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Morphological and molecular data on helminths of *Didelphis virginiana* and *Philander vossi* (Mammalia: Didelphidae) from the Yucatán Peninsula, southeast Mexico

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Abstract

In the present study, helminths from six *Didelphis virginiana* and one *Philander vossi* are reported using morphological techniques (clearing, staining, and scanning electron microscopy). Additionally, the 28S rRNA sequences of individuals from nine helminth taxa are provided. Phylogenetic analyses were performed with the new 28S rRNA sequences to confirm the identification and the genealogical relationships of the parasites. Thirteen helminth taxa were identified, comprising the trematodes *Brachylaima* sp. and *Platynosomum illiciens*, the cestode *Mathevotaenia* sp., the nematodes *Cruzia americana*, *Cruzia tentaculata*, *Viannaia arriaguensis*, *Viannaia* sp., *Travassostrongylus* sp., *Strongyloides* sp., *Turgida turgida*, *Trichuris minuta*, and *Trichuris* sp., and the acanthocephalan *Oligacanthorhynchus microcephalus*. All opossums were infected with at least four helminth taxa. In total, 17 new 28S rRNA sequences from nine helminth taxa were provided. These data, combined with previous records in Mexico, increase the number of helminth taxa parasitizing *D. virginiana* and *P. vossi* to 41 and 29, respectively. However, these reports are incompletes and concentered in localities of some states. It is possible that new surveys in the Nearctic and even Neotropical regions will reveal a higher helminth diversity in these mammals in the country.

Key words: helminths, integrative taxonomy, Neotropical region, opossums

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Introduction

Opossums are ancestral mammals with a widespread distribution in the Americas, from southern Canada to southern Argentina (Cruz-Salazar *et al.* 2014). In Mexico, opossums are represented by the Order Didelphimorphia, which contains 13 species: *Caluromys derbianus* (Waterhouse), *Chironectes minimus* (Zimmermann), *Didelphis marsupialis* Linnaeus, *Didelphis virginiana* Kerr, *Philander vossi* Gardner & Ramirez-Pulido, *Marmosa mayensis* Osgood, *Marmosa mexicana* Merriam, *Tlacuatzin canescens* (Allen), *Tlacuatzin insularis* (Merriam), *Tlacuatzin sinaloae* (Allen), *Tlacuatzin gaumeri* (Osgood), *Tlacuatzin balsasensis* Arcangeli, Light & Cervantes, and *Metachirus nudicaudatus* (È. Geoffroy Saint–Hilaire) (Ramírez-Pulido *et al.* 2014; Arcangeli *et al*. 2018). Of these, *D*. *virginiana* and *D*. *marsupialis* are the most common opossums in the country and occur in a wide variety of habitats, such as forests and cities (Cruz-Salazar *et al.* 2014). *Didelphis virginiana* has a widespread distribution in Mexico, with the exception of the Central Plateau and most of the Baja California Peninsula, while *D*. *marsupialis* occurs in the Gulf of coastal plain, the states of Chiapas and Oaxaca, and the Yucatán Peninsula (Albino Miranda *et al.* 2022).

The inventory of the helminth fauna of wild mammals in Mexico is incomplete, partly due to limited sampling in certain hosts and geographic areas (García-Prieto *et al.* 2012). Opossums are among the most studied mammals in parasitology, with 37 nominal species of helminths reported from five opossum species in 21 Mexican states (Acosta-Virgen *et al.* 2015; García-Prieto *et al.* 2012; García-Valle *et al.* 2023). However, these helminth records are incomplete because they have been limited to five host species and a few localities of some states. For example, in the Yucatán Peninsula (comprising the Mexican states of Campeche, Quintana Roo, and Yucatán) only seven species of helminths have been reported in *D*. *virginiana*, with records limited to Campeche and Yucatán (Acosta-Virgen *et al.* 2015; López-Caballero *et al.* 2015). In contrast, no helminths have been recorded from *P*. *vossi* (syn. *Philander opossum fuscogriseus* Allen) in the peninsula.

In the last two decades, the use of molecular markers has significantly enhanced our knowledge of the helminths of mammals by distinguishing closely related species, especially in helminths where morphological identification is often challenging (López-Caballero *et al.* 2019; Ramírez-Cañas *et al.* 2021). Unfortunately, the application of molecular methods in parasitological studies of terrestrial mammals remains limited, especially in some megadiverse developing countries (Poulin *et al.* 2019). In Mexico, most helminth records in opossums have been reported in *D*. *virginiana*, *D*. *marsupialis*, and *P*. *vossi* using morphological methods of adults and egg stages, which, in many cases, do not allow the identification of certain taxa at the species level (Aragón-Pech *et al.* 2018; López-Caballero *et al.* 2019; Ramírez-Cañas *et al.* 2021). This study addresses the application of an integrative taxonomic approach to explore the diversity of helminths in *D*. *virginiana* and *P*. *vossi* in the Yucatán Peninsula.

Materials and methods

Sample collection and morphological analysis

Six *D*. *virginiana* and one *P*. *vossi* were collected and examined for helminths from January 2022 to August 2023 (Table 1), under license from the Mexican Ministry of Environment (SGPA/DGVS/02974/22). Three opossums were found dead on roads, while four specimens were euthanized in local veterinary clinics due to the severity of spinal fractures. The heart, lungs, gastrointestinal tract (from the esophagus to the colon), liver, and mesenteries of each opossum were removed, immersed in 0.85% saline solution, and examined under a stereomicroscope. The research ethics committee of the Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán approved the protocols used in this study (protocol number CEI-02-2022), which adhered to the Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes & the Animal Care and Use Committee of the American Society of Mammalogists 2016).

Trematodes, cestodes, and nematodes were killed with hot water, while acanthocephalans were relaxed in distilled water for 12 h at 4°C until proboscides were evaginated. Helminths were preserved in 70% ethanol, 10% formol, and 100% ethanol for optic microscopy, scanning electron microscopy (SEM) and DNA extraction, respectively. For morphological identification, nematodes were cleared and temporarily mounted in lactophenol. As the study of the synlophe has proved its value not only at the species level but also at the generic level and above, transverse sections of trichostrongylid nematodes were conventionally taken at midbody (Durette-Desset *et al.* 2017). Trematodes, cestodes, and acanthocephalans were stained with Mayers' paracarmine, dehydrated in a graded

ethanol series, cleared in methyl salicylate, and mounted permanently in Canada balsam. Specimens were examined and drawn using a compound microscope equipped with a drawing tube. Measurements are given in micrometers. For SEM, selected helminths 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 at the Laboratorio de Microscopía y Fotografía de la Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico City.

TABLE 1. Localities of opossum samples in the Yucatán Peninsula.

Host	Locality	State	Geographical coordinates	Collection date
Didelphis virginiana	Campeche	Campeche	19°48'39.29"N, 90°29'35.58"W	January 2022
	Chencoh	Campeche	19°25'5.40"N, 89°48'58.52"W	July 2023
	Mérida	Yucatán	20°58'9.42"N, 89°38'24.57"W	May 2022
		Yucatán	20°56'13.27"N, 89°36'23.54"W	May 2022
		Yucatán	20°56'38.6"N, 89°35'1.29"W	March 2023
	Umán	Yucatán	20°53'21.8"N, 89°44'1.08"W	March 2023
Philander vossi	Chetumal	Quintana Roo	18°43'36.7"N, 88°22'13.9"W	June 2022

Morphological features were used for the identification of helminths at different taxonomic levels using keys for nematodes (Anderson *et al.* 2009; Schmidt-Rhaesa 2014), cestodes (Khalil *et al.* 1994), trematodes (Gibson *et al.* 2002; Bray *et al.* 2008), and acanthochephalans (Amin 1987), as well as original descriptions and redescriptions. The classifications used for Trematoda, Cestoda, Nematoda, and Acanthocephala were those of Bray *et al.* (2008), Caira & Jensen (2017), Hodda (2022), and Amin (2013), respectively.

Voucher specimens are deposited in the Colección Nacional de Helmintos (CNHE), IBUNAM (see below for accession numbers). Skulls and skins of opossums are deposited in the Mammal Collections of the Universidad Autónoma de Yucatán, Mérida, Yucatán (accession numbers: *D. virginiana* No. 1643 and 1644; *P. vossi* No. 1645) and the Universidad Autónoma de Campeche, Campeche, Campeche (*D. virginiana* No. 996 and 1029).

Molecular data and analysis

Total genomic DNA was extracted from individual specimens using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Partial fragments of the large subunit (28S) of the ribosomal RNA gene (domains D1–D3) were amplified using the forward primer 391 (Nadler *et al.* 2003) and the reverse primer 536 (García-Varela & Nadler 2005). Polymerase chain reaction (PCR) amplifications were conducted using the conditions described by Hernández-Mena *et al*. (2017). The PCR primers, along with additional internal primers 503 (Nadler *et al*. 2003) and 504 (García-Varela & Nadler 2005), 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) and submitted to GenBank.
Phylogenetic analyses were conducted using the newly obtained 28S rRNA sequences from each helminth taxa, as well as sequences from related taxa within the same family deposited in GenBank, to confirm the identification and the genealogical relationships of the parasites. 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 28S rRNA data sets was calculated using p-distances with MEGA 11 (Tamura *et al.* 2021).

Results

Thirteen species of helminths were identified including two trematodes (Brachylaimidae and Dicrocoeliidae), one cestode (Anoplocephalidae), nine nematodes (Kathlanidae, Physalopteridae, Trichostrongylidae, and Trichuridae),

and one acanthocephalan (Oligacanthorhynchidae). Unlike *D. virginiana*, which was infected with 11 helminth taxa, *P. vossi* harbored five helminth taxa. All opossums were infected with at least four helminth taxa.

List of species of helminths

Phylum Platyhelminthes Gegenbaur

Class Trematoda Rudolphi

Family Brachylaimidae Joyeux & Foley

Genus *Brachylaima* Dujardin

Brachylaima sp.

Site of infection: Small intestine.

Host species: *Didelphis virginiana*.

Localities: Umán and Mérida (Yucatán).

Prevalence: 33.3% (2/6).

Mean intensity: 28 (range 6–50).

Specimens deposited: CNHE 12898, 12899.

GenBank accession number: PP662453.

Comments: Specimens of *Brachylaima* with an elongated body (2,810–4,050 long by 450–700 in maximum width), covered with fine spines (Figure 1A). Oral (245–320 long by 220–290 wide) and ventral (250–315 long by 220–260 wide) suckers situated in the anterior part of body (Figure 1B). Gonads in tandem near to the posterior extremity of the body. Testes oval and similar in size; anterior testis 235–360 long by 150–370 wide, posterior testis 240–405 long by 150–375 wide. Ovary small, 100–170 long by 129–220 wide. Genital pore posterior, close to the anterior testis. Vitellaria in two lateral fields, reaching anteriorly to level of ventral sucker, posteriorly about level of anterior testis. Eggs numerous, ovoid, 29–30 long by 20–17 wide. These specimens are morphologically closest to *Brachylaima virginianum* (Dickerson), originally described from *D. virginiana* in Virginia, USA. However, *B. virginianum* differs from the studied specimens by a smaller body length (1,535–2,541 vs 2,810–4,050) and shorter anterior (177–205 vs 235–360) and posterior (123–204 vs 240–405) testes. The oral sucker is distinctly larger (246–286) than the ventral sucker (150–205) in *B. virginianum*, while in the studied specimens the suckers have somewhat similar size (245–320 and 250–315). *Brachylaima didelphus* Premvati & Bair, another trematode recorded in *D. virginiana* from Mexico (Acosta-Virgen *et al.* 2015), differs from the studied specimens by the vitellaria position (Premvati & Bair 1979) (from the pharynx to the end of the body vs. from the ventral sucker to anterior testis). Therefore, we consider that the specimens from Yucatán could represent a putative new specie.

