RESEARCH/INVESTIGACIÓN

A NEW SPECIES OF THE GENUS *DIASTOLAIMUS* AND REDESCRIPTION OF *D. MEXICANUS* CID DEL PRADO, 2012 (RHABDITIDA: CHAMBERSIELLIDAE) FROM MEXICO

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ABSTRACT

Cid del Prado-Vera, I., H. Ferris, and S. A. Subbotin. 2023. A new species of the genus *Diastolaimus* and redescription of *D. mexicanus* Cid del Prado, 2012 (Rhabditida: Chambersiellidae) from Mexico. Nematropica 53:30-40.

A new species, *Diastolaimus noffsingeri* sp. n., and redescription of *D. mexicanus* collected from mosses and lichens growing on tree branches in México were morphologically studied using light and electronic microscopy. Molecular characterization of these two *Diastolaimus* species with the D2-D3 expansion segments of 28S rRNA gene sequences was also conducted. Phylogenetic analysis of sequences of *Diastolaimus* species and other related nematodes revealed that that genus has a sister relation with the representatives of the genus *Geraldius*. Molecular analysis showed that 28S rRNA gene sequences of *D. mexicanus* collected from the type and other locations were different from those of *D. grossus*. The diagnosis of the genus *Diastolaimus* was emended.

Key words: 28S rRNA gene, *Diastolaimus*, lichen, moss, new species, phylogeny, SEM

RESUMEN

Cid del Prado-Vera, I., H. Ferris, and S. A. Subbotin. 2023. Nueva especie del género *Diastolaimus* y redescripción de *D. mexicanus* Cid del Prado, 2012 (Rhabditida: Chambersiellidae) de México. Nematropica 53:30-40.

Una nueva especie *Diastolaimus noffsingeri* sp. n. y *D. mexicanus* recolectadas de musgos y líquenes que crecen en las ramas de árboles en México se estudiaron morfológicamente usando microscopia de luz y electrónica. También se realizó una caracterización molecular de estas dos especies de *Diastolaimus* con los segmentos de expansión D2-D3 de las secuencias del gen 28S rARN. El análisis filogenético de las secuencias de especies de *Diastolaimus* y otros nematodos relacionados reveló que ese género posee una relación de hermanas con los representantes del género *Geraldius*. El análisis molecular mostró que las secuencias del gen 28S rARN de *D. mexicanus* recolectadas del tipo y de otras localidades eran diferentes de las de *D. grossus*. Se modificó el diagnóstico del género *Diastolaimus*.

Palabras clave: gen 28S rARN, *Diastolaimus,* líquen, musgo, nueva especie, filogenia, SEM

INTRODUCTION

Nematodes of the genus *Diastolaimus* are currently assigned to the subfamily Macrolaiminae of the family Chambersiellidae (Čermák *et al.*, 2022). Until now, the genus *Diastolaimus* Rahm, 1928 contained six species, described from North and Central America and Europe: *D. papillatus* Rahm, 1928 which is the type species, *D. croca* (Massey, 1963) Andrássy, 1984, *D. grossus* (Truskova and Eroshenko, 1977) Andrássy, 1984, *D. damalis* (Massey, 1966) Andrássy, 1984, *D. aculentus* (Von Daday, 1905) Andrássy, 1984, and *D. mexicanus* Cid del Prado, 2012. Recently, Čermák *et al.* (2022) redescribed *D. grossus* and proposed *D. mexicanus* as a junior synonym of *D. grossus*. Čermák *et al.* (2022) also provided a morphological key for identification of *Diastolaimus* species.

During a nematological survey in Mexico, several samples contained *Diastolaimus* spp. Based on morphological and molecular assessment of these samples; a new species is described here. We also redescribe *D. mexicanus* and emend the diagnostics of the genus *Diastolaimus*.

MATERIALS AND METHODS

Nematode samples. Several samples of mosses and lichens growing on tree branches of oak, pine, and other trees were collected from different locations in Mexico. Five samples were collected from moss growing on a *Buddleia cordata* Kunth tree in the type locality of *D. mexicanus* in San Diego Huehuecalco, Amecameca, Mexico State. Samples were placed in separate jars with water at room temperature and agitated at 12-hr intervals. The suspension was poured through 60- and 325-mesh sieves every 24 hr over a period of 3 days, using a method described by Cid del Prado (2012). Nematodes were processed to glycerine and then nematodes were handpicked for mounting on glass slides using the paraffin wax ring method (Cid del Prado, 2012).

Morphological study. Measurements and drawings were made using a drawing tube mounted on an American Optical Division compound microscope (New York, NY). The specimens for scanning electron microscope studies were washed in magnesium buffer solution, pH 7.2, for 20 min and dehydrated in a series of ethanol solutions, from 10 to 100% in 10 stages, for 15 min each. Samples were then critical-point dried before coating with gold/palladium (80/20%) for 4 min and observation in a Jeol JSM-6390 microscope (Tokyo, Japan) at 10 KV acceleration voltage (Cid del Prado, 2012).

Molecular study. Nematodes collected from three locations were used in the molecular study. DNA was extracted from one to five specimens. DNA extraction with the proteinase K, PCR and cloning protocols were used as described by Subbotin (2021a). The forward D2A (5′ - ACA AGT ACC GTG AGG GAA AGT TG – 3′) and reverse D3B (5′ - TCG GAA GGA ACC AGC TAC TA – 3′) primers were used for PCR amplification of the D2-D3 expansion segments of 28S rRNA gene (Subbotin, 2021a). Sequencing was conducted at Genewiz (California, USA). Sequences were submitted to the GenBank database under accession numbers: OQ286104, OP407671, OP407671 (*D. mexicanus*), and OP407673 (*D. noffsingeri* sp. n.). New sequences of the D2-D3 of 28S rRNA gene were aligned using ClustalX 1.83 (Chenna *et al.*, 2003) with modified alignment parameter (5:3) with published sequences (Nadler *et al.*, 2006; Holovachov *et al.*, 2015; Cid del Prado Vera *et al.*, 2021; Čermák *et al.*, 2022). The alignment was analyzed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) under the GTR + G + I model as described by Subbotin (2021b).

