RESEARCH/INVESTIGACIÓN

TWO NEW SPECIES OF *MACROLAIMUS* (NEMATODA: CHAMBERSIELLIDAE) WITH COMMENTS ON DIAGNOSTIC CHARACTERS FOR DISTINGUISHING SPECIES

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ABSTRACT

Cid del Prado Vera, I., H. Ferris, and S. A. Subbotin. 2020. Two new species of *Macrolaimus* (Nematoda: Chambersiellidae) with comments on diagnostic characters for distinguishing species. Nematropica 50:170-185.

Two new species of *Macrolaimus* are described; nematodes were examined with light and scanning electron microscopy (SEM). *Macrolaimus montrealensis* sp.n., collected from lichen growing on the bark of a maple tree in the Montreal Botanical Garden in Montreal, Canada, is described. The new species is characterized by the similarity in lengths of the cheilostom and gymnostom, 4.0 - 7.0 (5.5 ± 0.9) µm and 3.0 - 6.0 (4.6 ± 1.0) µm, respectively; the lateral field with three incisures, the external incisures evident throughout the body length and the middle incisure most visible in the region of the vulva and at the posterior end of the body; the vulval lips protruding, the anterior lip more than the posterior; the short postuterine sac, 5.0 – 12.0 (8.1 ± 2.3) µm; the female tail, which is elongate conoid, curved ventrally, ending in an acute tip with a dorsally-curved mucro; the anal lips slightly protruding, mainly the posterior lip; phasmids near the posterior end of the tail; the male with paired, symmetrical spicules, curved ventrad; a manubrium with rounded proximal end, short calamus and curved lamina; the arc-shaped gubernaculum; two pairs of subventral precloacal and five pairs of postcloacal genital papillae. The second new species, *Macrolaimus inecolensis* sp.n., is characterized by the cheilostom 1.4 - 2.5 (1.9) times the length to the gymnostom, length of the female tail 47.0 - 52.0 (50.3) µm, a postuterine sac 12.0 - 20.0 (17.2 ± 3.6) µm long; and in the male genital papillae, three pairs precloacal and five postcloacal. Collected from filamentous epiphyte on a tree branch at the Institute of Ecology in Jalapa, Veracruz, México. A revised identification key for the genus is provided.

Key words: Chambersiellidae, epiphyte, lichen, *Macrolaimus*, new species

RESUMEN

Cid del Prado Vera, I., H. Ferris, and S. A. Subbotin. 2020. Dos nuevas especies de *Macrolaimus* (Nematoda: Chambersiellidae) con comentarios de diagnóstico para distinguir especies. Nematropica 50:170-185.

Dos nuevas especies de *Macrolaimus* son descritas; los nematodos fueron examinados con el microscopio de luz y el microscopio electrónico de barrido (SEM). *Macrolaimus montrealensis* sp.n., colectada de liquen creciendo en la corteza de un árbol de maple, en el jardín Botánico de Montreal,

Canadá. Esta especie se caracteriza por la similitud en longitud del cheilostoma y gymnostoma (4.0 - 7.0 (5.5 ± 0.9) µm and 3.0 - 6.0 (4.6 ± 1.0) µm, respectivamente; el campo lateral con tres incisuras, las externas muy evidentes y la central fina y más visible en la región de la vulva y la región posterior del cuerpo; los labios vulvares proyectados, el labio anterior más que el posterior; el saco postuterino de 5.0 - 12 (8.1 ± 2.3) µm de largo; la cola de la hembra es elongada-conoide y curvada ventralmente, terminando en una agudo dorsalmente curvado mucrón; los labios del ano ligeramente proyectados, principalmente el labio posterior; fasmidios cerca del extremos porterior de la cola; el macho con un par de espiculas simétricas, curvadas ventralmente, un manubrio con estremo proximal redondeado, un calamus corto y lamina curvada; el gubernaculum en forma de arco; dos pares de papilas subventrales precloacales y cinco pares de papilas postcloacales. *Macrolaimus inecolensis*, se caracteriza por el cheilostoma es 1.4 – 2.5 (1.9) veces largo que el gymnostom, en la longitud de la cola de las hembras 47.0 – 52.0 (50.3) µm, el saco postuterino, 12.0 – 20.0 (17.2 ± 3.6) µm de largo y el macho por la papilas genitales, tres pares precloacales y cinco postcloacales. Esta especie fue colectada de una planta epifita filamentosa creciendo en ramas de un árbol en el jardín Botánico del Instituto de Ecologia de Jalapa, Veracruz, México. La clave de identificación para las especies del género es provista.

Palabras claves: Chambersiellidae, epiphyte, lichen, *Macrolaimus*, especies nuevas

INTRODUCTION

Currently, the genus *Macrolaimus* Maupas 1900 has nine valid species; four of the species are described from Europe (Czech Republic, Corsica (France), Bosnia and Herzegovina and the southern Iberian Peninsula of Spain) and two were redescribed from bark samples from several areas of natural vegetation in central Europe, (Abolafia and Peña-Santiago, 2014; Abolafia *et al*., 2019). Other species are described from Canada, Pakistan, and South Africa (Sanwal, 1960; Timm, 1960; Swart and Heyns, 1992). Four *species inquirendae* are tentatively associated with the genus (Abolafia and Peña-Santiago, 2014).

Nematodes of the genus *Macrolaimus* are characterized by the oral opening being surrounded by six triangle-shaped lips, the internal labial papillae not being evident; the six outer labial papillae being setiform and conical and the four cephalic papillae small and close to the outer labial papillae; the amphid apertures are oval shaped and located two cuticular striations posterior to the cephalic setae; and, the cheilostom and gymnostom are sclerotized with the cheilostom usually longer than the gymnostom. Some features vary among species: the presence or absence of a postuterine sac, the varied morphology of female and male tails and tail tips, the morphology of the spicules with a hook-shaped or rounded manubrium and fusiform gubernaculum, and, different numbers of precloacal and postcloacal genital papillae in males. The valid species have been collected from various habitats, including soil, moss, lichens, tree bark and insect galleries. Some of the species were poorly described and illustrated by the original authors with description of characters later determined to be of diagnostic importance were omitted. Consequently, the identification of species with the existing morphological and morphometric data is difficult. Fortunately, additional information was recently supplied in the redescriptions of *M. canadensis*, *M. ruehmi*, and *M. crucis* (Abolafia *et al*., 2019) and *M. arboreus* (Shokoohi *et al*., 2018).

MATERIALS AND METHODS

Samples of lichen from the bark of a maple tree in the Montreal Botanical Garden (Montreal, Canada) and from lichen on a tree at the Institute of Ecology (Jalapa, Veracruz, México) were placed in jars with water at room temperature and agitated every 12 hr. Nematodes were collected by decanting and sieving the suspension every 24 hr, over a period of 2 days, using 60 and 325 mesh sieves.

