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Intestinal helminths of bats in the States of Nayarit and Veracruz, Mexico, with redescription of *Bidigiticauda vivipara* Chitwood

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Abstract

Although surveys on bat parasites in the Americas have been conducted, information on helminths remains limited. In Mexican states such as Nayarit and Veracruz only 1 and 8 helminth species have been reported in bats, respectively. In this study, we provide new helminthological records for bats using morphological techniques. Also, the 28S rRNA gene of specimens from several helminth taxa was successfully amplified and sequenced. To confirm the identification at the generic level, and in some cases at the specific level, and the genealogical relationships of the parasites, we performed the phylogenetic analyses using the new 28S rRNA sequences. From March to May 2022, 16 bats of 10 species are captured and examined for helminths. Three bat species of two families, two Vespertillionidae (*Bauerus dubiaquercus* and *Rhogeessa parvula*) and one Phyllostomidae (*Glossophaga mutica*), are parasitized by helminths. Seven helminth taxa are morphologically identified: the trematodes *Urotrema scabridum* and *Anenterotrema* cf. *hastati*, the cestodes *Vampirolepis macroti*, *Vampirolepis* sp. (1), and *Vampirolepis* sp. (2), and the nematodes *Bidigiticauda vivipara* and Capillariidae gen. sp. The first helminthological records for *R. parvula* and *B. dubiaquercus* and the 28S rRNA gene data of *B. vivipara*, *V. macroti*, and *Vampirolepis* spp. are provided. The findings of the present study increase the number of helminth taxa recorded in Mexican bats from 78 to 79, as well as the number of bat species with helminthological records from 35 to 37.

Key words: Cestoda, Chiroptera, Morphology, Nematoda, Phylogenetic analyses, Trematoda

Introduction

Chiroptera is the second most diverse mammalian order, encompassing nearly 1,470 species (Simmons & Cirranello 2023). In Mexico, there are records of 144 bat species belonging to 8 families, representing 10% of the global diversity of bats (Simmons & Cirranello 2023; Garbino *et al.* 2024; López-Cuamatzi *et al.* 2024). This diversity of bats corresponds to the ecosystem services they provide, such as pollination, seed dispersal and agriculture pest control (Jones *et al.* 2009; Kunz *et al.* 2011). Despite the ecological importance of bats, certain aspects related to them, such as the parasites they harbor, remain poorly understood.

In recent years, there has been a notable increase in studies on bat parasites with zoonotic potential, such as viruses, particularly in the Americas (Góes *et al.* 2013; Bergner *et al.* 2021). This trend is expected to continue following the emergence of SARS-CoV-2. However, little attention has been paid to other parasites, such as helminths (Hayman *et al.* 2013). In Mexico, helminthological records of bats have primary focused on specific groups, such as mormoopid bats, while helminths of vespertilionid bats remain understudied (García-Prieto *et al.* 2012; Jiménez *et al.* 2017).

To date, 78 helminth taxa (Trematoda, Cestoda, and Nematoda) have been reported in 19 Mexican States (Caspeta-Mandujano *et al.* 2017; Salinas-Ramos *et al.* 2017; Luviano-Hernández *et al.* 2018; Martínez-Pérez 2021; Moguel-Chin *et al.* 2023, 2024). These helminthological records on bats show heterogeneity across regions. For example, in Nayarit (western Mexico), only the nematode *Biacantha desmoda* (Wolfgang) has been reported parasitizing *Desmodus rotundus* Geoffroy Saint-Hilaire (Wolfgang, 1954), while the remaining 57 bat species do not have helminthological records. Similarly, in Veracruz (eastern Mexico) only 11 helminth taxa (2 Trematoda, 2 Cestoda, and 4 Nematoda) have been reported in 8 of the 89 bat species recorded in this region (Caspeta-Mandujano *et al.* 2017; Clarke-Crespo *et al.* 2017; Coates *et al.* 2017; Martínez-Pérez 2021). In contrast, the bat *Mormoops megalophylla*, which has a wide distribution in the country, including Nayarit and Veracruz, has approximately 26 records of helminth species (Caspeta-Mandujano *et al.* 2017). This clearly shows that the inventory of helminths of some Mexican bats is poorly known.

Collections of voucher specimens have long been the backbone of taxonomy, evolutionary biology, biodiversity research, and conservation biology (Clemannnn *et al.* 2014). However, in recent years, concerns have been raised over the risk posed by unnecessary collection of organisms, such as bats, because in some cases this might add pressure to already small populations, threatening them further, thus pushing them to the brink of extinction (Russo *et al.* 2017). To address these issues, several authors have made various recommendations for the comprehensive use of voucher specimens (Russo *et al.* 2017; Galbreath *et al.* 2019; Thompson *et al.* 2021). These recommendations include the collection of additional sample types, in addition to the target samples regardless of the goals of the study (Thompson *et al.* 2021). Mammalogists who collect mammal specimens could expand the scope and impact of their work by collecting tissues for investigating parasite biodiversity (Galbreath *et al.* 2019). It is also important to make specimens and their tissues easily accessible to researchers from different disciplines so that, together, they can create integrated baseline series and current records of parasites in the face of deforestation, climate change, and species extinction scenarios (Russo *et al.* 2017; Galbreath *et al.* 2019; Thompson *et al.* 2021).

As a part of a study on DNA barcoding of bats from poorly studied States of Mexico, some voucher specimens and their gastrointestinal tract were examined for helminths. In the present study, a list of the intestinal helminths of bats is presented. In addition, the type species of *Bidigiticauda* Chitwood is redescribed based on morphological and molecular data.

Materials and methods

Collection and examination of bats. From March to May 2022, a total of 16 bats of 10 species belonging to three families were collected and examined for helminths in Nayarit and Veracruz (Figure 1): Phyllostomidae, *Glossophaga mutica* Merriam (3), *Artibeus lituratus* Olfers (1), *Artibeus intermedius* Allen (1), *Macrotus waterhousii* Gray (1); Vespertilionidae, *Lasiurus frantzii* Peters (1), *Bauerus dubiaquercus* Van Gelder (1), *Rhogeessa parvula* Allen (3), *Myotis findleyi* Bogan (1); and Emballonuridae, *Balantiopteryx plicata* Peters (1) from Nayarit, and Vespertilionidae, *B. dubiaquercus* (2) and Phyllostomidae, *Vampyrodes major* Allen (1) from Veracruz. Collecting permits were obtained from the Mexican Ministry of Environment [SEMARNAT] (SGPA/DGVS/08926/21 and

SGPA/DGVS/01532/22). The captured bats were removed from the nets, placed in cloth bags and identified using a field identification key for bats (Medellín *et al.* 2008). Animals were anesthetized and euthanized by placing them in a closed receptacle containing cotton soaked with isoflurane (Leary *et al.* 2020). The stomach, intestines, liver, and mesenteries of each bat were collected and stored in 96% ethanol. All collected organs were dissected and immersed in distilled water in Petri dishes using a stereo microscope (Olympus SZ2-ILST). Helminths were collected, counted, and preserved in 70% ethanol for light microscopy, 100% ethanol for DNA extraction, and 10% formalin for scanning electron microscopy (SEM).

