

# RESEARCH/INVESTIGACIÓN

## A NEW SPECIES OF *GERALDIUS INSERRAI* SP. N (RHABDITIDA: CHAMBERSIELLIDAE) FROM MEXICO

I. Cid Del Prado-Vera<sup>1\*</sup>, H. Ferris<sup>2</sup> and S. A. Subbotin<sup>3</sup>

<sup>1</sup>Programa de Fitopatología, Colegio de Postgraduados, Texcoco, México; <sup>2</sup>Department of Entomology and Nematology, University of California, Davis, California, USA; <sup>3</sup>Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, California, USA; \*Corresponding author: [icid@colpos.mx](mailto:icid@colpos.mx)

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### ABSTRACT

Cid Del Prado-Vera, I., H. Ferris, and S. A. Subbotin. 2021. A new species of *Geraldus inserrai* sp. n (Rhabditida: Chambersiellidae) from Mexico. *Nematropica* 51:67-77.

A new species, *Geraldus inserrai* sp. n. collected from lichens and epiphytic plants growing on branches of trees in Mexico State, México, is described and illustrated. *Geraldus inserrai* sp. n. is characterized by female body length = 1.0-1.2 mm, b = 4.3-5.1 and c = 8.5-15.3, lengths of pharynx = 215-247  $\mu$ m, and pharyngeal corpus = 123-143  $\mu$ m, distance between the phasmid and anus = 33-50  $\mu$ m and presence of a single pair of dorsal post-cloacal papillae at the end of the tail. An identification key is provided for the species of *Geraldus* Sanwal, 1957. Phylogenetic relationships of the representatives of the genus *Geraldus* with other related taxa are constructed using the D2-D3 of 28S rRNA gene sequences. The description of the genus is emended.

**Key words:** 28S rRNA gene, epiphytic plant, genus diagnosis, *Geraldus*, lichen, new species, phylogeny, SEM

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### RESUMEN

Cid Del Prado-Vera, I., H. Ferris, y S. A. Subbotin. 2021. Una nueva especie de *Geraldus inserrai* sp. n (Rhabditida: Chambersiellidae) de México. *Nematropica* 51:67-77.

Una nueva especie, *Geraldus inserrai* sp. n. colectada líquenes y plantas epifitas creciendo en ramas de árboles en el Estado de México, México, es descrita e ilustrada. *Geraldus inserrai* sp. n. se caracteriza por la longitud del cuerpo de la hembra = 1.0-1.2 mm, b = 4.3-5.1 y c = 8.5-15.3, longitud de la faringe = 215-247  $\mu$ m, y el corpus de la faringe = 123-143  $\mu$ m, distancia entre fasmidio y el ano = 33-50  $\mu$ m y presencia de un solo par de papilas dorsales post-cloacales al final de la cola. Se proporciona una clave de identificación para la especie de *Geraldus* Sanwal, 1957. Se construyeron relaciones filogenéticas con los representantes del género *Geraldus* con otros taxones relacionados utilizando secuencias del D2-D3 del gen 28S ARNr. Se modifica la descripción del género.

**Key words:** gen 28S ARNr, planta epifita, diagnóstico de género, *Geraldus*, líquen, nueva especie, filogenia, SEM

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### INTRODUCTION

Prior to this species description, the genus

*Geraldus* Sanwal, 1957 included only two valid species: *G. bakeri* (Sanwal, 1957) Sanwal, 1971, and *G. galapagoensis* Cid del Prado, 2012.

*Geraldus bakeri* was collected from galleries of the insects *Leperisinus aculeatus* and *Phloeosinus dentatus* in Canada and, later, from lichen on a tree in Costa Rica (Sanwal, 1957; Holovachov *et al.*, 2003). The second species, *G. galapagoensis*, was collected from mosses of the family Meteoriaceae, growing abundantly on endemic trees, *Scalesia pedunculata* Hook. (Asteraceae), in the tropical forest on the twin volcanoes of Isla Santa Cruz, Galápagos Islands, Ecuador (Cid del Prado, 2012). In 2016–2021, during nematode surveys in Mexico, a new species of genus *Geraldus* was found in samples collected from mosses, lichens and epiphytic plants growing on various trees, mainly pines and oaks. The objectives of the study described herein were to provide: i) a morphological description of the new species using light and scanning electron microscopy; ii) a species identification key for the genus, iii) molecular characterization using D2-D3 expansion segments of 28S rRNA gene sequences, and iv) an emended genus description for *Geraldus*.

## MATERIALS AND METHODS

### *Nematode population*

Nematodes were collected in two locations: i) from lichen growing on a branch of an oak tree in the road to Oaxtepec, Mexico State, Mexico and ii) from the epiphyte, *Tillandsia recurvata* L., growing on an avocado tree, *Persea americana* Mill., in La Purificación, Tepetitla, Texcoco, Mexico State, Mexico. Plant samples were placed in jars with water at room temperature and agitated every 12 hours by swirling the jar. The suspension was decanted and sieved every 24 hr for 3 days, using 60- and 325-mesh sieves, and nematodes were collected by backwashing the 325-mesh sieve. Nematodes were hand-picked under a dissecting microscope and placed in a vial with 5–7 ml water.

### *Light (LM) and Scanning Electron Microscopy (SEM) study*

Extracted nematodes were killed by heating to 65°C in two drops of water until movement ceased. They were transferred to water in glass bottles at room temperature. An equal volume of Golden fixative (Hooper, 1970) was added to the suspension for a final fixative concentration of 4%. The glass bottles were stored at room temperature

for 10 days. The nematodes were transferred to a Petri dish and the fixative was then carefully removed by pipetting from the surface, without disturbing the nematodes, until the depth in the dish was ~4 mm. The covered Petri dish was placed in a small desiccator over 96% ethanol and incubated at 40°C. After 3 days, when the odor of formalin was no longer detectable, the volume of liquid in the dish was reduced to half, without disturbing the nematodes, by removing liquid with a pipette under a microscope.