Extracted nematodes were hand-picked and killed by heating to 60ºC in a microwave oven for 6 sec in about 7 ml of water in a 15 ml vial. An equal volume of a solution of 8% formalin and 2% glycerol in water was added to achieve a final fixative concentration of 4% (Hooper, 1970). The nematodes were stored at room temperature for 10 days then transferred to a covered Petri dish. The

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

Samples were further processed to glycerin using a modification of the Seinhorst (1959) and De Grisse and Choi (1971) methods. An equal volume of Seinhorst A solution (1 part glycerin, 20 parts 96% ethanol, 79 parts water) was added to the dish, and it was incubated at 40ºC with the cover slightly open. When the solution level dropped to 1 mm, Seinhorst B solution (95 parts 96% ethanol, 5 parts glycerin) was added, and the dish and incubated at 40ºC. When the solution level again dropped to 1 mm, Seinhorst C solution (80 parts 96% ethanol, 20 parts glycerin) was added and was incubated at 40ºC. 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 optical compound microscope. The specimens for scanning electron microscope (SEM) studies were washed in magnesium buffer solution, pH 6.8 and 0.05 M, for 20 min and dehydrated in an ethanol solution 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 using a Jeol JSM - 6390 microscope at 10 KV acceleration voltage.

Macrolaimus montrealensis sp.n.

Drawings and measurements. See Table 1 and 2, Figure 1, Plates 1-3.

Female (n=10). Body an open C shape, curved ventrad after fixation; cylindrical, tapering at both extremities, more towards the posterior end at the conical tail. Cuticle with very fine striations. Head truncate, continuous with the body contour. Stoma surrounded by six triangular-shaped lips which are not fused; inner labial sensilla not visible, six conoid outer labial setae 2.0 - 3.0 (2.5 ± 0.5) µm long, and four small cephalic sensilla situated slightly posterior to the outer labial setae. Amphid apertures small, oval shape, located two cuticular striations posterior to the cephalic setae at 5.0 - 9.0 (6.7 ± 1.5) µm from the anterior end. Stoma heavily sclerotized, subdivided into cheilostom 4.0 - 7.0 (5.5 ± 0.9) µm long and gymnostom 3.0 - 6.0 (4.6 ± 1.0) µm long; the stegostom is funnel-shaped but not clearly visible. Lateral field with three incisures, the external incisures evident throughout the body length and the middle incisure most visible in the region of the vulva and at the posterior end of body; there is a single longitudinal line along the dorsal side of the body. Oesophagus cephaloboid: corpus cylindrical 75.0 - 112.0 (99.7 ± 12.6) µm long and 1.5 times longer than the isthmus; isthmus narrower 49.0 - 76.0 (63.0 ± 9.7) µm long; metacorpus nearly pyriform 20.0 - 27.0 (23.4 ± 2.4) µm long and 17.0 - 22.0 (19.0 ± 1.6) µm wide. Cardia conoid. Nerve ring in the first third of the isthmus at 85.0 - 145.0 (121.0 ± 20.6) µm from the anterior end. Excretory pore posterior to the nerve ring and anterior to the deirid at 94.0 - 166.0 (133.0 ± 22.9) µm from the anterior end. Deirid slightly posterior to excretory pore at 104.0 - 178.0 (158.0 ± 23.1) µm from the anterior end. Reproductive system monodelphic-prodelphic with the ovary reflexed, the germinal area at 83.0 - 229.0 (142.0 ± 50.2) µm from the anus, with a short postuterine sac, 5.0 - 12.0 (8.8 ± 2.3) µm long. Vulva with protruding lips oriented posteriad, anterior lip the larger. Two gland cells, 8 µm long by 4 µm wide with conspicuous nuclei, are located one anterior and the other posterior to the vagina. Vagina length 8.0 - 15.0 (11.0 ± 1.9) µm. Rectum length 1.1 - 2.3 (1.6 ± 0.3) times anal body diameter with two gland-like cells at its anterior end. Tail elongate conoid, curved ventrally and ending in an acute tip with a dorsally-curved mucro. Anal lips slightly protruding, mainly the posterior lip. Phasmids located in the posterior end of the tail 35.0 - 45.0 (41.0 ± 3.7) µm and 51.0 - 61.0 (56.2 ± 3.8) % of tail length from anus.

Male (n=4). Body an open C-shape, curved ventrad after fixation. Cuticle with very fine striations. Head similar to females; outer labial setae 2.0 - 3.0 (2.3 ± 0.5) µm long. Amphid aperture at 6.0 – 8.0 (7 ± 1.2) µm from the anterior end. Cheilostom as long as wide, 4.0 - 5.0

Table 1. Morphometrics of females of *Macrolaimus montrealensis* sp.n., *M. inecolensis* sp.n., *M. arboreus*, and *M. canadensis* (measurements in µm ± standard deviation unless otherwise indicated).

	M. montrealensis paratypes (n=10) ♀	*M. inecolensis* paratypes (n=4) ♀	*M. arboreus* (n=10) ♀	*M. canadensis* (n=10) ♀
L (mm)	0.9-1.2 (1.0±0.1)	0.8-1.1 (0.93±0.1)	1.0-1.04	0.99-1.2
a	28.3-36.6 (32.9±3.1)	33.6-40.3 (36.1±3.0)	33-52	34-36.6
b	5.1-6.1 (5.6±0.4)	4.9-6.1 (5.4±0.5)	4.0-5.0	4.5-4.9
c	11.5-16 (13.6±1.6)	17-20.9 (18.5±1.7)	13.0-17.0	13.5-14.8
c´	3.9-5.3 (4.3±0.4)	3.3-4.3 (3.6±0.5)	3.8	4.08
V%	54-62 (57±2.5)	51-58.8 (56.2±3.5)	50-57	55-57.6
Postuterine sac	5-12 (8.8±2.1)	12-20 (17.3±3.6)	0.6	0.5
Oesophagus	160-207 (183.8±18.7)	164-178 (173.8±6.6)	-	-
Corpus	75-112 (100.8±11.6)	97-106 (100.8±3.8)	-	-
Isthmus	49-70 (61±8.2)	50-55 (52.3±2.2)	-	-
Excretory pore	94-166 (132±30.1)	115-132 (125±7.3)	-	-
Tail	72-80 (75.3±2.4)	47-52 50.3±2.4)	45	76
Tail width	17-19 (18±0.8)	12-16 (14.3±1.7)	-	-
Rectum	20-31 (28.9±1.6)	20-28 (22.8±3.8)	-	-
Phasmid-anus	38-45 (42.7±3.0)	27-34 (31.5±3.1)	-	-
Deirid-anterior end	104-178 (141±32.2)	115-141 (132.3±15.0)	-	-
Body diameter at vulva	29-37 (34.5±2.9)	27-30 (28.5±1.7)	-	-
Body diameter	26-33 (30±2.1)	22-28 (25.8±2.6)	-	-

Table 2. Morphometric of paratypes of females and males of *Macrolaimus montrealensis sp.n.; M arboreus* and *M. canadiensis.* (measurements in µm ± standard deviation)

