DOLICHODORUS COSTARICENSIS N. SP. (TYLENCHIDA: DOLICHODORIDAE): A NEW AWL NEMATODE SPECIES FROM THE CENTRAL PACIFIC REGION OF COSTA RICA

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ABSTRACT

Esquivel, A., H. Ferris, I. Cid del Prado, and S.A. Subbotin. 2017. *Dolichodorus costaricensis* n. sp. (Tylenchida: Dolichodoridae): A new awl nematode species from the Central Pacific region of Costa Rica. Nematropica 47:143-154.

A new species, *Dolichodorus costaricensis* n. sp., was found in soil samples collected from a native tree in the Central Pacific Region of Costa Rica. It is the second species of the genus *Dolichodorus*, after *D. minor*, to be reported in our country. This new species is described and illustrated herein based on light and scanning electron microscopy. Molecular characterization of *D. costaricensis* n.sp. using the D2-D3 expansion fragments of *28S* rRNA gene is provided. The phylogenetic relationships of this species with other representatives of the genus *Dolichodorus* indicated that *D. costaricensis* n.sp. has a sister relationship with *D. mediterraneus*. *Dolichodorus costaricensis* n.sp. is distinguished from the other 18 species of the genus by combinations of morphological and morphometric characteristics, including total body length (1.9-2.4 mm); body width (32-45 µm); stylet length (74-117 µm); oesophagus length (202-258 µm); and tail length (62-86 µm). Important morphological characters of the species are: a rounded head flattened distally, lip region with a distinctive flat oral disc, vulva, and vagina without evident sclerotization and gubernaculum clearly bent, lobed, and forked at the distal end, a character not previously described for any *Dolichodorus* species. An updated key is provided for identification of species of the genus *Dolichodorus*.

Key words: awl nematode, Costa Rica, *Dolichodorus costaricensis*, *Inga ruiziana*, taxonomy

RESUMEN

Esquivel, A., H. Ferris, I. Cid del Prado and S. A. Subbotin. 2017. *Dolichodorus costaricensis* n. sp. (Tylenchida: Dolichodoridae): Una nueva especie de nematodo daga de la región del Pacífico Central de Costa Rica. Nematropica 47:143-154.

Una nueva especie *Dolichodorus costaricensis* n.sp. fue hallada en muestras de suelo colectadas en la rizosfera de un árbol nativo en el Pacífico Central de Costa Rica. Es la segunda especie del género *Dolichodorus*, después de *D. minor* encontrado hasta ahora en nuestro país. La nueva especie es descrita e ilustrada detalladamente por medio de microscopía de luz y microscopía electrónica de barrido. Además se brinda la caracterización molecular de *D. costaricensis* n. sp. utilizando los fragmentos de expansión D2-D3 del gen *28S* rRNA. Las relaciones filogenéticas de esta especie con otros representantes del género *Dolichodorus*, indican que *D. costaricensis* n.sp. tiene una relación hermana con *D. mediterraneus*. *Dolichodorus costaricensis* n.sp. se distingue de las otras 18 especies del género, por una serie de características morfológicas y morfométricas tales como: longitud total del cuerpo (1.9-2.4 mm), anchura del cuerpo (32-45 µm), longitud del estilete (74-117 µm), longitud del esófago (202-258 µm) y longitud de la cola (62-86 µm). Los caracteres morfológicos considerados importantes en la especie son: una cabeza redondeada aplanada distalmente y la región labial con un disco oral plano distintivo, la vulva y vagina sin esclerotización evidente y el gubernáculo claramente doblado, lobulado y bifurcado en el extremo distal, siendo una característica no descrita en ninguna especie de *Dolichodorus*. Se incluye la clave actualizada para la separación de especies del género *Dolichodorus*.

Palabras clave: Costa Rica, *Dolichodorus costaricensis*, *Nematodo daga*, *Inga ruiziana*, taxonomy.

INTRODUCTION

The awl nematode genus, *Dolichodorus* Cobb, 1914, currently consists of 18 species (Siddiqi, 2000; Geraert, 2011; Gagarin and Nguyen, 2015). They are found in different regions of the world, usually in moist soils (Robbins, 1982; Doucet, 1985; Germani, 1990) and in fresh water and brackish environments (Chow and Taylor, 1978; Smart and Khuong, 1985; Gagarin and Nguyen, 2015). The species are parasites of the roots of higher plants and have been found in forests (Golden *et al*., 1986), grasslands (Luc and Caveness, 1963), aquatic plants (Golden, 1958) and fruit tree orchards (Luc, 1960; Jiménez Guirado *et al*., 2007).

Nematodes of the genus *Dolichodorus* have been rarely found in Costa Rica. The records of *Dolichodorus* in the country are from an area in the southeast, near the Panamá border, where a population of *D. minor* Loof & Sharma, 1975 was detected in association with cacao plantations (López and Salazar, 1989; López 1994). López and Salazar (1989) also found a population of *Neodolichodorus rostrulatus* (Siddiqi, 1976) Siddiqi, 1977 around the roots of coconut palms and wild grasses in beach sand on the Caribbean coast. Those authors suggested that *D. minor* could become an important problem in cacao plantations in Costa Rica. However, *D. minor* has not been detected again since the initial reports. During a period from 1998 to 2002, the National Biodiversity Institute (INBio) in collaboration with the Nematology Department of the Universidad Nacional de Costa Rica (UNA), conducted an extensive nematode inventory of conservation and natural reserve areas of Costa Rica, collecting several hundred soil samples from random locations in a variety of ecosystems. A total of 74 families, 231 genera, and 105 species of nematodes were identified; however, *Dolichodorus* was not detected in any of the samples (Esquivel, 2003; Esquivel and Arias, 2004). Additionally, the Nematology Laboratory of UNA, through a period of more than 20 yr, has analyzed hundreds of soil and root samples collected from more than 140 crops across the country. During that period of time, the laboratory records include 33 genera and 48 species of plant-parasitic nematodes (Esquivel, 2015), but the awl nematode was not encountered in any of the samples. In 2015, a new awl nematode was found in soil samples from *Puntarenas*, in the Central Pacific of Costa Rica. The objective of this work is to describe and to illustrate the new species *Dolichodorus costaricensis* n. sp. from Costa Rica.