The specimens for LM studies were processed to glycerin using a modification of the Seinhorst (1959) and De Grisse and Choi (1971) methods as described by Cid Del Prado Vera and Subbotin (2012). An equal volume of Seinhorst A solution (1 part of glycerin, 20 parts of 96% ethanol, 79 parts of water) was added to the dish, which was then incubated at 40°C with the cover slightly open. When the solution level dropped to 2 mm, additional Seinhorst A solution was added, and the incubation continued. When the solution level dropped to 1 mm, Seinhorst B solution (95 parts 96% ethanol, 5 parts glycerin) was added and the dish again was incubated at 40°C. When the solution level again dropped to 1 mm, Seinhorst C solution (80 parts 96% ethanol, 20 parts glycerin) was added. Three days later, 1 ml of pure glycerin was added to the dish. Selected nematodes were hand-picked from the dish for mounting on glass slides using the paraffin wax ring method (de Maeseneer and d'Herde, 1963). Measurements and drawings were made using a drawing tube mounted on an American Optical compound microscope.

The specimens for SEM study were washed in magnesium buffer solution, pH 7.2, for 20 min and dehydrated in an ethanol solutions series, 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. Specimens were observed in a Jeol JSM-6390 microscope at 10 KV acceleration voltage.

### *Molecular study*

Nematodes collected from two locations were used in the molecular study. DNA was extracted from several specimens using the proteinase K protocol. DNA extraction, PCR and cloning protocols were used as described by Subbotin (2021a). The following primer set was used for PCR: the forward D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG – 3') or the *Geraldus*-D2F

(5' - AAC GGA TAG TGC CAA CGT ATC - 3') and reverse D3B (5' - TCG GAA GGA ACC AGC TAC TA - 3') primers for amplification of the D2-D3 expansion segments of 28S rRNA gene. Sequencing was conducted at Genewiz (California, USA). New sequences were submitted to the GenBank database under accession numbers: SUB10303626- SUB10303627. New sequences of the D2-D3 of 28S rRNA gene were aligned using ClustalX 1.83 with some published sequences

(2015). The alignment was analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) under the GTR + G + I model as described by Subbotin (2021b).

## RESULTS

*Geraldus inserrai* sp. n.

*Description of specimens from Oaxtepec, Mexico State:* See Figures 1-6 and Tables 1 and 2.

(Holovachov *et al.*, 2009; Holovachov and Nadler,

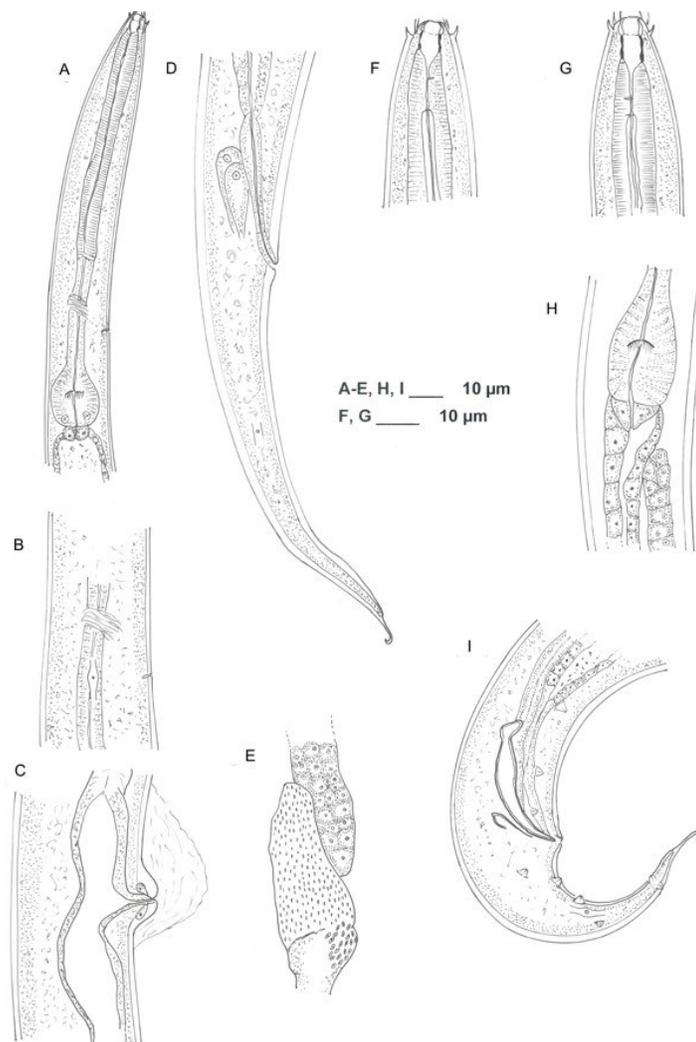


Figure 1. *Geraldus inserrai* sp. n. Female. (A-D): A. Anterior region of body; B. Nerve ring, deirids and excretory pore region; C. Vulva region, lateral view; D. Tail; Male (E-I): E. Testis germinal region and seminal vesicle; F and G. Anterior end, head; H. Posterior end of pharynx; I. Tail.

