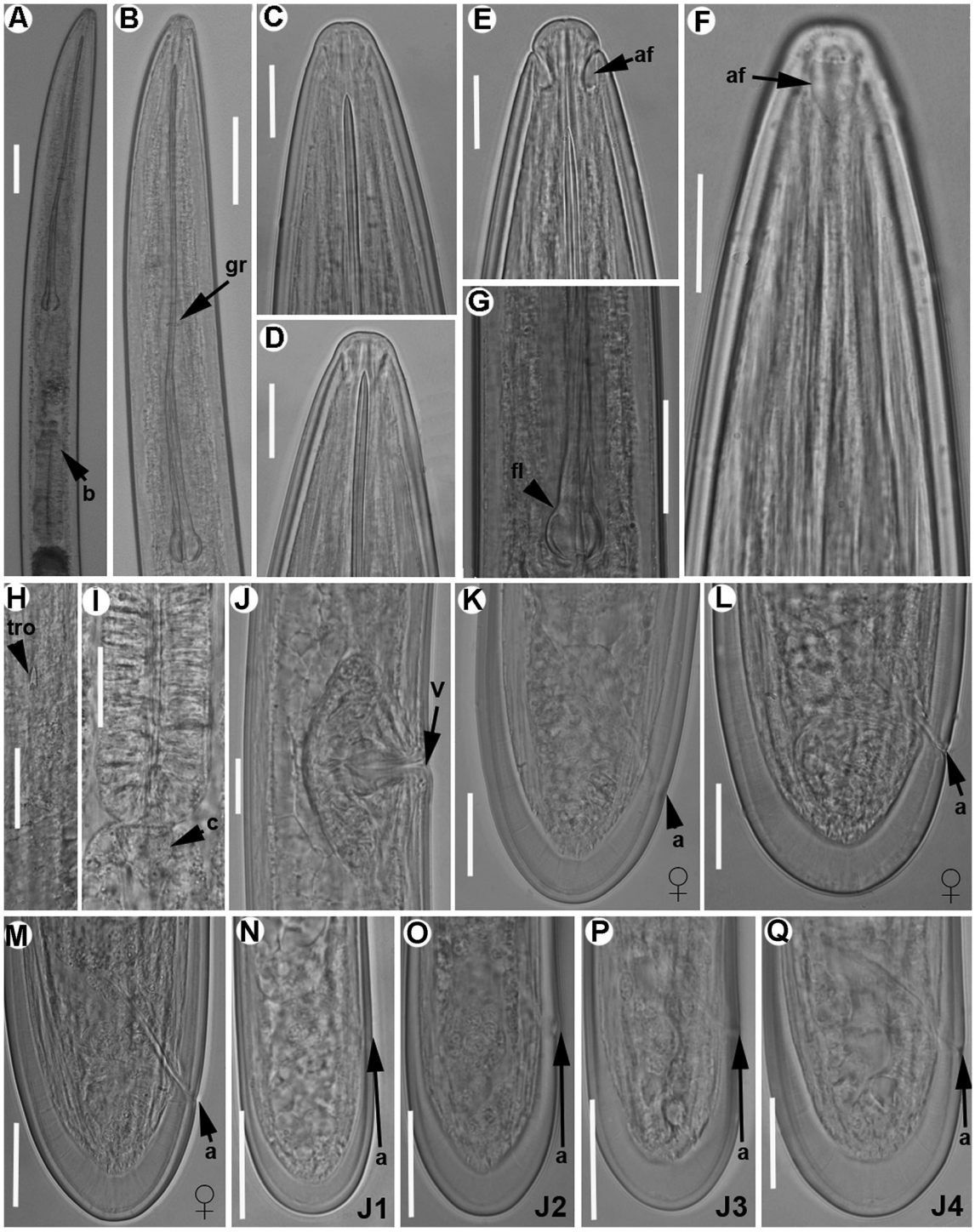

Fig. 3. Light micrographs of *Xiphinema poasense* n. sp. A: Female pharyngeal region; B-F: Female lip region; G: Detail of odontophore flanges; H: Detail of tip of reserve odontostyle; I: Detail of cardia; J: Vulval region; K-M: Female tail region; N-Q: First-, second-, third-, and fourth-stage juvenile tails (J1-J4), respectively. Abbreviations: bb = basal bulb; e = egg; c; V = vulva. (Scale bars: A, B = 50 μm; C-Q = 20 μm.)