Descriptions

Diastolaimus noffsingeri sp. n.
(Fig. 1-2)

Female. Body an open C-shape, curved ventrad when heat relaxed, attenuated towards both ends; cuticle very finely striated; head rounded, continuous with body contour, 7.0 - 13.2 (10.4 ± 1.3) µm wide and 3.8 µm high; labial region with six triangle-shaped lips, each with one filament-like cirrus at the base; six outer conoid, setose sensilla, 4 µm long; and four papilliform cephalic sensilla, located near the bases of the outer setae (Table 1). Cephalic papillae shorter than the anterior part of gymnostom. Stoma divisible into

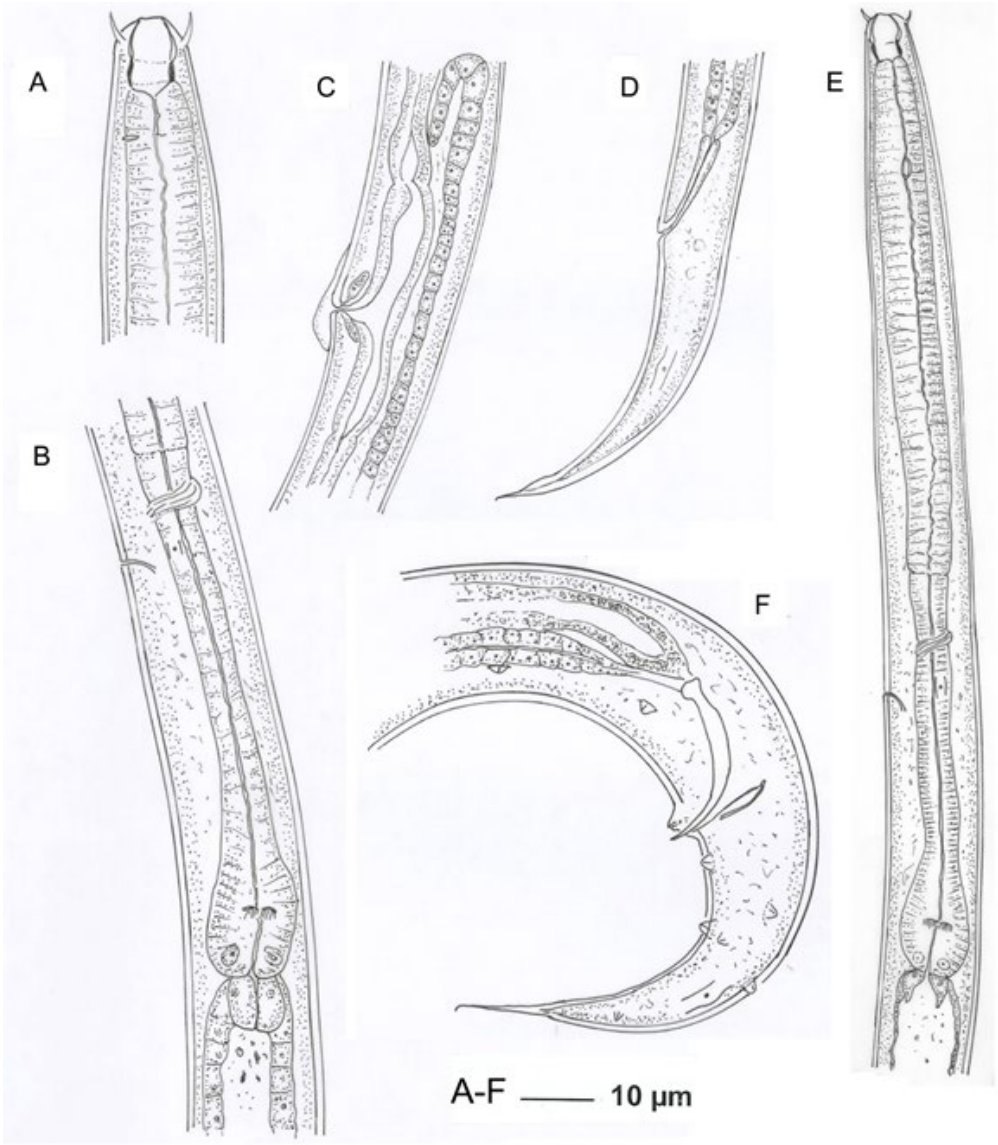

Figure 1. *Diastolaimus noffsingeri* sp. n. Female (A-D). A: Anterior end, head; B: Posterior end of pharynx; C: Vulva region, lateral view; D: Tail. Male. (E, F). E: Body anterior end, pharynx region; F: Tail lateral view.