MATERIALS AND METHODS

Nematode samples

The composite soil samples containing this new species were collected in July 2015 by H. Ferris around the roots of an *Inga ruiziana* G. Don tree growing on the bank of small stream near San Andrés Road, east of Matapalo, Puntarenas, Costa Rica. Females, males, and juveniles of *Dolichodorus* sp. were found in one of the samples. More specimens were collected in subsequent samples from the same location. Soil samples were analyzed at the Nematology Laboratory at UNA. Nematodes were extracted from samples using Baermann funnel technique and Cobb's modified decanting and sieving method (Barker, 1985). Species identification was carried out using an integrated approach combining morphological and morphometric analyses with molecular and phylogenetic analyses.

Light and scanning electron microscope observations

Nematodes were fixed with hot 4% formaldehyde and brought to pure glycerol by a modification of the method of Seinhorst (1959) for study by light microscopy. Specimens were permanently mounted on glass slides using the paraffin wax ring method (De Maeseneer and d´Herde, 1963), measured with an Olympus BX 50 light microscope and photographed with a Nikon DS-Fi1 digital camera. Drawings were made with a camera lucida.

For the Scanning Electron Microscope (SEM) study, nematodes were fixed in 8% formalin, rinsed twice with cold (5°C) phosphate buffer (pH =7.1; 0.1 M), and dehydrated by transfer through a graded ethanol series (10, 15, 20, 30, 40, 50, 70, 80, 90, 95, and 100 %), 15 min. at each concentration. Critical point drying with liquid CO_2 was performed by Leica EM CPD 300. The nematodes were mounted on aluminum bases, coated with gold to a thickness of 10.2 nm by EMS Quorum 150 RS. Photomicrographs of surface features were made using a Scanning Electron Microscope (Hitachi S-3700 N) at an acceleration voltage of 15 kV.

Molecular and phylogenetic analyses

Nematodes were fixed in ethanol for molecular study. Detailed protocols for DNA extraction, PCR, and sequencing are described by Maafi *et al*. (2003). The D2-D3 expansion segments of the *28S* rRNA

gene were amplified with the forward D2A (5'-ACA AGT ACC GT GAG GGA AAG TTG – 3') and the reverse D3B (5'- TCG GAA GGA ACC AGC TAC TA – 3') primers (Subbotin *et al*., 2006). The new sequence of the D2-D3 of *28S* rRNA gene was aligned using ClustalX 1.83 (Thompson *et al*., 1997) with published sequences of other *Dolichodorus* species and closely related sequences of Dolichodoridae and sequences of *Cephalenchus hexalineatus* and *Aglenchus agricola* were included as outgroups (Subbotin *et al*., 2006; Taheri *et al*., 2013; Stirling *et al*., 2013; Ghaderi *et al*., 2014). The alignment was analyzed with Bayesian Inference (BI) using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) under the GTR + I + G model. BI analysis was run with four chains for 1.0×10^6 generations. Two runs were performed for each analysis. After discarding burn-in samples, other trees were used to generate a 50% majority rule consensus tree. The new *Dolichodorus* sequence was submitted to the GenBank database under the accession number KY860480.

RESULTS

Dolichodorus costaricensis n. sp. (Figs. 1-5;Table 1)