	M. montrealensis holotype ♀	*M. montrealensis* paratypes (n=10) ♀	*M. montrealensis* lectotypes (n=4) ♂	*M. arboreus* (n=10) ♀	*M. arboreus* (n=10) ♂	*M. canadensis* (n=10) ♀	*M. canadensis* (n=10) ♂
L(mm)	0.924	0.88-1.2 (1.0±0.1)	0.79-0.98 (0.91)	1.0-1.04	1.0-1.2	0.99-1.2	0.95-1.07
a	33.9	28.3-36.6 (32.9±3.1)	38-42 (40)	33-52	39-52	34-36.6	38.8-43
b	5.03	5.1-6.1 (5.6±0.4)	4-5.4 (4.8)	4.0-5.0	4.1-5.7	4.5-4.9	4.3-4.7
c	12.2	11.5-16 (13.6±1.6)	16.5-17.8 (17.4)	13.0-17.0	17-21	13.5-14.8	16.7-19.8
c′	4.5	3.9-5.3 (4.3±0.4)	2.2-2.6 (2.4)	3.8	2.7	4.08	2.6
V% or T%	54.1	54-62 (57±2.5)	55-68 (62.7)	50-57	-	55-57.6	-
Postuterine sac	6.0	5.0-12 (8.8±2.1)	-	0.6		0.5	
Oesophagus	183.8	160-207 (183.8±18.7)	179-198 (192)	-	-	-	-
Corpus	102	75-112 (100.8±11.6)	100-107 (103)	-	-	-	-
Isthmus	75	49-70 (61±8.2)	65-70 (68)	-	-	-	-
Excretory pore	136	94-166 (132±30.1)	125-146 (137)	-	-	-	-
Tail	76	72-80 (75.3±2.4)	48-55 (52)	45	44	76	61
Tail width	17	17-19 (18±0.8)	19-22 (21)	-	-	-	-

Table 2. Continued.

	M. montrealensis holotype ♀	*M. montrealensis* paratypes (n=10) ♀	*M. montrealensis* lectotypes (n=4) ♂	*M. arboreus* (n=10) ♀	*M. arboreus* (n=10) ♂	*M. canadensis* (n=10) ♀	*M. canadensis* (n=10) ♂
Rectum	20	20-31 (28.9±1.6)	_	-	-	-	-
Phasmid-anus	40	38-45 (42.7±3.0)	27-33 (30.7)	-	-	-	-
Deirid-Anterior end	141	104-178 (141±32.2)	130	-	-	-	-
Body diameter at vulva	31	29-37 (34.5±2.9)	-	-	-	-	-
Body diameter	31	26-33 (30±2.1)	21-24 (22.7)	-		-	
Spicule	-	-	28-33 (30)	-	30	-	37
Gubernaculum		-	10-12 (11.3)	-	13	-	16

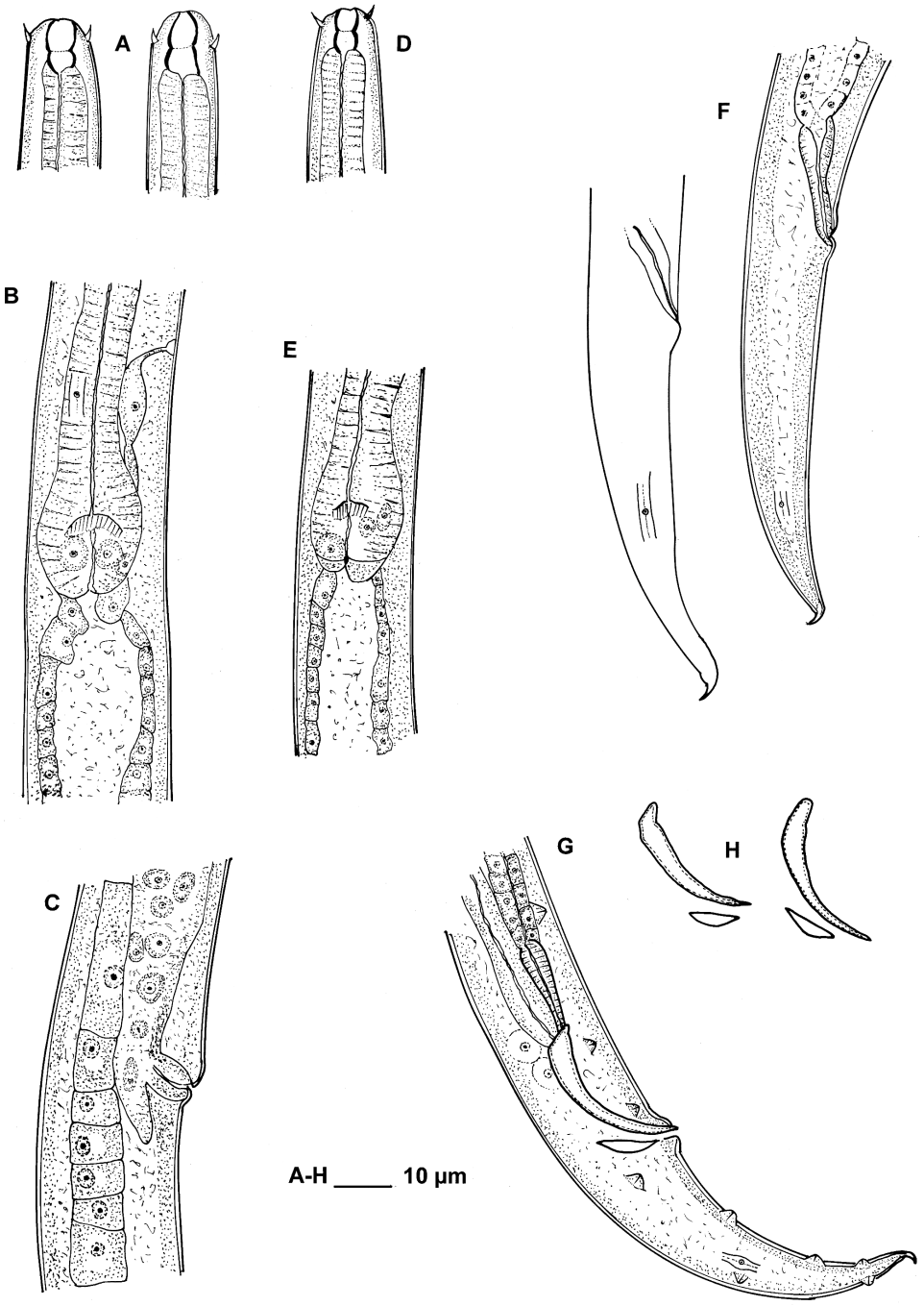

Figure 1. *Macrolaimus montrealensis* sp.n. Female: A, B, C and F. A. Head; B. Posterior end oesophagus; C. Vulva lateral view; F. Tails. Male: D, E, G and H. D. Head; E. Posterior end oesophagus. G. Tail and H. Spicules and gubernaculm.