Two known species of *Brachylaima* have been reported from *D. virginiana* in Mexico: *B. virginianum* in Mexico State (Monet-Mendoza *et al.* 2005) and *B. didelphus in* Campeche (Acosta-Virgen *et al.* 2015). In addition, an unidentified species of *Brachylaima* was reported from *D. virginiana* in Guerrero (Monet-Mendoza *et al.* 2005). Our finding represents the first record of the genus *Brachylaima* in the Yucatán Peninsula.

Family Dicrocoeliidae Looss

Genus *Platynosomum* Looss

Platynosomum illiciens (Braun)

Site of infection: Liver.

Host species: *Didelphis virginiana.*

Locality: Mérida (Yucatán).

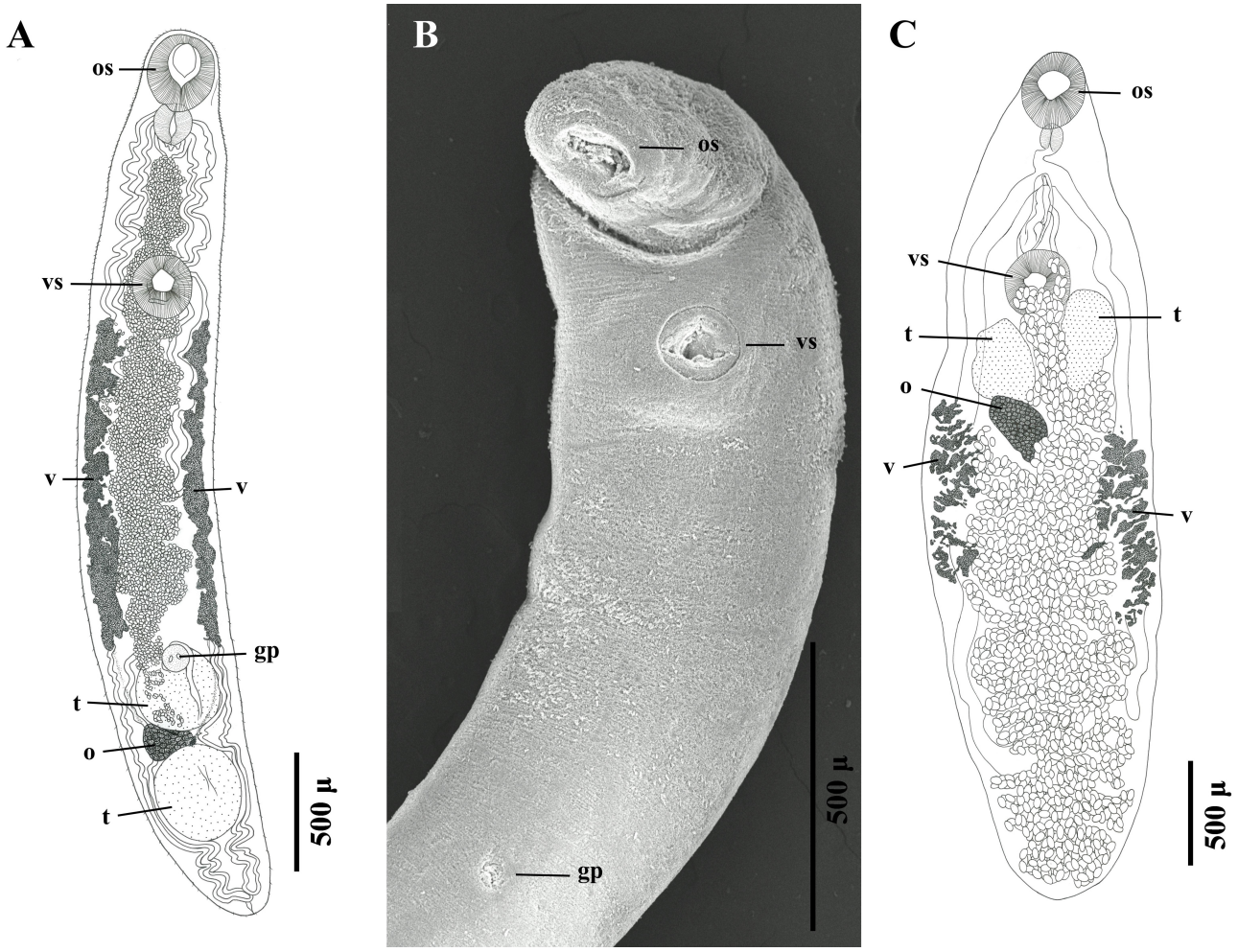

FIGURE 1. *Brachylaima* sp. and *Platynosomum illiciens* from *Didelphis virginiana*. A. Adult specimen of *Brachylaima* sp., ventral view. B. SEM micrograph of *Brachylaima* sp. anterior end, ventral view. C. Adult specimen of *Platynosomum illiciens* ventral view. Abbreviations: oral sucker (os), ventral sucker (vs), testis (t), vitellaria (v), ovary (o), genital pore (gp).

Prevalence: 16.7% (1/6).

Intensity: 1.

Specimen deposited: CNHE 12897.

Comments: Only one specimen was found, which conform to the morphological characteristics of *P. illiciens* reported by Nguyen *et al.* (2017) and Pinto *et al.* (2017). Body elongate (4,150 long by 1,240 in maximum width) (Figure 1C). Suckers subequal, situated in the anterior part of body; oral sucker 350 long by 330 wide, ventral sucker 340 long by 360 wide. Testes posterolateral to the ventral sucker; both testes anterior to midbody, left testis 320 long by 300 wide, right testis 440 long by 250 wide. Elongated cirrus sac, small (425 long by 110 wide), extending slightly posterior to margin of ventral sucker. Genital pore median, at level of the caecal bifurcation. Ovary dextral, small (190 long by 320 wide), immediately posterior to the right testis. Vitellaria in two lateral field, extending from the ovary to 3/4 of the body length. Eggs 32–33 long by 20–23 wide.

Platynosomum illiciens is a trematode with a worldwide distribution reported in several birds and mammals as definitive hosts (Pinto *et al.* 2022), including *D. virginiana* (Eckerlin & Leigh 1962). Molecular studies have confirmed its wide geographic distribution and low host specificity. Previously described species in cats, primates, and birds, such as *Platyonosomum fastosum* Kossack, *Platynosomum concinnum* (Braun), *Platynosomum amazonensis* Kingston & Cosgrove, and *Platynosomum marmoseti* Kingston & Cosgrove, are now considered synonyms of *P. illiciens* (Pinto *et al.* 2022). This is the first record of *P. illiciens* from *D. virginiana* in Mexico.

Class Cestoda Rudolphi

Family Anoplocephalidae Blanchard

Genus *Mathevotaenia* Akhumyan

***Mathevotaenia* sp.**

Site of infection: Small intestine.

Host species: *Didelphis virginiana.*

Locality: Mérida (Yucatán).

Prevalence: 16.7% (1/6).

Intensity: 6.

Specimen deposited: CNHE 12896.

GenBank accession number: PP662454.

Comments: The specimens of *Mathevotaenia* possess an unarmed scolex, 270 long by 310–330 wide (Figure 2A). Suckers 135–148 long by 127–132 wide. Proglottids craspedote. Genital pores alternate irregularly. Testes from 45 to 48 in number, arranged in two lateral fields between both ovary and vitelline gland, 40–45 long by 34–40 wide. Elongated cirrus pouch, reaching lateral osmoregulatory canals. Ovary 200 long by 460 wide, medial, in anterior half of proglottid (Figure 2B). Eggs spherical, 38–40 in diameter. Seven species of *Mathevotaenia* have been reported from numerous species of opossums in the Americas: *Mathevotaenia bivittata* (Janicki), *Mathevotaenia didelphidis* (Rudolphi), *Mathevotaenia marmosae* (Beddard), *Mathevotaenia pennsylvanica* (Chandler & Melvin), *Mathevotaenia surinamensis* (Cohn), *Mathevotaenia argentinensis* Campbell, Gardner & Navone, and *Mathevotaenia sanmartini* Jiménez, Braun, Campbell & Gardner. The number of testes (45–48) allowed the differentiation of the Yucatán specimens from *M. argentinensis* (vs 19–29) (Campbell *et al.* 2003), *M. bivittata* (vs 5–13) (Hughes, 1940; Campbell *et al*. 2003), *M. sanmartini* (26–41) (Jiménez *et al.* 2008), *M. surinamensis* (vs 90–100) (Buchanan 1956), *M. didelphidis* (vs 20) (Hughes 1940), *M. pennsylvanica* (30–40) (Campbell *et al.* 2003), and *M. marmosae* (vs >100) (Beddard 1914). Moreover, the studied specimens had a narrower scolex (310–330) than that of *M. argentinensis* (vs 392–536), *M. bivittata* (vs 460–600), *M. sanmartinensis* (vs 843–1246), *M. surinamensis* (vs 580–800), and *M. pennsylvanica* (vs 946). These differences suggest that the specimens from Yucatán may represent a species not described yet.

In Mexico, unidentified species of *Mathevotaenia* have been reported from *D. virginiana* in Chiapas (Monet-Mendoza *et al.* 2005) and Colima (García-Prieto *et al.* 2012; García-Valle *et al.* 2023). This is the first report of *Mathevotaenia* in the Yucatán Peninsula.