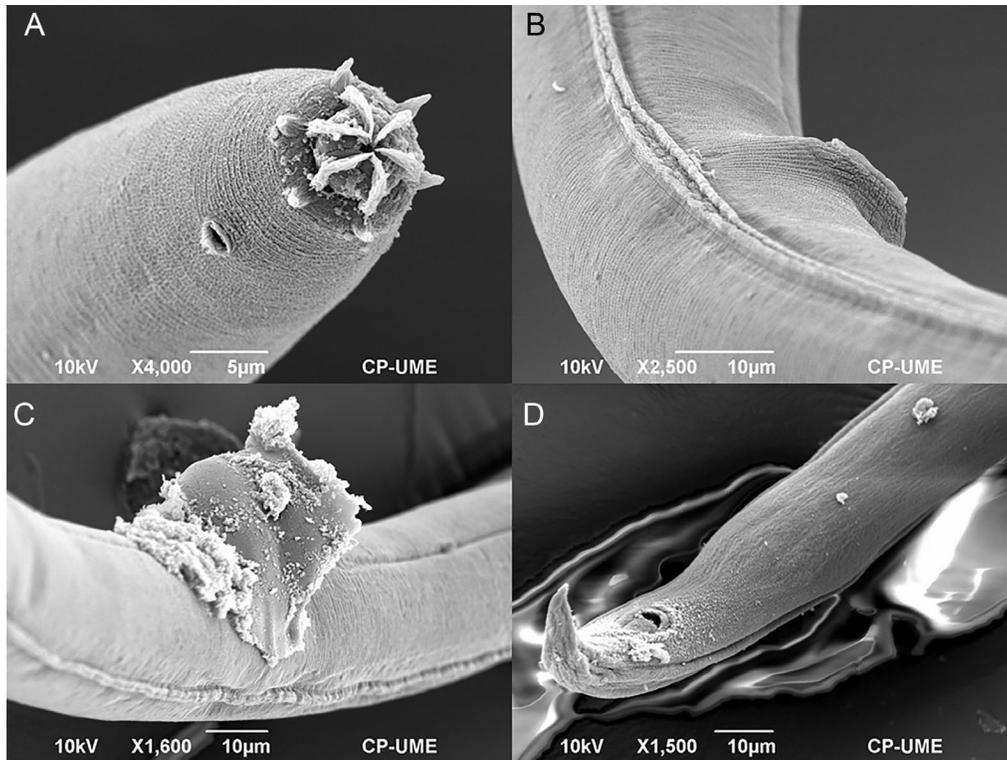


Figure 2. Female *Geraldius inserrai* sp. n. (Oaxtepec, Mexico State, Mexico): A. Anterior end, lateral view; B. Vulva lateral view; C. Vulva latero-ventral view; D. Tail ventral view.

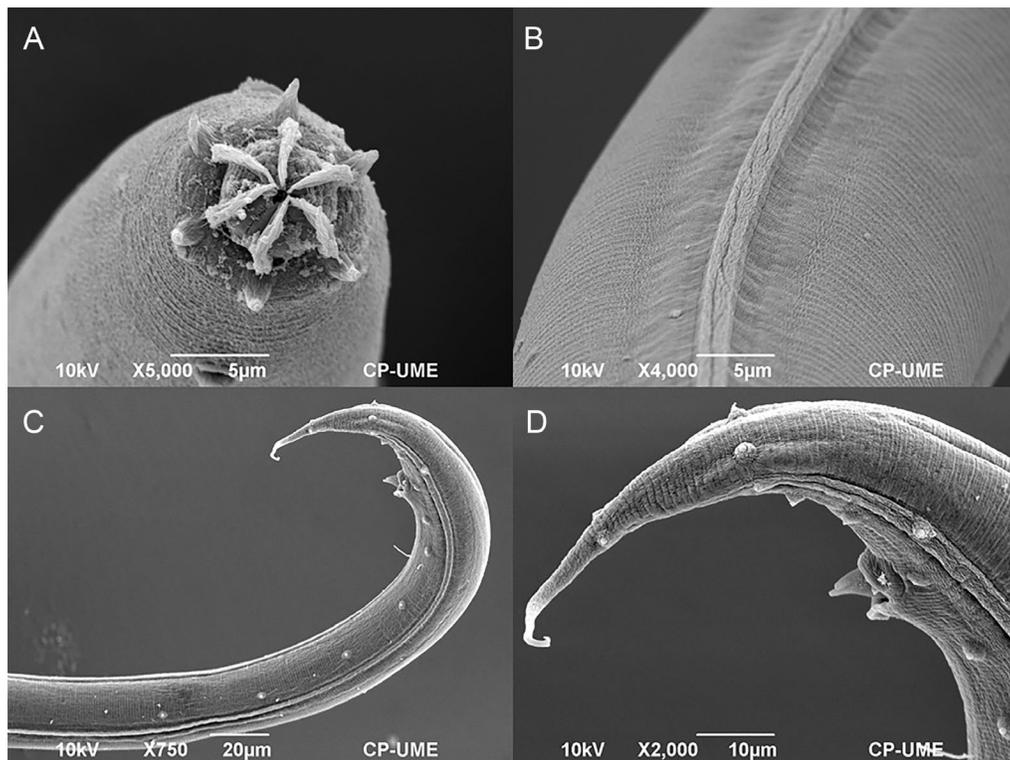


Figure 3. Male *Geraldius inserrai* sp. n. (Oaxtepec, Mexico State, Mexico): A. Head face view; B. Mid-body lateral view. C. Posterior end, lateral view; D. Tail, lateral view.