(4.7 ± 0.5) µm; gymnostom 4.0 - 6.0 (5.0 ± 0.8) µm long. Nerve ring at 120.0 - 136.0 (127.0 ± 8.1) µm from the anterior end. Excretory pore posterior to the nerve ring and anterior to the deirid, at 125.0 - 146.0 (137.0 ± 10.8) µm from the anterior end. Deirid slightly posterior to excretory pore at 130.0 µm from the anterior end. As in females, the lateral field has three incisures, and there is one line along the ventral side of the body. Reproductive system monarchic reflexed

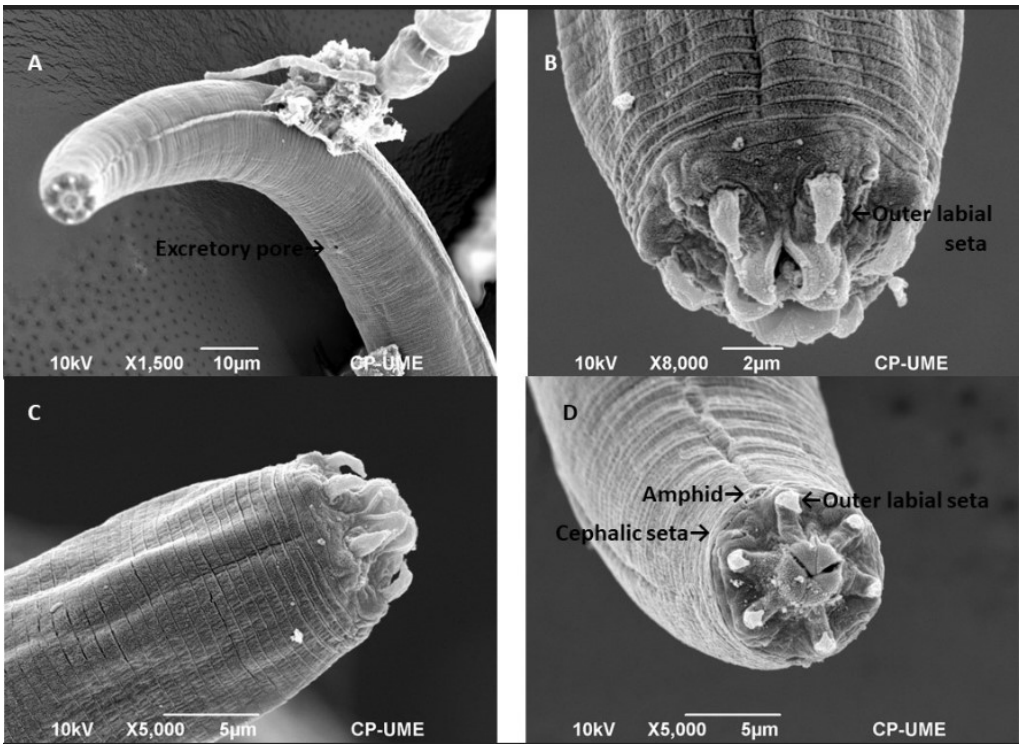

Plate 1. *Macrolaimus montrealensis* n.sp. Female A-D. A) anterior end, B) cephalic region, lateral view, C) anterior end, lateral view, and D) face view.

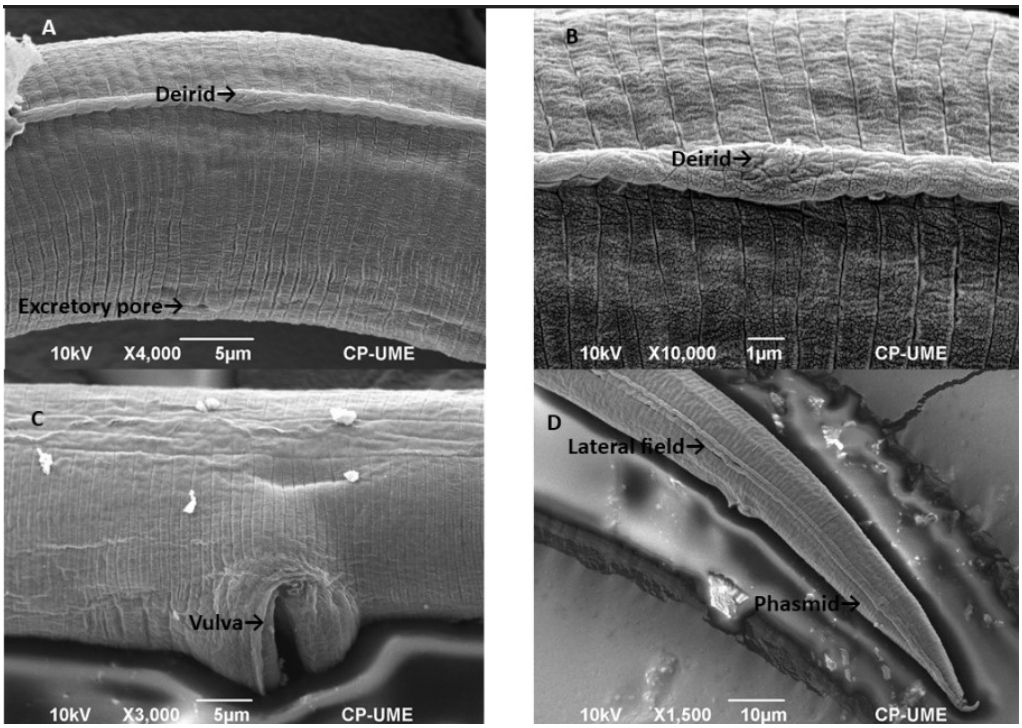

Plate 2. *Macrolaimus montrealensis* n.sp. Female A-D. A) Anterior region body, lateral view, B) close up deirid, lateral view, C) vulva, lateral view, and D) tail lateral view.