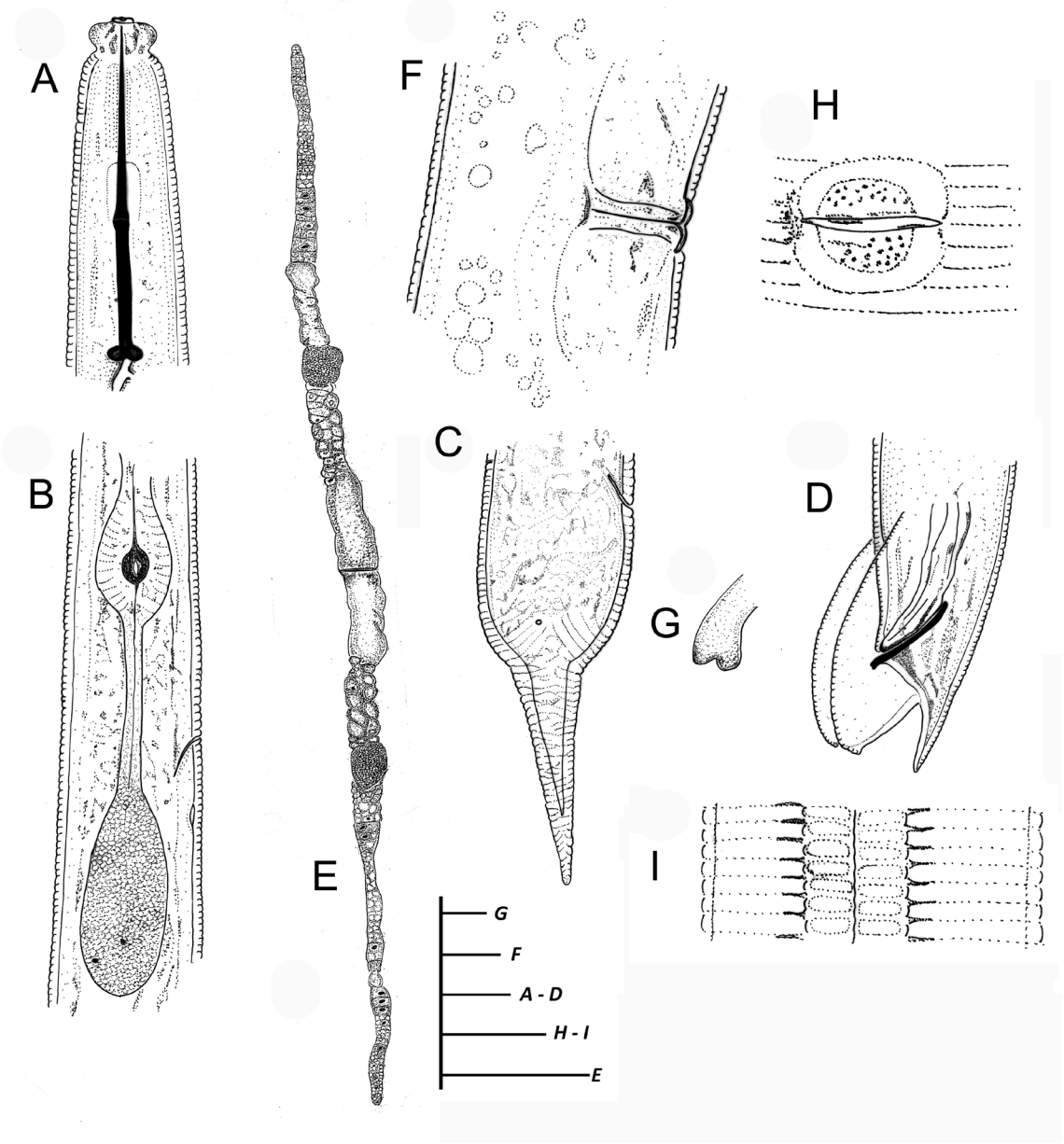

Fig. 1. *Dolichodorus costaricensis* n. sp. A: Female anterior region; B: Median and basal bulb; C: Female tail; D: Male tail, bursa, spicule, and gubernaculum; E: Female gonads (G1 and G2); F: Vulva and vagina in lateral view; G: Detail of gubernaculum distal part; H: Punctuations around vulva; I: Female lateral field, showing oval pattern between lateral lines. Scale bars - A-D: 15 µm; E: 100 µm; F: 10 µm; G: 1.5 µm; H-I: 10 µm.

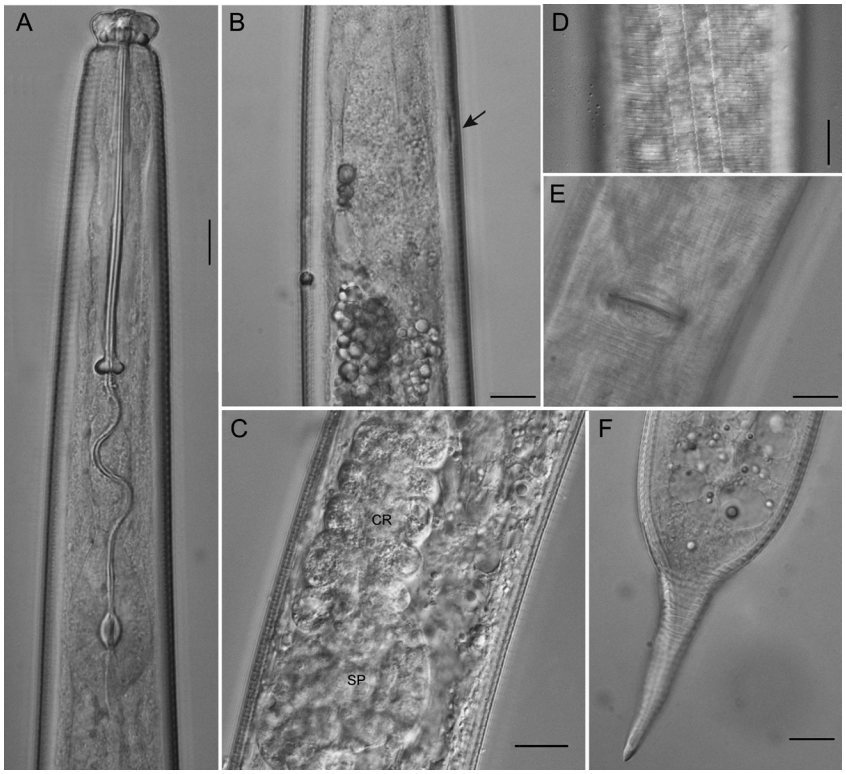

Fig. 2. *Dolichodorus costaricensis* n. sp. Female. A: Anterior body region; B: Basal bulb of the oesophagus and hemizonid (arrow); C: Spermatheca (SP) and crustaformeria cells (CR); D: Lateral field; E: Vulva; F: Tail. Scale bars: 10 µm.

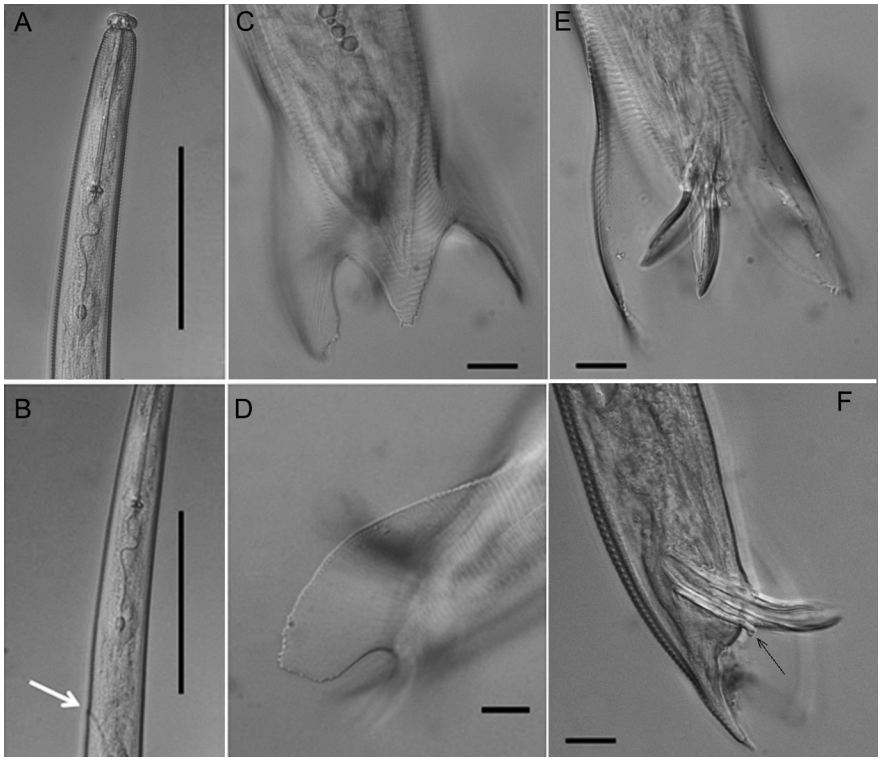

Fig. 3. *Dolichodorus costaricensis* n. sp. Male. A: Anterior body region; B: Excretory pore (arrow); C: Trilobed bursa; D: Striated cuticle; E: Spicules; F: Gubernaculum. Scale bars -A-B: 100 µm, C-F: 10 µm.