Trematodes and cestodes were stained with Mayers' paracarmine, dehydrated through an ethanol series, cleared in methyl salicylate, and mounted permanently in Canada balsam. Nematodes were cleared and temporarily mounted in lactophenol. Specimens were studied and drawn with the aid of a light microscope (Leica DM500) with a drawing tube (Leica Microsystems). For SEM, selected specimens were dehydrated using a graded ethanol series and 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 (IB), Universidad Nacional Autónoma de México (UNAM), Mexico City. Specimens were identified to the lowest taxonomic level possible using keys for nematodes (Moravec 1982; Anderson *et al.* 2009), cestodes (Khalil *et al.* 1994) and trematodes (Bray *et al.* 2008), as well as original descriptions and redescriptions of bat helminths (e.g., Zdzitowiecki & Rutkowska 1980; Cacique *et al.* 2023). When more than four helminth specimens were examined, we presented the mean followed by the range in parenthesis, otherwise only the range is given. All measurements are in micrometers unless otherwise indicated.

Helminth specimens were deposited in the Colección Nacional de Helmintos (CNHE), IB-UNAM, Mexico City (see below for accession numbers). Skulls and skins of infected bats were deposited in the mammal collection of the Museo de Zoología Alfonso L. Herrera, UNAM (catalog numbers: *G. mutica* MZFC-M-16343, *R. parvula* MZFC-M-16775 and MZFC-M-16776) and the mammal collection of the Instituto de Investigaciones Biológica, Universidad Veracruzana (catalog numbers: *B. dubiaquercus* IIB-UV-4381 and IIB-UV-4382).

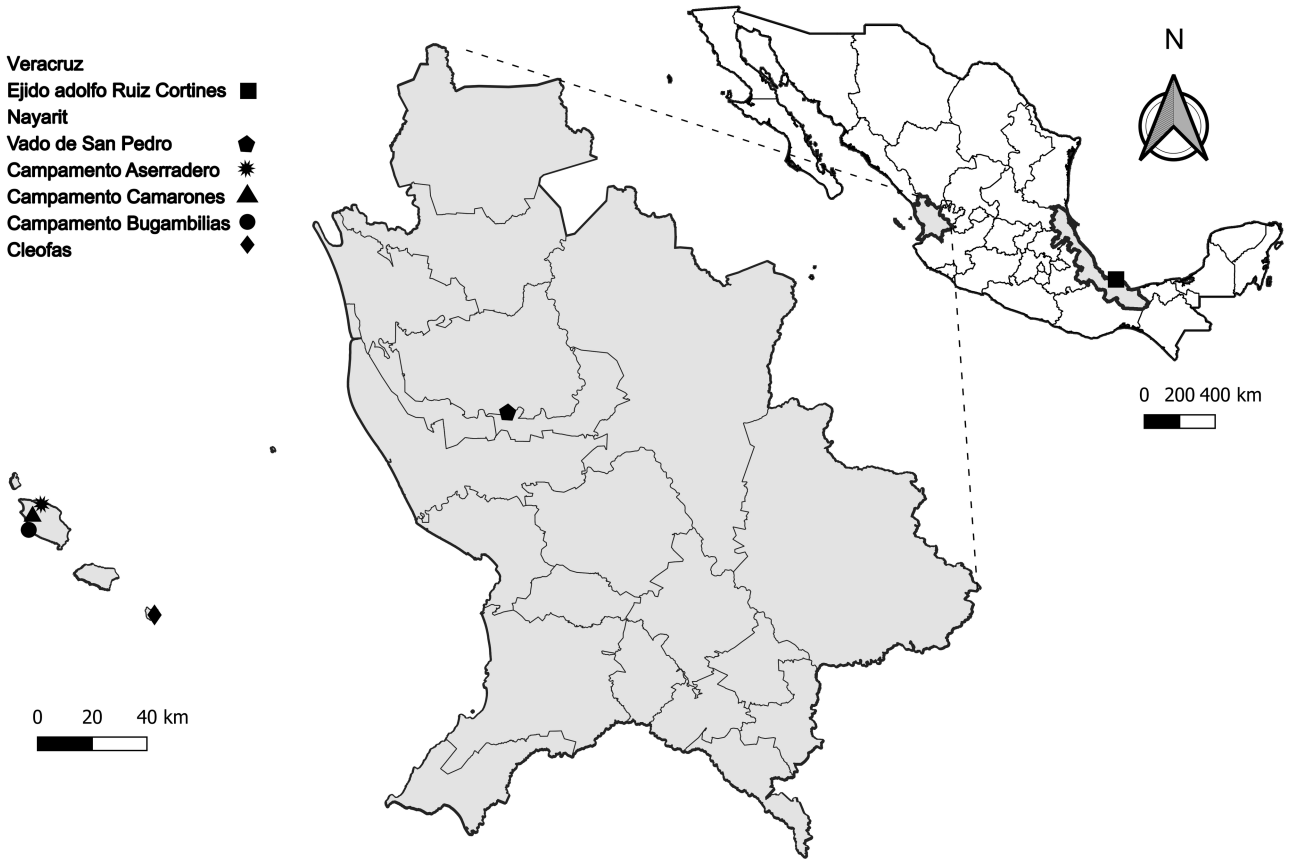

FIGURE 1. Map showing the distribution of the sampling locations.

DNA extraction and sequencing of helminth. Total genomic DNA of at least one specimen or fragment of each helminth taxon was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). A fragment of the 28S rRNA gene of ribosomal RNA (28S rRNA) was targeted using the forward primer 391 5′-AGCGGAGGAAAAGAAACTAA-3′ (Stock *et al.* 2001) and the reverse primer 536 5′-CAGCTATCCTGAGGGAAAC-3′ (Stock *et al.* 2001), which amplify a fragment of 700–1400 base pairs, depending on the helminth species. Polymerase chain reaction (PCR) amplifications were carried out using the conditions described by Hernández-Mena *et al*. (2017). Sanger sequencing was carried at the Laboratorio de Secuenciación Genómica de la Biodiversidad y la Salud, IB-UNAM, Mexico City. The resulting sequences were analyzed and edited in Geneious Pro 4.8.4 software (Biomatters Ltd., Auckland, New Zealand) and the consensus was obtained for each sequenced specimen.

Phylogenetic analysis. The generated sequences of 28S rRNA were compared with related species deposited in GenBank® (National Center for Biotechnology Information). Alignment was performed 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 dataset with jModelTest v2 (Darriba *et al.* 2012) under the Akaike information criterion. Phylogenetic analysis for each data set were assessed by Maximum Likelihood (ML) method analysis using RAxML v. 7.0.4 (Stamatakis 2006). Bootstrap support values were estimates by running 1,000 bootstrap resamples. Genetic variation within the 28S rRNA datasets was calculated using p-distances with MEGA 11 (Tamura *et al.* 2013).