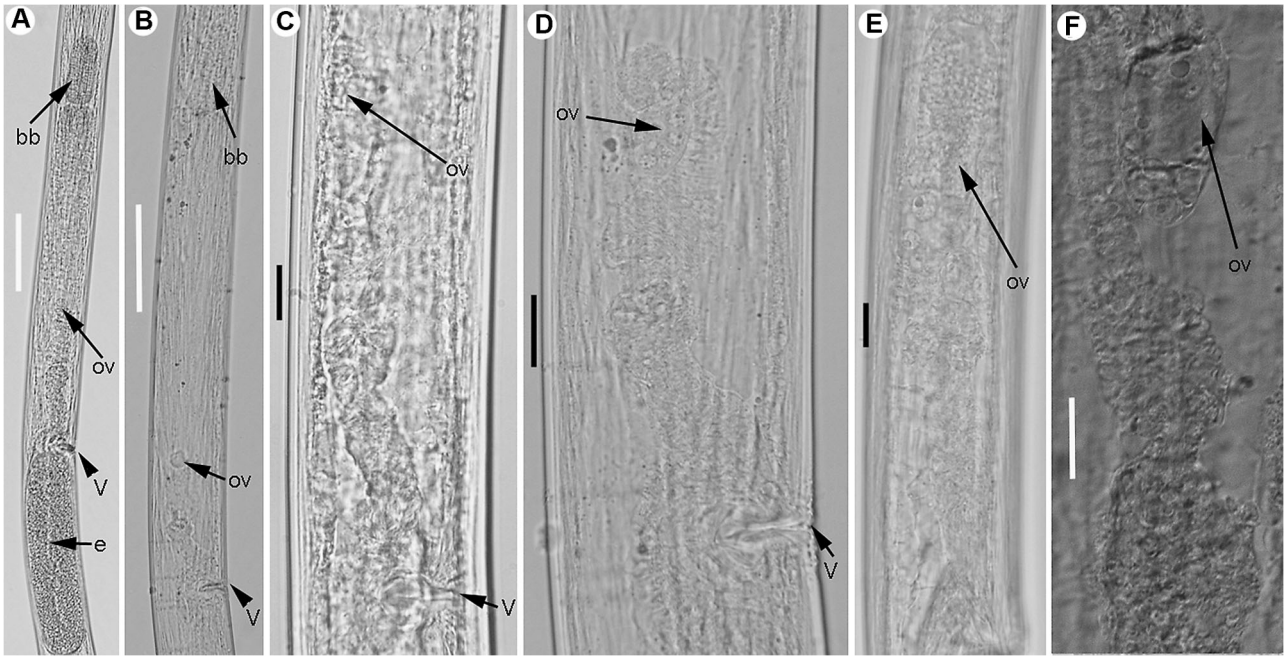

Fig. 4. Light micrographs of *Xiphinema poasense* n. sp. A, B: Detail of anterior genital branch showing position of basal bulb; C-F: Detail of anterior genital branch showing differences in development of anterior ovary. Abbreviations: bb = basal bulb; e = egg; ov = anterior ovary; V = vulva. (Scale bars: A, B = 100 μm; C-F = 20 μm.)

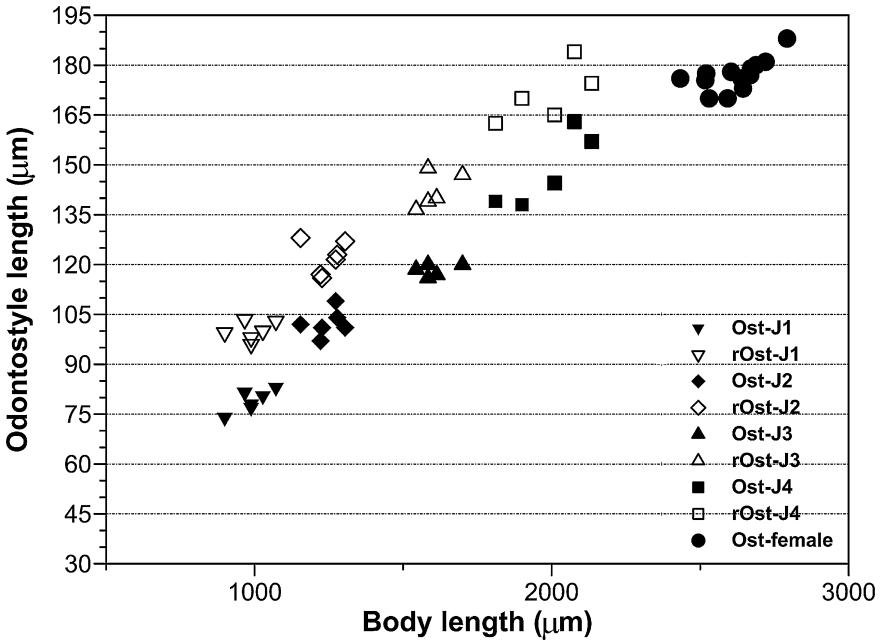

Fig. 5. Relationship of body length to length of functional and replacement odontostyle (Ost and rOst, respectively); length in all developmental stages from first-stage juveniles (J1) to mature females of *Xiphinema poasense* n. sp.