three regions: a slightly sclerotized cheilostom, a heavily sclerotized gymnostom, and a funnel-shaped stegostom with a thin cuticular wall and enveloped by muscular pharyngeal tissue. Oval amphid apertures located at the level of half the length of the stegostom and 14.0 - 26.0 (19.8 ± 3.5) µm from the anterior end. Lateral field with three incisures. Dorsal pharyngeal gland orifice found in the last third of stegostom. Pharyngeal corpus cylindrical without a median bulb, lumen with clearly visible cuticular lining. Narrow isthmus with thinner cuticular lining of the lumen than the corpus; basal pharyngeal bulb oval, with strong transverse butterfly valve apparatus and visible dorsal and subventral pharyngeal glands located in the posterior part of basal bulb. Nerve ring encircling the pharynx in the anterior part of the isthmus. Excretory pore posterior to nerve ring, clearly visible and deirids slightly anterior to excretory pore. Reproductive system didelphic-amphidelphic, ovaries opposed and reflexed with oocytes arranged in a single row, in some specimens crossing beyond the position of the vulva, vagina with thick transverse muscular layers. Two small vulval glands present, one anterior and the other posterior to the vulva lips

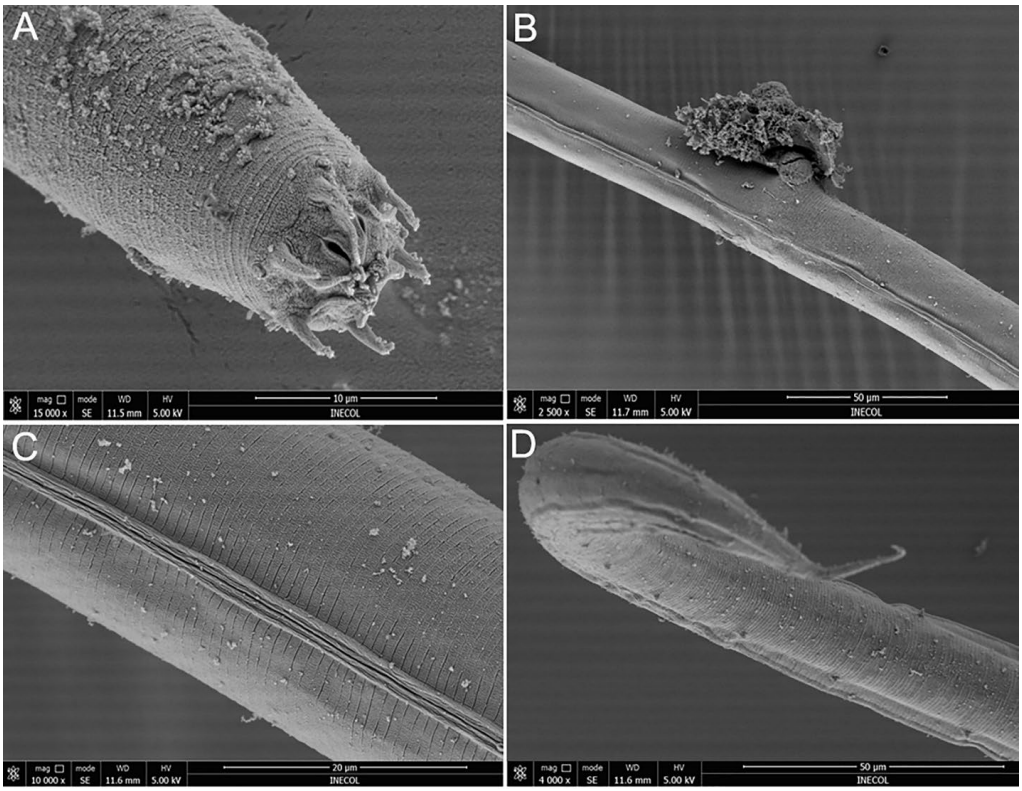

Figure 2. *Diastolaimus noffsingeri* sp. n. SEM. A: Female anterior end body; B: Vulva and gelatin plug; C: Lateral field. Male. D: Posterior end body. Ventral view.

which are 6.6 µm long and 2.8 µm wide and slightly protruding in mature females. Each vulval lip has a very small spine-like structure, on a slightly elevated cone, often covered with a dense gelatin-like secretion presumed to be a copulation plug. Rectum 1.2 - 1.8 (1.6 ± 0.2) times the anal body diameter and with two small gland-like cells beside it. Phasmids posterior to anus and at 32.1 - 50.6 (39.4 ± 5.6)% of tail length. Tail conoid narrowing gradually and ending in a pair of sclerotized mucros with terminal points.

Male. Body habitus J-shaped, almost straight, with posterior end curved ventrally when killed by gentle heat. Cuticle finely striated. Head rounded, continuous with body contour, 9.4 - 11.0 (10.1 ± 0.4) µm wide and 3.8 µm high (Table 1). Six cirri (filamentous papillae), one at the base of each lip; six outer conoid, setose sensilla 4 µm long; four cephalic setae difficult to measure, immediately posterior to the outer conical setae. Amphid aperture oval, 16 - 22 (19.2 ± 2.4) µm from the anterior end. Lateral field with three incisures. Pharynx as described for females. Single gonad extends anteriorly, reflexed in some specimens. Three rectal glands present with a single ventral aperture; in some specimens there was a secretion on the anterior cloacal lip. Tail, curved ventrally with dorsally hooked terminal mucro. Phasmid at 33.3 - 48.5 (40.4 ± 4.4)% of tail from the cloaca. Seven pairs of latero-ventral pre-cloacal papillae and five pairs of post-cloacal papillae arranged as follows: one pair lateral, two pairs latero-ventral and two pairs dorso-lateral. Two spicules, symmetrical, curved ventrally with elongate oval manubrium with an anterior extension; three subdorsal gland cells present anterior to the manubrium, gubernaculum conspicuous, sclerotized, anteriorly wide and reducing gradually to a thin curved tip.

Diagnosis and relationships. *Diastolaimus noffsingeri* sp. n. is similar to *D. mexicanus* Cid del Prado, 2012, in the size of the body, in the index a, b and c´, in the size of the cheilostom, isthmus and rectum and position of the vulva. Females differ by longer average stegostom length (7.3 vs 6.2-6.8 µm) in females; in the size of the pharynx and

Table 1. Morphometrics of females and males of *Diastolaimus* species (measurements in µm unless otherwise indicated).