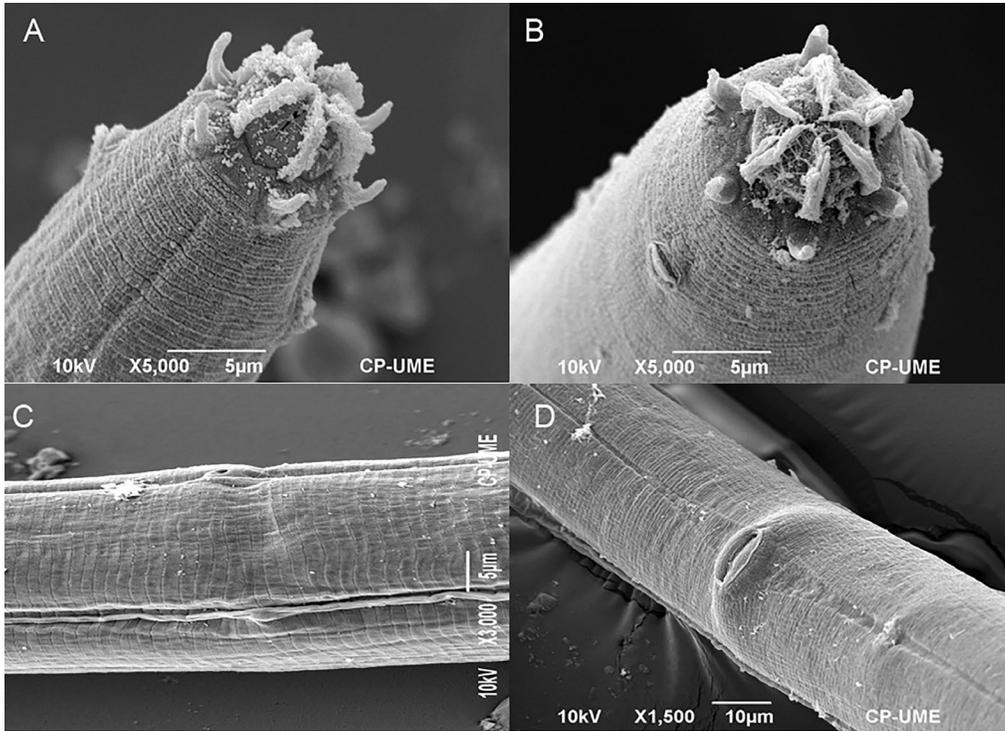


Figure 4. Female *Geraldus inserrai* sp. n. (La Purificación, Tepetitla, Texcoco, Mexico State, Mexico): A. Anterior end ventral view; B. Head face view; C. Lateral view at excretory and deirid level; D. Vulva, ventral view.

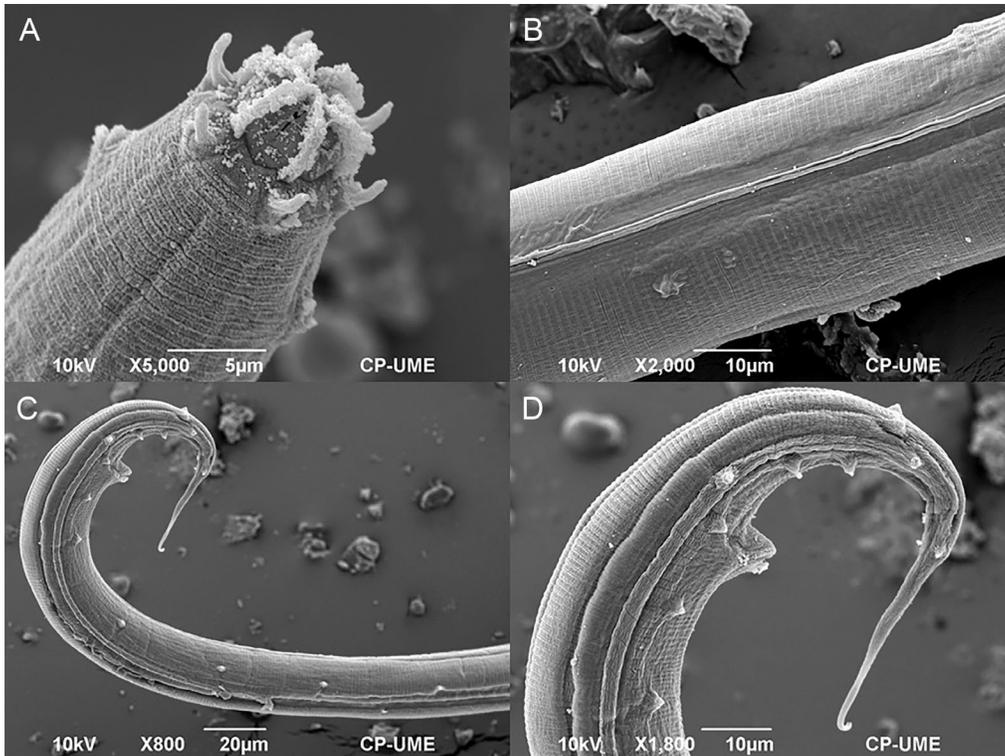


Figure 5. Male *Geraldus inserrai* sp. n. (La Purificación, Tepetitla, Texcoco, Mexico State, Mexico): A. Anterior end dorsal view; B. Lateral field; C. Posterior end lateral view, D. Tail lateral view.