Results

Seven helminth taxa were identified from the intestine of bats comprising two trematodes, three cestodes, and two nematodes. Among the 16 examined hosts, 5 bats (31.2%) of 3 species (*B*. *dubiaquercus*, *R*. *parvula*, and *G*. *mutica*) were infected with helminths, and 2 bats (*B*. *dubiaquercus* and *R*. *parvula*) harbored 2 helminth taxa. Below we present a list of the helminth species found in the infected bats with comments based on morphological and biometrical data, followed by the results of the phylogenetic analysis.

List of species of helminths

Phylum Platyhelminthes Gegenbaur

Class Trematoda Rudolphi

Subclass Digenea Carus

Order Plagiorchiida La Rue

Superfamily Plagiorchioidea Liihe

Family Anenterotrematidae Yamaguti

Anenterotrema Stunkard

Anenterotrema cf. *hastati* Cacique, Cruces & Chero

Site of infection: Small intestine
Host species: *Bauerus dubiaquercus*
Locality: Ejido Adolfo Ruiz Cortines (18°31'53.8" N, 95°08'01.7" W, 1061 MASL), Tuxtlas, Veracruz
Prevalence and intensity: 50% (1/2) and 151
Specimen deposited: CNHE 12134

Comments: Based on five trematodes. Body pyriform, minute, 225 (165–330) long and 123 (105–151) wide (Figure 2A). Tegument unarmed. Oral sucker spherical, subterminal, 50 (50–51) long by 52 (50–56) wide. Ventral sucker in middle third of body, 55 (50–60) long by 69 (62–74) wide. Pharynx, oesophagus, and intestinal caeca not observed. Gonads in anterior half of hindbody. Testes postero-lateral to ventral sucker; right testis 42 long by 70 wide, left testis 55 long by 62 wide. Ovary median, post-testicular, 32 long by 53 wide. Vitellaria in hindbody, concentrated in lateral region, occupying almost entire left testis. Eggs 27 (25–30) long by 13 (11–15) wide.

The closest species in morphology is *A*. *hastati* described from *Phyllostomus hastatus* (Pallas) in Peru. However, we were unable to observe the characteristic unicellular glands located anterolateral to cirrus sac due to the poor condition of preservation of our specimens. The remaining species, *Anenterotrema liliputianum* (Travassos), *Anenterotrema auritum* (Stunkard), *Anenterotrema eduardocaballeroi* (Freitas), *Anenterotrema freitasi* (Caballero), *Anenterotrema mesolecitha* (Marshall & Miller), *Anenterotrema iannaconei* (Achatz, Cardenaz-Callirgos & Tkach), *Anenterotrema megacetabulum* (Fernandes, Santos, Melo, Achatz, Greiman, Bonilla & Tkach), *Anenterotrema paramegacetabulum* (Cacique, Cruces & Chero), *Anenterotrema kawsayense* (Cacique, Cruces & Chero), *Anenterotrema stunkardi* (Caballero & Grocott), and *Anenterotrema peruense* (Cacique, Cruces & Chero), differ in shape and size of oral sucker, body length, and position of gonads (Cacique *et al.* 2023). This is the first record of *A*. cf. *hastati* in Mexico.

Family Urotrematidae Poche

***Urotrema* Braun**

***Urotrema scabridum* Braun**

Site of infection: Small intestine

Host: *Rhogeessa parvula*

Locality: Cleofas (21°19’19.3” N, 106°14’41.6” W, 98 MASL), Islas Marías, Nayarit

Prevalence and intensity: 33.3% (1/3) and 1

Specimen deposited: CNHE 12133

Comments: Based on a single specimen. Body elongate (3360 long and 485 wide), covered with fine spines (Figure 2B). Oral sucker 145 long by 100 wide, ventral sucker 200 long by 140 wide. Pharynx 60 long by 50 wide. Ovary 250 long by 190 wide. Anterior testis 260 long by 270 wide, posterior testis 250 long by 225 wide. Eggs 15–20 long by 10 wide.

Morphologically, the specimen fits the original description of *U. scabridum* (Braun 1900) and recent records (Martínez-Salazar *et al.* 2020). *Urotrema scabridum* has been recorded in several bat species in Mexico, such as *Tadarida brasiliensis* (Geoffroy Saint-Hilaire)*, Pteronotus mesoamericanus* Smith (syn. *Pteronotus parnellii* Gray), *B*. *plicata*, *Pteronotus fulvus* (Thomas) (syn. *Pteronotus davyi* Gray), *Pteronotus psilotis* (Dobson) (syn. *Pteronotus personatus* Wagner), *Myotis velifer* (Allen), *Mormoops megalophylla* (Peters) in Morelos (see the checklist of Jiménez *et al.* 2017 and references therein), *T*. *brasiliensis* in Durango, Mexico State, Nuevo León, Zacatecas and Puebla, and *Natalus mexicanus* Miller in Mexico City (Jiménez *et al.* 2017; Martínez-Salazar *et al.* 2020). This is the first record of *U*. *scabridum* in Nayarit and for *G*. *mutica*.

Class Cestoda Rudolphi

Subclass Eucestoda Southwell

Order Cyclophyllidea Van Beneden in Braun

Family Hymenolepididae Pierrier

***Vampirolepis* Spasskii**

Vampirolepis macroti **Zdzitowiecki & Rutkowska**

Site of infection: Small intestine

Host: *Bauerus dubiaquercus*

Locality: Ejido Adolfo Ruiz Cortines (18°31'53.8" N, 95°08'01.7" W, 1061 MASL), Tuxtlas, Veracruz

Prevalence and intensity: 50% (1/2) and 4

Specimen deposited: CNHE 12135

GenBank accession number: PQ476180

Comments: Based on two incomplete specimens. Scolex 260 in diameter. Suckers unarmed, 100–108 in diameter. Rostellum broad, 73–75 in diameter, armed with 28–29 hooks in single row (Figure 2C). Hook length 26–32. Although no mature proglottids were found, the number and length of hooks observed in the scolex of our specimens conformed to the original description of *V. macroti* from *M. waterhousii* in Cuba (*vs*. 29–34 hooks of 28–30 long) (Zdzitowiecki & Rutkowska 1980).

Vampirolepis macroti has been reported from *M. waterhousii* in Michoacán (Luviano-Hernández *et al.* 2018). This is the second record of *V. macroti* in bats from Mexico.

