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A new species of *Heligmostrongylus* Travassos 1917 (Nematoda: Heligmonellidae) in small rodents (Cricetidae and Heteromyidae) from Mexico

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Research Paper

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Abstract

A new species of *Heligmostrongylus* (Nematoda: Heligmonellidae) is described from the small rodents *Ototylomys phyllotis* (Cricetidae: Tylomyinae) and *Heteromys gaumeri* (Heteromyidae: Heteromyinae) in the Yucatan Peninsula, Mexico, based on studies of light and scanning electron microscopy, and partial sequences of COI, ITS1 and 28S rRNA. *Heligmostrongylus yucatanensis* n. sp. is characterized by a synlophe of 13 interrupted ridges (except those forming careen) in both sexes at midbody; males with a ventral cuticle inflation at anterior region of copulatory bursa, rays 9 and 10 long, comparable in length, and rays 9 strongly curved laterally at a right angle crossing ventrally rays 8; and females with a torsion of 180° to left of the posterior extremity. These characteristics were shared with *Heligmostrongylus* nematodes reported previously from *O. phyllotis* and *Peromyscus yucatanicus* (Cricetidae: Neotominae) also in the Yucatan Peninsula. The absence of intraspecific sequence variations in COI, and the low variation in D2-D3 expansion segments of 28S rRNA and ITS1 among the specimens obtained from the different hosts provided strong support that the worms found in the three rodent species belong to the same new species. The nine previously known species of *Heligmostrongylus* have been reported from caviomorph rodents of the families Cuniculidae, Dasyproctidae, Echimyidae, and Erethizontidae from the Neotropics. The occurrence of *H. yucatanensis* in three phylogenetically distant rodent species suggests that this nematode species could have the ability to expand its host range by colonizing new hosts.

Introduction

The genus *Heligmostrongylus* Travassos, 1917 (Heligmonellidae, Pudicinae) comprises nematodes characterized by a caudal bursa with a pattern of type 2-2-1 and a synlophe with a well-developed careen made up of two continuous ridges, plus 11–12 discontinuous ridges arranged in linear series (Durette-Desset *et al.*, 2017). This genus contains nine species described from rodents of the families Cuniculidae, Dasyproctidae, Echimyidae, and Erethizontidae from the Neotropics (Durette-Desset *et al.*, 2017): *Heligmostrongylus almeidai* (Durette-Desset & Tchéprakoff 1969), *Heligmostrongylus differens* Lent & Freitas, 1938, *Heligmostrongylus crucifer* (Travassos, 1943), *Heligmostrongylus elegans* (Travassos, 1921), *Heligmostrongylus sedecimradiatus* (Linstow, 1899), and *Heligmostrongylus squamastrongylus* (Travassos, 1937) in Brazil, *Heligmostrongylus chiarae* Durette-Desset, Deharo, Santiváñez-Galarza & Chabaud, 2001 in Bolivia, *Heligmostrongylus echimyos* Diaw, 1976 in French Guiana, and *Heligmostrongylus proechimysi* Durette-Desset, 1970 in Colombia.

In a previous helminthological survey conducted to explore the helminth diversity of wild small rodents (Cricetidae and Heteromyidae) from the Yucatan Peninsula (Mexico), we identified nematodes that showed characteristics of *Heligmostrongylus* from *Ototylomys phyllotis* Merriam, 1901 and *Peromyscus yucatanicus* Allen & Chapman, 1897 (Rodentia: Cricetidae), a finding that extended the host range of this genus of nematodes in the Neotropics (Panti-May *et al.*, 2023). Their morphological characteristics revealed *a priori* that they belonged to an undescribed species of *Heligmostrongylus*; however, we did not describe it due to the few specimens found and their poor condition. In the present study, we describe this new species based on new material collected in the Yucatan Peninsula.

Materials and methods

Nematode collection and morphological characterization

Nineteen specimens of *O. phyllotis* and nine of *Heteromys gaumeri* Allen & Chapman, 1897 were trapped in a cattle ranch in the state of Yucatan, Mexico, from July 2022 to May 2023, as a part of a

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larger study described elsewhere (Chan-Casanova, 2024). Nematodes were collected from the small intestine, washed in 0.85% sodium chloride solution, and then fixed in 10% buffered formalin or preserved in 70% ethanol for morphological study. For the molecular study, some specimens were preserved in 100% ethanol and stored at –4 °C.

Nematodes were cleared and temporarily mounted in lactophenol for morphological examination. Specimens were studied/drawn using a Leica DM750 light microscope equipped with a drawing tube. Some nematodes preserved in 10% formalin were dehydrated using a graded ethanol series, critical point–dried with carbon dioxide, sputter-coated with a gold-palladium mixture, and examined at an accelerating voltage of 10 kV with a Hitachi SU1510 scanning electron microscope (SEM) at the Laboratorio de Microscopía y Fotografía de la Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico City. The synlophe was studied following the method of Durette-Desset (1985). The description of the caudal bursa follows Durette-Desset & Digiani (2012) and was based on fully extended bursae and observation of individual lobes. All measurements are given in micrometres unless otherwise stated. For the description of the new species, the measurements of the holotype and allotype are presented first, followed by the mean, standard deviation, and the range in parentheses of the paratypes. Classification used above the family Heligmonellidae follows (Beveridge *et al.*, 2014). The nomenclature and synonymy of the hosts' species follows the American Society of Mammalogists Biodiversity Committee (2023). Prevalence (expressed as a percentage) and mean intensity with their 95% confidence intervals (CI) were estimated following Bush *et al.* (1997). Specimens were deposited in the Colección Nacional de Helmintos (CNHE), IBUNAM, Mexico City, Mexico. Vouchers of hosts were deposited in the Colección Mastozoológica, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán (FMVZ-UADY).