*Female.* Body open or C-shaped when heat relaxed, attenuated towards both ends, cuticle very finely striated, head marked from the rest of the body by a constriction. Labial region with six triangle-shaped lips with six clearly-branched cirri. In a second labial ring are six cone-shaped setae, 3.0  $\mu\text{m}$  long and, at the same level, four small cone-shaped cephalic setae. Oval amphid apertures located  $17 \pm 1.4$  (16.0-18.0)  $\mu\text{m}$  from the anterior end. Lip region  $8.0 \pm 0.7$  (7.0-9.0)  $\mu\text{m}$  wide, heavily sclerotized. Lateral field with three incisures, the internal incisures very fine. A single longitudinal incisure on both the ventral and dorsal sides of the body, terminating in the cervical and tail regions. Stoma divisible into three regions: cheilostom  $4.8 \pm 0.5$  (4.0-5.0)  $\mu\text{m}$  long by  $5.0 \pm 0.7$  (4.0-6.0)  $\mu\text{m}$  wide, gymnostom  $6.4 \pm 0.9$  (6.0-8.0)  $\mu\text{m}$  long, and stegostom  $13.2 \pm 0.8$  (12.0-14.0)  $\mu\text{m}$  long with conspicuous cuticular lining and

enveloped by muscular pharyngeal tissue. Dorsal pharyngeal gland orifice in the posterior third of the stegostom. Pharyngeal corpus cylindrical, with prominent cuticular lining and without a median bulb. Narrow pharyngeal isthmus, with thinner cuticular lining than the corpus. Basal pharyngeal bulb oval,  $33.6 \pm 2.5$  (30.0-36.0)  $\mu\text{m}$  long and  $25.4 \pm 1.5$  (23.0-27.0)  $\mu\text{m}$  wide, with strong transverse butterfly valve apparatus and visible pharyngeal glands. Nerve ring encircling the pharynx in the anterior part of the isthmus. Excretory pore clearly visible between the first third and mid-length of the isthmus, and posterior to the nerve ring. Deirids slightly anterior to the excretory pore. Reproductive system didelphic-amphidelphic, ovaries opposed and reflexed with oocytes arranged in single file; in some specimens the

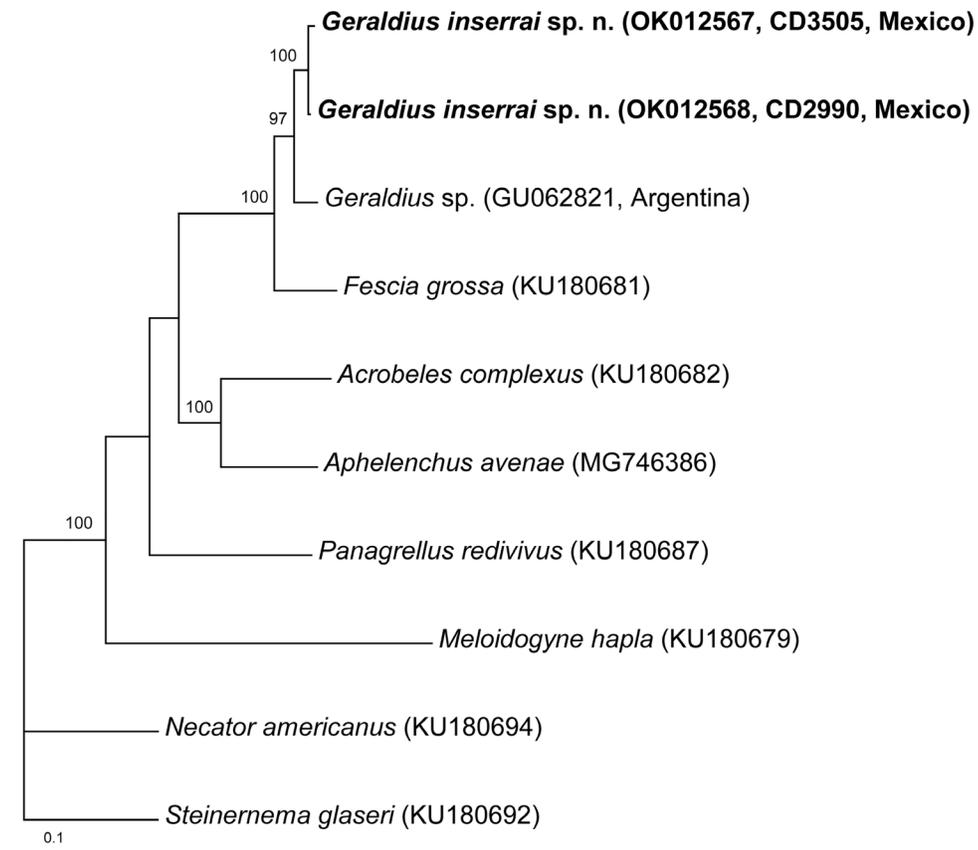


Figure 6. Phylogenetic relationships of *Geraldius* spp. with other related nematodes as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequences under the GTR + I + G model. Posterior probabilities greater than 70% are given for appropriate clades. New sequences are indicated in bold.

Table 1. Morphometric of holotype and paratype females of *Geraldus insertai* sp. n. and *Geraldus* spp.