Species Character	*D. noffsingeri* Holotype female	*D. noffsingeri* Allotype male	*D. noffsingeri* Paratype females	*D. noffsingeri* Paratype males	*D. mexicanus* Tecolapan, Jilotepec County females	*D. mexicanus* Tecolapan, Jilotepec County males
n	1	1	18	12	19	17
L (mm)	1.065	1.31	0.92-1.2 (1.1±0.1)	0.86-1.19 (1.04±0.1)	0.9-1.27 (1.1±0.1)	0.95-1.25 (1.14±0.1)
a	38.0	39.0	32.4-42.2 (37.4±2.9)	31.4-43 (39.4±3.3)	27.9-44.2 (34.9±4.1)	28.2-41.7 (37.3±4.2)
b	4.8	4.7	4.3-5.8 (4.8±0.4)	3.4-7.3 (4.9±0.9)	3.9-6.6 (4.8±0.6)	4.2-5.2 (4.8±0.3)
c	13.3	15.9	11.2-17.8 (13.4±1.9)	11.7-15.9 (14.4±1.3)	9.2-21.2 (12.9±2.6)	12.7-18.9 (16.3±2.1)
c′	4.7	2.4	3.3-7.4 (4.7±0.9)	2.4-2.8 (2.7±0.1)	3.2-6.3 (4.5±0.7)	2.0-2.8 (2.4±0.2)
V% / T%	52.2	66.7	47.3-53.9 (50.9±1.9)	52.9-80.7 (70.1±7.2)	36.9-55.2 (47.5±4.6)	30-64.3 (42.5±12.4)
Pharynx	220	239	181-262 (222.4±18.7)	193-248 (223.5±15.3)	177-254 (226.4±21.1)	129-253 (230.8±29.5)
Corpus	122	135.0	114-145 (127±10.3)	114-139 (123.9±8.4)	115-142 (129.9±7.4)	76-144 (130.7±16.2)
Isthmus	75.0	82.0	60-100 (75.8±10.7)	63-109.9 (76.8±12.9)	40-87 (71.6±13.7)	70-98.7 (80.1±7.2)
Bulb length	-	-	18.0-31.0 (22.6 ±3.5)	-	20.7-30.1 (24.6 ±2.6)	20.7-27 (23.6 ± 2.0)
Bulb width	-	-	15.0-27.3 (18.7 ±2.9)	-	14.1-20.7 (18.5 ±1.7)	14.1-19.0 (17.4±1.5)
Cheilostom length	-	-	3.0-7.0 (6.1 ±1.2)	5.6-7.0 (6.4±0.6)	5.6 -7.5 (6.7 ±0.6)	5.6-9.0 (7.1±1.1)
Cheilostoma width	-	-	5.0-7.5 (5.8 ± 0.7)	5.0-7.5 (5.8±0.8)	6.6-7.5 (7.1 ± 0.5)	5.0-8.0 (6.5±0.9)
Gymnostom length	-	-	2.0-6.0 (3.4±1.0)	3.0-4.0 (3.3±0.5)	2.8-4.0 (3.3 ± 0.5)	2.8-4.7 (3.4±0.5)
Stegostom length	-	-	6.0-10.3 (7.3 ±1.1)	5.0-7.0 (6.4±0.8)	4.7-7.5 (6.2 ± 0.7)	4.7-9.0 (6.1 ± 1.1)
Nerve ring			122-160 (138.7 ±10.9)	-	141.0-185.2 (157.2±14.5)	-
Excretory pore	145.0	167.0	131.6-172 (153.6±12.1)	125-177 (159.8±14.5)	143.8-181 (159±113.3)	147.6-273.0 (176.9±30.9)
Tail	80.0	71.0	61.1-104 (80.6±12.2)	64.9-78.0 (72.5±4.3)	60-105 (85.5±11.8)	57-91 (70.5±9.4)
Tail width	17.0	30.0	15-19 (17.5±1.4)	22.6-30 (27.0±2.3)	14-23 (19.2±1.9)	25-34 (29.8±2.9)
Rectum	28.0	-	25-35 (27.4±4.0)	-	20-35 (29.3±3.9)	-
Phasmid-anus	33.0	30.0	28-45 (34.1±5.6)	24.4-34 (29.2±3.0)	29-47 (35.4±5.1)	24.0-28.0 (25.7±1.4)
Deirid-anterior end	143.0	165	140-168 (154.5±10.1)	128.8-179 (160.6±14.0)	136-174 (156.6±14.2)	148.5-277 (180.2±33.9
Body diameter at vulva	31.0	-	24-35 (29.8±3.3)	-	25-47 (36.0±5.9)	-
Maximum body diameter	31.0	29.0	-	23-29 (26.6±1.7)	-	27-37 (29.9±2.3)
Vagina	-	-	7.0-14 (10.4 ±1.8)	-	6.0-18.8 (11.7±2.9)	-
Spicules	-	-	-	32.9-52 (40.9±5.6)	-	31-50 (41.4±6.5)
Gubernaculum	-	-	-	15-21 (18.3±1.6)	-	12-24 (19.2±2.9)

corpus 181 - 262 (222) µm vs. 189 - 236 (216) µm and 114 - 145 (127) µm vs. 115 - 159 (134) µm, respectively, and position of the excretory pore to anterior end 131.6 - 172 (153.6) vs 118 - 185 (149) µm. Males differ in the size of pharynx 193-248 (223.5) vs. 210-273 (233) µm long and spicule size 37 - 52 (42) µm vs. 30 - 40 (36) µm long.