Molecular data and analysis

We produced partial sequences of three molecular genetic markers, the cytochrome c oxidase subunit 1 (COI), the Internal Transcribed Spacer region 1 (ITS1), and the 28S rRNA, from individual worms collected from *O. phyllotis* and *H. gaumeri.* Genomic DNA was extracted from individual specimens using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). A fragment of the COI was amplified using primers LCO1490/ HC02198 (Folmer *et al.*, 1994). Partial sequences of the ITS1 region were amplified using the primers NC16 (Chilton *et al.*, 2003)/NC13R (Chilton & Gasser, 1999). A fragment of the 28S rRNA gene was amplified using the primers 391 (Nadler *et al.*, 2003)/536 (García-Varela & Nadler, 2005). Thermal conditions of the polymerase chain reaction (PCR) amplifications were those described by Folmer *et al.* (1994), Sukee *et al.* (2018), and Hernández-Mena *et al.* (2017) for COI, ITS1, and 28S rRNA, respectively. The PCR primers, along with additional internal primers 503 (Nadler *et al.*, 2003) and 504 (Hernández-Mena *et al.*, 2017) for 28S rRNA, were used for Sanger sequencing at Macrogen (Seoul, Korea). Contiguous sequences were assembled and edited using Geneious Pro 4.8.4 (Kearse *et al.*, 2012). Consensus sequences obtained in this study and other sequences of Heligmosomoidea available in GenBank were used for phylogenetic analyses; for the 28S rRNA gene, trimmed sequences of the domains D2–D3 were used. The alignment was generated using ClustalW (http://www.genome.jp/tools/clustalw/) with the "SLOW/ACCURATE" approach and weight matrix "CLUSTALW (for DNA)" (Thompson *et al.*, 1994). The best-fitting nucleotide substitution model was selected for each data set with jModelTest v2 (Darriba *et al.*, 2012) under Akaike information criterion. Phylogenetic affinities for each data set were evaluated by maximum likelihood (ML) analysis using RAxML v. 7.0.4 (Stamatakis, 2006). Bootstrap support values were estimated by running 1000 bootstrap resamples. Genetic variation within the COI, ITS1, and the D2–D3 of the 28S rRNA data sets was calculated using p-distances with MEGA 11 (Tamura *et al.*, 2021).

Results

Thirteen specimens of *O. phyllotis* and six specimens of *H. gaumeri* were infected with nematodes of the genus *Heligmostrongylus.* *Ototylomys phyllotis* was usually coinfected with Heligmosomoidea gen. sp. cf. *Vexillata*, *Strongyloides* sp., *Syphacia* spp., and *Raillietina* sp., while *H. gaumeri* had coinfections with *Vexillata vexillata* (Hall, 1916), *Strongyloides* sp., and *Trichuris silviae* Panti-May & Robles, 2016. Next we provide a taxonomic description of the new species of *Heligmostrongylus* based on specimens from the type host (*O. phyllotis*). Measurements of the new species isolated from *O. phyllotis* and *H. gaumeri*, as well as measurements of other species of *Heligmostrongylus* are presented in Table 1 for comparative purposes.

Heligmostrongylus yucatanensis n. sp. (Figures 1–2 and Table 1)

Description

General: Medium-sized nematodes. Cephalic vesicle present (Figure 1a). In apical view, triangular oral opening surrounded by thin rim; two amphids and six external labial papillae visible (Figure 2a). Deirids small, situated usually posterior to nerve ring (Figure 1a). Excretory pore located slightly anterior to distal end of oesophagus (Figure 1a). Ventral cuticle at anterior region of copulatory bursa with inflation (Figures 1j–k, 2d). Posterior extremity of female twisted approximately 180° to left (Figure 2e).

Synlophe (studied in three males and three females): Cuticle with longitudinally interrupted ridges (except those forming careen), arranged in linear series (Figure 2b–c). Careen present, supported by two hypertrophied ridges similar in size. Ridges appearing posterior to cephalic vesicle and disappearing just anterior to prebursal papillae in male (Figure 2d) and at level of vestibule in female. In both sexes, 11–12 (careen, 4–5 dorsal, 5 ventral) ridges at level of distal oesophagus (Figure 1b, e) and 13 ridges (careen, 5 dorsal, 6 ventral) at mid-body (Figure 1c, f). Within distal third of body length, size of ridges forming the careen decreasing progressively; 13 ridges (careen, five dorsal, six ventral) at level of distal uterus in female (Figure 1d) and same at level of the middle portion of the spicules in male (Figure 1g). Right side (opposite to careen) free of ridges (Figure 1b-g, Figure 2b-c). Axis of orientation of ridges sub-frontal, directed from right to left (Figure 1b–g).

Male (based on holotype and 19 paratypes): 10.7, 8.7 ± 0.9 (6.6–10.2) mm long and 118, 107.1 ± 13.8 (85–138) wide (careen included) at mid-body. Cephalic vesicle 80, 70.4 ± 7 (60–88) long and 40, 32.5 ± 4.2 (25–40) wide. Nerve ring, deirids, and excretory pore situated at 245, 214.1 ± 20.8 (170–250), 362, 278 ± 32.5 (220–335), and 455, 386.3 ± 42.7 (310–485) from apex, respectively. Oesophagus 408, 346.8 ± 30.8 (295–400) long.

Caudal bursa subsymmetrical with dorsal ray well developed (Figure 1j). Pre-bursal papillae observed (Figures 1j, 2d). Pattern of

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Table 1. Host and main morphological features and measurements of *Heligmostrongylus* species

	H. sedecimradiatus	*H. almeidai*	*H. chiarae*	*H. crucifer*
References	Travassos, 1921, Durette-Desset, 1970a	Durette-Desset & Tchéprakoff, 1969	Durette-Desset *et al*., 2001	Travassos, 1943
Type host	*Cuniculus paca*	*Thrichomys apereoides*	*Proechimys longicaudatus*	*Thrichomys apereoides*
Other hosts	*Dasyprocta leporina*, *Coendou spinosus*	–	–	–
Location	Brazil	Brazil	Bolivia	Brazil
Host family	Dasyproctidae, Erethizontidae	Echimyidae	Echimyidae	Echimyidae
Male (N)	–	1	5	–
Body length (mm)	8–10	4.5	2.7–3.0	4.4
Body width	140	150	90	60
Cephalic vesicle length	70–80	80	30	45
Cephalic vesicle width	–	50	27	–
Oesophagus length	360	390	250–280	310
Nerve ring (dae)	–	210	150	–
Deirids (dae)	–	330	200	–
Excretory pore (dae)	–	310	205–220	–
Spicule length	500–530	840	200–240	380–590
Genital cone	Triangular	Triangular	Conical	Triangular
Ridges at mid-body	13	13	13	12
Spicule length/body length (%)	5.5	19	9	11
Female (N)	–	–	10	–
Body length (mm)	14–16.2	5.9	3.6–4.3	6.1
Body width	180	150	70–90	70
Cephalic vesicle length	–	50	30	49
Cephalic vesicle width	–	50	27	–
Oesophagus length	–	390	260 (240–290)	380
Nerve ring (dae)	–	220	140	–
Deirids (dae)	–	330	205	–
Excretory pore (dae)	–	340	196 (170–225)	–
Ridges at mid-body	–	13	14	12
Vulva (dpe)	140	122	55–90	230–240
Vagina vera length	–	–	20–30	–
Vestibule length	–	175	40–55	–
Sphincter length	–	38	18–22	–
Infundibulum length	–	150	80–130	–
Uterus length (mm)	–	–	0.4–0.8	–
Egg length	59	68	50–80	57–76
Egg width	31	32	20–40	23–24
Tail length	42	42	35–40	70–90