Character	<i>G. insertai</i> sp. n.		<i>G. insertai</i> sp. n.		<i>G. insertai</i> sp. n.		<i>G. bakeri</i>		<i>G. bakeri</i>		<i>G. galapagoensis</i>	
	Holotype, Oaxtepec, State, Mexico	Paratype, Oaxtepec, State, Mexico	Holotype, Oaxtepec, State, Mexico	Paratype, Oaxtepec, State, Mexico	La Purificación, Tepetitla, Texcoco, Mexico State, Mexico	(Sanwal, 1971)	(Holovachov et al. 2003)	(Sanwal, 1971)	(Holovachov et al. 2003)	(Sanwal, 1971)	(Holovachov et al. 2003)	(Cid del Prado, 2012)
n	1	5	1	5	13	-	10	-	10	-	19	19
L	1.244	1.0-1.2 (1.1)	1.244	1.0-1.2 (1.1)	0.97-1.2 (1.07)	1.1-1.3	1.1-1.2 (1.16)	1.1-1.3	1.1-1.2 (1.16)	1.1-1.3	1.1-1.2 (1.16)	0.8-1.2 (1.0)
a	25.9	24.6-28.8 (26.9)	25.9	24.6-28.8 (26.9)	20.8-29 (26.5)	-	17.6-23.5 (20.7)	-	17.6-23.5 (20.7)	-	17.6-23.5 (20.7)	19.3-35 (25.0)
b	5.0	4.5-5.1 (4.9)	5.0	4.5-5.1 (4.9)	4.3-5.0 (4.7)	4.4-5.3	4.4-5.3 (4.9)	4.4-5.3	4.4-5.3 (4.9)	4.4-5.3	4.4-5.3 (4.9)	4.9-6.8 (5.7)
c	9.1	8.9-14.5 (10.3)	9.1	8.9-14.5 (10.3)	8.5-15.3 (9.9)	8.0-9.5	8.0-9.5 (8.9)	8.0-9.5	8.0-9.5 (8.9)	8.0-9.5	8.0-9.5 (8.9)	8.1-10.8 (9.6)
é	5.0	3.3-5.8 (5.0)	5.0	3.3-5.8 (5.0)	2.9-5.4 (4.7)	4.1-6.3	4.1-6.3 (5.1)	4.1-6.3	4.1-6.3 (5.1)	4.1-6.3	4.1-6.3 (5.1)	3.7-6.3 (4.7)
V%	53.9	47.3-53.9 (49.9)	53.9	47.3-53.9 (49.9)	38.5-51.2(48.9)	48.8-52.3	48.8-52.3 (50.2)	48.8-52.3	48.8-52.3 (50.2)	48.8-52.3	48.8-52.3 (50.2)	39-50 (47)
Pharynx	247.0	215-247 (228.0)	247.0	215-247 (228.0)	55-70 (63.9)	224-248	224-248 (238.0)	224-248	224-248 (238.0)	224-248	224-248 (238.0)	141-207 (179)
Corpus	141.0	134-143 (139.4)	141.0	134-143 (139.4)	155-185 (163.8)	120-138	120-138 (131)	120-138	120-138 (131)	120-138	120-138 (131)	112-145 (125)
Isthmus	70.0	50-70 (59.0)	70.0	50-70 (59.0)	79-124 (109.0)	52-63	52-63 (58.6)	52-63	52-63 (58.6)	52-63	52-63 (58.6)	32-45 (39)
Excretory pore	185.0	155-185 (166.5)	185.0	155-185 (166.5)	21-27 (23.3)	171-190	171-190 (178)	171-190	171-190 (178)	171-190	171-190 (178)	73-161 (133)
Tail	136.0	116-127 (121.3)	136.0	116-127 (121.3)	29-45 (37.8)	121-144	121-144 (131)	121-144	121-144 (131)	121-144	121-144 (131)	81-121 (105)
Tail width	27.0	21-27 (22.6)	27.0	21-27 (22.6)	140-167 (149.5)	23-31	23-31 (26)	23-31	23-31 (26)	23-31	23-31 (26)	18-28 (24.4)
Rectum	45.0	30-45 (39.6)	45.0	30-45 (39.6)	33-50 (41.9)	-	34.5-43 (39.3)	-	34.5-43 (39.3)	-	34.5-43 (39.3)	24-35 (30)
Nerve ring	166.0	145-166 (154.5)	166.0	145-166 (154.5)	152-185 (165.8)	-	144-165 (157)	-	144-165 (157)	-	144-165 (157)	110-145 (130.5)
Phasmid-anus	55.0	40-47 (43.2)	55.0	40-47 (43.2)	36.0-58 (40.5)	33-46	33-46 (39.8)	33-46	33-46 (39.8)	33-46	33-46 (39.8)	32-51 (40.5)
Deitrid-anterior end	-	154-166 (159.0)	-	154-166 (159.0)	152-185 (165.8)	166-190	166-190 (178)	166-190	166-190 (178)	166-190	166-190 (178)	129-152 (138)
Body diam. at vulva	50.0	42-50 (45.6)	50.0	42-50 (45.6)	36.0-58 (40.5)	-	-	-	-	-	-	35-64 (49)
Rectum/wide tail	1.7	1.4-2.0 (1.8)	1.7	1.4-2.0 (1.8)	1.1-1.9 (1.6)	-	1.3-1.8 (1.5)	-	1.3-1.8 (1.5)	-	1.3-1.8 (1.5)	0.7-4.7 (1.6)
Body diam.	48.0	38-48 (41.4)	48.0	38-48 (41.4)	36-58 (40.5)	46-67	46-67 (55.8)	46-67	46-67 (55.8)	46-67	46-67 (55.8)	30-55 (41)

Table 2. Morphometric of lectotype and paratype males of of *Geraldus inserrai* sp. n. and *Geraldus* spp.