Etymology. This new species is named in memory of Miss Ella Mae Noffsinger who provided outstanding scientific services as curator of the University of California, Davis (UC Davis) Nematode Collection, Davis, CA. She is gratefully acknowledged for providing important advice and orientation to the first author during his Ph.D. studies at UC Davis.

Type locality and habitat. *Diastolaimus noffsingeri* sp. n. was collected from lichen growing on dead branches of oak and pine trees on the road 113 Xochimilco to Oaxtepec, México State, Mexico, 19° 6' 8.4"N, 98° 56' 2.1"W, 2,456 m above sea level. Collector I. Cid del Prado-Vera in January 2016 and May 2021.

Type materials. The type specimens were deposited in the Laboratorio de Helmintología del Instituto de Biología, National University of Mexico (UNAM), Mexico City Mexico: holotype female - CNHE 11592, allotype male - CNHE 11593, paratype females - CNHE 11593. Other paratype materials, 5 females and 5 males were deposited in the Colegio de Postgraduados Mexico State, Nematode Collection CPNC A-111. The LSID code of the new species is urn: lsid: zoobank. org:act:B2D8FAE8.DC1B-4033-8231-738F5A36C308.

Diastolaimus mexicanus Cid del Prado, 2012
(Fig. 3-4)

Three populations of this species were analyzed. A population from Spanish moss growing on a branch of *Quercus peduncularis* in Texcoco County was morphologically and molecularly characterized. Two populations, one from lichen growing on a dead branch pf *Q. peduncularis* in Cerro Jusda 'El Diablo', Tecolapan, Jilotepec, Mexico State, Mexico and in an epiphyte Spanish moss "heno" *Tillandsia usneoides* growing on a branch of *Q. peduncularis* in Parque Ecoturistico "El Cedral", San Pablo Ixzayo, Texcoco County, Mexico State, México were molecularly characterized. Another population from moss and lichen *Buddleia cordata* Kunth in Amecameca County, the type locality of *D. mexicanus*, was molecularly characterized.

Female. Body an open C-shape with a few specimens almost straight when heat relaxed, curved ventrad, attenuated towards both ends, cuticle very finely striated, head rounded, continuous with body contour, 2.8 - 3.8 (3.4 ± 0.5) µm high and 9.4 - 11.3 (10.1 ± 0.8) µm wide; labial region with six triangle-shaped lips, each with a filamentous cirrus at the base, six setose, conoid outer labial sensilla, 2.8 - 3.8 (3.4 ± 0.5) µm long; and four papilliform cephalic sensilla, located near bases of outer labial setae (Table 1). Cephalic papillae shorter at the anterior part of the gymnostom. Oval amphid apertures located level with the anterior end of the stegostom, 11.3 - 18.8 (16.1 ± 2.8) µm from the anterior end; stoma with three regions: slightly sclerotized cheilostom, heavily sclerotized gymnostom, and funnel-shaped stegostom enveloped by muscular pharyngeal tissue, with slightly conspicuous cuticular lining. Lateral field with three incisures. Dorsal pharyngeal gland orifice in the posterior third of the stegostom. Pharyngeal corpus cylindrical without a median bulb, with prominent cuticular lining. Narrow isthmus with thinner cuticular lining than the corpus; basal pharyngeal bulb oval, with strong transverse/butterfly valve apparatus and visible dorsal and subventral pharyngeal glands located on the posterior part of the basal bulb. Nerve ring encircling the pharynx in the anterior part of the isthmus. Excretory pore clearly visible posterior to the nerve ring. Deirids slightly posterior to excretory pore. Reproductive system didelphic-amphidelphic, ovaries opposed and reflexed with oocytes arranged in single file, in some specimens crossing beyond the position of the vulva; vagina with thick transverse muscular layers. Two small vulval glands present, one anterior and the other posterior to the vulval lips which are 5.6 - 18.8 (11.3 ± 5.0) µm long and 3.8 - 9.4 (5.0 ± 1.8) µm wide and slightly protruding in mature females, on a slight elevated valval cone, often covered with a dense gelatin-like secretion, presumably a copulation plug. Rectum is 0.3 - 1.7 (1.3 ± 0.4) times the anal body diameter with two associated small gland-like cells. Phasmids 17.6 - 67.8 (40.2 ± 13.4)% of the tail length. Tail conoid, narrowing gradually and ending in a pair of sclerotized mucros.

Male. Body habitus J-shaped, almost straight, or C-shaped with posterior end curved ventrally