	H. differens	*H. echimyos*	*H. elegans*	*H. proechimysi*
References	Lent & Freitas, 1938	Diaw, 1976	Travassos, 1921, Lent & Freitas, 1938	Durette-Desset, 1970b
Type host	*Coendou insidiosus*	*Makalata didelphoides*	*Coendou spinosus*	*Proechimys semispinosus*
Other hosts	–	–	–	–
Location	Brazil	French Guiana	Brazil	Colombia

(*Continued*)

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Table 1. (*Continued*)

	H. differens	*H. echimyos*	*H. elegans*	*H. proechimysi*
Host family	Erethizontidae	Echimyidae	Erethizontidae	Echimyidae
Male (N)	1	1	5	1
Body length (mm)	6.8	7.4	3.7–4.6	2.5
Body width	88	140	80–140	60
Cephalic vesicle length	72	62	57–67	22
Cephalic vesicle width	32	38	38–40	20
Oesophagus length	–	380	376–440	235
Nerve ring (dae)	240	140	224–240	135
Deirids (dae)	–	290	–	190
Excretory pore (dae)	–	390	–	195
Spicule length	608	735	370–424	245
Genital cone	Triangular	Bulbous	Triangular	Triangular
Ridges at mid-body	–	13	13	13
Spicule length/body length (%)	9	10	10	10
Female (N)	–	6	4	5
Body length (mm)	–	10.4–13.9	5.9–8.5	2.9
Body width	–	170	96–175	90
Cephalic vesicle length	–	60	50–73	22
Cephalic vesicle width	–	40	30–40	25
Oesophagus length	–	395	324–432	230
Nerve ring (dae)	–	145	232–240	135
Deirids (dae)	–	280	220	188
Excretory pore (dae)	–	350	280	190
Ridges at mid-body	–	13	13	13
Vulva (dpe)	–	390	210–310	52
Vagina vera length	–	70	–	19
Vestibule length	–	200	150	30
Sphincter length	–	60	40	20
Infundibulum length	–	280	200	72
Uterus length (mm)	–	1.8–2	–	0.5
Egg length	–	80	70	52
Egg width	–	40	40	30
Tail length	–	105	110	36
	H. squamastrongylus	*H. yucatanensis*	*H. yucatanensis*	
References	Travassos, 1937	This study	This study	
Type host	*Proechimys roberti*	*Ototylomys phyllotis*		
Other hosts	–		*Heteromys gaumeri*	
Location	Brazil	Mexico	Mexico	
Host family	Echimyidae	Cricetidae	Heteromyidae	
Male (N)	1	20	4	
Body length (mm)	4.5	6.6–10.7	3.6–5.7	
Body width	130	85–138	85–106	
Cephalic vesicle length	–	60–88	55–62	

(*Continued*)

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Table 1. (*Continued*)

	H. squamastrongylus	*H. yucatanensis*	*H. yucatanensis*
Cephalic vesicle width	–	25–40	25–40
Oesophagus length	300	295–408	292–315
Nerve ring (dae)	–	170–250	155–200
Deirids (dae)	–	220–362	192–235
Excretory pore (dae)	–	310–485	262–308
Spicule length	450	580–700	505–600
Genital cone	Inconspicuous	Triangular	Triangular
Synlophe mid-body	–	13	13
Spicule length/body length (%)	10	6.2–9.5	9.7–15.9
Female (N)	1	20	4
Body length (mm)	5.3	11.8–17.7	6.9–10.7
Body width	130–150	92–150	95–125
Cephalic vesicle length	–	55–75	55–65
Cephalic vesicle width	–	30–45	30–38
Oesophagus length	330	300–450	346–368
Nerve ring (dae)	–	170–280	190–235
Deirids (dae)	–	105–330	235–265
Excretory pore (dae)	–	290–462	310–345
Ridges at mid-body	–	13	13
Vulva (dpe)	90	80–140	100–128
Vagina vera length	–	50–80	42–60
Vestibule length	–	115–160	100–110
Sphincter length	–	35–50	40–45
Infundibulum length	–	110–185	140–185
Uterus length (mm)	–	2.5–4.1	1.5–2.3
Egg length	64	55–75	55–78
Egg width	40	28–44	28–38
Tail length	37	30–45	35–42

Measurements given in micrometres unless otherwise specified. General measurements are presented as a single range.
Abbreviations: dae, distance from anterior end; dpe, distance from posterior end.

type 2-2-1. Rays 2 smallest, slightly curved inwards. Rays 3, 5 and 6 approximately equal in length. Rays 4 and 5 diverging at distal third of common trunk of rays 3–6, rays 4 slightly shorter. Rays 8 long, arising symmetrically from the proximal third of dorsal ray. Dorsal ray long, divided at proximal third, proximally to arising of rays 8, into two branches, each one bifurcated at distal third into 2 sub-branches, rays 9 (external) and rays 10 (internal) (Figure 1j–k). Rays 9 and 10 long, comparable in length, rays 9 slightly shorter. Rays 9 strongly curved laterally at a right angle and crossing ventrally rays 8. Genital cone poorly developed, not sclerotized, 40, 41 ± 5.4 (30–50) long and 40, 40.4 ± 3.5 (32–45) wide, hidden by the ventral cuticular inflation. Spicules subequal, alate, with pointed tips curved in a right angle, enclosed in an expanded membrane (Figure 1l), 665, 655 ± 32.1 (580–700) long and representing 6.2, 7.6 ± 0.8 (6.7–9.5) % of body length. Gubernaculum absent. Telamon not observed.