Character	<i>G. inserrai</i> sp. n. Lectotype, Oaxtepec, Mexico State, Mexico	<i>G. inserrai</i> sp. n. Paratypes, Oaxtepec, Mexico State, Mexico	<i>G. inserrai</i> sp. n. La Purificación, Tepetilla, Texcoco, Mexico State, Mexico	<i>G. bakeri</i> (Sanwal, 1971)	<i>G. bakeri</i> (Holovachov <i>et al.</i> 2003)	<i>G. galapagoensis</i> Cid del Prado, 2012
n	1	7	9	-	2	7
L	1.084	0.91-1.1 (1.0)	0.9-1.3 (1.1)	1.1-1.3	1.1; 1.2	0.8-1.1 (1.0)
a	28.5	24.7-34.3 (28.5)	27.2-30.0 (28.7)	26.9-31.5	42.4; 40.5	18.6-31.5 (25)
b	4.6	3.9-5.5 (4.6)	4.3-5.3 (4.8)	4.8-5.3	4.8; 5.3	4.6-8.3 (5.8)
c	12.0	9.9-13.5 (11.4)	10.7-15.0 (12.0)	9.5-11.1	9.5; 11.1	9.5-14.1 (11.6)
é	2.6	2.0-3.1 (2.5)	2.2-3.3 (2.8)	2.8	2.8	1.8-2.6 (2.2)
T%	26.1	25.1-29.9 (27.0)	65.3-76.1 (71.4)	60.5-55.5	60.5; 55.5	23.3-65 (45)
Pharynx	236.0	198-224 (223.4)	211-243 (223.0)	240-242	240; 242	122.5-226 (183)
Corpus	138.0	126-150 (136.0)	123-141 (129.3)	135-136	135; 136	101-136 (125)
Isthmus	63.0	39-65 (56.4)	56-69 (62.0)	58	58	32-57 (43)
Excretory pore	173.0	138-183 (160.3)	144-173 (159.1)	194-195	194; 195	108-169 (145)
Tail	90.0	77-101 (89.4)	80-98 (88.0)	115-121	121; 155	78-98 (88.7)
Tail width	35.0	31-40 (34.9)	29-42 (34.0)	41.5-43	41.5; 43	33-50 (41)
Nerve ring	168.0	138-168 (153.8)	134-158 (146)	170	170	107-157 (133.6)
Phasmid-anus	35.0	30-35 (33)	14-40 (23.4)	41.5-43	40.5; 54	32-40 (35.7)
Deirid-anterior end	173.0	141-184 (162)	142-181 (165.7)	-	193	113-174 (147)
Spicules	45.0	39-49 (42.7)	39-54 (47.0)	50-52	50; 52	44-59 (49)
Gubernaculum	20.0	18-21 (19.6)	18.0-22.0 (20.1)	24	24	20-25 (22)
Body diameter	38.0	34-40 (36.6)	31-45 (37.0)	42.5-40.5	40.5; 42.5	32-55 (43)

reflexed anterior or posterior ovary crosses the position of the vulva. Vulva in mature females on an elevated vulval cone, often covered with a dense gelatin-like secretion, presumably a copulatory plug; vulva lips with one small spine on the anterior border. Small vulval glands present. Vagina with thick transverse muscular layers,  $12.6 \pm 4.2$  (8.0-18)  $\mu\text{m}$  long. Phasmid in the posterior half of the tail. Tail conoid, narrowing gradually and ending in a pair of sclerotized mucros with pointed ends.

*Male.* Body habitus an open C-shape, posterior end curved ventrally when killed by gentle heat. Cuticle very finely striated. Incisures in lateral field and on dorsal and ventral side of body as described in females. Head offset by a constriction from the rest of the body. Cirri and setae similar to those of female: six outer cone-shaped setae, 2  $\mu\text{m}$  long, and four small cephalic setae that are difficult to measure. Oval amphid apertures,  $15.3 \pm 1.4$  (13.0-17.0)  $\mu\text{m}$  from the anterior end. Head  $7.4 \pm 0.5$  (7.0-8.0)  $\mu\text{m}$  wide and  $4.3 \pm 0.5$  (4.0-5.0)  $\mu\text{m}$  long. Cheilostom 5.0  $\mu\text{m}$  length and width, anterior margin not projecting from the oral opening. Gymnostom  $6.4 \pm 0.8$  (6.0-8.0)  $\mu\text{m}$  long and the stegostom  $12.1 \pm 1.8$  (10.0-15.0)  $\mu\text{m}$  long. Pharyngeal parts as described for female. Single gonad extends anteriorly, reflexed or not,  $27.0 \pm 1.8$  (25.1-29.9) % of body length. Two rectal glands present, and one conspicuous glandular orifice present on the anterior cloaca lip. In a lateral view of the tail, a secretion from the ventral side of the pore located on anterior lip of the cloaca may be visible. Tail curved ventrally, with a dorsally-hooked end. Seven pairs of latero-ventral pre-cloacal papillae present, the first pair adcloacal in position and five pairs post-cloacal in the following positions: one pair lateral ad-cloacal, two pairs latero-ventral, one pair dorso-lateral and one small pair dorso-lateral, almost at the end of the tail. Phasmid at same level as the pair of latero-dorsal papillae and at  $38.1 \pm 3.6$  (31.6-42.8)% of the tail length. Two spicules, symmetrical, curved ventrally, each with an elongate oval manubrium with an anterior extension,  $42.7 \pm 3.5$  (39.0-49.0)  $\mu\text{m}$  long; conspicuous gubernaculum present,  $19.6 \pm 1.1$  (18.0-21.0)  $\mu\text{m}$  long, anterior part wide and reducing gradually to a narrow tip.

*Differential diagnosis.* *Geraldus inserrai* sp. n. is characterized by length of female body = 1.0-1.2 mm, b = 4.3-5.1 and c = 8.5-15.3, lengths of pharynx = 215-247  $\mu\text{m}$ , and pharyngeal corpus = 123-143  $\mu\text{m}$ , distance between the phasmid and

anus = 33-50  $\mu\text{m}$  and presence of a single pair of dorsal post-cloacal papillae at the end of the tail.

*Geraldus inserrai* sp. n. is similar to *G. bakeri* in the position of the nerve ring, position of the vulva and lengths of the pharynx, corpus and isthmus and in having three incisures in the lateral field; it differs in the length of the tail, 73-127  $\mu\text{m}$  vs 121-144  $\mu\text{m}$ , in the position of the excretory pore, 155-185  $\mu\text{m}$  vs 171-190  $\mu\text{m}$  and the position of the deirid 152-185  $\mu\text{m}$  vs 166-190  $\mu\text{m}$  from the anterior end in females.