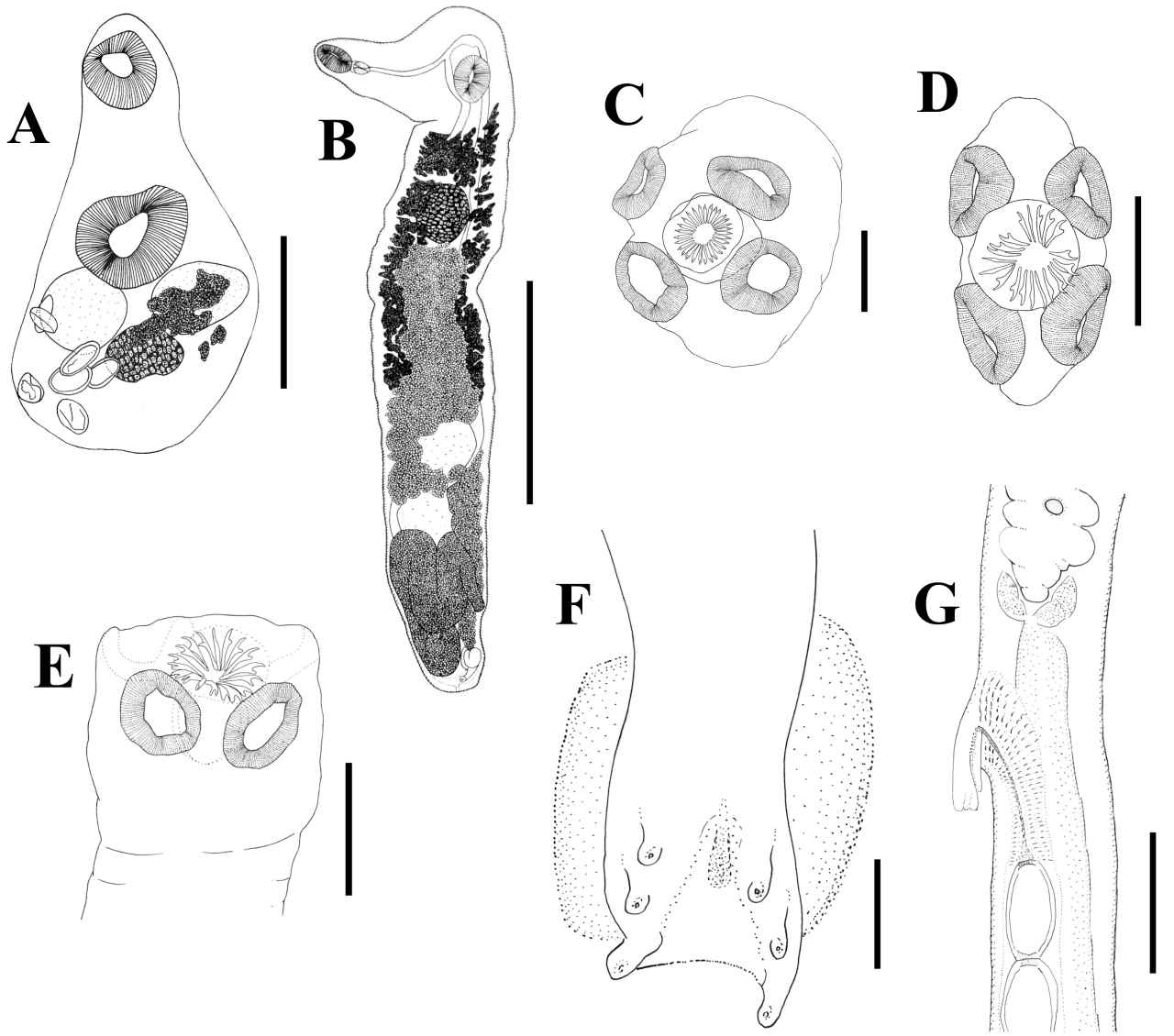

FIGURE 2. Helminths of bats from Nayarit and Veracruz, Mexico. A) Adult of *Anenterotrema* cf. *hastati*, ventral view. B) Adult of *Urotrema scabridum*, ventral view. C) Scolex of *Vampirolepis macroti*, apical view. D) Scolex of *Vampirolepis* sp. (1), apical view. E) Scolex of *Vampirolepis* sp. (2), lateral view. F) Male posterior extremity of Capillariidae gen. sp., ventral view. G) Esophagus-intestine junction and vulva of female Capillariidae gen. sp., lateral view. Scale bars: A, C, D, E = 100 μm; B = 1000 μm; F = 10 μm; G = 65 μm.

***Vampirolepis* sp. (1)**

Site of infection: Small intestine

Host: *Rhogeessa parvula*

Locality: Campamento Camarones (21°37'27.2" N, 106°37'50.4" W, 42 MASL), Islas Marías, Nayarit

Prevalence and intensity: 33.3% (1/3) and 7

Specimen deposited: CNHE 12136

GenBank accession number: PQ476178

Comments: Based on three immature specimens and one scolex observed at SEM. Scolex oval to rounded, 140–200 in diameter (Figures 2D, 3A). Suckers unarmed, cup-shaped, 58–68 in diameter. Rostellum broad, 60–70 in diameter, armed with 17–20 hooks in single row (Figures 2D, 3B). Hook length 27–30.

The specimens described here were included in the genus *Vampirolepis* based on the presence of armed rostellum, because they are parasites of bats (Makarikova 2018), and by the molecularly established phylogenetic position (see below). Based on the number of rostellar hooks, the most similar species are *Vampirolepis artibei* (Zdzitowiecki & Rutkowska) (vs. 20–23) and *Vampirolepis bidentatus* (Zdzitowiecki & Rutkowska) (vs. 18–22) (Zdzitowiecki & Rutkowska 1980); however, the hooks of our specimens are longer than those of *V. artibei* (vs. 19–20) and *V. bidentatus* (16–17). The remaining 18 species recorded in the Americas, *Vampirolepis bihamata* (Sawada & Harada), *Vampirolepis chiropterophila* (Pérez-Vigueras), *Vampirolepis christensoni* (Macy), *Vampirolepis crassihamata* (Sawada & Harada), *Vampirolepis dalvae* (dos Santos, Simões, D´Andrea, Verde, Maldonado Júnior, Cartagena, Ubiali & Luque), *Vampirolepis decipiens* (Diesing), *Vampirolepis elongatus* (Rego), *Vampirolepis gertschi* (Macy), *Vampirolepis guarany* (Rego), *Vampirolepis longisccata* (Sawada & Harada), *V. macroti*, *Vampirolepis mazanensis* (Vaucher), *Vampirolepis pandoensis* (Sawada & Harada), *Vampirolepis phyllostomi* (Vaucher), *Vampirolepis promopsis* (Vaucher), *Vampirolepis roudabushi* (Macy & Rausch), *Vampirolepis santacruzensis* (Sawada & Harada), and *Vampirolepis temmincki* (Vaucher), have differences in the number and the length of rostellar hooks compared with the studied specimens. This study adds the first record of the genus *Vampirolepis* for *R. parvula* in Mexico.