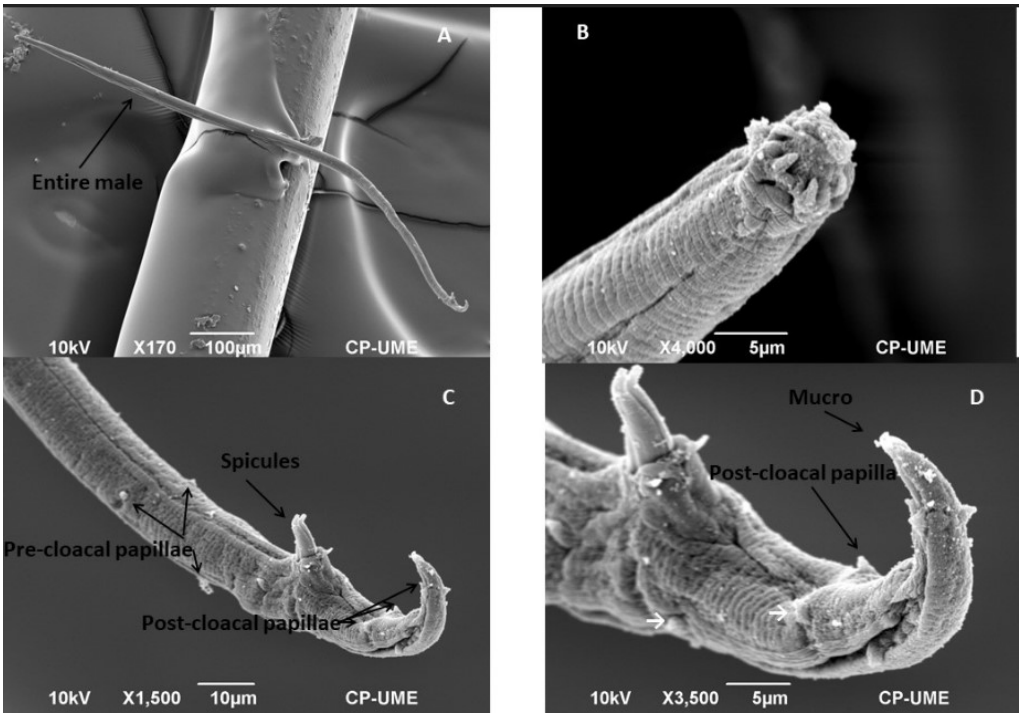

Plate 3. *Macrolaimus montrealensis* n.sp. Male A-D. A) Body, B) anterior region body, dorsal view, C) posterior region body, ventral view, and D) tail lateral view.

ventrad. Spicules paired and symmetrical, curved ventrad; manubrium with rounded proximal end, short calamus and curved lamina. Gubernaculum arc-shaped. Three gland-like cells are present anterior to the spicules. There are two pairs of subventral precloacal genital papillae; one pair is 6.0 - 10.0 (8.3 ± 2.1) µm and the second 31.0 - 45.0 (38.0 ± 6.6) µm from cloacal aperture. There are five postcloacal pairs of papillae. Phasmids are located in the posterior half of the tail at 53.8 - 61.0% (57.3 ± 3.1) of tail length from anus. Tail curved more ventrad than in females, ending in an acute tip with a dorsally- curved mucro.

Diagnosis. Macrolaimus montrealensis sp.n. is characterized by the heavily sclerotized stoma subdivided into a cheilostom and gymnostom, which are almost the same length and width, the lateral field with three incisures, the middle line only slightly evident. Available SEM images reveal a line along the dorsal side of the body in females and a line on the ventral side in males.

Images of the dorsal side of males and the ventral side of females were not obtained, however, it seems probable that both males and females have both dorsal and ventral lines along the body. The ovary is reflexed, with the germinal area at 83.0 - 229.0 (142.0 ± 50.2) µm from the anus; the vulva has protruding lips oriented posteriad and there is a short postuterine sac. The male has paired, symmetrical spicules, curved ventrad and manubrium with rounded proximal end; the gubernaculum is arc-shaped. There are two pairs of subventral precloacal, and five pairs of postcloacal, genital papillae.

Macrolaimus montrealensis sp.n. is close to *M. arboreus* Truskova and Eroshenko, 1977, *M. canadensis* Sanwal, 1960, and *M. taurus* Thorne, 1937, in the presence of a postuterine sac, in the size of body and position of vulva and prominent vulval lips. It differs from *M. arboreus* in that the cheilostom is similar in length to the gymnostom 4.0 - 7.0 (5.5) and 3.0 - 6.0 (4.6) µm long, respectively vs. the gymnostom half as long as the cheilostom, in the length of the female tail 63.0 - 80.0 (73.2) µm vs. 49.0 µm long, and in the five pairs vs. three pairs of postcloacal genital papillae. *Macrolaimus montrealensis* sp.n. differs from *M. canadensis* in the absence of thorn-like spines on the spicules, in the index b, 4.1 - 6.1 (5.3) vs. 4.5 - 4.9 in females, in the size and shape of cheilostom and gymnostom, almost the same size vs. the cheilostom long and wide and the gymnostom narrow and less than one-third of the length of the cheilostom; in the length of the postuterine sac

relative to the body width at the vulva (0.2 - 0.5 (0.29) vs. 1.1 - 1.8 µm). It also differs from *M. canadensis* in the length of the male tail, 48.0 - 55.0 (52.0) µm vs. 61.0 µm, in the number of postcloacal pairs of papillae in males: five vs. four in *M. canadensis*, and it differs in the position of the deirid, at the same level vs. posterior to the excretory pore. Finally, the new species differs from *M. canadensis* in the habitat from which it was collected, lichen on a tree trunk vs. the gallery of a bark beetle. *Macrolaimus montrealensis* sp.n. differs from *M. taurus* mainly in the shape of the curved, elongate conoid tail vs. subacute tail and in the number and position of the postcloacal genital papillae in males, five vs. six, and in the length of the postuterine sac, almost 0.3 vs. 0.8 times body diameter at the vulva.

Type locality and habitat. Montreal Botanical Garden, Montreal, Quebec, Canada, N 45° 55' 94.9 ", W 73° 56' 27. 2", 40 m above sea level, collected on July 22, 2016 from lichen on the trunk of a maple tree, *Acer* sp.

Type specimens. Holotype female on slide 11263; allotype 11264 and five paratype females on slide 11265, in the Colección Nacional de Helmintos Instituto de Biología, Laboratorio de Helmintología, Universidad Nacional Autónoma de México (CNHE). Other type material is deposited in the University of California, Riverside, Nematode Collection (UCRNC) and the Colegio de Postgraduados Nematode Collection (CPNC) on slide number A-097.

Etymology. The species name recognizes the type locality, Montreal, Quebec, Canada.

Macrolaimus inecolensis sp.n.

Drawings and measurements. See Table 1 and 2, Figure 2-3.