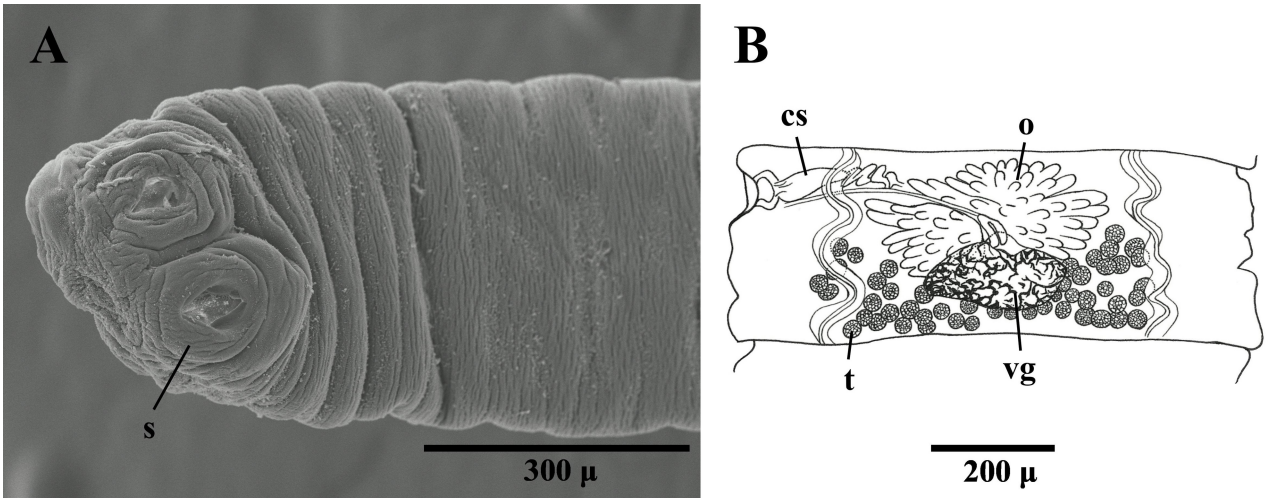

FIGURE 2. *Mathevotaenia* sp. from *Didelphis virginiana*. A. SEM micrograph of scolex in lateral view. B. Mature proglottid in dorsal view. Abbreviations: sucker (s), cirrus sac (cs), ovary (o), testis (t), vitelline gland (vg).

Phylum Nematoda Cobb

Class Chromadorea Inglis

Family Kathlaniidae Lane

Genus *Cruzia* Travassos

***Cruzia americana* Maplestone**

Site of infection: Caecum.

Host species: *Didelphis virginiana* and *Philander vossi.*

Localities: Mérida, Umán (Yucatán) and Chetumal (Quintana Roo).

Prevalence: *Didelphis virginiana* 66.7% (4/6), *Philander vossi* 100% (1/1).

Mean intensity: *Didelphis virginiana* 273 (range 11–606), *Philander vossi* 60.

Specimens deposited: CNHE 12870–12872.

GenBank accession numbers: PP662455–PP662457.

Comments: The characteristics observed in studied nematodes agree with the redescription by Li (2019). Cephalic extremity with three small lips, almost equal in size, each with one pair of small teeth at inner margin; dorsal lip with two submedian papillae and each subventral lip with one subventral papilla and one prominent amphid (Figure 3A). Male 7,050–14,000 in body length. Pharynx (160–220 long) armed with three longitudinal rows of 13–15 pharyngeal lamellae each (Figure 3B). Esophagus (1,720–2,500 long) consisting of cylindrical corpus (gradually becoming narrower toward posterior part and forming a small anterior bulb at end), inconspicuous isthmus and terminating in conspicuous valved posterior bulb. Intestinal diverticulum present. Posterior extremity of body slightly curved ventrally, with 11 pairs of caudal papillae (three pairs precloacal, three pairs paracloacal and five pairs postcloacal) plus a single precloacal medioventral papilla (Figure 3C–D). Spicules subequal, 820–1,060 long. Gubernaculum 140–205 long with two sharp edges (Figure 3E). Female 7,250–16,230 in body length. Vulva slit-like, 3750–6,900 from the anterior end. Eggs oval, 105–120 long by 50–68 wide. According to Li (2019), *C. americana* can be differentiated from *Cruzia tentaculata* (Rudolphi) by the number of pharyngeal lamellae, 13–17 in *C. americana* and 10 in *C. tentaculata*. However, these structures are not easy to observe under light microscopy (Li 2019). In the studied nematodes the exact number of pharyngeal lamellae was observed only by cutting the pharynx. In all *C. americana* males dissected to examine the pharyngeal lamellae, the gubernaculum had sharp edges. This characteristic was reported by Crites (1956) in *C. americana* and Adnet *et al.* (2009) in *C. tentaculata*, while Li (2019) described a blunt gubernaculum at distal end in *C. americana*. The taxonomic status of *C. americana* has been under debate due to the morphological similarities with *C. tentaculata*. Wolfgang (1951) considered *C. americana* synonym of *C. tentaculata*. In contrast, Crites (1956) and, more recently, Li (2019) redescribed *C. americana* based on newly collected specimens from the type-host *D. virginiana* in the USA. We agree with the proposal of Li (2019) on the necessity of clarifying the taxonomical relationships of *C. americana* and *C. tentaculata* using an integrative approach that includes molecular markers in the future.

Cruzia americana has been recorded from *D. virginiana* in Colima, Guerrero, Oaxaca, Veracruz (Monet-Mendoza *et al.* 2005), and Zacatecas (Martínez-Flores & Martínez-Salazar 2016). This is the first report of *C. americana* in the Yucatán Peninsula.

***Cruzia tentaculata* (Rudolphi)**

Site of infection: Caecum.

Host species: *Didelphis virginiana.*

Locality: Mérida (Yucatán).

Prevalence: 16.7% (1/6).

Intensity: 1.

Specimen deposited: CNHE 12873.

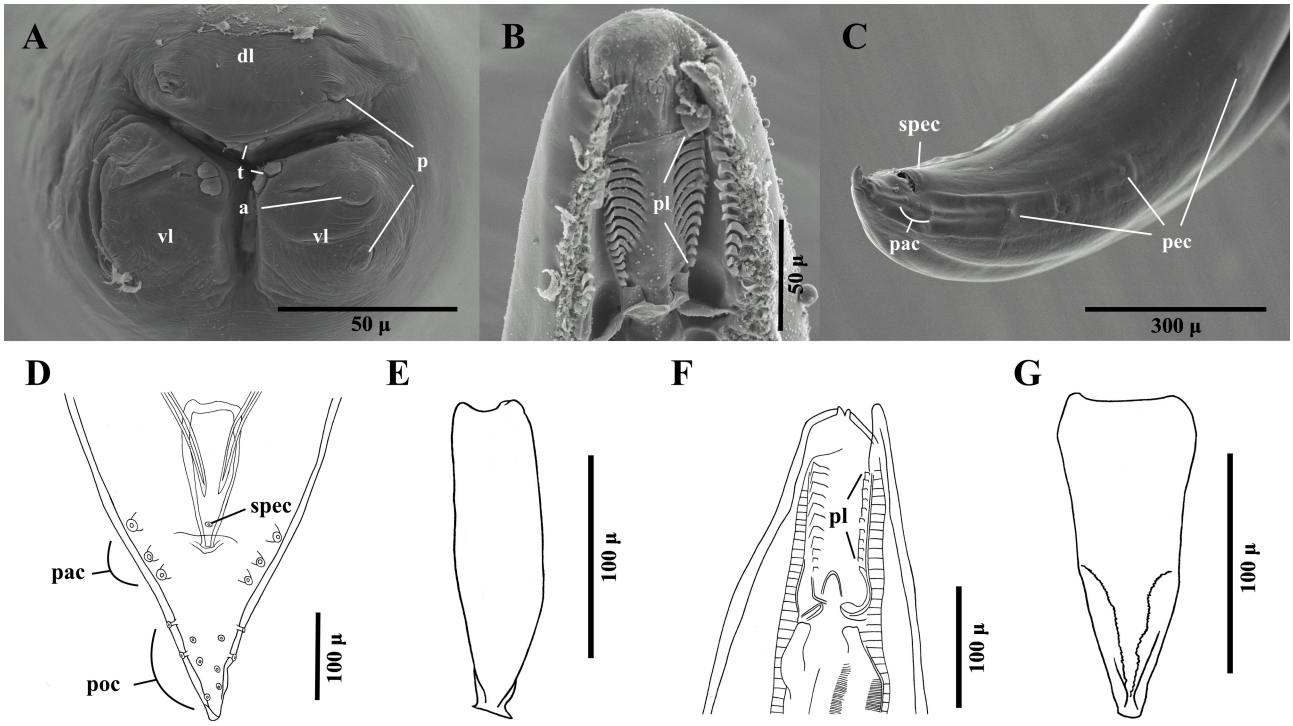

FIGURE 3. Males of *Cruzia americana* and *Cruzia tentaculata* from *Didelphis virginiana*. A. SEM micrograph of *C. americana* anterior end, apical view. B. SEM micrograph of internal structures of pharynx (columnar structures of cuticular lamellae) of *C. americana*, longitudinal section. C. SEM micrograph of *C. americana* posterior end, lateral view. D. *Cruzia americana* posterior end, ventral view. E. Gubernaculum of *C. americana*, ventral view. F. Internal structures of pharynx (columnar structures of cuticular lamellae) of *C. tentaculata*, longitudinal section. G. Gubernaculum of *C. tentaculata*, ventral view. Abbreviations: amphid (a), dorsal lip (dl), ventral lip (vl), papillae (p), pharyngeal lamellae (pl), paracloacal papillae (pac), precloacal papillae (pec), poscloacal papillae (poc), single precloacal papilla (spec), teeth (t).

Comments: The single male specimen has 11,400 in body length. Pharynx (160 long) with three longitudinal rows of 11 pharyngeal lamellae each (Figure 3F). Esophagus 2,000 long. Spicules subequal, 1,050 long. Gubernaculum (165 long) with a blunt terminal end (Fig. 3G).

In Mexico, *C. tentaculata* has been widely reported from *D. virginiana*, *D. marsupialis*, and *P. vossi* in 16 states (García-Prieto *et al.* 2012; Acosta-Virgen *et al.* 2015; Ramírez-Cañas *et al.* 2019), including Yucatán.

Family Trichostrongylidae Leiper

Genus *Viannaia* Travassos

Viannaia arriaguensis Ramírez-Cañas, López-Caballero & Mata-López

Site of infection: Small intestine.

Host species: *Didelphis virginiana* and *Philander vossi*.

Localities: Mérida, Umán (Yucatán), Campeche (Campeche), and Chetumal (Quintana Roo).

Prevalence: *Didelphis virginiana* 83.33% (5/6), *Philander vossi* 100% (1/1).

Mean intensity: *Didelphis virginiana* 93 (range 47–129), *Philander vossi* 1043.

Specimens deposited: CNHE 12874–12877.

GenBank accession numbers: PP662458–PP662460.

Comments: The morphology of the specimens matches the original description by Ramírez-Cañas *et al*. (2021). Synlophe with three sub-ventral cuticular ridges at midbody oriented from right to left, with the absence of lateral

and dorsal cuticular ridges. Male body length 2,650–9,040. Bursa sub-symmetrical, rectangular with pattern of type 2-1-2. Dorsal ray short and slender. Spicules simple, long (360–428), thing, equal in length and shape (Figure 4A), representing 7.3–8.7% of the body length. Gubernaculum 32–55 long. Monodelphic female 4,850–7,370 in total body length. Eggs ovoid, 52–70 long by 32–40 wide.

Viannaia arriaguensis was described based on specimens found in *P. vossi* (originally reported as *P. opossum*) from Chiapas, Mexico (Ramírez-Cañas *et al.* 2021). This is the first record of *V. arriaguensis* in *D. virginiana* as well as the first report in the Yucatán Peninsula.

Viannaia sp.

Site of infection: Small intestine.

Host species: *Didelphis virginiana* and *Philander vossi.*

Localities: Campeche (Campeche) and Chetumal (Quintana Roo).

Prevalence: *Didelphis virginiana* 16.7% (1/6), *Philander vossi* 100% (1/1).