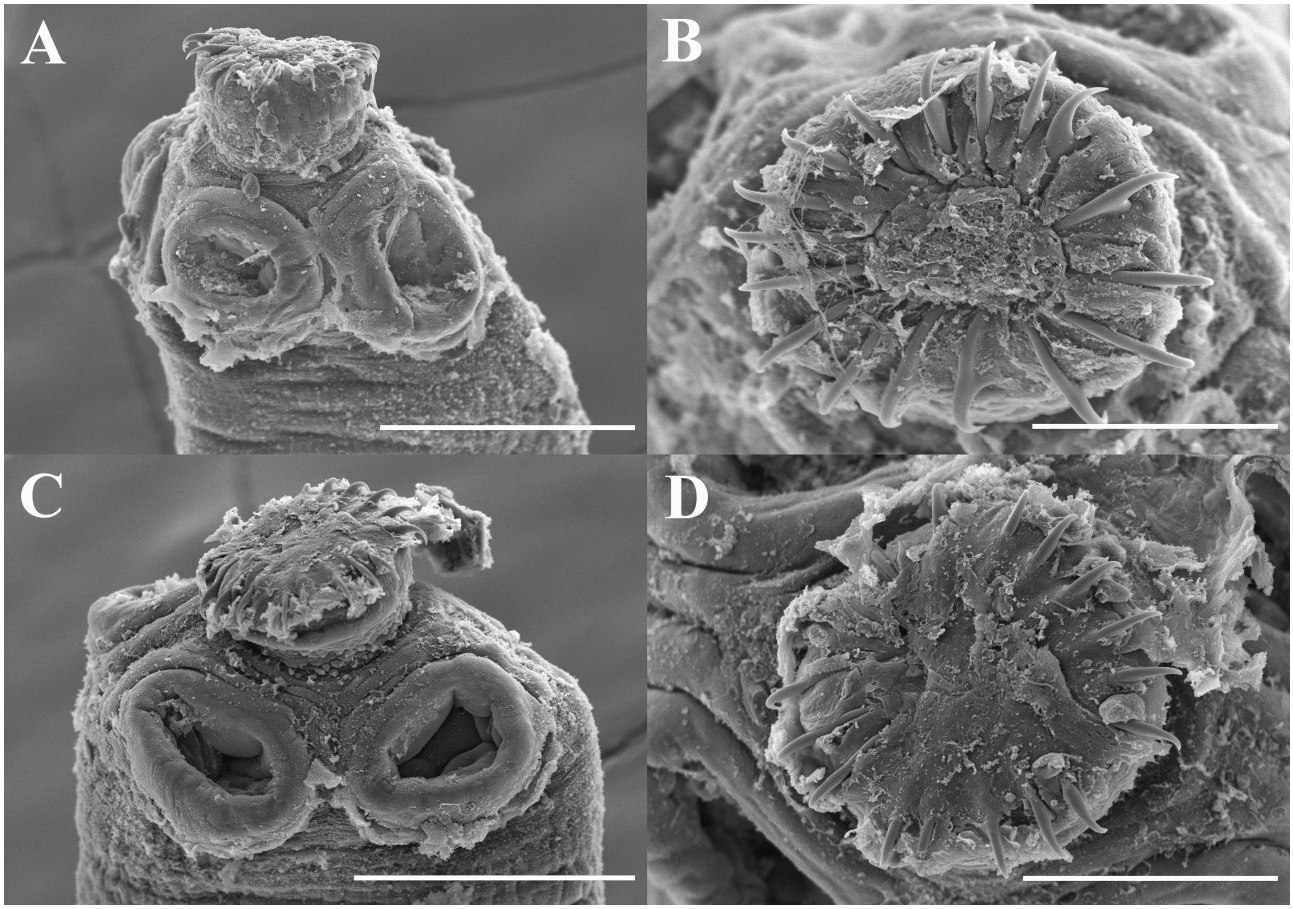

FIGURE 3. Scanning electron micrographs of *Vampirolepis* sp. (1) (A–B) and *Vampirolepis* sp. (2) (C–D). A) Scolex, lateral view. B) Rostelum, apical view. C) Scolex, lateral view. D) Rostelum, apical view. Scale bars: A, C = 100 μm; B = 40 μm; D = 50 μm.

***Vampirolepis* sp. (2)**

Site of infection: Small intestine

Host: *Rhogeessa parvula*

Locality: Cleofas (21°19’19.3” N, 106°14’41.6” W, 98 MASL), Islas Marías, Nayarit

Prevalence and intensity: 33.3% (1/3) and 3

Specimen deposited: CNHE 12137

GenBank accession number: PQ476179

Comments: Based on one immature specimen and one scolex observed at SEM. Scolex 168 in diameter. Suckers unarmed, 66 in diameter (Figures 2E, 3C). Rostellum broad, 65–70 in diameter, armed with 17–18 hooks in single row (Figures 2E, 3D). Hook length 30–32. These characteristics closely resemble *Vampirolepis* sp. (1); however, the phylogenetic position and genetic distance (see phylogenetic section) of the 28S rRNA sequences confirm that they are different species.

Phylum Nematoda Rudolphi

Class Adenophorea Chitwood

Subclass Enoplia Pearse

Order Enoplida Baird

Superfamily Enoploidae Dujardin

Family Capillariidae Railliet

Capillariidae gen. sp.

Site of infection: Small intestine

Host: *Bauerus dubiaquercus*

Locality: Ejido Adolfo Ruiz Cortines (18°31’53.8” N, 95°08’01.7” W, 1061 MASL), Tuxtlas, Veracruz

Prevalence and intensity: 50% (1/2) and 18

Specimens deposited: CNHE 12132

Comments: Based on three males and four females. Male body length 5,982–6,310 and body width 36–38 at esophagus-intestine level. Muscular esophagus 188–198 long and glandular esophagus 2,680–2,860 long. Anterior cloaca 560–770 long and posterior cloaca with terminal portion attenuated, 650–736 long. Cirrus (internal cuticular lining) forming a few longitudinal crests, terminal end with thin spines (eventually observed coming out through the cloacal opening). Spicule not observed. Caudal end composed of two dorsal lobes with three pedunculated papillae on each dorsal lobe (Figure 2F). Dorsal lobes joined with well-developed membranous bursa. Lateral alae developed. Female body length 7,782–9,030 and body width 50–55 at esophagus-intestinal junction. Muscular esophagus 139–240 long and glandular esophagus 2,786–3,295 long. Vulva close to posterior end of esophagus. Vulvar appendage present, arising basally as a protrusion of the anterior vulvar lip and continuing as a heart-shaped cuticular fold (Figure 2G). Vagina short, with thick musculature. Posterior end of body slightly constricted laterally and ventrally at level of end of intestine. Eggs 45–50 long by 23–26 wide, symmetric, or not, depending on orientation, poles slightly convex.