Female (based on allotype and 19 paratypes): 16.6, 14.8 ± 1.8 (11.8–17.7) mm long and 110, 122.5 ± 15.9 (92–150) wide (careen included) at mid-body. Cephalic vesicle 66, 65.6 ± 5.2 (55–75) long and 40, 35.3 ± 4.5 (30–45) wide. Nerve ring, deirids, and excretory pore situated at 250, 212.2 ± 31.5 (175–280), 322, 245.6 ± 57.9 (105–330), and 462, 367.7 ± 45.5 (290–458) from apex, respectively. Oesophagus 430, 363 ± 33.6 (300–450) long. Monodelphic. Vulva situated at 120, 109.9 ± 16.2 (80–140) from caudal extremity. Vagina vera 68, 63.9 ± 7.6 (50–80) long, vestibule 152, 134.2 ± 13.9 (115–160) long, sphincter 50, 41.9 ± 4 (35–50) long, infundibulum 165, 151.6 ± 25.8 (110–185). Uterus 3.4, 3.3 ± 0.5 (2.5–4.1) mm long, representing 20.8, 22.7 ± 2.9 (17.6–26.8)% of body length, containing 108, 107 ± 19 (77–148) eggs. Eggs 55–62, 64 ± 4 (58–75) long and 32–40, 35 ± 4 (28–45) wide; based on five eggs measured from the allotype and 66 from the paratypes. Tail conical, 45, 38.9 ± 4.9 (30–45) long. Posterior extremity (approximately from ovejector level, coincident with reduction in size of ridges of the careen) undergoes a torsion of 180° to left, or counterclockwise; resulting in vulva and anus opening on the functionally dorsal surface of the worm (Figures 1h, 2e). This torsion is evidenced through the

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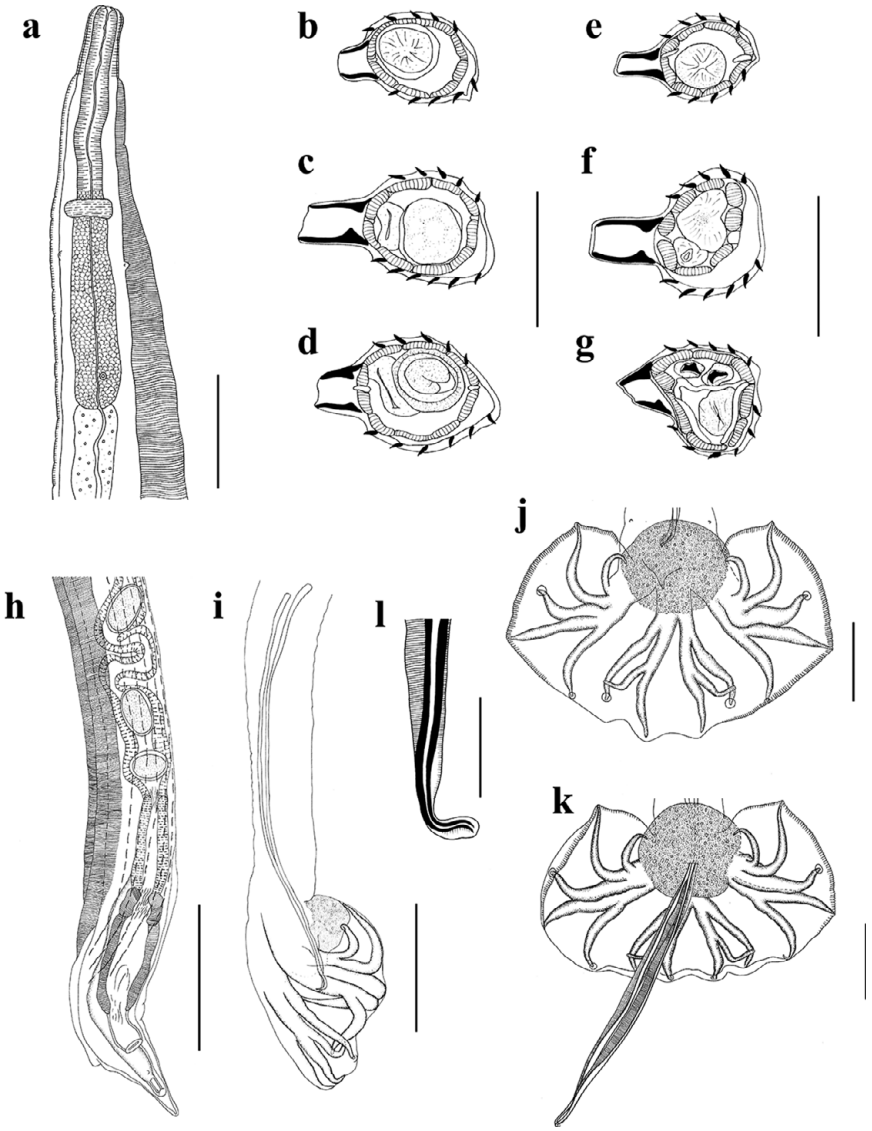

Figure 1. Line drawings of *Heligmostrongylus yucatanensis* n. sp. (a) Anterior part of female, ventral view. (b–d) Transverse sections of the body of female, at level of the oesophagus (b), mid-body (c), and the uterus (d). (e–g) Transverse sections of the body of male, at level of the oesophagus (e), mid-body (f), and spicules (g). (h) Posterior part of female showing cuticular ridges, tail, ovejector, and distal uterus, dorsal view. (i) Posterior part of male, right lateral view. (j) Male caudal bursa, ventral view. (k) Male caudal bursa, ventral view. (l) Tip of spicule, lateral view. Scale bars: (a–g, j–k) 100 μm; (h–i) 200 μm; (l) 30 μm.

rotation of the cuticular ridges and the position of the vulva and anus in relation to the careen.

Type host: Ototylomys phyllotis Merriam, 1901 (Rodentia, Cricetidae, Tylomyinae), male, voucher specimen (No. 1720) deposited at FMVZ-UADY. Other specimens FMVZ-UADY–1694,1696, 1698, 1719.

Other hosts: Heteromys gaumeri Allen & Chapman, 1897 (Rodentia, Heteromyidae, Heteromyinae) (FMVZ-UADY–1686, 1695, 1697, 1718). *Peromyscus yucatanicus* Allen & Chapman, 1897 (Rodentia, Cricetidae, Neotominae) in Panti-May *et al.* (2023).

Type locality: Rancho Santa María (21°15' 48.2"N, 88°16' 31.4"O), municipality of Panabá, state of Yucatan, Mexico.

Other localities: Vallazoo, municipality of Valladolid, Santa Cruz cattle ranch, municipality of Tizimin, state of Yucatan, and Balam Nah eco-hotel, municipality of Felipe Carrillo Puerto, state of Quintana Roo, Mexico, in Panti-May *et al.* (2023).