Female (n=4). Body an open C shape, curved ventrad after fixation; cylindrical, tapering at both extremities, more towards the posterior end at the conical tail. Cuticle with very fine striations. Head truncate, continuous with the body contour 10.0 - 12 (11.0 ± 1.2) µm wide. Stoma surrounded by six triangular-shaped lips that are not fused; inner labial sensilla not visible, six conoid outer labial setae 2.0 µm long, and four small cephalic sensilla situated slightly posterior to the outer labial setae. Amphid apertures small, oval shape, located two cuticular striations posterior to the cephalic setae at 3.0 - 6.0 (4.8 ± 1.3) µm from the anterior end. Stoma heavily sclerotized, subdivided into cheilostom 5.0 - 6.0 (5.7 ± 0.5) µm long and gymnostom 2.0 - 3.0 (2.7 ± 0.5) µm long; cheilostom/gymnostom 1.4 - 2.5 (1.9 ± 0.5) times longer than gymnostom; the stegostom is funnel-shaped but not clearly visible. Lateral field with two fine incisures. Oesophagus cephaloboid: corpus cylindrical 97.0 - 106.0 (101.0 ± 4.6) µm long and 1.8 - 2.1 (1.9 ± 0.2) times longer than the isthmus; isthmus narrower 50.0 - 55.0 (52.0 ± 2.2) µm long; metacorpus nearly pyriform, 16.0 - 23.0 (20.8 ± 3.2) µm long and 15.0 - 28.0 (19.5 ± 5.8) µm wide. Cardia conoid. Nerve ring in the first third of the isthmus at 111.0 - 131.0 (119.5 ± 8.7) µm from the anterior end. Excretory pore posterior to the nerve ring and anterior to the deirid at 155.0 - 132.0 (125.0 ± 7.3) µm from the anterior end. Deirid difficult to see. Reproductive system monodelphic-prodelphic with the ovary reflexed, the germinal area at 53 - 130 µm from the anus, with a postuterine sac, 12.0 - 20.0 (17.2 ± 3.6) µm long. Vulva with protruding lips oriented posteriad, anterior lip the larger, the vulva is 471.3-555.0 (518.8 ± 34.9) µm from the anterior end. Two gland cells, 5 µm long by 3 µm wide with conspicuous nuclei, are located one anterior and the other posterior to the vagina. Vagina length 7.0 - 10.0 (8.3 ± 1.5) µm. Rectum 20 - 28 (22.8) 3.8) µm long length and 1.3 - 2.3 (1.6 ± 0.3) times anal body diameter with two gland-like cells at its anterior end. Tail elongate conoid, curved ventrally and ending in an acute tip with a dorsally-curved mucro, and 4.8 - 5.9% (5.4 ± 0.5) of the body length. Anal lips slightly protruding, mainly the posterior lip. Phasmids located in the posterior end of the tail 27.0 - 34.0 (31.0 ± 3.1) µm from the anus and 57.4 - 66.0% (62.6 ± 4.0) of tail length.

Male (n=3). Body an open C shape, curved ventrad after fixation. Cuticle with very fine striations. Head similar to female, 9.0 - 10.0 (9.3 ± 0.6) µm wide; outer labial setae 2.0 µm long. Amphid aperture at 4.0 - 6.0 (5.0 ± 1.4) µm from the anterior end. Cheilostom 4.0 µm long and 4.0 - 5.0 (4.3 ± 0.6) µm wide; gymnostom 3.0 µm long. Nerve ring at 70.0 - 105.0 (87.5 ± 24.8) µm from the anterior end. Excretory pore posterior to the nerve ring and anterior to the deirid, at 84.0 - 121.0 (102.5 ± 26.2) µm from the anterior end. Deirid slightly posterior to excretory pore at 84 µm from the anterior end. As in females, the

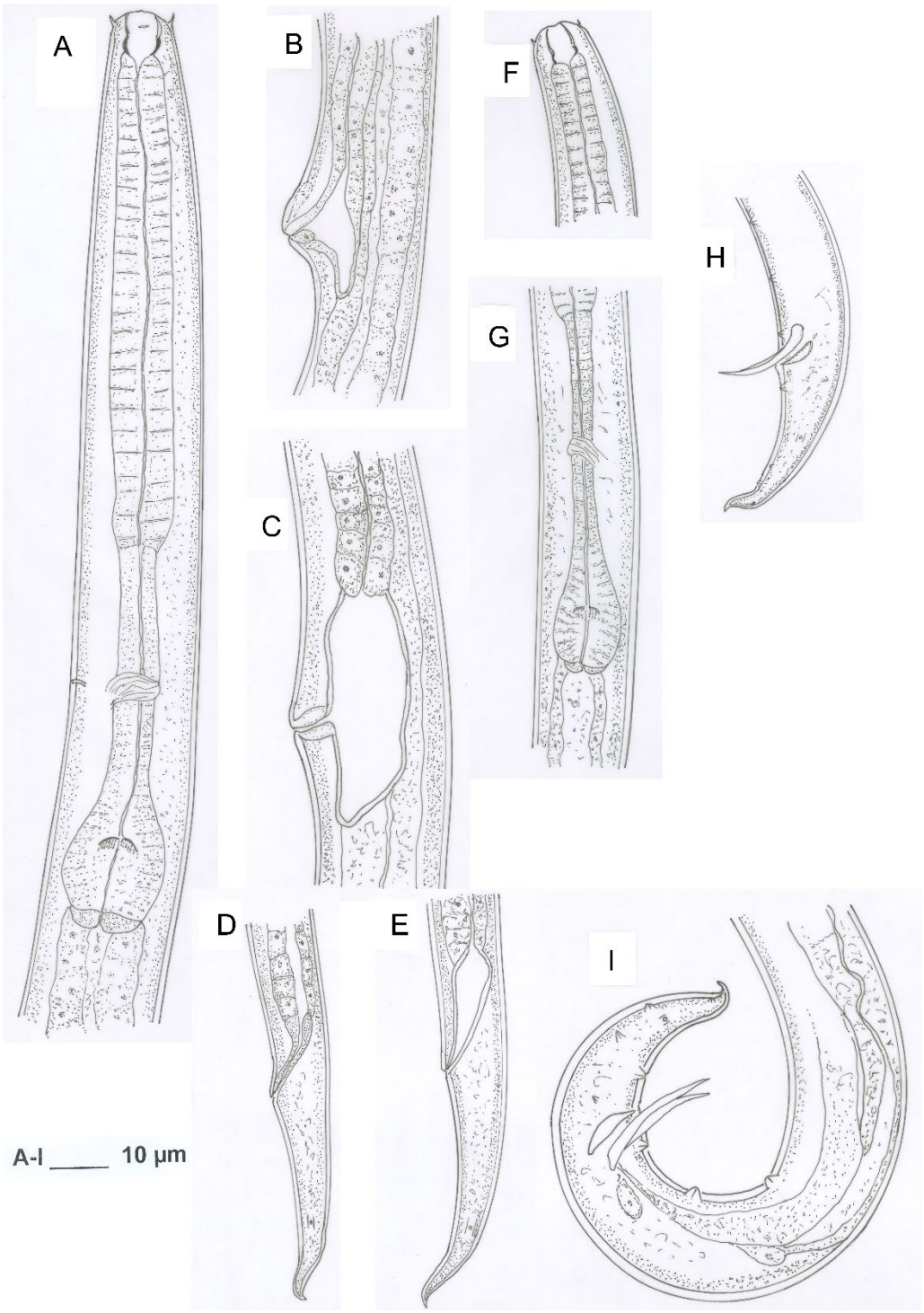

Figure 2. *Macrolaimus inecolensis* sp.n. Female: A-E. A) Anterior end, B, C) vulva region, and D, E) tails. Male F-I. F) Head, G) posterior end oesophagus, and H, I) tails.

lateral field has two incisures. Reproductive system monorchic reflexed ventrad. Spicules paired and symetrical, curved ventrad, 22 µm long; manubrium with rounded proximal end, short calamus and curved lamina. Gubernaculum arc-shaped 10.0 – 11.0 (10.7 ± 0.6) µm long.