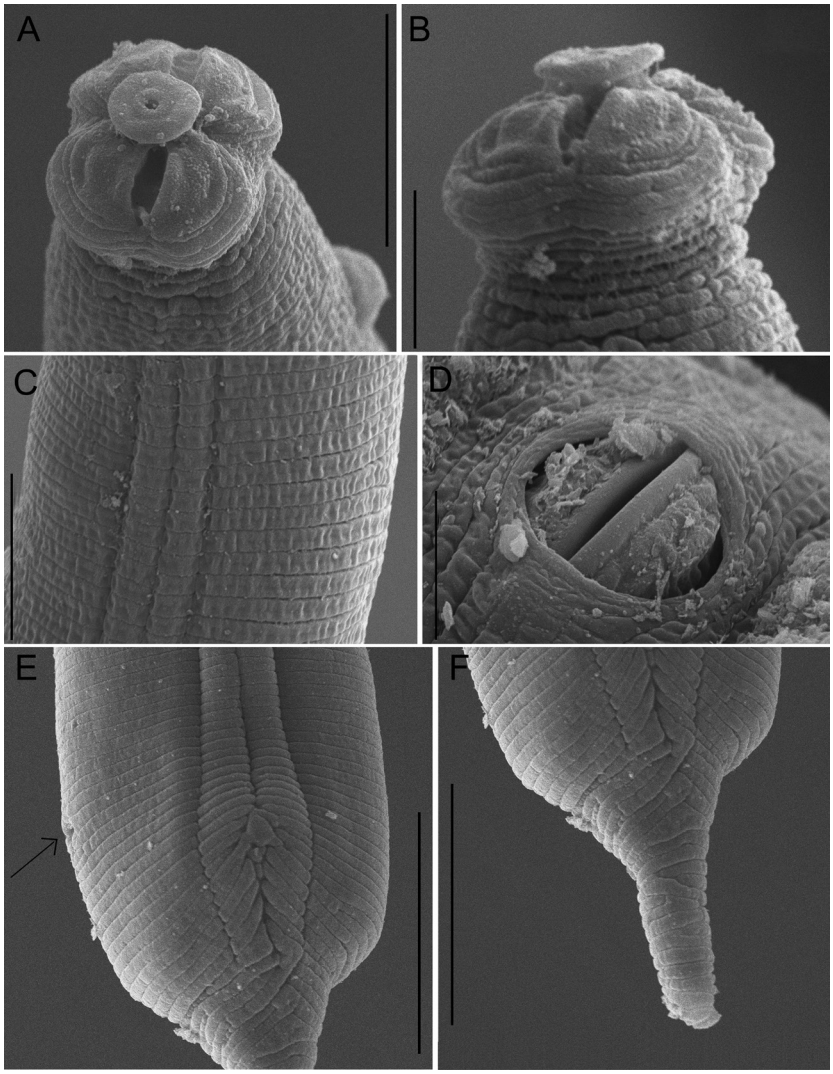

Fig. 4. SEM pictures of *Dolichodorus costaricensis* n. sp. Female. A and B: Anterior end; C: Lateral field; D: Vulva; E: Posterior end and anus (arrow); F: Tail tip. Scale bars - A, C: 10 µm, B, D: 5 µm, E, F: 20 µm.

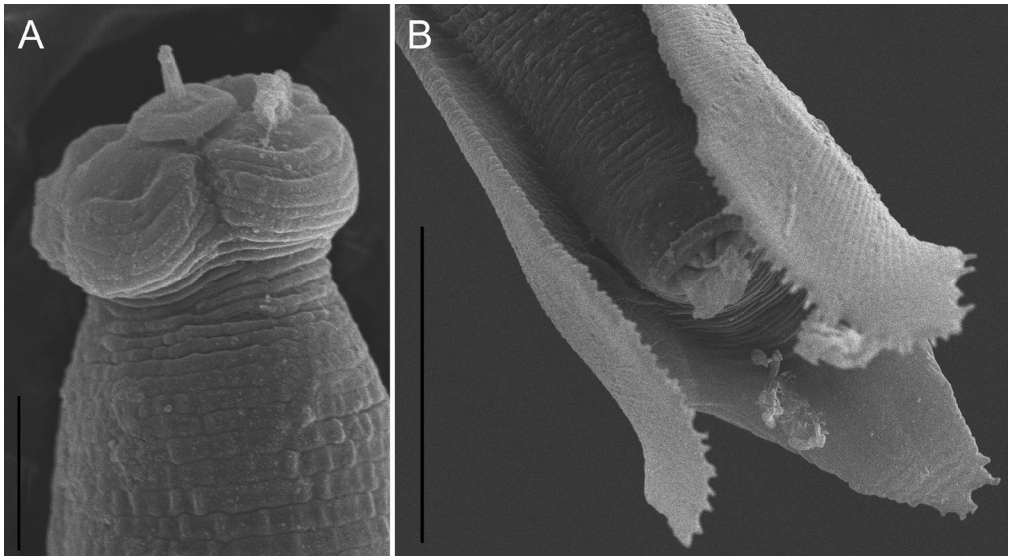

Fig. 5. SEM pictures of *Dolichodorus costaricensis* n. sp. Male. A: Anterior end; B: Bursa. Scale bars - A: 5 µm, B: 20 µm.

Table. 1. Measurements and ratios of *Dolichodorus costaricensis* n. sp. All measurements are in µm and in form: mean ± SD and range.