Site of infection: Small intestine.

Prevalence (CI) and mean intensity (CI): 68.4% (43.4–87.4%) and 44 (25.9–69.1) in *O. phyllotis*, and 66.7% (29.9–92.5%) and 9 (4.5–15.8) in *H. gaumeri.*

Material deposited: Holotype male (No. 13039), allotype female (No. 13040), and a total of 46 paratypes (*O. phyllotis*: No. 13041, 13042; *H. gaumeri*: No. 13043, 13044) were deposited at CNHE.

GenBank accession numbers: COI (PQ429005, PQ429006), ITS1 (PQ472082, PQ472083), 28S rRNA (PQ454599, PQ454600, PQ454601).

ZooBank Life Science Identifier: 735B0E1F-B5D4-4FEC-B969-294F63FB2799.

Etymology: The species epithet refers to the geographic region where the species was detected (Yucatan Peninsula, Mexico).

Diagnosis

The genus *Heligmostrongylus* includes nine species parasitic in Neotropical rodents. The presence of developed rays 9 in *H.*

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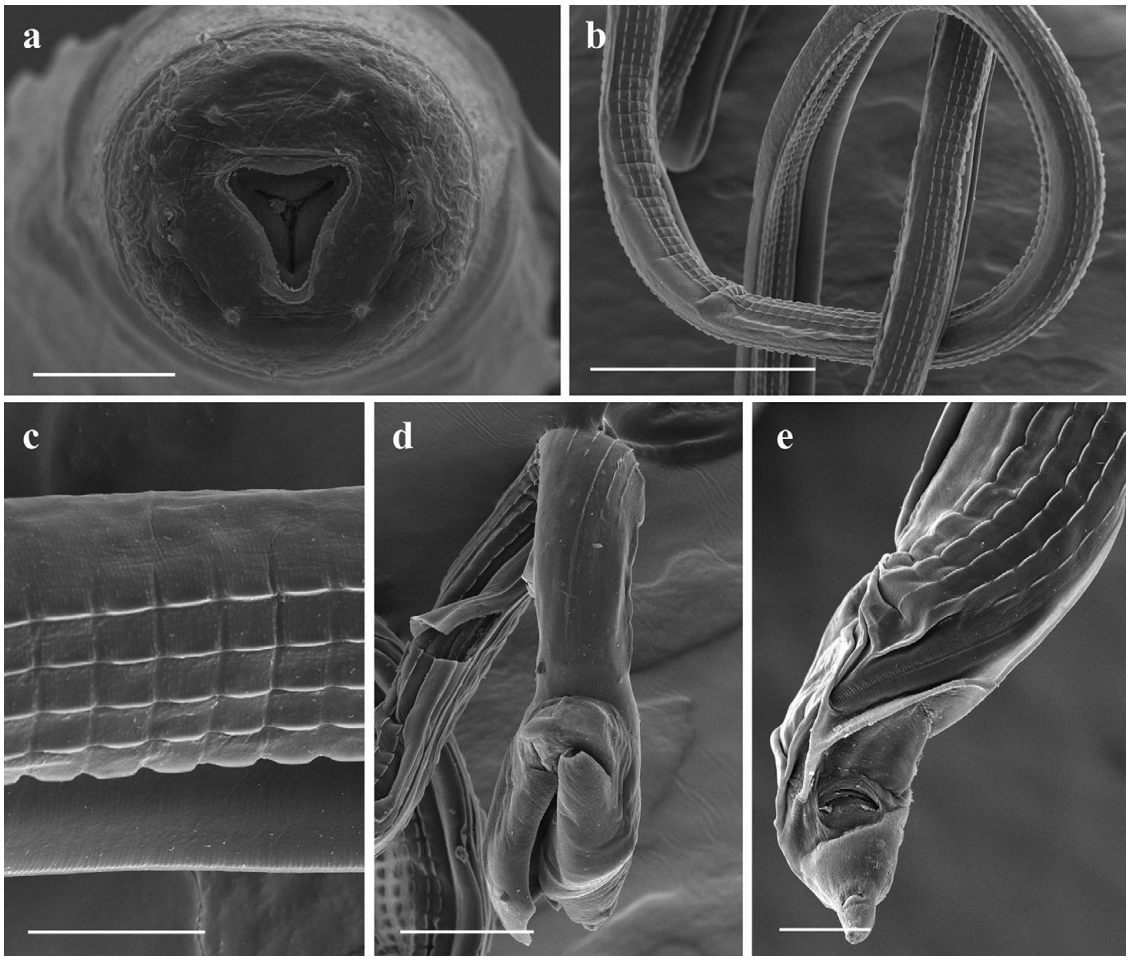

Figure 2. SEM micrographs of *Heligmostrongylus yucatanensis* n. sp. (a) Head of female, apical view. (b) Section of female at mid-body, dorso-lateral view. (c) Middle part of female body, dorsal view (left side towards the bottom of the page). (d) Posterior part of male, ventral view, showing closed caudal bursa and ventral cuticular inflation. (e) Posterior part of female, dorsal view, showing torsion of the posterior extremity. Scale bars: (a) 10 μm, (b) 300 μm, (c) 50 μm, (d–e) 100 μm.

yucatanensis allows its discrimination from *H. squamastrongylus* and *H. echimyos*, which have rays 9 minute or merged with rays 10. The new species has rays 9 comparable in length to rays 10, which allows it to be distinguished from *H. sedecimradiatus*, *H. differens*, and *H. elegans*, which have rays 9 much shorter than rays 10. The new species is also distinguished from *H. chiarae* and *H. proechimysi* by having dorsal ray dividing proximally, about the same level at which rays 8 arise, while in these two species the fork of dorsal ray is distal to the arising of rays 8. Additionally, *H. chiarae* and *H. proechimysi* rays 9, although well developed, are straight and directed posteriorly. *Heligmostrongylus almeidai* differs from the new species by having rays 4 hypertrophied and well separated from rays 5 and by having each spicule ending into dissimilar tips.