Figure 3. *Macrolaimus inecolensis* sp.n. Fermale A-C. A) Anterior region, B) vulva region, and, C) tail. Male D-F. D) Anterior region, and, E, F) Tails.

Three gland-like cells are present anterior to the spicules. There are three pairs of subventral precloacal genital papillae; one pair is 4.0 - 5.0 (4.73 ± 0.6) µm and the second 16.0 - 25.0 (21.0 ± 4.6) µm and the third 30.0 – 45.0 µm from cloacal aperture. There are five postcloacal pairs of papillae. Phasmids are located in the posterior half of the tail at 16.0 - 29.0 (23.3 ± 6.7) µm from the anus. Tail curved more ventrad than in females, 28.0 - 36.0 (33.0 ± 4.4) µm long ending in an acute tip with a dorsally-curved mucro.

Diagnosis. *Macrolaimus inecolensis* sp.n. is characterized by the heavily-sclerotized stoma subdivided into a cheilostom and gymnostom with the cheilostom approximately twice as long as the gymnoston; the lateral field with two incisures; the postuterine sac 12.0 – 20.0 µm long and half the body width at vulva level. The ovary is reflexed, with the germinal area at 53.0 – 130.0 µm from the anus; the vulva has protruding lips oriented posteriad. The male has paired, symmetrical spicules, curved ventrad and manubrium with rounded proximal end; the gubernaculum is arc-shaped. There are three pairs of subventral precloacal, and five pairs of postcloacal, genital papillae.

Macrolaimus inecolensis sp.n. is close to *M. arboreus* Truskova and Eroshenko, 1977, *M. canadensis* Sanwal, 1960, *M. taurus* Thorne, 1937, and *M. montrealenisis* sp.n. in the presence of a postuterine sac and prominent vulval lips. It differs from *M. arboreus* in that the cheilostom is 1.4 - 2.5 (1.9) times the length to the gymnostom vs. 3.0 times, in the length of the female tail 47.0 - 52.0 (50.3) µm vs. 45.0 µm long, and in the male genital papillae, three pairs precloacal and five postcloacal vs, two precloacal and three postcloacal.

Macrolaimus inecolensis sp.n. differs from *M. canadensis* in the size of the spicules, 22.0 µm vs 27.0 µm long in the of pairs genital papillae of male vs. three pairs precloacal and five postcloacal vs two precloacal and four postcloacal; in the length of the postuterine sac relative to the body width at the vulva, 0.4 - 0.6 (0.55) vs. 1.4 times long. It also differs from *M. canadensis* in the length of the male tail, 28.0 - 36.0 (33.0) µm vs. 40.0 – 54.0 µm. *Macrolaimus inecolensis* sp.n. differs from *M. taurus*, in the size of the body in females and males, on average less than 1.0 mm vs. more than 1.0 mm; in the position of vulva 51.0 - 58.8 (55) vs. 60% of the body length, and in the length of the postuterine sac relative to the body width at the vulva, 1.6 - 2.3 (1.6) vs. 1.2 times. Finally, *Macrolaimus inecolensis* sp.n. differs from *M. montrealensis* sp.n.in the size of the potvulval sac, 12.0 – 20.0 (17.2) µm vs 5.0 – 12.0 (8.8) µm long; in the size of the tail in females and males 47.0 – 52.0 (50.0) µm and 28.0 – 36.0 (33.0) µm long vs 63.0 – 80.0 (73.0) µm and 48.0 – 55.0 (52.0) µm, respectively, and finally in the number of male genital papillae, three precloacal and five postcloacal vs. two precloacal and five postcloacal.

Type locality and habitat. Collected on February 20, 2020, from a filamentous epiphyte, *Misodendrum* sp. (Misodendraceae) growing on a tree in the Botanical Garden of the Ecology Institute of Jalapa, Veracruz, México, N 19°30'47.4", W 96°56'32.9", elevation 1400 m asl.

Type specimens. Accession numbers of type specimens deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, México, are holotype female CNHE: 11288 allotype male CNHE: 11289, paratype females CNHE: 11230. Other paratype material is deposited in the University of California Riverside Nematode Collection (UCRNC), and the Colegio de Postgraduados Nematode Collection (CPNC).

Etymology. The species name recognizes that the type locality National Institute of Ecology Jalapa, Veracruz State, México.

DISCUSSION

The subfamily Macrolaimnae of the family Chambersiellidae consists of three genera *Diastolaimus* Rahm, 1928, *Macrolaimellus* Andrássy, 1966 and *Macrolaimus* Maupas, 1900. Some species of *Macrolaimus* have been described from terrestrial environments, associated with lichen, moss and insect galleries in tree trunks (Thorne, 1937; Sanwal, 1960) while others, including *M. crucis*, *M. richteri*, *M. natator* and *M. somniorum* have been described from soil samples (Abolafia *et al*., 2019; Swart and Heyns, 1992; Timm, 1960; Andrassy, 1984).

Species of *Macrolaimus* may be carried between habitats by insects (Massey, 1974). Azizoglu *et al*. (2016) noted that *Macrolaimus* species are commonly recovered from bark infested with beetles; *M. canadensis* was isolated from the frass of the bark beetle Phloeosinus canadensis (Sanwal, 1960), *M. crucis* was isolated from the pine - top weevil gallery Pissodes piniphilus (Poinar, 1975), and *M. taurus*, was isolated from gallery of Ips confusus on *Pinus edulis* (Thorne, 1937). *Macrolaimus* spp. are assumed to be bacterivores based on the unarmed stoma and the cephaloboid oesophagus. Given their frequent association with insects, besides the advantage of transportation to new resources by phoresy, some species may acquire resources through necromeny, that is, by feeding on bacteria that are exploiting the bodies of dead insects.

As more species have been added to the genus *Macrolaimus*, their separation based on morphometric characteristics has become increasingly difficult. Features such as the relative dimensions of components of the buccal cavity, the length of a postuterine sac relative to body width at vulva level and the presence of projections on spicules involve some subjectivity of judgement. Further, in earlier descriptions of species, the importance of such characteristics may not have been anticipated and details were not provided. Several of the species were poorly described and poorly illustrated by the original authors, with important diagnostic characters omitted. Examples are *M. crucis* described by Maupas (1990) from a single female specimen and *M. natator* described by Timm (1960) from a single male specimen. Other species described from a single female and single male include: *M. hamatus* and *M. taurus* (Thorne, 1937) and *M. somniorum* (Andrássy, 1984). In a few cases, recent authors have provided detailed redescriptions of many of the morphological characters that have become increasingly important in species separation, including details of the components of the buccal cavity and the

male genital structures (Abolafia and Peña-Santiago, 2014; Abolofia *et al*., 2019; Shokoohi *et al*., 2018). For species separation within *Macrolaimus*, molecular diagnostics are and will be important. Unfortunately, in the case of *M. montrealensis* sp.n., despite repeated attempts, we were unable to obtain useful molecular sequences.