Character	Holotype female	Paratypes 20 females	Paratype 20 males
L	1898	2162 ± 170 (1867-2457)	1841± 183 (1507.5-2270)
a	48.7	59 ± 6 (48.7-71.3)	60.2 ± 6.2 (53.2-73.2)
b	9.4	9 ± 1 (8.0-10.4)	8.4 ±1.1 (6.4-10.7)
c	30.6	30 ± 2 (23.0-33.3)	60.9 ± 6.4 (52.0-73.0)
c′	1.9	2 ± 0.3 (1.7-2.6)	1.4 ± 0.1 (1.2-1.7)
V (%)	49.3	53 ± 2 (49.3-57.6)	-
Lip region width	15	15 ± 1 (12.0-17.0)	13.5 ± 1.6 (11.0-17.0)
Head height	8	8 ± 1 (7.0-9.0)	7.9 ± 0.5 (7.0-9.0)
Stylet length	74	87± 8 (74.0-117)	80.0 ± 3.4 (75.0-87.0)
Oesophagus length	202	234 ± 17 (202-258)	221.5± 22.3 (172-285)
Exc. pore to anterior	174	182 ± 12 (168-204)	178± 13.9 (151-205)
Body width at midbody	39	37 ± 3 (32.0-45.0)	30.8 ± 3.3 (26.0-40.0)
Anterior ovary	335	473 ± 173 (335-711)	-
Posterior ovary	340	415 ± 109 (355-581)	-
Vulva to anterior end	936	1150 ± 88 (936-1283)	-
Body width at anus or cloaca	32	34 ± 3 (30.0-38.0)	21.7 ± 2.3 (18.0-27.0)
Tail	62	73 ± 8 (62-86)	30.4 ± 3.2 (25.0-36.0)
Spicules	-	-	38.8 ± 2.1 (36.0-44.0)
Gubernaculum	-	-	20 ± 1.7 (17.0-22.0)

Description

Female. Slender nematodes of medium length, 1.8-2.4 mm. Habitus almost straight to slightly curved in the posterior part of the body after fixation. Body cylindrical, tapering abruptly in the tail region. Outer cuticle layer with distinct transverse striations separating annuli that are 1.5 µm wide at mid-body. Lateral field with three incisures demarcating two lateral longitudinal lines, areolated 10 µm wide or occupying 28% of the corresponding mid-body diameter. Head rounded, flattened distally, formed by four lobes, each with four annuli, distinctly offset from the body contour by a deep depression with four very fine annuli. Lip region has a distinctive flat oral disc, 4-5 µm wide. SEM micrographs reveal the conspicuous amphidial openings having longitudinal slit shapes (Fig. 4A). Stylet long and well developed, with the conus occupying more than 50 % of the stylet length and slightly curved in some specimens. Stylet knobs strong and oviform. Dorsal pharyngeal gland orifice (DGO) located 2.0-4.0 µm behind the stylet base. Lumen distinctive throughout the entire procorpus. Median bulb is well developed, ovate with a strong valvular apparatus, and occupies around 50% to 60% of the corresponding body diameter. Isthmus long and straight. Excretory pore difficult to observe in fixed specimens, located posterior to the median bulb in the last third of the isthmus, in average 182 ± 12 µm from the anterior end. Hemizonid appears as a dark slit (Fig. 1B, 2B), 5 µm long, located between 5-15 µm posterior to the excretory pore. Basal bulb of the oesophagus is elongate-saccate, 1.3-1.7 times as long as the body diameter at its base and does not overlap the intestine. Cardia not seen. Reproductive system didelphic -, amphidelphic. Ovaries outstretched and similar in length. Ovary occupies about 60% of total length of each genital branch and has oocytes in a single row. Spermatheca rounded, 17-20 µm wide, filled with rounded sperm cells 2.5–3.0 µm in diameter. Uterus complex, crustaformeria present and composed of two or three rows of rounded cells. In some specimens, eggs were observed in the uterus. Vulva a transverse slit, without evident sclerotization, located equatorially. Under SEM, the vulva shows a distinct characteristic not described before. A circular area bordered by a well-demarcated cuticular ring, surrounds the vulva slit (Fig. 4D). Under the light microscope, punctuations appear in the cuticular depression surrounding the vulva. These do not appear in the SEM images, suggesting that they are

within the cuticle rather than on the surface. Intestine dark, rectum 16-19 µm long. Phasmids (Fig. 1C) very small, pore like, located 19-22 µm from the anus, almost at the beginning of the tail projection. Tail abruptly tapering, tail projection 35-45% of tail length.

Male. Similar to female but smaller and thinner, 1.5-2.3 µm in length. Habitus straight to slightly curved in the posterior part of the body after fixation. Body distinctly tapering just after the cloaca. Outer cuticle layer with distinct transverse striations that separate annuli about 1.0 µm wide at mid-body. Lateral field with three incisures and two longitudinal lines, areolated, 7-8 µm wide or occupying 18% to 27% of the corresponding mid-body diameter, ending at the beginning of the caudal alae. Head rounded but flattened anteriorly, consisting of four lobes, each with four annuli. Lobes clearly offset from the body by a deep depression with four very fine annuli. Lip region has a flat oral disc, 3.5-5 µm wide. Stylet is well developed with the conus occupying 55% to 60% of the total stylet length. Stylet knobs well developed, oval shaped. DGO located 1.5-2.0 µm posterior to the stylet base. Median bulb well developed, oval shaped, with a strong valvular apparatus. Isthmus long and straight. Excretory pore difficult to observe but opens posterior to the median bulb in the last third of the isthmus, on average 178 ± 13.9 µm from the anterior end. Hemizonid located just behind excretory pore. Basal bulb elongate-saccate, not overlapping the intestine. Reproductive system is monorchic with the gonad outstretched. Spicules well developed, slightly curved ventrally, blades with membranous extension (velum), manubrium plain, telamon (a cuticularized guiding thickening of the ventral wall of the cloaca) absent. Gubernaculum straight, projecting outside of the body with the distal end clearly bent, lobed and forked (Fig. 1G). Caudal alae trilobed, typical for the genus, with very fine transverse striations. SEM images of the male tail, show serrated edges of the edges of the caudal alae. Phasmids very small, pore like. On average, the tail tip projection is 65% of the total tail length.