Intensities: *Didelphis virginiana* 73, *Philander vossi* 22.

Specimens deposited: CNHE 12878, 12879.

GenBank accession number: PP662461.

Comments: The nematodes were identified as belonging to the genus *Viannaia* based on the characteristics of synlophe and the bursa. Male 4,260–4,840 in body length. Bursa subsymmetrical, heart-shaped, with 2-1-2 pattern. Rays 8 long, reaching the margin of the bursa (Figure 4B). Rays 10 simple. Spicules short, subequal, 130–145 long, representing 3–3.1% of the body length. Monodelphic female 4,650–5,570 in body length. Eggs ovoid, 60–65 long by 30–40 wide. The genus *Viannaia* comprises 19 species: *Viannaia conspicua* Travassos, *Viannaia didelphis* Travassos, *Viannaia hamata* Travassos, *Viannaia pusilla* Travassos, *Viannaia viannai* Travassos, *Viannaia skrjabini* Lent & Freitas, *Viannaia philanderi* Wolfgang, *Viannaia monodelphisi* Durette-Desset, *Viannaia metachirops* Durette-Desset, *Viannaia bisbali* Guerrero, *Viannaia reigi* Guerrero, *Viannaia tenorai* Guerrero, *Viannaia minispicula* Guerrero, *Viannaia guayanensis* Guerrero, *Viannaia venezuelensis* Guerrero, *Viannaia gabaldoni* Guerrero, *Viannaia barusi* Guerrero, *V. arriaguensis*, and *Viannaia angelae* Ramírez-Cañas, López-Caballero & Mata-López. *Viannaia hamata* is morphological closest to the species found in the Yucatán Peninsula, with which it shares the length of the spicules and the esophagus (Guerrero 1985; Lopes de Jesus 2020). However, *V. hamata* differs from the studied specimens in having a smaller body length (880–2,300 vs. 4, 260–5570), a shorter rays 4 and rays 10 forked (Guerrero 1985; Vicente *et al.* 1997). Based on these findings, the nematodes collected in the Yucatán Peninsula likely represent an undescribed *Viannaia* species.

In addition to *V. arriaguensis*, three species of *Viannaia* have been reported in Mexico: *V. didelphis* from *D. virginiana* in Colima (Monet-Mendoza *et al.* 2005) and Morelos (Ortíz-Villaseñor 2000), *V. viannaia* from *D. virginiana* and *D. marsupialis* in Campeche, Chiapas, Colima, Oaxaca, Puebla, Tabasco, Veracruz (Acosta-Virgen *et al.* 2015), and Guerrero (Monet-Mendoza *et al.* 2005), and *V. angelae* from *D. virginiana* in Colima (Ramírez-Cañas *et al.* 2021). Further, unidentified species of *Viannaia* have also been reported in Chiapas (Acosta-Virgen *et al.* 2015), Colima (García-Valle *et al.* 2023), Guerrero (Monet-Mendoza *et al.* 2005), and Veracruz (Cañeda Guzman 1997; Monet-Mendoza *et al.* 2005). This is the first of *Viannaia* in Campeche and Quintana Roo.

Genus *Travassostrongylus* Orloff

Travassostrongylus sp.

Site of infection: Small intestine.

Host species: *Didelphis virginiana.*

Localities: Umán, Mérida (Yucatán) and Chencoh (Campeche).

Prevalence: 50% (3/6).

Mean intensity: 7.3 (range 2–17).

Specimens deposited: CNHE 12880–12882.

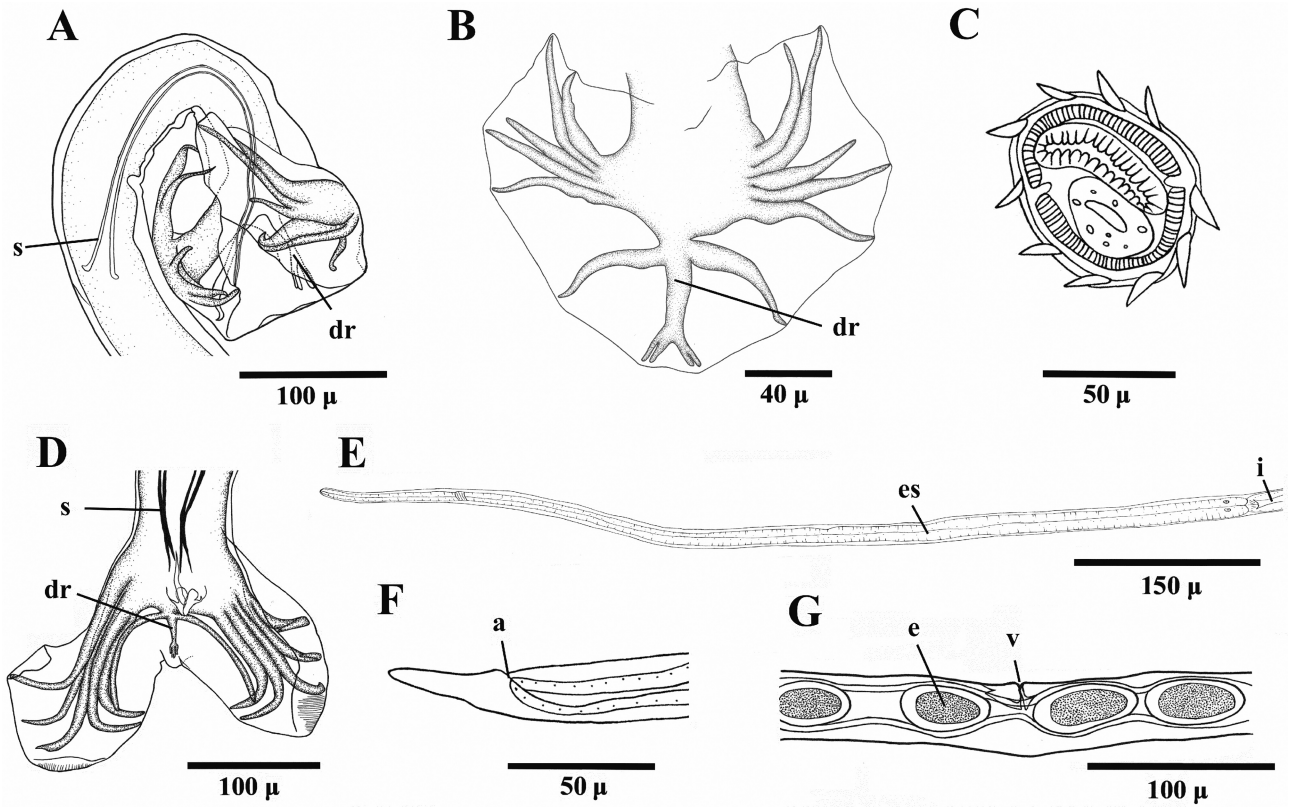

FIGURE 4. A. Male caudal bursa of *Viannaia arriguensis* from *Philander vossi*, ventral view. B. Male caudal bursa of *Viannaia* sp. from *Didelphis virginiana*, ventral view. C. Cross section at midbody showing features of the synlophe of male *Travassostrongylus* sp. from *Didelphis virginiana*. D. Male caudal bursa of *Travassostrongylus* sp. from *Didelphis virginiana*, ventral view. E. Anterior end of female *Strongyloides* sp. from *Philander vossi*, ventral view. F. Female tail of *Strongyloides* sp. from *Philander vossi* showing the anus, lateral v*iew. G.* Vulva of *Strongyloides* sp. from *Philander vossi*, lateral view. Abbreviations: anus (a), egg (e), intestine (i), spicule (s), vulva (v), dorsal ray (dr), esophagus (es).

GenBank accession number: PP662462.

Comments: The characteristics of the studied nematodes agree with those established for the genus *Travassostrongylus* (Durette-Desset 2009). Coiled nematodes with 10 continuous ridges at midbody in both sexes. Five dorsal and five ventral ridges, orientated from right to left and symmetrically arranged with respect to frontal axis; ridges absent from lateral fields (Figure 4C). Male 4,190–5,040 in body length. Male with caudal bursa subsymmetrical and a pattern type of 2-1-2. Dorsal ray short. Rays 10 shorter than rays 9 (Figure 4D). Spicules subequal, short, bi-radiate at end point, 110–135 long. Gubernaculum 35–40 long. Female body length 5,050–6825. Didelphic female with prominent anterior lip. Eggs ovoid, 60–65 long by 32–40 wide. The genus *Travassostrongylus* comprises 11 species (Hodda 2022): *Travassostrongylus callis* (Travassos), *Travassostrongylus orloffi* Travassos, *Travassostrongylus tertiuos* Travassos, *Travassostrongylus quatour* Texeira de Freitas, *Travassostrongylus quintus* Texeira de Freitas, *Travassostrongylus sextus* Texeira de Freitas, *Travassostrongylus travassosi* Durette-Desset, *Travassostrongylus paranquintus* Durette-Desset, *Travassostrongylus tourei* Diaw, *Travassostrongylus yungaensis* Navone, Suriano & Pujol, and *Travassostrongylus scheibelorum* Scheibel, Catzeflis & Jiménez. The studied specimens closely resemble *T. callis*. However, *T. callis* has a larger gubernaculum (94 vs 35–40). Also, the distal end of the spicules of our specimens is simple whereas that of *T. callis* end with exposed lamina (Diaw 1976). Based on these differences, the specimens from the Yucatán Peninsula could represent a species not described yet.

Only one species of *Travassostrongylus* has been reported from opossums in Mexico: *T. orloffi* from *D. marsupialis* in Veracruz (Scheibel *et al.* 2014). Also, an unidentified species of *Travassostrongylus* has been recorded in *Didelphis* sp. in Chiapas (Acosta-Virgen *et al.* 2015). The present study adds the first record of *Travassostrongylus* in the Yucatán Peninsula.

Family Strongyloididae Chitwood & MacIntosh

Genus *Strongyloides* Grassi

***Strongyloides* sp.**

Site of infection: Small intestine.

Host species: *Philander vossi.*

Locality: Chetumal (Quintana Roo).

Prevalence: 100% (1/1).

Intensity: 5.

Specimen deposited: CNHE 12883.

Comments: The specimens consist of five females with general morphology of the genus *Strongyloides* (dos Santos *et al.* 2010). The anterior end was truncated and presented stoma; however, details of this structure could not be observed due to poor preservation. Absence of cephalic vesicle and caudal spines. Slender nematodes, gradually tapering anteriorly from the region of the esophagus (Figure 4E); tapering more so posteriorly from region of anus (Figure 4F). Bucal capsule and synlophe absent. Body length 2,600–3,480 and body width 30–40. Didelphic females (Figure 4G). Vulva no prominent, 1,170–1,230 from the posterior end. Anus opens 40–50 from tip of tail (Figure 4F). Eggs ovoid, 48–62 long by 25–38 wide. The morphological similarity among species of *Strongyloides* has frustrated taxonomic work on the genus (Viney *et al.* 1991). While features of the parasitic female such as the shape of the stoma, the type of ovary, the shape of the tail, and the number of lobes on the circumoral elevation have been useful to differentiate many species of the *Strongyloides*, other species cannot be separated (Speare 1989). On the other hand, features of free-living males such as the shape of spicules and gubernaculum, arrangement of caudal papillae are important criteria for separating some species (Viney *et al.* 1991). Considering the present morphological data, the specimens were designed as *Strongyloides* sp. until more morphological and molecular evidence is generated.