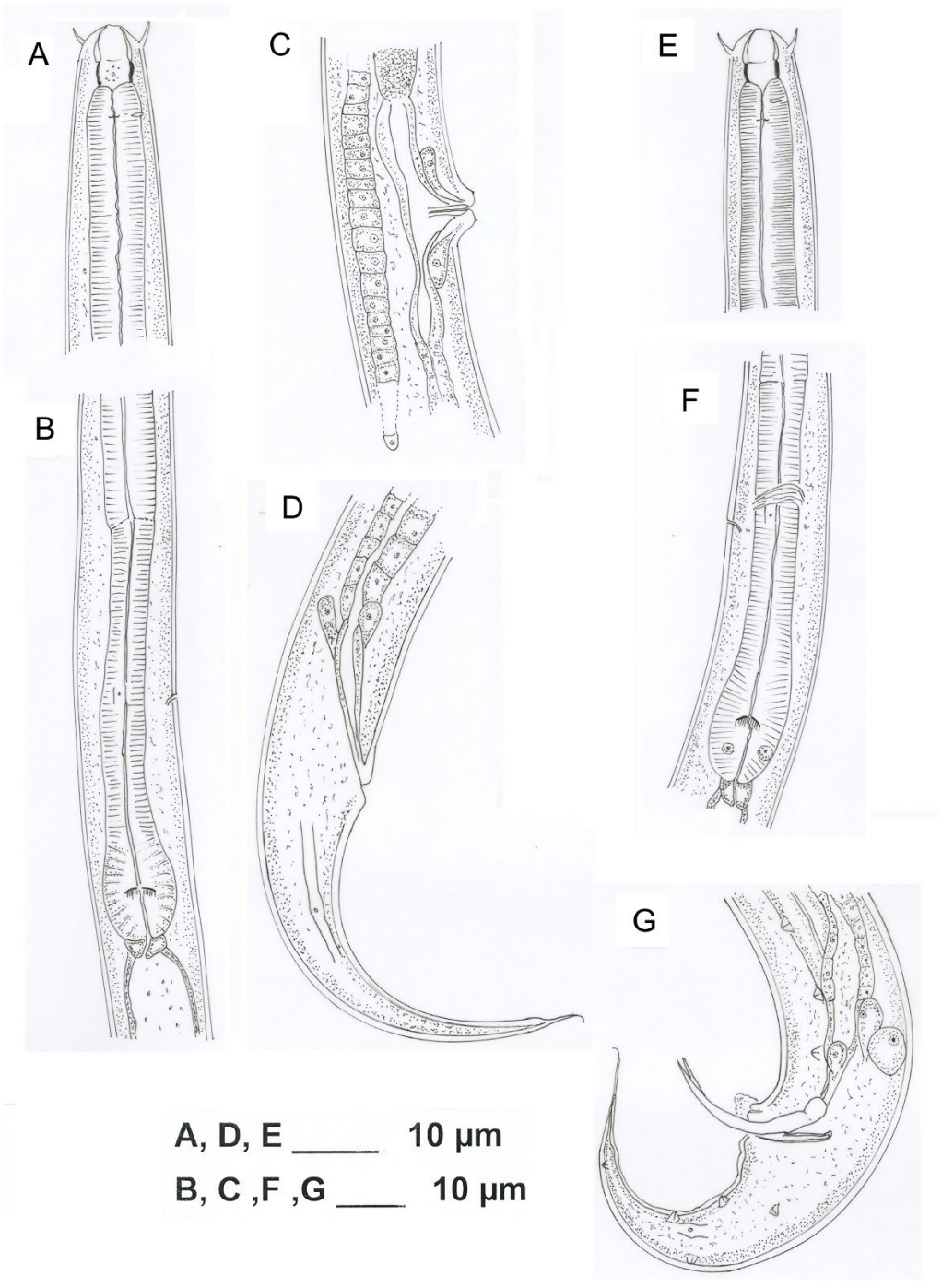

Figure 3. *Diastolaimus mexicanus*. Female (A-D). A: Anterior end, head; B: Posterior end of pharynx: C. Vulva region, lateral view; D: Tail lateral view. Male. (E-G). E: Anterior end head, pharynx region; F: Posterior end of pharynx. G: Posterior end and tail lateral view.

when killed by gentle heat. Head rounded, continuous with body contour, 3.8 µm high and 9.4 – 11 (10.2 ± 0.6) µm wide (Table 1). Cuticle finely striated. Six filamentous cirri, one at the base of each lip; six setose, conoid outer labial sensilla, 3.8 – 5.0 (4.4 ± 0.5) µm long; four cephalic setae, difficult to measure, immediately posterior to the outer labial setae. Oval amphid aperture, 13.0 - 15.9 (12.4 ± 2.2) µm from the anterior end. Pharynx parts as described for females, basal pharyngeal