Fig. 6. Phylogenetic relationships within the *Xiphinema* non-*americanum* group complex. Bayesian 50% majority rule consensus tree as inferred from D2 and D3 expansion segments of 28S rRNA sequence alignment under the transversion model with invariable sites and a gamma-shaped distribution (TVM + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site.)

Table 2. Morphometrics of *Xiphinema poasense* n. sp. females and juveniles (J1-J4) from the rhizosphere of several crops and wild plants from Costa Rica. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Toro Amarillo, Valverde Vega, San Carlos, Alajuela (CTF01)						Toro Amarillo, San Carlos, Alajuela (tropical pre-montane forest) (CTF02)	Cerro Dantas, San Rafael de Heredia (ACC28)
	Female		J1	J2	J3	J4	Female	Female
	Holotype	Paratypes	Paratypes	Paratypes	Paratypes	Paratypes		
n	–	12	6	6	5	5	10	4
L	2668	2612 ± 97 (2433-2792)	990 ± 58 (900-1072)	1243 ± 53.8 (1155-1305)	1605 ± 58.6 (1544-1700)	1986 ± 131 (1811-2135)	2703 ± 175 (2416-3042)	2735 ± 106 (2622-2775)
a	39.3	35.8 ± 2.4 (32.6-41.1)	31.8 ± 3.5 (27.3-35.7)	37.8 ± 3.4 (33.3-42.3)	38.4 ± 2.3 (35.5-41.1)	37.8 ± 5.0 (32.8-45.8)	37.5 ± 4.5 (30.6-46.5)	40.4 ± 5.8 (34.5-46.5)
b	4.5	4.8 ± 0.3 (4.1-5.3)	3.6 ± 0.6 (2.9-4.6)	3.3 ± 0.4 (2.7-3.8)	3.8 ± 0.4 (3.5-4.5)	4.0 ± 0.1 (3.8-4.1)	5.0 ± 0.4 (4.6-5.7)	5.1 ± 0.6 (4.7-6.0)
c	86.1	79.7 ± 8.4 (66.0-91.0)	30.5 ± 2.5 (27.7-33.1)	37.7 ± 2.0 (34.5-40.2)	54.8 ± 8.3 (47.6-66.0)	52.9 ± 10.6 (45.3-71.2)	80.2 ± 9.3 (64.2-93.2)	87.5 ± 4.5 (84.7-94.2)
c′	0.7	0.7 ± 0.1 (0.6-0.8)	1.3 ± 0.1 (1.2-1.4)	1.2 ± 0.1 (1.1-1.2)	1.0 ± 0.2 (0.7-1.1)	0.9 ± 0.1 (0.8-1.0)	0.7 ± 0.1 (0.6-0.8)	0.6 ± 0.01 (0.6-0.7)
V	38.5	38.2 ± 1.5 (35.9-40.5)	–	–	–	–	37.6 ± 1.3 (35.5-39.7)	38.2 ± 1.3 (36.4-39.4)
G_1	3.9	6.6 ± 1.2 (4.8-8.6)	–	–	–	–	6.6 ± 0.8 (5.6-8.1)	–
G_2	9.7	12.0 ± 3.1 (7.8-17.6)	–	–	–	–	8.6 ± 0.6 (8.0-9.6)	–
Odontostyle	177	177 ± 4.7 (170-188)	79 ± 3.4 (74-83)	102 ± 4.0 (98-109)	118 ± 1.7 (116-120)	148 ± 11.3 (137-163)	177 ± 5.7 (166-185)	172 ± 0.8 (164-175)
Odontophore	102	100 ± 2.2 (97-105)	51 ± 4.2 (43-55)	64 ± 2.7 (59-66)	73 ± 3.4 (69-78)	84 ± 5.7 (75-90)	105 ± 2.4 (101-108)	103 ± 3.6 (99-107)
Total stylet	279	277 ± 5.3 (269-289)	–	–	–	–	282 ± 6.6 (269-291)	275 ± 4.4 (270-280)
Replacement odontostyle	–	–	100 ± 2.8 (96-103)	122 ± 4.8 (116-128)	142 ± 5.4 (136-148)	171 ± 8.6 (162-184)	–	–
Lip region diam.	15.0	15.0 ± 0.9 (13.5-16.5)	10.0 ± 1.1 (8.0-11.0)	11.0 ± 0.8 (10.0-12.0)	13.0 ± 1.1 (12.0-14.5)	13.5 ± 0.7 (12.5-14.5)	15.8 ± 0.7 (15.0-17.0)	16.5 ± 0.9 (16.0-17.5)
Oral aperture to guide ring	172	167 ± 6.5 (152-177)	74 ± 5.4 (67-80)	105 ± 14.1 (94-132)	116 ± 2.4 (113-120)	143 ± 8.4 (132-153)	173 ± 5.3 (166-180)	158 ± 7.9 (136-173)
Tail length	31.0	33.0 ± 3.3 (29.0-40.0)	32.5 ± 1.8 (30.0-35.0)	33.0 ± 0.8 (32.0-34.0)	30.0 ± 5.0 (24.0-35.5)	38.0 ± 5 (30.0-43.5)	34.0 ± 3.2 (30.0-40.0)	31.5 ± 1.3 (29.5-33.0)
Hyaline region	11.0	11.0 ± 0.7 (10.0-12.0)	6.0 ± 0.9 (5.0-7.0)	7.5 ± 0.5 (6.5-8.0)	9.0 ± 1.0 (8.0-10.0)	9.5 ± 1.1 (8.0-10.5)	11.5 ± 0.9 (10.0-13.0)	14.0 ± 0.6 (13.0-14.5)