This is the first record of *Strongyloides* from *P. vossi* in Mexico.

Family Physalopteridae Railliet

Genus *Turgida* Schulz

***Turgida turgida* (Rudolphi)**

Sites of infection: Esophagus and stomach.

Host species: *Didelphis virginiana.*

Localities: Umán, Mérida (Yucatán), Campeche, and Chencoh (Campeche).

Prevalence: 100% (6/6).

Mean intensity: 14.8 (range 2–33).

Specimens deposited: CNHE 12884–12887.

GenBank accession numbers: PP662463–PP662465.

Comments: The specimens conformed to the descriptions of *T. turgida* by Gray & Anderson (1982) and Matey *et al*. (2001). The nematodes with oral opening surrounded laterally by two symmetrical, semidomed pseudolabia; each pseudolabia composed of a single external tooth, a single tripartite tooth, an amphid, two papillae, and two spongelike areas (Figure 5A). Ventral surface of the male tail with 21 caudal papillae (three precloacal papillae, four pairs of externolateral papillae associated with the caudal alae, five pairs of postcloacal papillae, the first and second pairs are located directly behind the cloaca in a transverse row) and two phasmids (Figure 5B). Male body length 13,240–38,095. Spicules unequal in length, right 242–330 long, left 280–380. Female body length 21,030–27805. Females with nine uterine branches (Figure 5C). Eggs ovoid 42–50 by 20–22 wide.

Turgida turgida has been widely reported from *D. marsupialis*, *D. virginiana* and *P. vossi* in Mexico, including the states of Campeche, Chiapas, Colima, Guanajuato, Guerrero, Hidalgo, Jalisco, Michoacán, Mexico City, Mexico

State, Morelos, Nayarit, Nuevo León, Oaxaca, Puebla, Tabasco, Veracruz, and Zacatecas (see García-Prieto *et al.* 2012; Acosta-Virgen *et al.* 2015; Ramírez-Cañas *et al.* 2019; García-Valle *et al.* 2023). This is the first record of *T. turgida* in Yucatán.

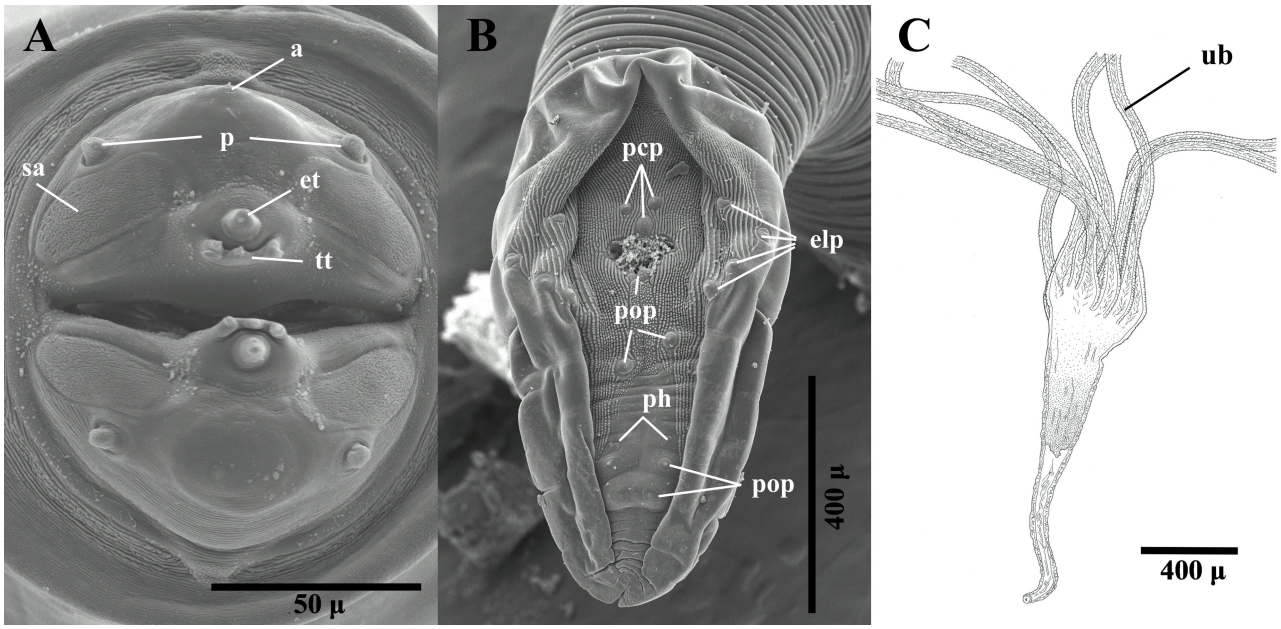

FIGURE 5. *Turgida turgida* from *Didelphis virginiana*. SEM micrograph of male cephalic region, apical view. B. SEM micrograph of male posterior end, ventral view. C. Uterus with nine uterine branches, ventral view. Abbreviations: amphid (a), external tooth (et), externolateral papillae (elp), precloacal papillae (pcp), postcloacal papillae (pop), phasmids (ph), spongelike area (sa), tripartite tooth (tt), uterine branch (ub).

Class Dorylaimea Hodda

Family Trichuridae Ransom

Genus *Trichuris* Roederer

***Trichuris minuta* Rudolphi**

Site of infection: Caecum.

Host species: *Didelphis virginiana.*

Localities: Mérida (Yucatán), Campeche and Chencoh (Campeche).

Prevalence: 83.3% (5/6).

Mean intensity: 23.3 (range 1–83).

Specimens deposited: CNHE 12888–12890.

GenBank accession numbers: PP662466, PP662467.

Comments: The specimens examined have characteristics of *T. minuta* described by Babero (1960) from *D. virginiana* in the USA. Anterior part of body long, narrow, tapered, and whip-like; posterior part of body broad, and handle-like. Male 14,150– 22,740 in body length. Males with spicular tube. Proximal cloacal tube (800–1,310 long) united laterally to a distal cloacal tube (412– 855 long). Spicule (920–1,080 long) covered in its distal portion with a cylindrical and spiny sheath (Figure 6A). Female body length 15,810–18,300. Female with non-protrusive vulva. Eggs oval, with bipolar plugs, 62–80 long by 32–35 wide.

Trichuris minuta has only been reported from *D. virginiana* in Morelos (Eslava-Araujo 2005). This is the second record of *T. minuta* in Mexico and the first record in the Yucatán Peninsula.

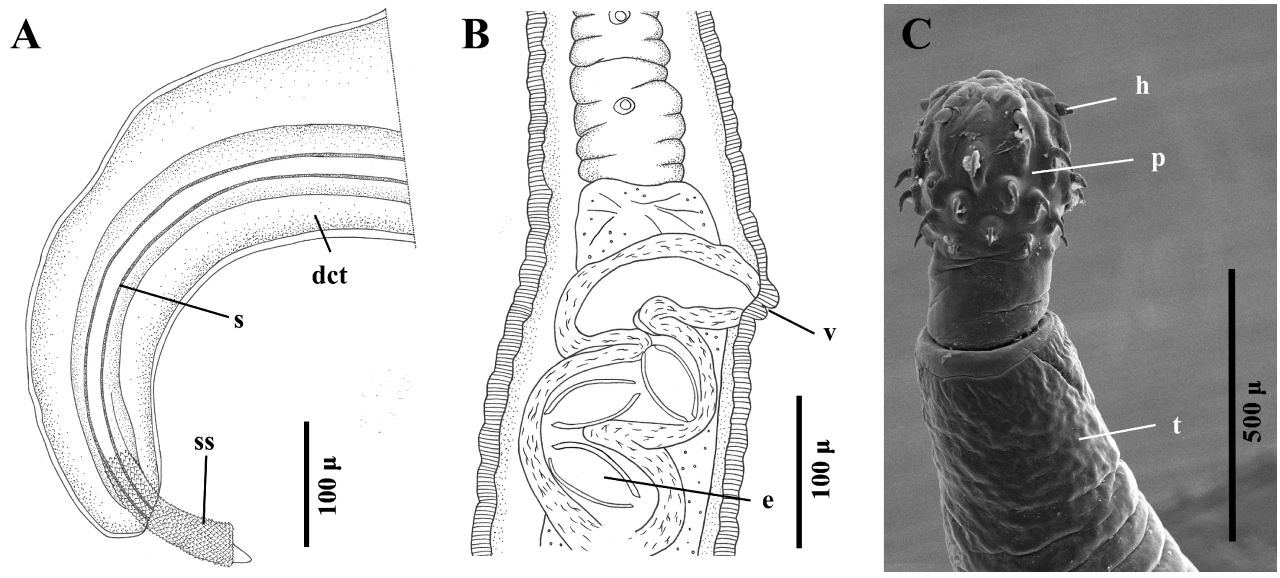

FIGURE 6. A. Posterior end of male *Trichuris minuta* from *Didelphis virginiana* showing the cilindrical spicular sheath, lateral view. B. Esophagus-intestine junction, and vulva of female *Trichuris* sp. from *Philander vossi*, lateral view. C. SEM micrograph of female proboscis of *Oligacanthorhynchus microcephalus*, lateral view. Abbreviations: egg (e), hook (h), proboscis (p), spicule (s), trunk (t), vulva (v), spicule sheath (ss), distal cloacal tube (dct).

***Trichuris* sp.**

Site of infection: Caecum.

Host species: *Philander vossi*.

Locality: Chetumal (Quintana Roo).

Prevalence: 100% (1/1).

Mean intensity: 1.

Specimen deposited: CNHE 12891.

Comments: The single female whipworm is 9,680 in body length, with anterior portion of body 6,980 long and thick portion of body 2,700 long. The specimen has a non-protrusive vulva (Figure 6B). Eggs oval, with bipolar plugs, 72–75 long by 30 wide.

In addition to the records of *T. minuta*, *Trichuris didelphis* Babero has been reported in Mexico from *D. virginiana* and *D. marsupialis* in Campeche, Colima, Hidalgo, Morelos, Yucatán (Acosta-Virgen *et al.* 2015; García-Valle *et al.* 2023), and Veracruz (Cañeda Guzman 1997). Unidentified species of *Trichuris* has also been reported from *D. virginiana* in Guerrero (Monet-Mendoza *et al.* 2005). New specimens, mainly males, should be collected to identify this taxon to the species level.

Phylum Acanthocephala Rudolphi

Class Archiacanthocephala Meyer

Family Oligacanthorhynchidae Southwell & Macfie

Genus *Oligacanthorhynchus* Travassos

***Oligacanthorhynchus microcephalus* (Rudolphi)**

Site of infection: Intestines.