Type host, locality, and habitat: Soil around the roots of *Inga ruiziana* G. Don (Fabaceae) tree, on the bank of a small stream near San Andrés Road in the hills east of Matapalo, Puntarenas, Costa Rica (GPS: 9˚ 20' 3.8" N, 83˚ 56' 22.8" W), 106 m above sea level. The area is characterized by a mixture of trees, vines, and understory. According to the Holdridge Life Zone system (Holdridge, 1967), the region corresponds to a tropical wet forest, premontane transition. The average rainfall is 3500 mm per year, with a range of temperature between 22.7ºC and 31ºC. The soil texture at the sampling site is rocky sandy clay loam as determined by the Bouyoucos method (Cervantes and Mojica, 1995).

Type material: Holotype female on slide L25-2016, paratypes on slides L5, L6, L9, and L11-2016, males on slides L2, L4, and L10-2016 are in the Nematode Reference Collection of the Laboratorio de Nematología, Escuela de Ciencias Agrarias, Universidad Nacional, Costa Rica. Paratype females and males on slides L5, L6, and L7-2015 are deposited in the USDA Nematode Reference Collection in Beltsville, MD, USA.

Etymology: The specific epithet is derived from the country of origin, Costa Rica, and the latin suffix ensis, meaning belonging to or from. Zoobank number: 7F39FB44-A993-4C2B-9265-B025D50BADC4.

Diagnosis and relationship

Eighteen nominal species of *Dolichodorus* have been described so far. The most important morphological diagnostic characters of species for this genus include: stylet length, position of the excretory pore, female and male tail characteristics, shapes and sizes of caudal alae, spicules and gubernaculum. Geraert´s key (2011) separated the species of *Dolichodorus* based on the shape of labial region and position of the excretory pore in relation to the median bulb. Of the 18 species, only 6 have the lip region flattened (*D. pulvinus* Khan *et al*., 1971; *D. kishansinghi* Jairajpuri and Rahmani, 1977; *D. nigeriensis* Luc and Caveness, 1963; *D. profundus* Luc, 1960; *D. silvestris* Gillespie and Adams, 1962; and *D. orientalis* Gagarin and Nguyen, 2015). *Dolichodorus costaricensis* n. sp. shares the flattened lip region characteristic with these species. It is similar to *D. kishansinghi* and *D. orientalis* in the excretory pore position being located posterior to the median bulb, but it differs from *D. kishansinghi* by the shorter tail (62-86 µm vs. 103-105 µm), shorter oesophagus (202-258 µm vs. 255-328 µm) and higher c value (23-33 vs. 23-25), and from *D. orientalis* by the shorter and narrower body (1.9-2.4 mm, 32-45 µm vs. 2.9-3.0 mm, 72-83 µm), shorter oesophagus (202-258 µm vs. 259-281 µm)and tail (62-86 µm vs. 103-108 µm) and values of De Man´s ratios (a: 49-71, c: 23-33, c´: 1,7-2,6 vs. a: 35-47, c: 59-70, c´: 0.8-1.0).

The following list contains all the species of *Dolichodorus* currently described: (*D. heterocephalus* Cobb, 1914; *D. similis* Golden, 1958; *D. profundus* Luc, 1960; *D. silvestris* Gillespie and Adams, 1962; *D. nigeriensis* Luc and Caveness, 1963; *D. pulvinus* Khan *et al*., 1971; *D. minor* Loof and Sharma, 1975; *D. kishansinghi* Jairajpuri and Rahmani, 1977; *D. aestuarinus*

Chow and Taylor, 1978; *D. longicaudautus* Doucet, 1981; *D. marylandicus* Lewis and Golden, 1981; *D. grandaspicatus* Robbins, 1982; *D. aquaticus* Doucet, 1985; *D. miradvulvus* Smart and Nguyen, 1985; *D. cobbi* Golden *et al*., 1986; *D. pellegrini* Germani, 1990; *D. mediterraneus* Jiménez *et al*., 2007; *D. orientalis* Gagarin and Nguyen, 2015).

The new species, *D. costaricensis* n. sp., differs from the species listed above in the following characteristics: A) Total body length (1.9-2.4 mm) vs *D. aestuarius* (2.5-2.9 mm), *D. aquaticu*s (2.5-3.5 mm), *D. longicaudatus* (2.4-2.8 mm), *D. minor* (1.3-1.9 mm), *D. nigeriensis* (1.8-1.9 mm), *D. orientalis* (2.9-3.0 mm), *D. silvestris* (2.6-3.6 mm), *D. similis* (2.9-3.1 mm); B) Body width (32-45 µm) vs. *D. aestuarius* (65 µm), *D. orientalis* (72-83 µm), *D. profundus* (50 µm), *D. pulvinus* (27 µm), *D. silvestris* (52 µm), *D. similis* (58 µm); C) Stylet length (74-117 µm) vs. *D. aestuarius* (62-76 µm), *D. cobbi* (114-129 µm), *D. pulvinus* (105-129 µm), *D. silvestris* (132-162 µm), *D. miradvulvu*s (105-120 µm); D) Oesophagus length (202-258 µm) vs. *D. aquaticus* (240-405 µm), *D. cobbi* (303 µm), *D. kishansinghi* (255-328 µm), *D. nigeriensis* (267 µm), *D. orientalis* (259-281 µm), *D. profundus* (270 µm), *D. silvestris* (360 µm); E) Tail length (62-86 µm) vs. *D. cobbi* (32-40 µm), *D. kishansinghi* (103-105 µm), *D. longicaudatus* (83-100 µm), *D. orientalis* (103-108 µm), *D. similis* (78-99 µm).

Dolichodorus costaricensis n. sp. is separated from the remaining described species on the basis of combinations of the following characteristics. It differs from *D. heterocephalus* in having a shorter oesophagus (234 vs. 306 µm), smaller c value (23-33 vs. 36-47) and shorter gubernaculum (17-22 vs. 23-32 µm); from *D. marylandicus* in having a shorter gubernaculum (17-22 vs. 24-29 µm), a smaller DGO distance from the base of the stylet (2-4 vs. 4-6 µm) and longer tail (62-86 vs. 50-64 µm); from *D. mediterraneus* in having a greater b (8.0-10.4 vs. 7.3) and c values (23-33 vs. 14-26), shorter c' value (1.7-2.6 vs. 2.7- 4.5), and the excretory pore located posteriorly vs. at same level with the median bulb; from *D. pellegrini* in having a shorter c' value (1.7-2.6 vs. 2.8-3.5), and the excretory pore located posteriorly vs. anteriorly to the median bulb; from *D. miradvulvus* in having shorter c value (23-33 vs. 34-45), excretory pore posterior, vs. level with to, the median bulb, males with shorter spicules (36-44 vs. 45-52 µm) and shorter gubernaculum (17-22 vs. 25-28 µm).