TYPE HABITAT AND LOCALITY

Rhizosphere of a combined species plantation including eucalyptus (*Eucalyptus* sp.), cypress (*Cupressus lusitanica* Mill.) and fountain grass (*Pennisetum* sp.). The plantation is located at Toro Amarillo, Valverde Vega, San Carlos, Alajuela province, Costa Rica (10°14′54.2″N latitude, 84°16′32 longitude, altitude 1256 m a.s.l).

Other habitat and locality

The species was also detected in the rhizosphere of trees in a pre-montane tropical forest in the same region (10°15′18.4″N latitude, 84°16′20.9″W longitude, altitude 1190 m a.s.l), and in Cerro Dantas, San Rafael de Heredia, Costa Rica.

Type material

Holotype female and 17 female paratypes deposited in the Nematode Collection at the Laboratorio de Nematología, Escuela de Agronomía, Instituto Tecnológico de Costa Rica, Costa Rica. One female paratype deposited in each of the collections located in the Institute for Sustainable Agriculture, CSIC, Córdoba, Spain, the Royal Belgian Institute of Natural Sciences, Brussels, Belgium, and the USDA Nematode Collection, Beltsville, MD, USA.

Diagnosis and relationships

Xiphinema poasense n. sp. is an apparently parthenogenetic species characterised by a rounded lip region separated from the body contour by a shallow depression; odontostyle 164-188 μm long and odontophore 97-108 μm long, female didelphic-amphidelphic with the anterior branch complete but reduced and probably not functional, vulva located at 35-40% of body length, and female tail short and hemispherical to convex-conoid. Specific D2-D3 segments, ITS1, 18S rRNA and *coxI* sequences were deposited in GenBank with accession numbers MF461347-MF461350, MF461339-MF461340, MF461337-MF461338, and MF461334-MF461336, respectively.

This species belongs to the *X.* non-*americanum* morphospecies Group 3, having an anterior genital branch complete but reduced when compared to the posterior branch, and has the following specific α-numeric codes: A3, B4, C7b, D6, E3(4), F3, G4, H2, I12, J7b, K7b, L1, numbers between parentheses indicating that few individuals presented that characteristic (Loof & Luc, 1990, 1993; Loof *et al.*, 1996; Peraza-Padilla *et al.*, 2017b). To date, eight *Xiphinema* species have been described as species belonging to this morphospecies group (Table 3), it being one of the smallest groups in body length within the *X.* non-*americanum* group (Loof & Luc, 1990; Coomans *et al.*, 2001).

Xiphinema poasense n. sp. can be differentiated from all known species of the *X.* non-*americanum* Group 3 by its long odontostyle that can reach more than 180 μm in length, the longest in Group 3 (Loof & Luc, 1990; Loof *et al.*, 2001; Ganguly *et al.*, 2002; Faye *et al.*, 2012), combined with its short hemispherical to conoid tail.

Morphologically, *X. poasense* n. sp. resembles *X. hygrophilum* Southey & Luc, 1973, but differs from it in having longer odontostyle and body (177 (164-188) μm, 2612 (2433-2792) *vs* 149 (136-164) μm, 1800 (1500-2190) μm; respectively), and in the tail shape of the J1 and J2 (hemispherical *vs* filiform). *Xiphinema poasense* n. sp. can also be differentiated from *X. arcum* Khan, 1964 by the size of its odontostyle (177 (164-188) *vs* 105 (101-108) μm), and vulval position (V = 38 (35-40) *vs* 34 (32-35)). *Xiphinema poasense* n. sp. also resembles *X. costarricense* Lamberti & Tarjan, 1974, *X. surinamense* Carvalho, 1955 (Loof & Mass, 1972) and *X. krugi* Lordello, 1995, from the *X.* non-*americanum* Group 2, but these species have no ovary in the anterior genital branch, have a shorter odontostyle and the juvenile tail shape differs from that of the adult female.