Host species: *Didelphis virginiana.*
Localities: Umán, Mérida (Yucatán), Campeche, and Chencoh (Campeche).
Prevalence: 83.3% (5/6).
Mean intensity: 11.6 (range 1–29).
Specimens deposited: CNHE 12892–12895.
GenBank accession numbers: PP662468, PP662469.
Comments: The present material conformed to the descriptions by Richardson *et al*. (2014) and López-Caballero *et al*. (2015). Worms elongated and narrowly constricted at anterior end. Proboscis ovoid, 300–320 long by 300 wide, armed with six longitudinally arranged spiral rows of six shallowly rooted hooks each. The length of hooks decreases posteriorly: first hooks 75–80, second hooks 50–65, third hooks 45–58, fourth hooks 40–45, fifth hooks 30–32, sixth hooks 20–25 (Figure 6C). Male trunk 99,200–140,600 long by 4,000–7,000 wide. Female trunk 106,500–222,000 long by 4,000–10,000 wide. Eggs ellipsoid, 75–85 long by 30–42 wide.

Oligacanthorhynchus microcephalus has been previously reported from opossums (*D. marsupialis*, *D. virginiana* and *P. vossi*) in Campeche, Chiapas, Colima, Guanajuato, Hidalgo, Michoacán, Morelos, Oaxaca, Tabasco, Veracruz, and Yucatán (see García-Prieto *et al*. 2012; Acosta-Virgen *et al*. 2015; López-Caballero *et al*. 2015). This study adds new locality records in Yucatán and Campeche.

Molecular identification and phylogenetic relationships

A total of 17 28S rRNA sequences were obtained of nine helminth taxa. Representative sequences of *P. illiciens*, *Trichuris* sp., *C. tentaculata*, and *Strongyloides* sp. could not be obtained due to the small numbers of specimens collected.

The alignment of the Brachylaimidae dataset comprised 23 sequences, including the 28S rRNA sequence of *Brachylaima* sp. from the Yucatán Peninsula. The sequence of *Brachylaima* sp. was grouped with other sequences of the genus *Brachylaima* from several host species (bootstrap = 100) (Figure 7). Our sequence was recorded as sister species of *B. virginianum* from *D. virginiana* (bootstrap = 100), showing a genetic difference of 0.3%.

The phylogenetic analysis of the 28S rRNA sequence of *Mathevotaenia* sp., along with 21 other anoplocephalid cestodes, reveled that the isolate from *D. virginiana* was a sister species of *Mathevotaenia symmetrica* (Baylis) from *Musca domestica* De Geer and *Mastomys erythroleucus* (Temminck) (Figure 8). The genetic difference between *Mathevotaenia* sp. and *M. symmetrica* was 3.6–4.9%.

Phylogenetic relationships of 15 28S rRNA sequences of kathlaniid nematodes showed that the two sequences of *C. americana* from the Yucatán Peninsula were nested in a subclade with other two sequences, one from the same species isolated from *D. virginiana* and another from an unidentified species of *Cruzia* from *Salvator merianae* Duméril & Bibron (bootstrap= 100) (Supplementary Material Figure S1). The genetic variation among the three sequences of *C. americana* from the Yucatán Peninsula ranged from 0.4 to 0.6%.

The alignment of the Physalopteridae dataset comprised 12 28S rRNA sequences, including the three sequences of *T. Turgida*. The sequences of *T. turgida* were nested as a sister group formed by species of the genera *Physaloptera* and *Turgida* (bootstrap = 99) (Supplementary Material Figure S2). The genetic differences between the three sequences of *T. turgida* ranged from 0.5 to 1.3%.

The sequences of *Viannaia arriaguensis*, *Viannaia* sp., and *Travassostrongylus* sp. were aligned with 33 other sequences of trichostrongylid nematodes. The phylogenetic tree showed that *Travassostrongylus* sp. was a sister species of the subclade formed by the *Viannaia* species (bootstrap = 65) (Figure 9). The genetic difference between the three sequences of *V. arriaguensis* ranged from 0 to 0.1%, and between these sequences and that of *Viannaia* sp. was 0.8–1%. The genetic variation between *Travassostrongylus* sp. and the *Viannaia* species ranged from 15.5 to 15.7%.

The 28S rRNA sequences of *O. microcephalus* from the Yucatán Peninsula were aligned with 22 other oligacanthorhynchid acanthocephalans. The two sequences from the present study were grouped with five sequences of *O. microcephalus* isolated from *D. marsupialis* and *D. virginiana* (Supplementary Material Figure S3). The genetic difference among our sequences was 0.2%, and between these sequences and others of *O. microcephalus*, it was 0%.

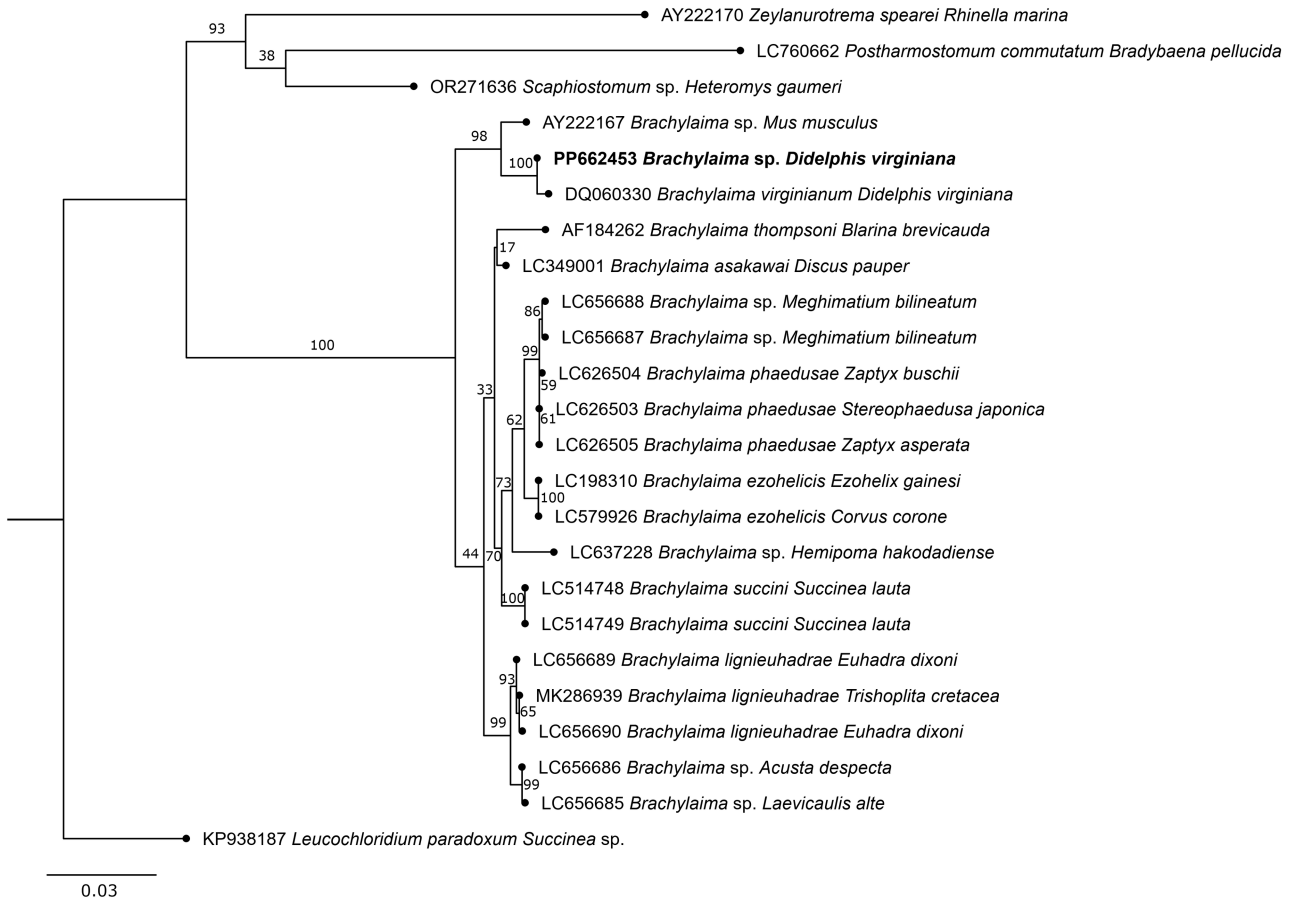

FIGURE 7. Maximum-likelihood (ML) phylogenetic tree of Brachylaimidea inferred with 28S rRNA sequence data using the TVM + G (ln likelihood -4739.195144). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

The two 28S rRNA sequences of *T. minuta* were similar to other trichurid nematodes, with similarity values ranging from 84.4% to 93%, albeit with poor coverage (33–34%), which prevented the generation of a resolved phylogeny. The two 28S rRNA sequences of *T. minuta* (PP662466 and PP662467) had a genetic difference of 0.2%.

Discussion

In the two last decades, the use of DNA sequences has enhanced our knowledge of parasite biodiversity, particularly in differentiating morphologically indistinguishable species (Poulin *et al.* 2019). The integration of morphological and molecular data has revealed unexpected species diversity of helminths infecting opossums in Mexico. Using partial sequences of the cytochrome c oxidase subunit 1 mitochondrial (COI) gene and the nuclear ribosomal internal transcribed spacer region (ITS1-5.8S-ITS2) alongside morphological evidence, López-Caballero *et al.* (2019) described *Rhopalias oochi* López-Caballero, Mata-López & Pérez-Ponce de León in *D. marsupialis*. This species originally identified as *Rhopalias coronatus* (Rudolphi) by Acosta-Virgen *et al.* (2015) based solely on morphological grounds. Similarly, Ramírez-Cañas *et al*. (2021) utilized the COI gene and the ITS1-5.8S-ITS2 region to describe *V. angelae* in *D. virginiana*, based on specimens morphologically identified as *V. viannai* by Acosta-Virgen *et al*. (2015). Despite these examples, the use of this integrative approach in parasitological research on terrestrial mammals in Mexico remains limited, as reflected in the scarcity of DNA sequences available in GenBank. In this study, we provided DNA sequences of several helminth taxa, including those that likely represent undescribed species.