Several morphometric characters and De Man ratios of *D. costaricensis* n. sp. are similar to those of *D. grandaspicatus*. Analyses of these characters are not useful for separating the two species; however, the morphology of these two species is different (Robbins, 1982). *Dolichodorus costaricensis* n. sp. differs from *D. grandaspicatus* in having a rounded head that is flattened distally with a distinctive flat and circular oral disc vs. rounded lip region and oval oral disc; the vulva and vagina without sclerotization vs. strongly sclerotized; the female tail tip without mucron vs. tail ending in a small mucron; the male with simple conical terminus vs. bifurcate tail terminus. The gubernaculum of *D. costaricensis* n. sp. is shorter (17-22 vs. 22-27 µm) and has a characteristic not described in any *Dolichodorus* species, the distal end is clearly bent, lobed and forked, resembling the distal head of a mammalian femur (Fig. 1G and Fig. 3F) vs. an extrudable, slightly-curved distal end that is characteristically hook-shaped and has an accessory structure interposed with the adjacent spicule. This accessory structure does not appear in *D. costaricensis* n. sp. Comparison of the SEM images of *D. costaricensis* n. sp. and *D. grandaspicatus* reveals clear differences between both species. *Dolichodorus costaricensis* n.sp has a wider amphidial opening, the female´s tail is pointed, but the tip rounded, and the male bursa has serrated edges. These characteristics are not observed in *D. grandaspicautus*.

Phylogenetic relationships and molecular characterization

The relationships of *D. costaricensis* n. sp. with other species and genera are depicted in Fig. 6. The new species forms a highly supported clade as a sister species with *D. mediterraneus* in the tree. The D2-D3 expansion segments of the *28S* rRNA gene sequence of *D. costaricensis* n. sp. differs from those of *D. mediterraneus* by 15% or 113 bp.

DISCUSSION

Dolichodorus costaricensis n.sp. is a species well defined morphologically. It shows large morphometric differences from *D. aestuarinus, D. aquaticus, D. cobbi, D. kishansinghi, D. longicaudatus, D. minor, D. miradvulvus, D. nigeriensis, D. orientalis, D. profundus, D. pulvinus, D. silvestris* and *D. similis*. Some difference with *D. heterocephalus, D. marylandicus, D. mediterraneus, D. pellegrini* and little difference with *D. grandaspicautus*. In this last case, anatomical characters observed with the light and scanning electron microscope allowed us to clearly separate both species.

Most of the *Dolichodorus* species described to date were done before the molecular era. This is a strong limitation if we want to know how close or far they are between them at molecular base. Our study,

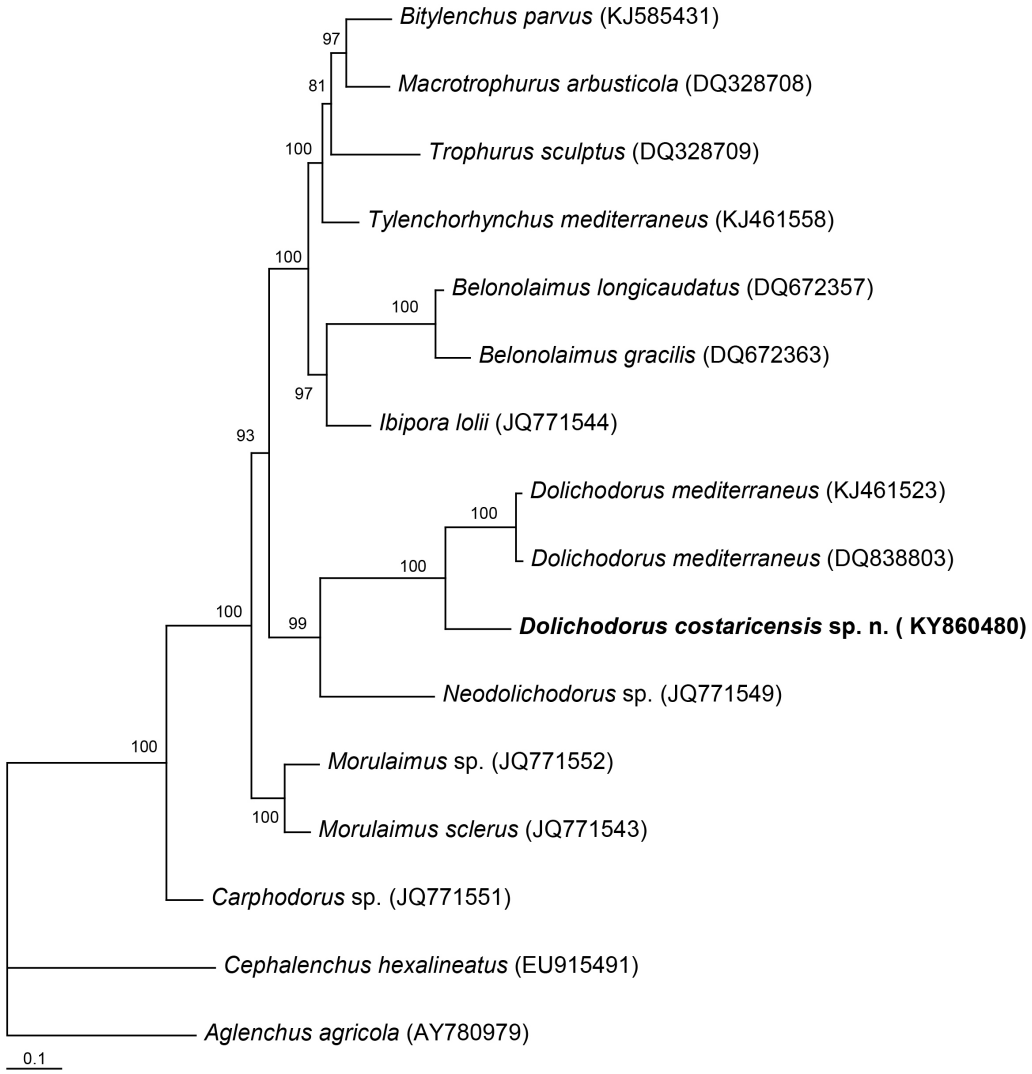

Fig. 6. Phylogenetic relationships of *Dolichodorus costaricens*is n. sp. within related species and genera. Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the D2-D3 of *28S* rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal or more than 70% are given for appropriate clades. New sequence is indicated by bold font.