Table 3. Specific α-numeric code of each *Xiphinema* spp. belonging to *X.* non-*americanum* morphospecies Group 3[*].

Xiphinema spp. / Code[**]	A	B	C	D	E	F	G	H	I	J	K	L
X. poasense n. sp.	3	4	7b	6	3(4)	3	4	2	1	7b	7b	1
X. arcum	3	4	7b	56	2	23	2	2	2	–	–	1
X. hygrophilum	3	4	7b	6	34	(1)2	34	1	2	7b	–	1
X. larliani	3	4	12	12	2	2	12	2	1	–	–	1
X. mali	3	4	2	23	12	3	2	2	3	–	–	1
X. mounporti	3	4	2	23	2	23	12	2	2	2	2	2
X. orbum	3	4	23	34	1	3	1	2	34	23	(2)	1
X. simillimum	3	4	2	3	2	2	2	2	3	–	–	1

[*] Specific α-numeric code according to Loof & Luc (1990).
[**] Codes in parentheses are exceptions.

Molecular characterisation and phylogenetic relationships of *Xiphinema poasense* n. sp. with other *Xiphinema* species

The PCR amplification of D2-D3 segments, ITS1 region, the partial 18S rRNA and partial *coxI* regions yielded single fragments of *ca* 900, 1100, 1800 and 500 bp, respectively, based on gel electrophoresis. Sequences from *X. poasense* n. sp. matched well with the *X.* non-*americanum* group species sequences deposited in GenBank, all being clearly different. Four new D2-D3 of 28S rRNA gene sequences were obtained in the present study. The D2-D3 segments of *X. poasense* n. sp. showed a 84-87% nucleotide similarity values (range from 100 to 112 nucleotides) with several *Xiphinema* species such as *X. costaricense* (KX931066), *X. krugi* (KX931063), and *X. brasiliense* (Lordello, 1951) Carvalho, 1962 (AY601616). The two new ITS1 rRNA sequences from *X. poasense* n. sp. had a low nucleotide similarity and coverage values with *Xiphinema* species deposited in GenBank, only accessions from *X. krugi* (KX931070-KX931071, KX931073-KX931074) showing a coverage value above 30%. By contrast, the partial 18S rRNA from *X. poasense* n. sp. showed a high nucleotide similarity with the 18S rRNA sequences from *X.* non-*americanum* group species, being 98-99% similar to all (differing by 24-101 nucleotides). Finally, three new *coxI* from *X. poasense* n. sp. were obtained in this study, these being clearly different to the other accession from *X.* non-*americanum* group species deposited in GenBank and being 78-80% similar to some (differing from 70-80 nucleotides), including *X. chambersi* (KU764412-KU761419), *X. italiae* Meyl, 1953(FJ713151) and *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964 (EF614265). Low intraspecific nucleotide variability was found in the four studied markers: no variability for the 18S rRNA and *coxI*, one nucleotide for the 28S rRNA, and finally three nucleotides for the ITS1 region.

Phylogenetic relationships among *X.* non-*americanum* group species inferred from analyses of D2-D3 segments, the partial 18S rRNA and *coxI* gene sequences using BI are given in Figures 6-8, respectively. The D2-D3 segments gene tree (Fig. 6) based on a multiple edited alignment, 66 sequences of 758 total characters revealed two major well-supported clades (PP = 1.00). The basal clade was formed only by species from Group 5, except for one species from Group 4 (*X. tica*-KY623485), one from Group 7 (*X. bakeri*-KU052864) and finally, one from Group 8 (*X. index*-KX244910). The other major clade clustered *X.* non-*americanum*-group species from all morphospecies groups where molecular data was available (Groups 1, 2, 3, 5, 6, 7 and 8) except for Group 4. *Xiphinema poasense* n. sp. was related phylogenetically with other pseudomonodelphic species, including *X. costaricense* (KX931059) and *X. krugi* (KX931063), which clustered in a well-supported clade (PP = 1.00) yet clearly separated from them. Also, the monodelphic species *X. brasiliense* (AY601616) seems to be related to these three species, although this relationship was not well resolved (PP = 0.64). For partial 18S rRNA and the *coxI* genes (Figs 7, 8), the 50% majority-rule BI trees of a multiple sequence alignment (55 sequences and 1682 characters and 46 sequences and 387 characters, respectively) showed similar topologies, and in both trees *X. poasense* n. sp. did not cluster with any *X.* non-*americanum* group species, occupying a basal and well-supported (PP = 1.00) position. The low similarity and small coverage between the ITS1 region from *X. poasense* n. sp. and the rest of the ITS1 sequences available in GenBank made it impossible to perform a phylogenetic analysis of this region.