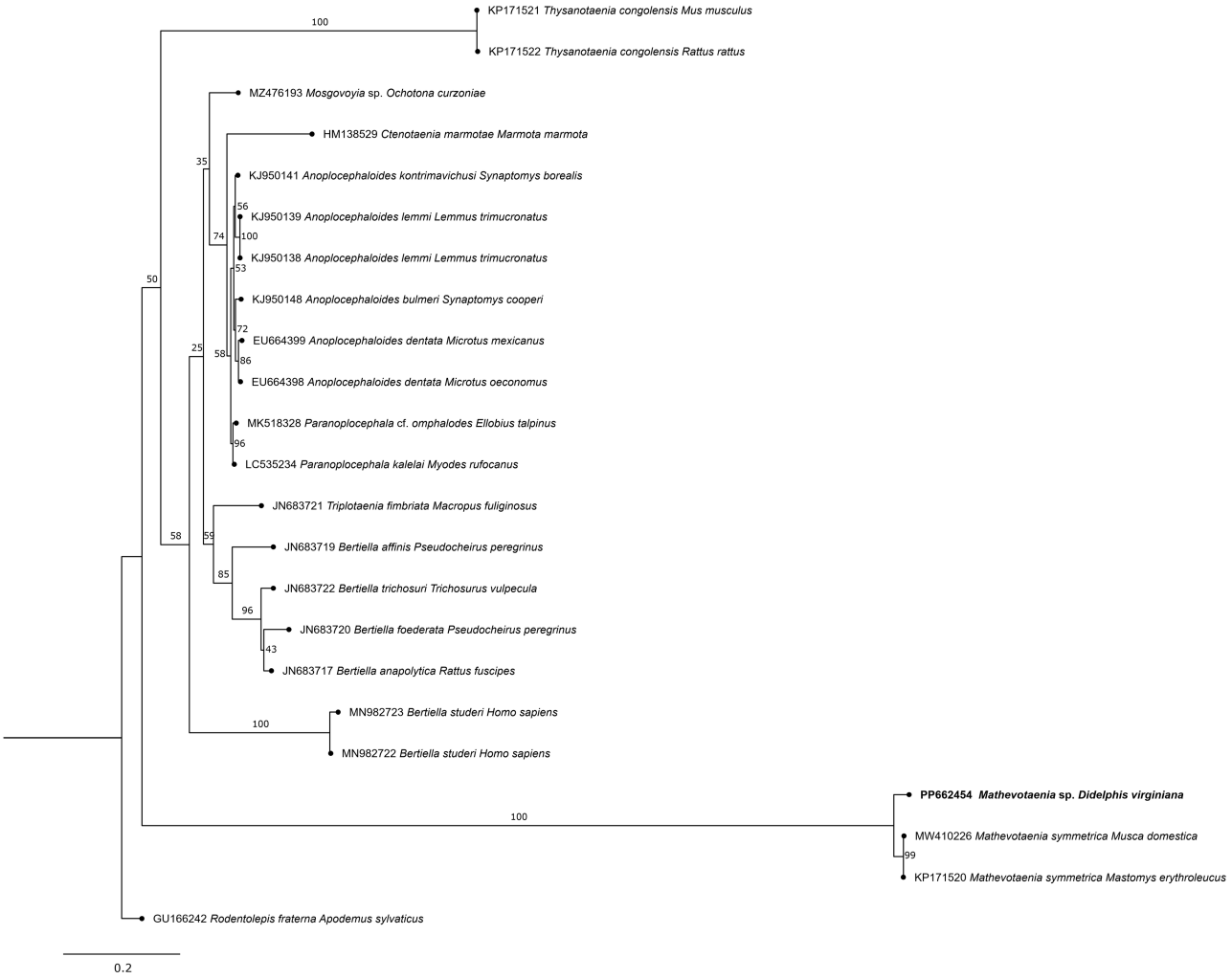

FIGURE 8. Maximum-likelihood (ML) phylogenetic tree of Anoplocephalidae inferred with 28S rRNA sequence data using the GTR + G model (ln likelihood -10764.655700). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

Except for the city of Mérida, all sampled localities in the Yucatán Peninsula had not been previously studied for helminths of opossums. At the state level, only seven and three taxa of helminths had been reported in *D. virginiana* from Campeche and Yucatán (Acosta-Virgen *et al.* 2015; López-Caballero *et al.* 2015), respectively. In Quintana Roo, only *Rhopalias* trematodes had been reported in *D. marsupialis* (Kingston & Tay 1968). Our study increases to 15 and 5 the number of helminth species reported in *D. virginiana* and *P. vossi*, respectively, in this region.

In the present study, we identified 13 helminth taxa, with *C. americana*, *V. angelae* and *Viannaia* sp. being found in both *D. virginiana* and *P. vossi*. In a previous study, Acosta-Virgen *et al.* (2015) examined *D. marsupialis*, *D. virginiana*, *Didelphis* sp., and *P. vossi* (reported as *P. opossum*), across 12 Mexican states, including Campeche and Yucatán, and recorded 21 helminth taxa. Notably, *R. coronatus*, *Aspidodera raillieti* Travassos, *C. tentaculata*, *Gnathostoma turgidum* Stossich, *T. turgida*, *T. didelphis*, and *V. viannaia* were found in more than two didelphid opossums across several states. Differences between these studies may be attributable to variations in sample size, the number of states included, the recent description of *V. angelae*, and the challenge of morphological differentiation between *C. americana* and *C. tentaculata* through light microscopy.

The present study was limited to a small sample size of opossums (six *D. virginiana* and one *P. vossi*) in the Yucatán Peninsula. Further studies including more sites and hosts in the Neotropical region, especially in Quintana Roo, will probably increase the richness of the helminthological inventory of this group of mammals (Acosta-Virgen *et al.* 2015). The molecular characterization of helminths was carried out with the 28S rRNA gene. This genetic marker is conserved across widely divergent taxa, and only the first 600–900 bases contain three divergent

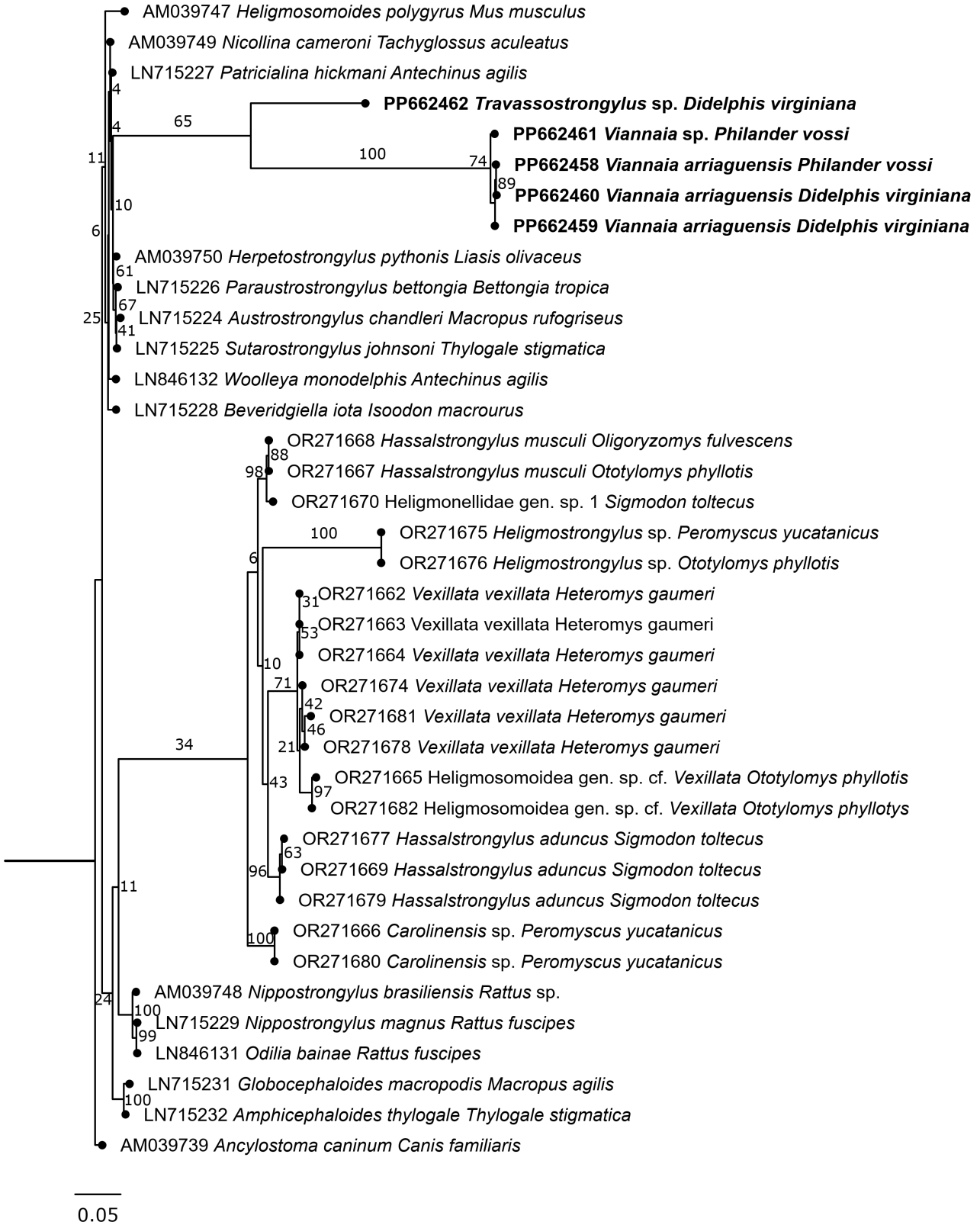

FIGURE 9. Maximum-likelihood (ML) phylogenetic tree of Trichostrongylidea inferred with 28S rRNA sequence data using the GTR + G model (ln likelihood -6392.437537). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

domains (D1–D3) that are useful for phylogenetic study of eukaryotic organisms, such as parasitic worms (Liu 2013; Thaenkham *et al.* 2022). Although, the 28S rRNA gene has been used for phylogenetic analysis at the suprageneric level, several studies have showed that this gene is able to successfully differentiate between species (Lee *et al.* 2004; Shylla *et al.* 2013; Řežábková *et al.* 2019). The present phylogeny of *Brachylaima* based on 28S rRNA sequences showed that the isolate from Yucatán and *B*. *virginianum* represent two distinct species that need further scrutiny for a better characterization. However, it should be considered the availability of partial reference sequences in nucleotide databases when utilizing the 28S rRNA gene (Thaenkham *et al.* 2022).

Using an integrative taxonomic approach based on morphological techniques and molecular phylogenetic analysis, we present a comprehensive overview of the helminth fauna of two species of opossums in the Yucatán Peninsula. We reported 13 helminth taxa, including the first record of *P*. *illiciens* from *D*. *virginiana* in Mexico, and the first record of *Mathevotaenia* sp., *T*. *minuta*, *C*. *americana*, *V*. *arriaguensis*, and *Travassostrongylus* sp. in the Yucatán Peninsula. Before the present study, the number of helminth species reported in *D*. *virginiana* and *P*. *vossi* in Mexico was 38 and 26, respectively. With the new records in the Yucatán Peninsula, the number of helminth taxa increase to 41 and 29 for *D*. *virginiana* and *P*. *vossi*, respectively. According to Acosta-Virgen *et al.* (2015), new studies in the Nearctic region of the country will probably increase the helminth fauna of this group of mammals. Despite extensive studies on helminths of *D*. *virginiana* and *P*. *vossi* in Mexico, the present study confirms that the inventory of these opossums is far from complete.