reveals that *D. costaricensis* n. sp. has a phylogenetic close relationship with *D. mediterraneus*. They share important morphological characters such as body size and stylet length of the females and the length of the spicule and gubernaculum of the males, but differ in the configuration of the lips, position of the excretory pore, tail length, and De Man ratios. It is very interesting that of all *Dolichodorus* species described, 13 of them are from America (8 from USA, 2 from Argentina, 1 from Brazil, and 1 from Costa Rica), 2 from India, 2 from Africa, 1 from Asia, 1 from Oceania, and 1 from Europe. This low number of described species of the genus is probably due to the dearth of specialized laboratories and taxonomic expertise in some areas of the world. Thus, it is not surprising that Costa Rica, as a Neotropical Country, has hundreds of new nematode species that have not been discovered yet, even though the country is considered one of the most biologically-diverse regions on the planet (Obando, 2002). Therefore, for groups such as nematodes, the inventory is very incomplete.

Species of *Dolichodorus* have commonly been described as moist and wet, coarse-textured or well-aggregated soils, which provide adequate pore space for the activity of these large nematodes (Golden,

1958; Chow and Taylor, 1978; Robbins, 1982; Doucet, 1985; Golden *et al*., 1986; Jiménez Guirado *et al*., 2007; Silva *et al*., 2008a, 2008b; Jagdale *et al*., 2013; Gagarin and Nguyen, 2015). In Costa Rica, *D. costaricensis* n.sp. was found in sandy clay loam soil confirming the ability of *Dolichodorus* species to adapt to soils having different texture. Apart from the studies on *D. heterocephalus*, there has been no work on the life history attributes of nematodes in the genus.

Members of *Dolichodoridae* are obligate ectoparasites of plant roots. Those species with short stylets feeding from cells in epidermal and superficial layers of the root, those with a long stylet feeding from cells in deeper tissues. During feeding of *D. heterocephalus*, Paracer *et al*. (1967) observed discrete root galls, curvature at the root tips, localized lesions in the root cortex and epidermis, and some enlargement of nuclei in cortical cells at the feeding site. Feeding of *D. miradvulvus* at roots of the aquatic plant *Anubias nana*, can result in complete destruction of the root system (Smart and Khuong, 1984, 1985; Smart and Nguyen, 1991). The damage caused by *D. heterocephalus* on a range of plants, including celery, garden balsam and sweet corn, planted in the moist sandy soils are well documented (Christie, 1952; Tarjan *et al*., 1952; Perry, 1953; Paracer *et al*., 1967). A list of terrestrial plants and agricultural crops considered hosts for *Dolichodorus* spp. was assembled by Smart and Nguyen (1991). The finding of a new species from *Inga ruiziana* in Costa Rica provided an additional host record for the genus. However, the role played by this species in the ecosystem where it was found it is not known.

Key to the species of *Dolichodorus* after Jiménez Guirado *et al.* (2007) and Geraert (2011) with modifications

1a. Lip region flattened 2
1b. Lip region rounded 7
2a. Excretory pore anterior to median bulb ..... *D. pulvinus*
2b. Excretory pore posterior to median bulb 3
2c. Excretory pore opposite median bulb 5
3a. Female tail 62-86 µm ..... *D. costaricensis* n.sp
3b. Female tail > 100 µm 4
4a. Female stylet length 87-90 µm, spicules length 44 µm ..... *D. kishansinghi*
4b. Female stylet length 77-80 µm, spicules length 63-68 µm ..... *D.orientalis*
5a. Female c' < 3.2; L < 2mm ..... *D. nigeriensis*
5b. Female c' = 1-2; L > 2mm 6
6a. L = 2.1-2.5 mm; stylet length = 105-117 µm ..... *D. profundus*
6b. L = 2.6-3.6 mm; stylet length = 132-162 µm ..... *D. silvestris*
7a. Excretory pore opposite median bulb 8
7b. Excretory pore opposite isthmus 11
7c. Excretory pore opposite basal bulb ..... *D. similis*
8a. Cuticle anterior and posterior to vulva with deep grooves ..... *D. miradvulvus*
8b. Cuticle anterior and posterior to vulva without deep grooves 9
9a. Excretory pore opposite anterior region of median bulb; female tail tapering smoothly to a relatively short attenuated posterior part ..... *D. pellegrini*
9b. Excretory pore opposite posterior region of median bulb; female tail tapering abruptly 10
10a. Female tail = 49-92 µm; stylet length = 90-110 µm ..... *D. heterocephalus*
10b. Female tail = 72-122 µm; stylet length = 78-106 µm ..... *D. mediterraneus*
11a. Stylet length = 114-129 µm ..... *D. cobbi*
11b. Stylet length < 105 µm 12
12a. L = 1.3-1.9 mm ..... *D. minor*
12b. L > 2mm (but *D. marylandicus* has some shorter females) 13
13a. Stylet length = 62-76 µm ..... *D. aestuarius*
13b. Stylet length > 79 µm 14
14a. Female tail = 50-64 µm, tapering abruptly to an acuminate, often spicate, terminus ..... *D. marylandicus*
14b. Female tail = 60-145 µm 15
15a. Female tail tapering gradually from anus to terminus ..... *D. longicaudatus*
15b. Female tail narroring suddenly at one third to half its length 16
16a. L = 2.5-3.5 mm; male spicule length 48-67 µm ..... *D. aquaticus*
16b. L = 2.1-2.7 mm; male spicule length 36-44 µm ..... *D. grandaspicautus*

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