Discussion

The new species showed morphological and molecular characteristics that support the identity of *X. poasense* n. sp. as a new species, based on integrative taxonomy and phylogenetic relationships based on nuclear rRNA and mtDNA. This is the first molecular characterisation for a species of the *X.* non-*americanum* Group 3, although more information is needed to get a better understanding of the relationship of this group with the other groups. In this first attempt, the D2-D3 segments, *coxI* and 18S reveal that *X. poasense* n. sp. is clearly different from all *Xiphinema* species by forming a separate clade. In addition, this study contributes to a better knowledge of the diversity of dagger nematodes in Costa Rica, an area that has recently been enriched by new species discoveries and descriptions (Lamberti & Tarjan, 1974; Doucet *et al.*, 1998; Peraza-Padilla *et al.*, 2017a, b).

As mentioned above, morphospecies Group 3 (Loof & Luc, 1990) comprises eight nominal species (including *X. poasense* n. sp.) distributed in tropical and subtropical regions around the latitude of the Tropic of Cancer, and that have only been found in northern Africa and southern Asia (Luc, 1961; Siddiqi, 1963; Khan, 1964; Loof & Yassin, 1970; Southey & Luc, 1973; Lamberti *et al.*, 1996; Dhanam & Jairajpuri, 1997; Loof *et al.*, 2001; Ganguly *et al.*, 2002; Faye *et al.*, 2012). India is the country with