The most similar known species to *H. yucatanensis* is *H. crucifer* by having long developed rays 9; dorsal ray dividing at proximal third about the same level at which rays 8 arise; and rays 9 arising from mid-length of dorsal ray and crossing over rays 8 ventrally. However, the pathway of rays 9 is different in both species: in *H. crucifer* rays 9 are first directed latero-posteriorly in an angle of *ca.* 45°, crossing over rays 8, and then bent towards the posterior bursal margin becoming parallel and external to rays 8; while in the new species rays 9, though first directed latero-posteriorly in an angle of ca. 45°, then bent laterally, or even antero-laterally, crossing over rays 8 in a nearly right angle. The spicule tips of *H. yucatanensis* are bent in a nearly right angle and enclosed in an expanded membrane while in *H. crucifer* the spicule tips are simply pointed. Finally, *H. yucatanensis* is the only species in the genus presenting a cuticle inflation in the anterior region of the bursa.

Molecular confirmation

Specimens assignable to *H. yucatanensis* were found not only in rodents of two different species, but also in different families during this study. Nematodes provisionally identified as *Heligmostrongylus* sp., isolated from *P. yucatanicus* in the Yucatan Peninsula (Panti-May *et al.*, 2023), shared morphological and morphometrical characters with the specimens isolated from *O. phyllotis* and *H. gaumeri*, such as the pattern of the bursal rays, the tips of the spicules, the ventral inflation of the bursa, spicule length (570–620) and the torsion of the female posterior end. Characteristics that, in the present study, are considered diagnostic of the new species.

With the purpose to test if the worms isolated from O. *phyllotis* and *H. gaumeri* were conspecific or represented a complex of morphologically similar species, we obtained and compared sequences of the COI, ITS1, and the D2–D3 expansion domains

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of the 28S rRNA. Published sequences of the 28S rRNA of provisionally identified as *Heligmostrongylus* sp., isolated from *P. yucatanicus* and *O. phyllotis* in the Yucatan Peninsula (Panti-May *et al.*, 2023) were also included in the phylogenetic analysis.

Seven nucleotide sequences of *H. yucatanensis* were obtained in this study: three from worms collected from *O. phyllotis* (one for each genetic marker, COI, ITS1, and 28S rRNA) and four from worms collected from *H. gaumeri* (one for both COI and ITS1, and two for 28S rRNA). Details of each dataset used to construct phylogenetic trees are given in Supplementary material (Table S1).

The amplified COI fragments of the new species were aligned with other nine sequences of heligmosomoid nematodes; alignment of 460 base pairs. The sequences of *H. yucatanensis* isolated from *O. phyllotis* and from *H. gaumeri* were identical, and both were positioned as a sister taxon to the clade formed by the sequences of *Vexillata convoluta* (Caballero & Cerecero, 1943) and *Nippostrongylus* sp., although with low support (bootstrap = 15) (Figure 3). The new species had a genetic difference of 11% and 12% compared with those of *Nippostrongylus* sp. and *V. convoluta*, respectively. Unfortunately, there were no COI sequences available from other representatives of the Pudicinae which could have produced a resolved phylogeny.

The ITS1 dataset of heligmosomoid nematodes from rodents included 27 sequences, with an alignment length of 488 pb. In the phylogenetic tree, the two sequences of *H. yucatanensis* were placed as a sister clade to another one formed by nematodes of the genera *Nippostrongylus*, *Stilestrongylus*, *Hassalstrongylus*, *Vexillata*, *Carolinensis*, and *Guerrerostrongylus* (bootstrap = 100) (Figure 4). The genetic difference among the sequences of *H. yucatanensis* from *O. phyllotis* and from *H. gaumeri* was 0.2% when compared to each other. As with the results obtained for the COI sequences, analysis of the ITS1 sequences recovered a similar tree topology but with most nodes highly supported (Figure 4).