*Geraldus inserrai* sp. n. is similar to *G. galapagoensis* in the size of the body in females and males, in the size of cheilostom and gymnostom and in the position of the vulva. In females, it differs in the length of the pharynx, 214-247  $\mu\text{m}$  vs 141-207  $\mu\text{m}$ , in the lengths of corpus and isthmus, 123-143  $\mu\text{m}$  and 50-70  $\mu\text{m}$  vs 112-145  $\mu\text{m}$  and 32-45  $\mu\text{m}$ , respectively, and in the position of excretory pore and deirid 155-185  $\mu\text{m}$  and 152-185  $\mu\text{m}$  vs 73-161 and 129-152  $\mu\text{m}$  from the anterior end, respectively. In males, it differs in the lengths of the pharynx and corpus 198-244  $\mu\text{m}$  and 123-150  $\mu\text{m}$  vs 123-226  $\mu\text{m}$  and 101-136  $\mu\text{m}$ , respectively, and also by three incisures vs two in the lateral field.

*Etymology.* This new species is named in honour of Dr. Renato N. Inserra, for his outstanding scientific contribution in systematics of nematodes and great role in development of the Organization of Nematologists of Tropical America (ONTA).

*Type locality and habitat.* *Geraldus inserrai* sp. n. was collected from lichen growing on a branch of an oak tree in the road D113 Juchitepec to Oaxtepec, Mexico State, Mexico, N19°18'37.1" W98°29'10.5", 2456 m above sea level. Collector I. Cid del Prado-Vera on January 2016.

*Other location.* Another population was collected from the epiphyte, *Tillandsia recurvata* L., growing on an avocado tree, *Persea americana* Mill., in La Purificación, Tepetitla, Texcoco, Mexico State, Mexico, N19°31'02.5" W98°48'38.0", 2421 m above sea level. Collector I. Cid del Prado-Vera on July 2016.

*Type materials.* Accession numbers of type specimens deposited in the Laboratorio de Helmintología del Instituto de Biología, Universidad Nacional Autónoma de México: holotype female CNHE 11594, allotype male CNHE 11595, paratype females CNHE 11596. Other paratype materials are deposited in the

University of California Riverside Nematode Collection (UCRNC), and the Colegio de Postgraduados Nematode Collection (CPNC): paratypes A-110.

*Molecular characterization.* The D2-D3 of the 28S rRNA gene sequence alignment contained ten sequences, including three sequences of *Geraldus*. Two new sequences of *G. inserrai* sp. n. were obtained in this study from two populations in Mexico State, Mexico. The sequences of *G. inserrai* sp. n. differed from each other in 1.1% (7 bp) and from that of *Geraldus* sp. from Argentina by 5.7 and 7.0% (41 and 47 bp). The phylogenetic position of *Geraldus* within other nematodes is given in Figure 6.

*Geraldus* Sanwal, 1971. Diagnosis (emended). Small nematodes, between 0.8 and 1.1 mm in length. Lip region rounded, continuous with body contour or offset by a constriction. Six separate triangle-shaped lips, with six small cephalic cirri; six conical outer labial setae and four papilliform cephalic setae at the same level as the outer labial setae; stoma strongly sclerotized, divided into three sections: cheilostom, gymnostom and stegostom, and enveloped by muscular pharyngeal tissue. Subventral pharyngeal glands located in posterior region of basal pharyngeal bulb and dorsal pharyngeal gland orifice in posterior part of stegostom. Amphid apertures oval, located at level of 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; a single incisure on ventral and dorsal side of body is present or absent. Two ovaries, reflexed to beyond level of vulva, vulva with protruding lips with small spine-like projections, two small glands next to vulval lips. Male monorchid, testis outstretched or reflexed. Rectal glands present with an aperture on the anterior lip of cloaca, in a ventral position,

presumably for secretion of the rectal glands. Male with seven pairs of precloacal and five or six pairs of postcloacal papillae, varying in position. Spicules paired; gubernaculum tapering from a wide anterior to a thin tip. Tail similar in both sexes with two mucros at the terminus. Habitats: moss, lichen and epiphytic plants, and galleries of insects.

*Type species:* *Geraldus bakeri* (Sanwal, 1957) Sanwal, 1971 = *Chambersiella bakeri* Sanwal, 1957

*Other species:*

*G. galapagoensis* Cid del Prado, 2012

*G. inserrai* sp. n.

## DISCUSSION

We have described a new species, *G. inserrai*, sp. n., which can be separated on the basis of fairly standard morphometric characteristics. Nematodes of the genus *Geraldus* are classified as bacterivores (Yeates *et al.*, 1993). They have an open stoma and lack teeth, either fixed or protrusible, that might have indicated predation or plant feeding. Therefore, it would seem that any habitat that supports bacteria, and perhaps fungi would suffice for *Geraldus* spp.

This interesting genus of nematodes successfully inhabits environments that would normally be considered inhospitable for an aquatic organism. The biology and physiology of these nematodes warrant further study. The open stoma, surrounded by elaborate cirri and with a muscular pharyngeal sheath, devoid of tooth-like structures, seems adapted to ingestion of organisms of a limited size. Undoubtedly, there are more nematode species with these habitat and trophic characteristics yet to be discovered. The development of reliable molecular diagnostic techniques will be critical tools.

### Identification key for the genus *Geraldus*

1. Females and males with head continuous with the rest of body.....*G. bakeri*  
Females and males with head separated from body by a constriction.....2
2. Pharynx in females and males < 200  $\mu$ m long on average; excretory pore and deirids in females and males < 150  $\mu$ m from the anterior end on average..... *G. galapagoensis*  
Pharynx in females and males > 200  $\mu$ m long on average; excretory pore and deirids in females and males > 150  $\mu$ m from the anterior end on average..... *G. inserrai* sp. n.

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