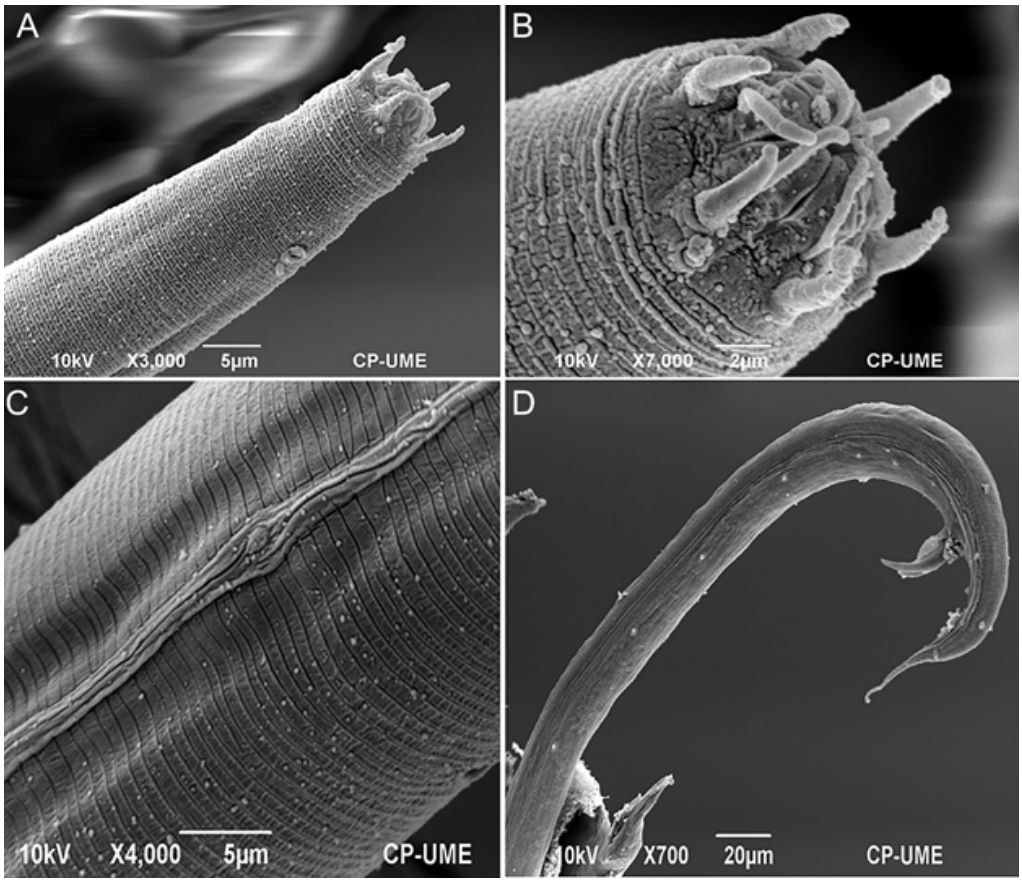

Figure 4. *Diastolaimus mexicanus*. SEM. Female (A, C). A: Anterior end body; C: Lateral field and deirid. Male. (B, D). B: Head and cephalic setae; D: Posterior end body. Lateral view.

bulb oval. Lateral field with three incisures, the middle very fine. Single gonad extends anteriorly, sometimes reflexed, 30.0 – 64.3 (42.5 ± 12.2)% of the body length. Tail curved ventrally, ending in a dorsally hooked terminus. Phasmid at 18.8 - 46.0 (28.9 ± 5.6)% from the anus. Seven pairs of genital latero-ventral precloacal and five pairs of postcloacal, papillae in the following positions: one pair lateral, two pairs latero-ventral and two pairs dorso-lateral. An aperture on the anterior lip of the cloaca in a ventral position, presumably for the secretion of the three glands, 0.4 - 10.3 (7.3 ± 3.4) µm long and 4.7 - 8.5 (6.0 ± 1.3) µm wide, that are anterior to the manubrium. Spicules paired, symmetrically curved ventrally, with an elongate oval manubrium, which has an anterior extension; gubernaculum conspicuous, sclerotized, its anterior part wide and reducing gradually to a thin, slightly curved, tail tip.

Locations. *Diastolaimus mexicanus* was collected from: i) the type locality and habitat, from moss and lichen on tree *B. cordata* Kunth, in San Diego Huehuecalco, Amecameca County, Mexico State 19° 5’ 47.1”N, 98° 44’ 44.46”W. Collector: I. Cid del Prado-Vera on November 28, 2022; ii) epiphyte Spanish moss “heno” *T. usneoides* growing on a branch of an oak tree *Q. peduncularis* Née in Parque Ecoturistico “El Cedral”, San Pablo Ixzayo, Texcoco County, Mexico State, México, 19° 28' 7.0”N, 98° 47’ 15.9”W, 2587 m above sea level. Collector: I. Cid del Prado-Vera on August 18, 2018; iii) lichen growing on a dead branch of oak tree *Q. peduncularis* Née on “El Diablo” hill in Tecolapan, Jilotepec, next to the freeway 57D, Mexico to Queretaro State, Km 105 Mexico State, México, 20° 4’ 55.6”N, 99° 37’ 29.4”W, 2670 m above sea level. Collector: I. Cid del Prado-Vera on August 12, 2019.

Materials. Several adult specimens were deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, Mexico City, Mexico: female - CNHE 8196, male - CNHE 8203, females - CNHE 8203. Other materials, 9 females and 6 males were deposited in the Colegio de

Postgraduados, Mexico State, Nematode Collection CPNC A-112.

Remark. Diastolaimus mexicanus is morphologically similar to *D. grossus* (Truskova and Eroshenko, 1977) Andrassy, 1984. It differs from this species by relatively smaller V% - 37-55 vs 50-57 in females and more posteriorly located excretory pore (134-273 µm vs 107-148 µm) in males and by number of pairs of genital papillae (12 in. *D. mexicanus* vs 14 in. *D. grossus*).

Molecular characterization. The D2-D3 of the 28S rRNA gene sequence alignment contained 17 sequences, including four new sequences of *Diastolaimus*. The alignment was 794 bp in a length. Sequence of *D. noffsingeri* sp. n differed from those of *D. grossus* in 6.6-6.9% (48-49 bp) and from those of *D. mexicanus* in 8.1-8.3% (56-59 bp). Three new sequences of *D. mexicanus* were obtained in this study from three populations in Mexico, and they differed up to 0.4% (3 bp). The phylogenetic position of the genus *Diastolaimus* within other nematodes is provided in Fig. 5.

DISCUSSION

Characterization of *D. noffsingeri* sp. n., and *D. mexicanus* and the recent redescription of *D. grossus* by Čermák *et al.* (2022), reveals high variations in morphometrics and morphology of many characters used for taxonomy of these nematodes. Čermák *et al.* (2022) concluded that *D. mexicanus*, described by Cid del Prado (2012) from Mexico, had morphology and morphometry identical to *D. grossus*, and thus, proposed it as a new junior synonym of *D. grossus*. Our analysis showed that although these two species are very similar and distinguished from each other in some morphometrical characters, they can be clearly differentiated based on partial 28S rRNA gene sequences and thus, *D. mexicanus* should be considered a valid species. Unfortunately, descriptions of the other five species are rather poor and based on insufficient number of specimens and all these species require molecular characterization of topotype materials. Based on review of the previous descriptions of the species of *Diastolaimus*, and our analysis of the new species, we emend the description of the genus.