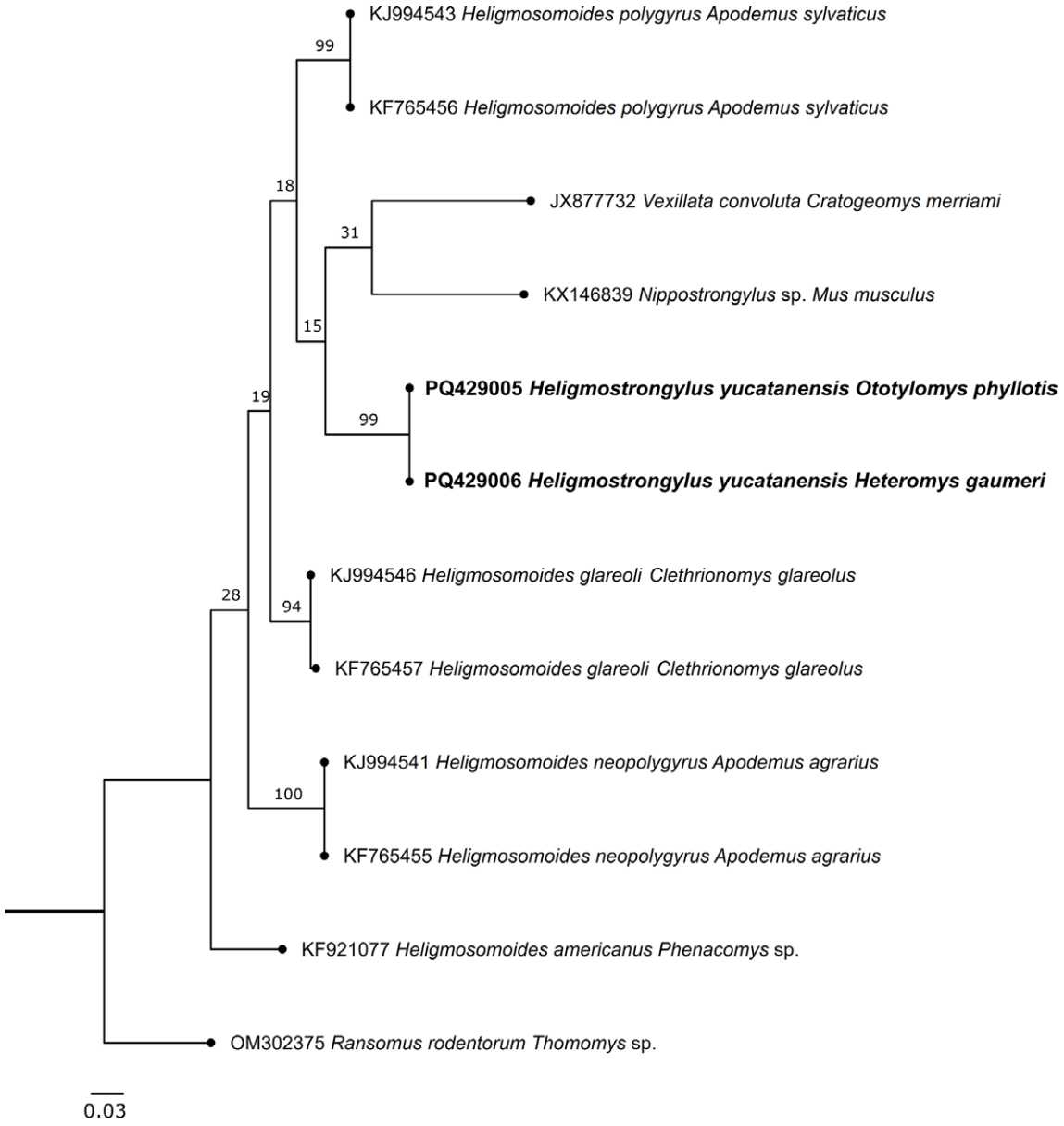

Figure 3. Maximum likelihood (ML) phylogenetic tree of Heligmosomoidea inferred with COI sequence data. GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

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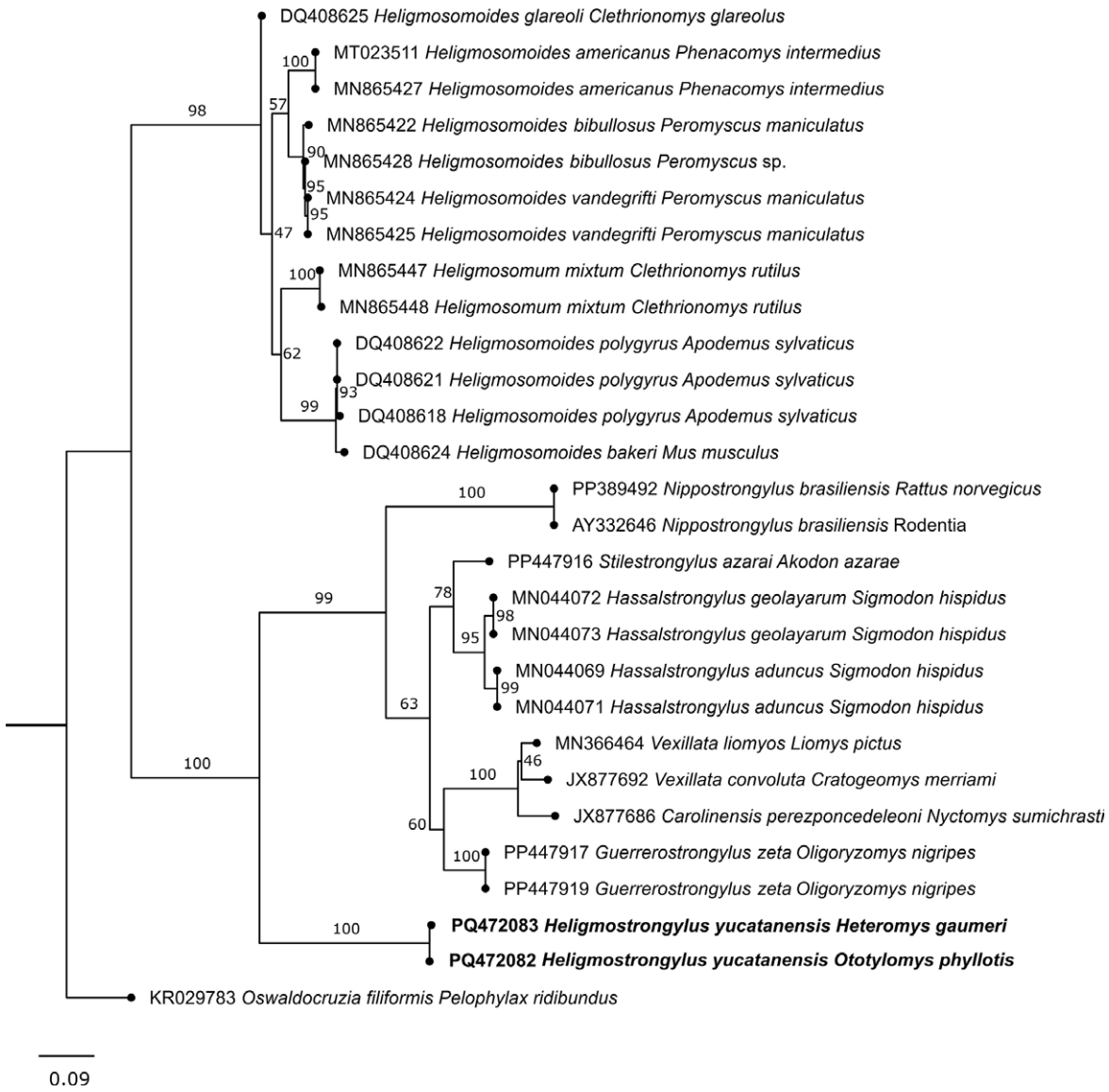

Figure 4. Maximum likelihood (ML) phylogenetic tree of Heligmosomoidea inferred with ITS1 sequence data. GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

The dataset of the D2-D3 expansion domains of the 28S rRNA of heligmosomoid nematodes from rodents included 23 sequences, with an alignment length of 714 pb. The phylogenetic tree of 28S rRNA clustered the three new sequences of *H. yucatanensis* from *O. phyllotis* and from *H. gaumeri* in a high supported clade (bootstrap = 100) together with the sequences previously reported for the same species (originally reported as *Heligmostrongylus* sp.) isolated from *O. phyllotis* and from *P. yucatanicus* (Figure 5). The genetic distance among the five sequences of *H. yucatanensis* ranged from 0 to 0.1%.

The absence of intraspecific sequence variations in COI, and genetic distances (0–2%) in the D2-D3 expansion segments of 28S rRNA and ITS1 provide strong support that the specimens isolated from *O. phyllotis*, *H. gaumeri* and *P. yucatanicus* are conspecific.

Discussion

Although morphologically and molecularly identical, male and female specimens isolated from *H. gaumeri* were smaller than those from *O. phyllotis* (Table 1). These differences may be related to the smaller size of *H. gaumeri* compared to *O. phyllotis* (39–67 g *vs.* 65–99 g) but also to the fact that *H. gaumeri* is frequently parasitized by another dominant nematode, *Vexillata vexillata* (Hall, 1916). Intensity of infection, size, age and diet of the host, recent or previous infections and coinfections with other worms, are factors that may also cause variations in the body size of helminths (Saldanha *et al.*, 2009; González *et al.*, 2019).