The most important distinguishing features among capillariid genera are related to the structure of the posterior end of the male, such as the presence or absence and characteristics of the caudal papillae, lobes, dorsal cuticular membrane, and caudal lateral alae (Moravec 1982). According to Moravec (1982), capillariids belonging to the genera *Pterothominx* and *Aonchotheca* exhibit well-developed caudal lateral alae and possess a membranous bursa. These genera can be distinguished by characteristics of the cirrus and spicule. *Pterothominx* has a cirrus covered by minute spines and a well sclerotized spicule, whereas *Aonchotheca* possess an unspiny cirrus, and its spicule

may sometimes be indiscernible due to insufficient sclerotization. In the studied specimens, the spicule was not observed, but spines were noted on the terminal portion of the cirrus. Considering the morphology observed in studied specimens, we prefer to adopt a conservative position and not assign them to any of these genera until we have more morphological and molecular evidence.

Three capillariid species have been reported from bats in Mexico: *Aonchoteca martinezi* (Caballero) from *N. mexicanus* in Mexico City (Caballero & Caballero 1942), *Aonchoteca speciosa* (Beneden) from *N. mexicanus*, *Leptonycteris yerbabuenae* (Martínez & Villa-Ramírez), *M. waterhousii*, *G. mutica*, *M. velifer*, *M. megalophylla*, *T. brasiliensis*, and *Dermanura azteca* (Andersen) in Morelos (Peralta 2012), and *Capillaria palmata* (Chandler) from *T. brasiliensis* in Morelos (Martínez 2009). Additionally, undescribed species of *Capillaria* have been reported from *Micronycteris microtis* Miller in Yucatan (Chitwood 1938) and *M. megalophylla*, *P. fulvus* and *Pteronotus mexicanus* Miller (syn. *P. parnellii*) in Jalisco (Salinas-Ramos *et al.* 2017), and *P. psilotis* in Veracruz (Clarke 2008), as well as nematodes of the genus *Pterothominx* from *P. mexicanus* in Jalisco (Lamothe-Argumedo *et al.* 1997) and *P. fulvus*, *P. mesoamericanus*, and *M. megalophylla* in Morelos (Peralta 2012; Ramírez 2015). This is the first record of Capillariidae gen. sp. in *B. dubiaquercus* in Mexico.

Class Secernentea Von Linstow

Subclass Rhabditia Chitwood

Order Strongylida Diesing

Superfamily Strongyloidea Weinland

Family Molineidae Skrjabin & Schultz

***Bidigiticauda* Chitwood**

***Bidigiticauda vivipara* Chitwood**

Site of infection: Small intestine

Host: *Glossophaga mutica*

Locality: Campamento Bugambilias (21°39’41.2” N, 106°36’58.3” W, 164 MASL), Islas Marías, Nayarit

Prevalence and intensity: 33.3% (1/3) and 131

Specimens deposited: CNHE 12131

GenBank accession numbers: PQ476176, PQ476177

Redescription: Based on 11 males, 11 females, and six third-stage larvae. Nematodes with cephalic vesicle (Figures 4A, 5A), usually wider than long (Figure 4A). Presence of four double cephalic papillae, two dorsolateral and two ventrolateral, as well as two amphids (Figure 5B). Esophageal tooth present (Figure 4A). Male and female lack longitudinal ridges (Figures 4B, C, 5A, C, F).

Males: Body length 3746 (3480–4250) and width at midbody 124 (100–140). Cephalic vesicle 103 (85–118) long and 117 (95–135) wide. Nerve ring, excretory pore and deirids situated at 199 (175–220), 306 (235–368), and 301 (232–368) from anterior end, respectively. Esophagus 319 (280–370) long. Caudal bursa subsymmetrical (Figures 4D, 5D). Rays 2, 3, and 4 arising independently, ray 4 slightly separated from ray 3. Rays 5 and 6 long, originating from common trunk (Figure 4D). Dorsal ray long, divided at its distal third into 2 branches (Figure 4D). In two specimens, bursal rays presented variations: right rays 4 and 5 fused (Figure 4E), left ray 2 and 3 fused and dorsal ray’s right branch long (Figure 4F). Spicules subequal, alate, 182 (160–200) long with sharp tips enclosed in a spatulate membrane (Figure 4H). Ratio spicule length/body length: 4.8% (4–5.3%). Gubernaculum absent. Genital cone with dorsal lip more developed than ventral lip (Figure 5D); papillae 7 situated on dorsal lip (Figure 5E).

Females: Body length 6330 (5480–7670) and width at midbody 186 (170–200). Cephalic vesicle 111 (90–130) long and 145 (115–170) wide. Nerve ring, excretory pore, and deirids situated at 201 (140–245), 291 (228–365), and 290 (220–380) from anterior end, respectively. Esophagus 315 (240–360) long (Figure 4A). Viviparous. Didelphic.