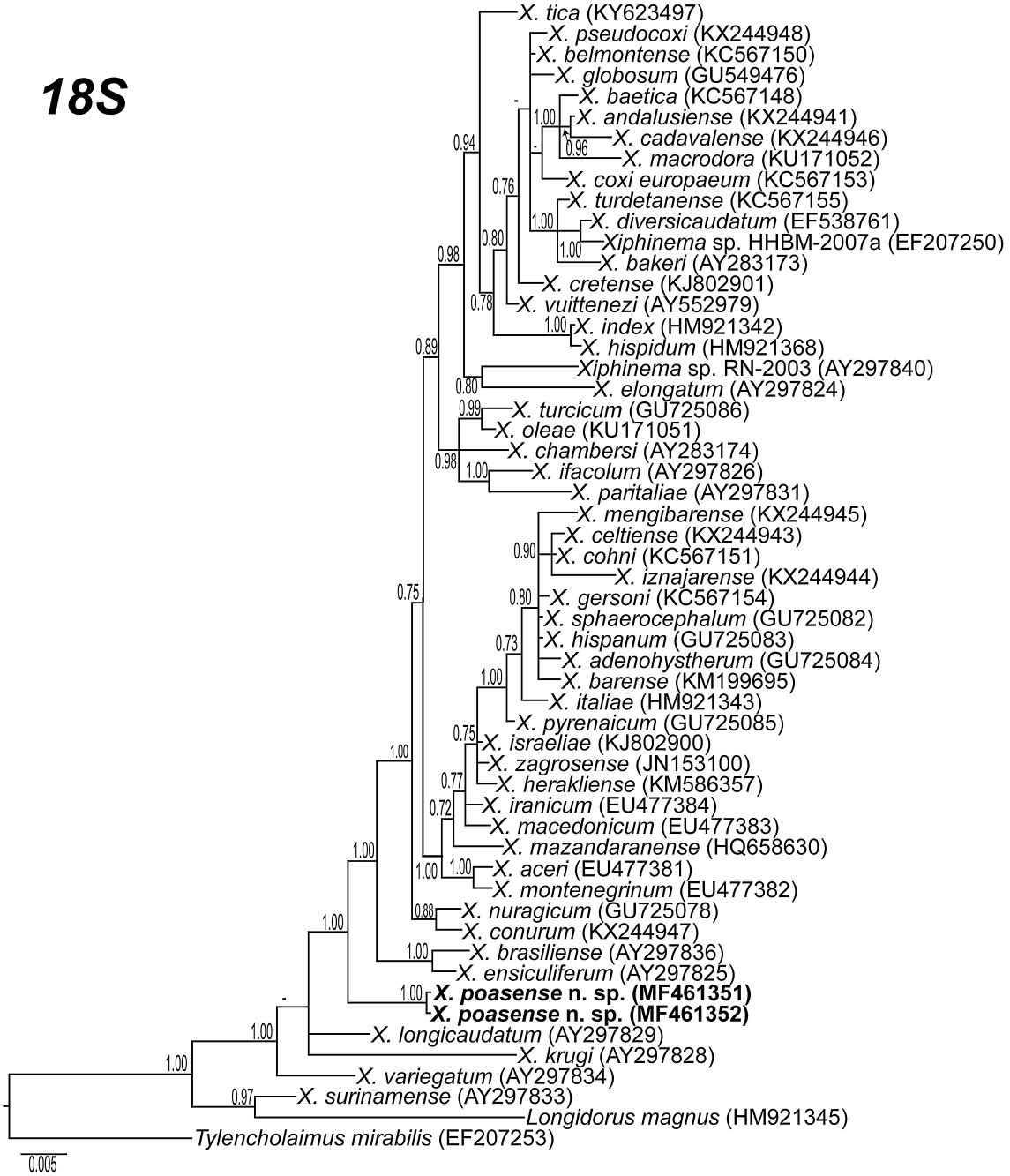

Fig. 7. Phylogenetic relationships within the *Xiphinema* non-*americanum* group complex. Bayesian 50% majority rule consensus tree as inferred from partial 18S rRNA gene sequence alignment under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site.)

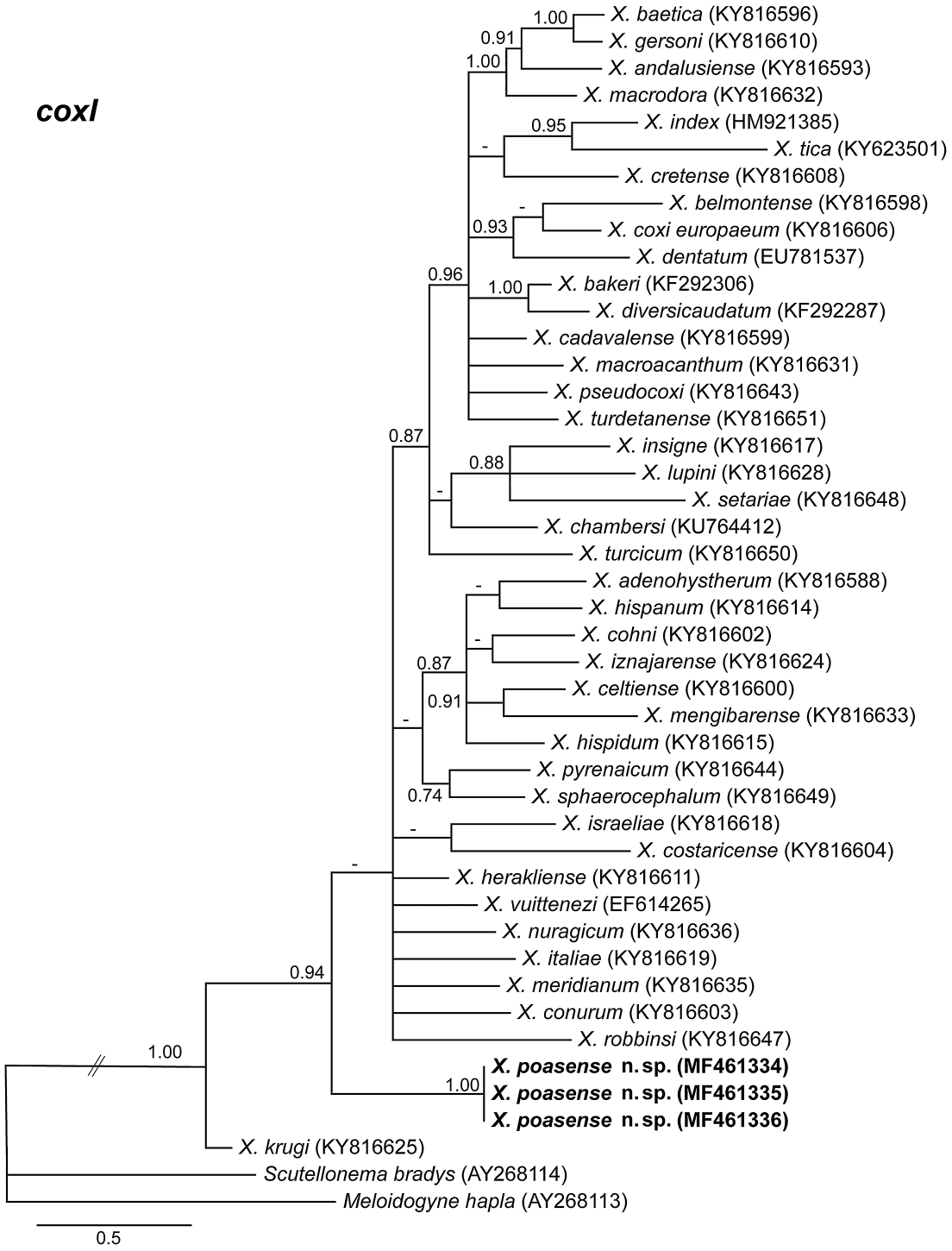

Fig. 8. Phylogenetic relationships within the *Xiphinema* non-*americanum* group complex. Bayesian 50% majority rule consensus tree as inferred from partial cytochrome c oxidase subunit I (*coxI*) sequence alignment under transversion model with invariable sites and a gamma-shaped distribution (TVM + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site.)