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Supplementary Materials

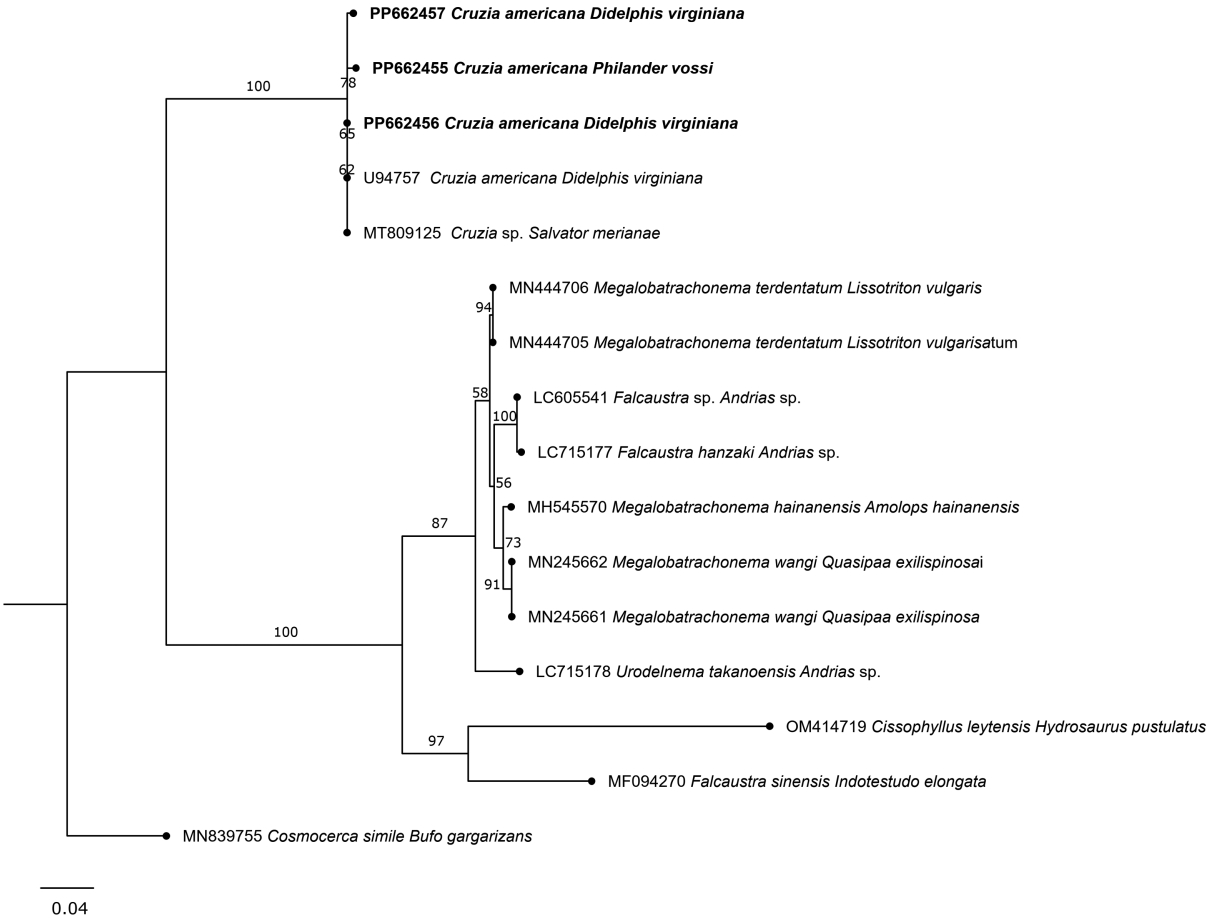

FIGURE S1. Maximum-likelihood (ML) phylogenetic tree of Kathlaniidae inferred with 28S rRNA sequence data using the GTR + G model (ln likelihood -4273.141975). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

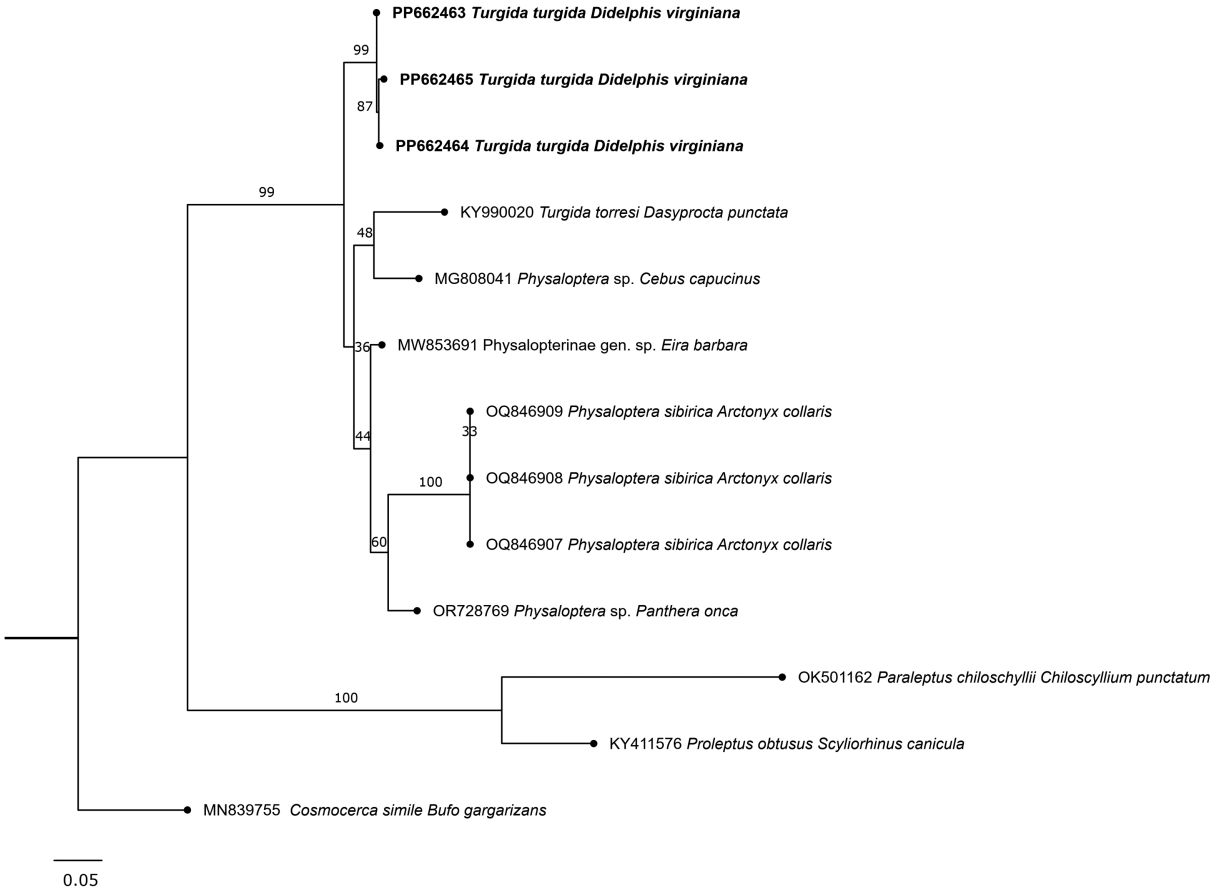

FIGURE S2. Maximum-likelihood (ML) phylogenetic tree of Physalopteridae inferred with 28S rRNA sequence data using the GTR + G model (ln likelihood -5866.434194). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.

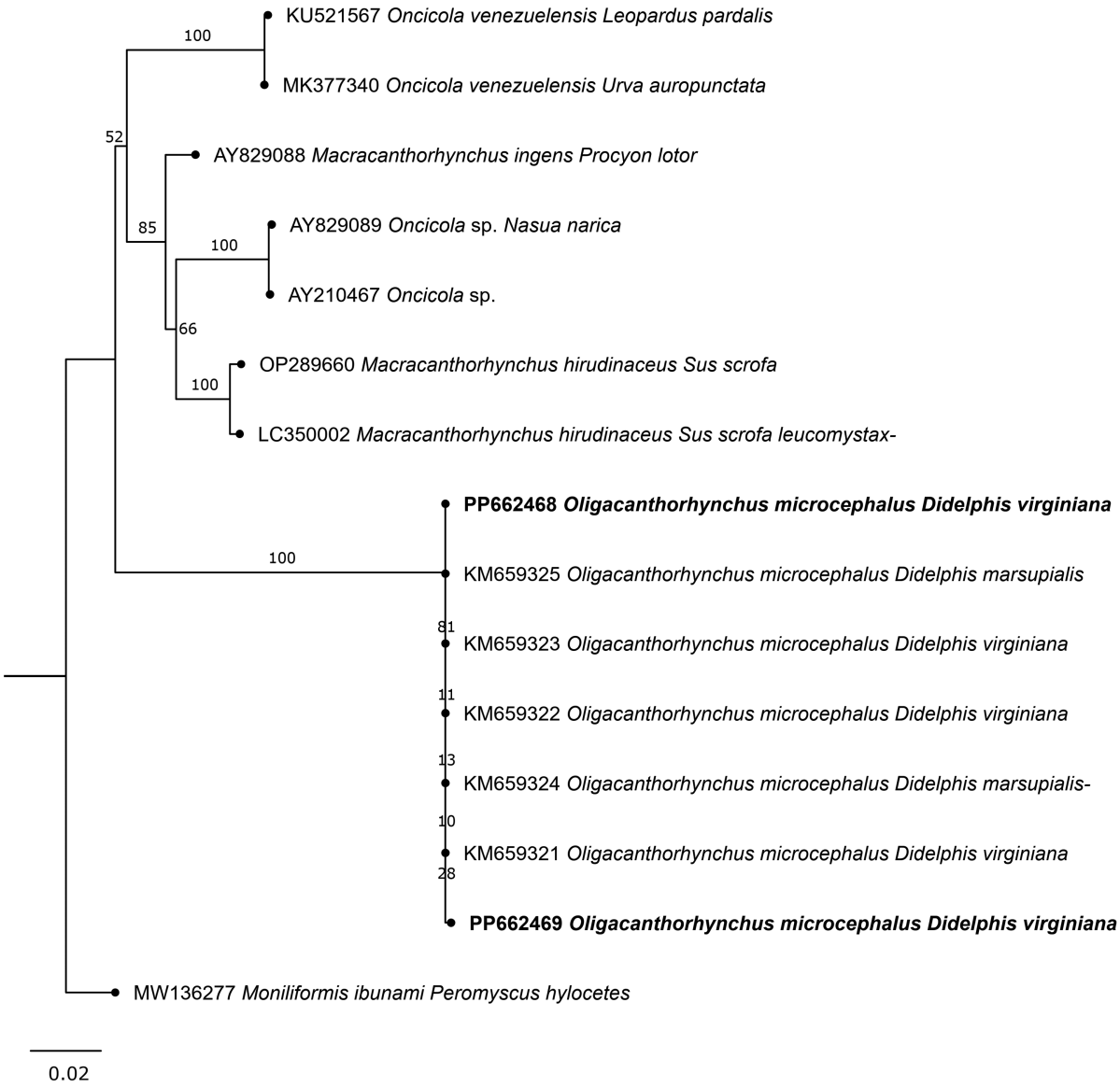

FIGURE S3. Maximum-likelihood (ML) phylogenetic tree of Oligacanthorhynchidae inferred with 28S rRNA sequence data using the GTR + G model (ln likelihood -6392.437537). GenBank accession numbers precede species name, followed by host name. Bootstrap support values for ML are provided at the nodes. The new sequences of the present study are in bold.