Genus Diastolaimus Rahm, 1928

Diagnosis. Chambersiellidae: Macrolaiminae (emended). Small to medium-sized nematodes, between 0.8 and 2.3 mm in length. Cuticle finely striated. Cephalic region rounded, continuous with body contour or offset by a slight constriction. Six triangular shape lips, separate, each with single long setiform inner labial setae; six conical shaped outer labial setae and four small papilliform cephalic setae almost at same level as the outer setae; stoma sclerotized, divided into three sections cheilostom, gymnostom and stegostom enveloped by muscular pharyngeal tissue and not divided; cheilostom slight sclerotized and longer than the gymnostom 1.5 - 2.7 times. Dorsal pharyngeal gland orifice at posterior part of stegostom. Amphids oval shaped, located at level of the anterior half of stegostom. Pharynx divided into three parts: a cylindrical corpus, narrow isthmus and terminal muscular valvate bulb. Lateral field marked by two or three incisures. Two ovaries reflexed, crossing beyond the position of the vulva; vulva with protruding lips covered with transparent gelatinous-like material; two small glands beside vulva lips. Testis single straight or reflexed. Male with one aperture in the anterior lip of cloaca in ventral position, presumably for the secretion of the three glands that are anterior to the manubrium. Precloacal and postcloacal genital papillae variable in number and position. Spicules paired; gubernaculum anterior part narrow and getting narrower gradually with thin tip. Tail similar in both sexes, ending in a pair of sclerotized mucros with ends pointed dorsad.

Type species. *Diastolaimus papillatus* Rahm, 1928

Habitats. galleries of insects, surfaces of lichens and epiphytic plants

Biology and ecology of Diastolaimus spp.

Nematodes of the genus *Diastolaimus* are clearly grouped with a large group of bacteriophagous nematodes. Many species in the Cephalobina, including those in the Chambersiellidae, have been described as associated with insects, especially with beetles that establish galleries beneath tree bark (Massey, 1963, 1964). We extracted substantial numbers of nematodes from the tissue of epiphytic plants and lichens. In the apparent absence of insects or insect galleries, we found an abundance of Macrolaiminae (*Diastolaimus* and *Macrolaimus*) and other Chambersiellidae, including *Geraldius*

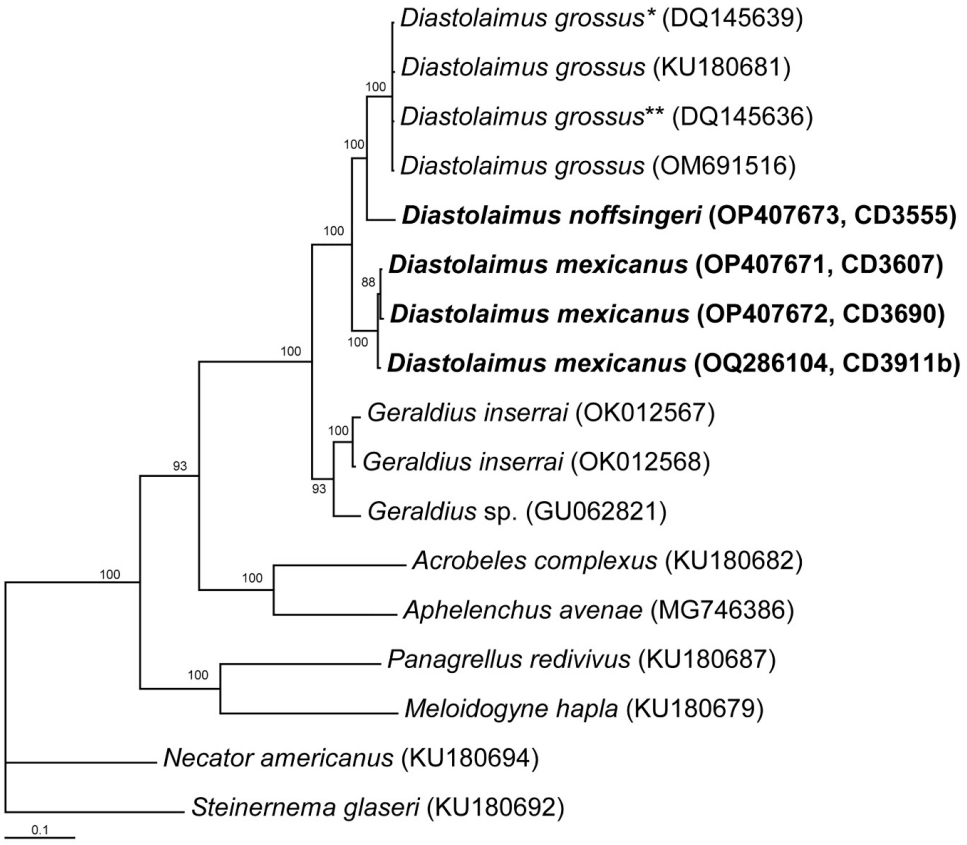

Figure 5. Phylogenetic relationships of *Diastolaimus* spp. with other related nematodes as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. New sequences are indicated in bold. * - originally identified as *Macrolaimus* sp. in the GenBank, ** - originally identified as *Fescia grossa* in the GenBank.

(Cid del Prado-Vera *et al.*, 2021).

In the intestines of nematodes of the Chambersiellidae we have observed bacteria, organic detritus and fungal spores. Therefore, while probably being predominantly bacteriophagous, they are also omnivorous in the absence of preferred resources. Besides being phoretic, the relationship with insects may be necromenic, whereby the nematodes feed on bacteria that exploit insect cadavers without being either actively (as in predatory) or passively (as in the transmission of toxic bacteria) involved in the death of insects. In species of the Chambersiellidae that we have studied, although we have not seen evidence of a necromenic association with insects, we believe that this is an area that may warrant further study.

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