the greatest number of species of *X.* non-*americanum* Group 3, including *X. simillimum* Loof & Yassin, 1970; *X. arcum* Khan, 1964; *X. larliani* Loof, Coomans, Baujard & Luc, 2001 and *X. orbum* Siddiqi, 1963 (Siddiqi, 1963; Khan, 1964; Dhanam & Jairajpuri, 1997; Loof *et al.*, 2001). In Egypt, *X. hygrophilum* Luc, 1961 (Southey & Luc, 1973) and *X. simillimum* have been reported (Southey & Luc, 1973; Lamberti *et al.*, 1996). *Xiphinema hygrophilum* was also reported in Ivory Coast (Southey & Luc, 1973). Furthermore, *X. simillimum* Loof & Yassin, 1970 was reported in Sudan (Loof & Yassin, 1970), *X. mounporti* Faye, Barsi & Decraemer, 2012 in Senegal and *X. mali* Ganguly, Singh & Kaushal, 2002 in Nepal. This distribution suggest that morphospecies Group 3, like morphospecies Group 4, is associated with warm and humid conditions (Archidona-Yuste *et al.*, 2016b). This new finding in Costa Rica is the first report of a *X.* non-*americanum* Group 3 for America, confirming the geographical distribution of this group in American tropical regions.

Xiphinema poasense n. sp. has a comparatively long odontostyle for the moderate length of its body (2.4-3.0 mm) and was detected associated with woody plants (eucalyptus, cypress, forest trees). This supports the hypothesis by Archidona-Yuste *et al.* (2016b) that a long odontostyle suggests an adaptive change and a selective advantage in order to exploit a wide host-range of woody roots, and that long nematodes with a long odontostyle could be adapted to live in deeper soils than smaller nematodes. This is in accordance with Yeates (1986), who proposed that the relationship between stylet and nematode body length in several genera of plant-parasitic nematodes is generally positive and significant.

D2-D3 segments, partial 18S and *coxI* were useful markers for species identification as they showed adequate variability among species, confirming *X. poasense* n. sp. as a new species and increasing the known variability within *Xiphinema* (Archidona-Yuste *et al.*, 2016a, b, c; Palomares-Rius *et al.*, 2017). No significant molecular differences among *X. poasense* n. sp. populations from different hosts or localities were found. The present study on the phylogeny based on D2-D3 segments supported a weak correlation in the phylogenetic relationships among the different morphospecies groups within *Xiphinema*, a finding already reported by several authors, *viz.*, Gutiérrez-Gutiérrez *et al.* (2013), De Luca *et al.* (2014), Tzortzakakis *et al.* (2014, 2015) and Archidona-Yuste *et al.* (2016a, b, c). In this case, however, the phylogenetic analysis based on D2-D3 segments showed that *X. poasense* n. sp. was related to pseudomonodelphic species, such as *X. costarricense* and *X. krugi*, both from *X.* non-*americanum* Group 2 (dagger nematodes with the anterior genital branch reduced and incomplete), thereby supporting the following statements of 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 and are related. In any case, more studies of other species from morphospecies Group 3 are necessary in order to resolve the phylogenetic relationships within the genus *Xiphinema*. Phylogenetic relationships in the *X.* non-*americanum* group based on *coxI* coincided with those provided by Palomares-Rius *et al.* (2017), although in the phylogeny of D2-D3 segments and *coxI* and partial 18S rRNA gene, *X. poasense* n. sp. did not cluster with those species having a reduced anterior genital branch, but was in a basal position well separated from *X. costaricense* and *X. krugi*, and from *X. krugi* and *X. brasiliense* in *coxI* and partial 18S rRNA, respectively.

In summary, the present study enlarges the biodiversity of dagger nematodes in Costa Rica and confirms that morphospecies Group 3, previously associated with the tropical and subtropical regions of Africa and Asia, is now known to be present in Central America. The new sequences for molecular markers (D2-D3 segments, ITS1, and *coxI*) were useful for a precise and unequivocal diagnosis of this new species, but further research is required to clarify the evolutionary process involved in the reduction of the anterior genital branch towards the pseudomonodelphic and monodelphic forms of *Xiphinema*.

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