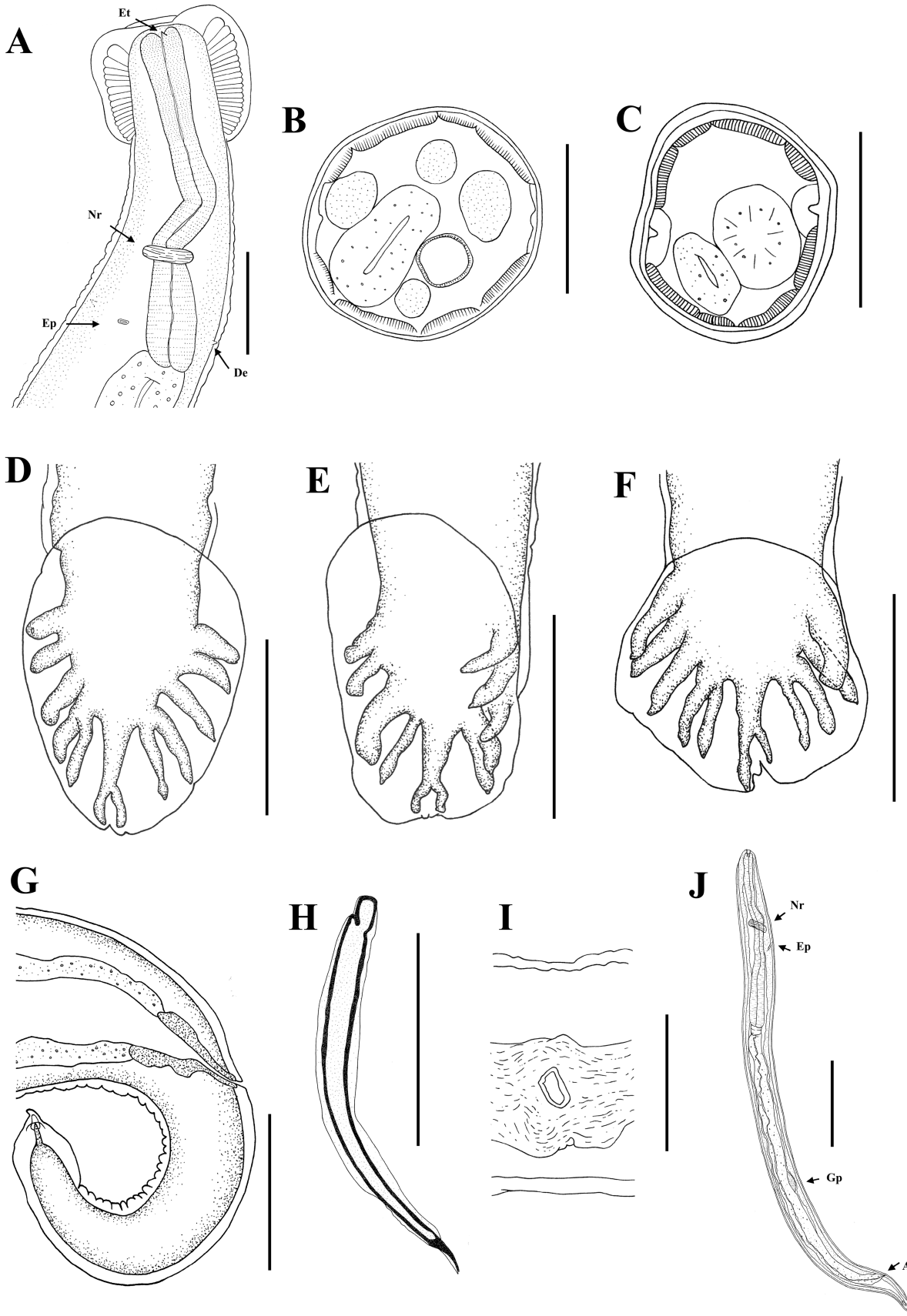

FIGURE 4. *Bidigiticauda vivipara*. A) Female anterior extremity, ventral view. B) Female transverse body section, at mid-body. C) Male transverse body section, at mid-body. D–F) Male caudal bursae, ventral views. G) Female posterior extremity, lateral view. H) Right spicule, lateral view. I) Vulvar aperture, ventral view. J) Third-stage larvae, lateral view. Abbreviations: Et, esophageal tooth; Nr, nerve ring; Ep, excretory pore; De, deirid; Gp, genital primordium; An, anus. Scale bars = 100 μm.

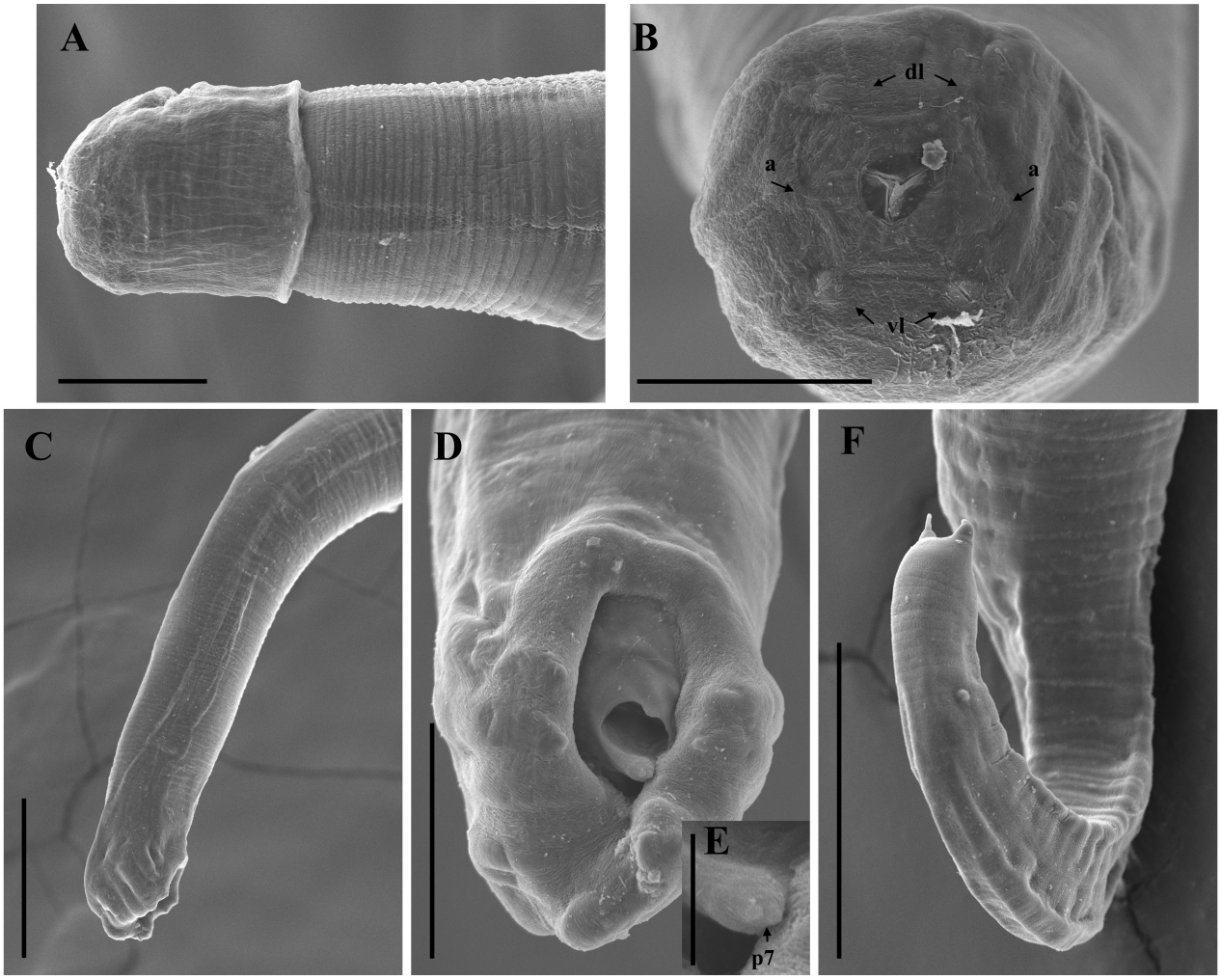

FIGURE 5. Scanning electron micrographs of *Bidigiticauda vivipara*. A) Female anterior extremity, lateral view. B) Female head, apical view. C) Male posterior extremity, lateral view. D) Male caudal bursa, ventral view. E) Magnified image of the dorsal lip of the genital cone. F) Female posterior extremity, dorsal view. Abbreviations: dl, dorsolateral papillae; vl, ventrolateral papillae; a, amphids; p7, papillae 7. Scale bars: A, F, 50 = μm; B, D = 40 μm; C = 100 μm; E = 8 μm

Vulva situated at 3093 (2500–3750) from caudal extremity (Figure 4I). Ratio distance vulva-posterior extremity/ body length 48.9% (39.3–55.6%). Anus 245 (180–300) from caudal extremity (Figure 4G). Tail elongated, sub-cylindrical with two small terminal digitiform processes (Figures 4G, 5F).