The presence of a ventral cuticular inflation anterior to the bursa had not been previously recorded in species of *Heligmostrongylus* or any other nematodes of Pudicinae. There are reports of similar structures in species of *Heligmosomoides* (Heligmosomidae) parasitizing North American rodents, such as *Heligmosomoides bullosus* Durette-Desset, 1967, *Heligmosomoides montanus* Durette-Desset, 1967, and *Heligmosomoides bibullosus* Alnaqeb, Greiman, Vandegrift, Campbell, Meagher & Jiménez, 2022 (Durette-Desset *et al.*, 1972; Alnaqeb *et al.*, 2022). Although this structure might initially seem to interfere with mating, it is notable that in at least two of the

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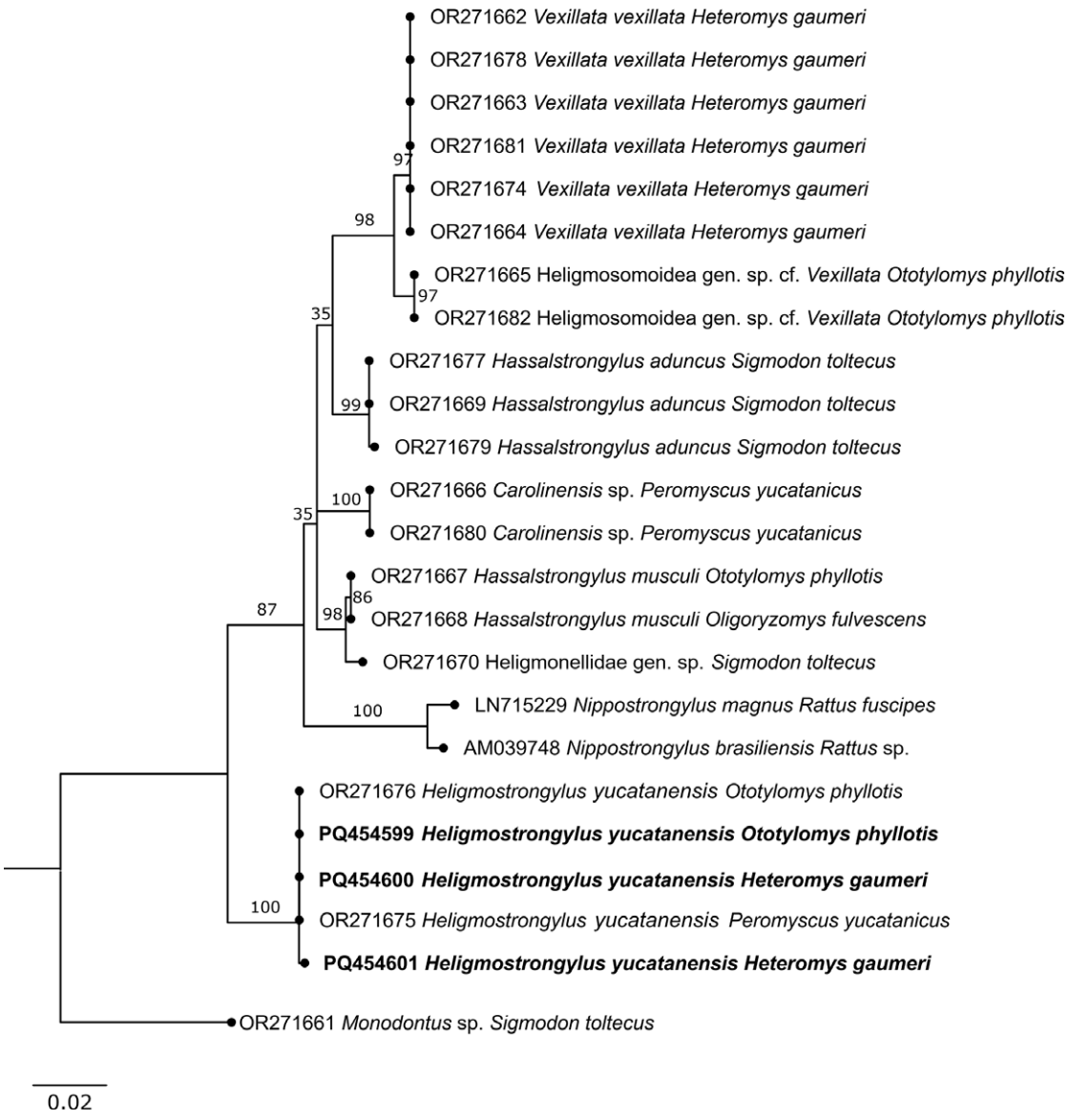

Figure 5. Maximum likelihood (ML) phylogenetic tree of Heligmosomoidea inferred with D2-D3 expansion domains of the 28S rRNA sequence data. GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

aforementioned species (*H. bullosus* and *H. montanus*), as well as in *H. yucatanensis*, the presence of this anteroventral inflation of the bursa is also accompanied by varying degrees of torsion in the posterior end of the females (Durette-Desset *et al.*, 1972). It is possible that in all these species, the torsion of the posterior end of the female parallels the presence of the ventral prebursal inflation in the male, so that both modifications may interact to ensure better attachment during copulation (Digiani & Kinsella, 2014).

Heligmostrongylus yucatanensis occurs in cricetid and heteromyid rodents from the Yucatan Peninsula. It is important to highlight that before the present record, all the species of this genus were only known from caviomorph rodents. Of the nine known species of *Heligmostrongylus*, six have been reported in Echimyidae, two in Erethizontidae, and one in Erethizontidae, Cuniculidae, and Dasyproctidae (Table 1). The occurrence of *H. yucatanensis* in three phylogenetically distant rodent species suggests that this nematode species could have the ability to expand its host range by colonizing new hosts. In disturbed environments (e.g., crop fields, ranches) where food resources and vegetation cover are limited, rodents can share burrows (Durán-Antonio & González-Romero, 2018). As a result, infective stages of parasites may be acquired by rodents that share burrows which could favour the transmission of parasites among hosts.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X24000750.

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Competing interest. The authors declare none.

Ethical approval. Protocols used in this study were approved by the Bioethics Committee of the Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán (protocol number CB-CCBA-L-2022-001). Rodent trapping was conducted under license from the Mexican Ministry of Environment (license number SGPA/DGVS/02974/22).

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