Macrolaimus crucis Maupas, 1900. The type species of the genus. Lateral field with three incisures; postuterine sac absent; female tail conical with acute terminal mucro; phasmid located at 60-70% of tail length from anus; male tail conical, curved ventrad; three precloacal and four postcloacal papillae; spicules 26.0 - 38.0 (30.0) µm and gubernaculum 10.0 - 13.0 µm with fusiform shape. Habitat: sandy soil.

Macrolaimus taurus Thorne, 1937. Lateral field with two incisures; postuterine sac 0.8 times vulval body diameter; female tail conical with acute terminus; phasmid in the last third of the length of tail >70%; male tail curved ventrad with conical end like mucro; spicules 29.0 µm, without ventral spine in manubrium; gubernaculum arch-shaped. Habitat: insect galleries in pinyon pine.

Macrolaimus arboreus Truskova and Erosenko, 1977. Lateral field with three incisures; postuterine sac 0.4 times vulval body diameter; female tail conoid with dorsally curved terminus; male tail conical, curved ventrally with dorsally curved terminus; two precloacal and three postcloacal pairs of papillae. Habitat: tree bark

Macrolaimus canadensis Sanwal, 1960.

Key to the species of *Macrolaimus* - adapted and modified from Abolafia and Peña-Santiago (2014) to include *M. montrealensis* sp.n. and *M. inecolensis* sp.n.

1. Cheilostom length < 2 times or equal length of gymnostom.....2
   Cheilostom length > 2 times or equal length of gymnostom.....5
2. Postuterine sac absent 3
   Postuterine sac present.....4
3. Lateral field with three incisures.....*M. crucis*
   Lateral field with five incisures..... *M. richteri*
4. Male with 3 precloacal and 6 postcloacal pairs of papillae.....*M. somniorum*
   Lateral field with two incisures; postuterine sac length 1.25 times body diameter at vulva.....*M. taurus*
   Lateral field with three incisures; postuterine sac length 4.2 times body diameter at vulva..... ***M. montrealensis* sp.n.**
5. Postuterine sac length < 2 vulval body diameter.....6
   Postuterine sac length > 2 vulval body diameter.....8
6. Lateral field with two incisures.....7
   Lateral field with three incisures.....8
7. Male with 2 precloacal and 4 postcloacal pairs of papillae.....*M. canadienses*
   Male with 3 precloacal and 5 postcloacal pairs of papillae..... ***M. inecolensis* sp.n.**
8. Lateral field with three incisures; male with 2 precloacal and 3 postcloacal pairs of papillae; Cheilostome/Gymnostome 1.7-2.0 .....*M. arboreus*
   Male with 2 precloacal and 5 postcloacal pairs of papillae; Cheilostome/Gymnostome 1.7-1.8.....*M. ruehmi*
   Male with 4 precloacal and 4 postcloacal pairs of papillae; Cheilostome/Gymnostome 1.6-4.2.....*M. natator*
   Male with 2 precloacal and 3 postcloacal pairs of papillae; Cheilostome/Gymnostome 2.0-2.8.....*M. hamatus***

** Thorne (1937) provided an inadequate description of *M. hamatus*, which omitted many of the characters currently considered diagnostically important.

Species *inquirendae* not included in the above key are: *M. citri* (Rahm, 1928) Andrássy, 1984, *M. crucis* var. *gracilis* (Rahm, 1929), Andrássy, 1984 = *Seleneella gracilis* (Rahm, 1929), Rahm, 1932, *M. crucis* var. *tenuis* (Rahm, 1928), Andrássy, 1984, *M. maipoensis* (Rahm, 1932), Andrássy, 1984 = *Seleneella maipoensis* Rahm, 1932.

Lateral field with two incisures; postuterine sac about one-half to one body diameter long. Female tail conical with acute tip bent dorsad; phasmid in the last third of the length of tail >70% in females and in the first third in males; male tail conical curved ventrally with dorsally curved terminus; two precloacal and four postcloacal papillae; spicule with thorn-like ventral spine and gubernaculum arch-shaped. Habitat: insect galleries in tree bark.

Macrolaimus richteri Swart and Heyns, 1992. Lateral field with five incisures; postuterine sac very small or absent; tail elongate - conoid with conical point - like mucro. Phasmid in the last third of length of tail. Male unknown. Habitat: sandy soil.

Macrolaimus natator Timm, 1960. Male with lateral field with three incisures; tail 28.0 µm long with conical dorsally-curved end, 1.6 times anal body diameter long; eight pairs of genital papillae, four sub ventral precloacal and four post cloacal papillae. Spicules 28.0 µm long; gubernaculum 11.0 µm long. Phasmids at 0.5x anal body diameter posterior to anus. Female unknown. Habitat: soil.

Macrolaimus somniorum Andrassy, 1984. Lateral field narrow; postuterine sac shorter than the body width. Cheilostom 2 - 2.5 times longer than gymnostom. Spicule 22.0 µm long; gubernaculum 18.0 µm long. Three pairs of precloacal and six pairs of postcloacal papillae; tail conical, bent ventrally at the tip. Habitat: sandy soil.

Macrolaimus ruehmi Andrassy, 1966. Cheilostom length 1.8 times that of gymnostom; lateral field with three incisures; postuterine sac 1.0 - 1.8 times vulval body diameter; female and male tail conoid, ventrally-curved in posterior part with short conoid mucro. Phasmids in posterior half of tail at 53-65% of tail length from anus in females and 56-76% in males; two subventral precloacal pairs and five postcloacal pairs of genital papillae. Habitat: tree bark.

Macrolaimus hamatus Thorne, 1937. Body length of female and male less than 1.0 mm; vulva at 55% of body length; female tail elongate - conoid and shorter in males, both with acutely dorsally-bent tail tip and terminal mucro; male with two pairs of subventral precloacal and three pairs of postcloacal genital papillae. Spicules 29.0 µm long, without ventral spine; gubernaculum arch-shaped. Habitat: moss.

Macrolaimus montrealensis sp.n. Body lengths of female and male on average 0.1 and 0.9 mm, respectively; lateral field with three incisures; vulva at 57% of body length; postuterine sac 0.2 - 0.5 (0.3) times vulval body diameter, female tail elongate conoid, curved ventrally and ending in an acute tip with a dorsally - curved mucro; male with two pairs of subventral precloacal and five pairs of postcloacal genital papillae; spicules paired and symmetrical, curved ventrally; manubrium with rounded proximal end. Habitat: lichen on tree bark.

Macrolaimus inecolensis sp.n. Body lengths of female and male on average 0.9 and 0.7 mm, respectively; lateral field with two fine incisures; vulva at 56% of body length; postuterine sac 12.0 - 20.0 (17.3) µm long and 1.6 - 2.3 (1.6) times vulval body diameter, female tail elongate conoid, curved ventrally and ending in an acute tip with a dorsally- curved mucro; male with three pairs of subventral precloacal and five pairs of postcloacal genital papillae; spicules paired and symmetrical, curved ventrally; manubrium with rounded proximal end. Habitat: filamentous epiphyte plant on branch tree.

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