Third-stage larvae: Body length 538 (510–608) and width 35 (35–37). Esophagus 176 (165–200) long. Anterior end showing rod-like structure, 11 (10–12) long. Nerve ring and excretory pore situated at 76 (70–80) and 91 (70–110) from apex, respectively (Figure 4J). Genital primordium and anus located 185 (138–512205) and 48 (45–50) from posterior extremity, respectively (Figure 4J).

Remarks: The bursal pattern and dorsal lobe of the male specimens from Nayarit agrees with the brief description of *B. vivipara* provided by Chitwood (1938) and the redescription by Caballero-Deloya (1971). In Mexico, *B. vivipara* has been reported from *A. jamaicensis* in Yucatan (Chitwood 1938), *A. lituratus* in Guerrero (Caballero-Deloya 1971), and *A. jamaicensis* and *A. lituratus* in Chiapas (Ubelaker *et al.* 1977). However, the present specimens were smaller than those reported by Chitwood, (1938) and Caballero-Deloya (1971) (male body length 3480–4250 vs 5120 and vs 10384–10554; female body length 5480–7670 vs 8020–8060 and vs 11932–11967). Similarly, the spicule length of our specimens was smaller than those described by Chitwood and Caballero-Deloya (160–200 vs 256–270 and vs 235–252). Nevertheless, the ratio spicule length/body length was similar between the Nayarit and Yucatan specimens (4–5.3 vs 5). In contrast, this ratio was longer compared to Caballero-Deloya's material (4–5.3 vs 2.2–2.4). The difference in size among these *B. vivipara* isolates may be attributed to their occurrence in different

host species (*A. jamaicensis*, *A. lituratus*, and *G. mutica*), as well as the fact that each morphological description is based on nematodes isolated from a single individual host. Furthermore, Chitwood's original description was based on one male and two females, whereas Caballero-Deloya redescribed the species without specifying the number of specimens measured, although he collected 49 specimens (Caballero-Deloya 1971). Based on these data, it is difficult to determine at which point along this morphological and morphometrical continuum a *B. vivipara* isolate may be considered a different species. Further studies incorporating morphological and molecular data from several host species and geographical locations are necessary to investigate whether *B. vivipara* is a species complex.

The genus *Bidigiticauda* contains two other species: *Bidigiticauda embryophilum* (Freitas & Dobbin) described from *Glossophaga soricina* (Pallas) in Brazil, and *Bidigiticauda serrafreirei* de Oliveira Simões, Fraga-Neto, Vilar Maldonado Júnior & Vilela described from *Artibeus planirostris* (Spix), in Brazil. *Bidigiticauda embryophilum* differs from *B. vivipara* by having rays 5 and 6 arising independently, the branches of the dorsal ray not reaching the edge of the caudal bursa, and deirids situated posterior to the esophagus-intestinal junction (Vicente *et al*.,1997). *Bidigiticauda serrafreirei* can be distinguished from *B. vivipara* by the bifurcation of the dorsal ray in the middle of the trunk and the distance of the excretory pore from the anterior end that is longer (de Oliveira Simões *et al*., 2019).

The new record of *B. vivipara* from *G. mutica* in Nayarit expands the host and geographical range of this nematode in the country.

Phylogenetic analysis

In total, we generated new sequences for the 28S rRNA gene of *B. vivipara*, *V. macroti*, *Vampirolepis* sp. (1), and *Vampirolepis* sp. (2). These sequences were used for calculating genetic distances and conducting phylogenetic analysis. Unfortunately, DNA samples of Capillariidae gen. sp. and *A.* cf. *hastati* were not successfully amplified. Details of each dataset used to construct ML phylogenetic trees are given in Table S1.

The data alignment of nematodes included 29 28S rRNA sequences of the subfamily Trichostrongylina (Figure 6). Our sequences of *B. vivipara* were grouped with other anoplostrongyline nematodes, such as *Tricholeiperia* cf. *proencai* Travassos and *Anoplostrongylus* sp., isolated from *Noctilio leporinus* (Linnaeus) and *Nyctinomops laticaudatus* (Geoffroy Saint-Hilaire) in Mexico (bootstrap = 100). Our sequences of *B. vivipara* had genetic differences 0.3% and show a high genetic difference of 16.6–17.3% compared to the sequences of other anoplostrongyline nematodes found in bats (Table S2).

The data set of cestodes included 36 28S rRNA sequences of the family Hymenolepididae (Figure 7). Our 28S rRNA sequences of *Vampirolepis* were grouped with other *Vampirolepis* species from Finland, China, and Mexico, with high support values (bootstrap = 94). Our sequences of *Vampirolepis* had genetic difference values ranging from 1.3 to 3.4% compared to each other. Similar differences (1.7–5.6%) were found when comparing them with other *Vampirolepis* sequences (Table S3).

Discussion

In this study, seven helminth taxa were identified in bats: *A.* cf. *hastati*, *V. macroti*, and Capillariidae gen. sp., from *B. dubiaquercus* in Veracruz, *U. scabridum*, *Vampirolepis* sp. (1), and *Vampirolepis* sp. (2) from *R. parvula*, and *B. vivipara* from *G. mutica* in Nayarit. This study provides the first record of *A.* cf. *hastati* in Mexico, as well as the first helminthological records for the bats *B. dubiaquercus* and *R. parvula* in the country. The number of helminth taxa recorded in *G. mutica* increases from eight to nine. Previous records in this bat species include *A. speciosa*, *Litomosoides guiterasi* (Pérez Vigueras), *V. elongatus*, *Physocephalus sexalatus* (Molin) in Morelos (Peralta 2012), *Litomosoides hamletti* (Sandground) (Chitwood 1938), *A. auritum* (Stunkard 1938), *Vampirolepis* sp. in Yucatan (Moguel-Chin *et al.* 2023), and *Linustrongylus pteronoti* (Vaucher & Durette-Desset) in Jalisco (García-Vargas *et al.* 1996).

Life cycles of most gastrointestinal helminths of bats have not been described (Hilton & Best 2000). A major group of nematodes infect the host directly without the intervention of an intermediate host. Viviparity in *Bidigiticauda* females is likely an adaptation to the social grooming of bats, enhancing the probability of helminth transmission,

given that the infective third-stage larvae would not need to reach the ground to infect new hosts (de Oliveira Simões *et al.* 2019). Eggs of Capillariinae species are expelled in the excretory products of the host, depending on the parasite' location (i.e., feces in species parasitizing the viscera) and are either autoinfective or require an earthworm host (Anderson 2000). Social grooming in bats in a communal roost-site may facilitate transmission of infective capillarid eggs (McAllister *et al.* 2005). In contrast, cestodes and trematodes require the participation of intermediate hosts in their life cycles. Although the life-histories of urotrematids and anenterotrematids are unknown, the trophic ecology of their known definitive hosts, insectivorous and generalist bats, indicates that insects are likely the second intermediate hosts (Bray *et al.* 2008). On the other hand, the genus *Vampirolepis*, belonging to the family Hymenolepididae, is characterized by its use of arthropods as intermediate hosts (Busch *et al.* 2001).

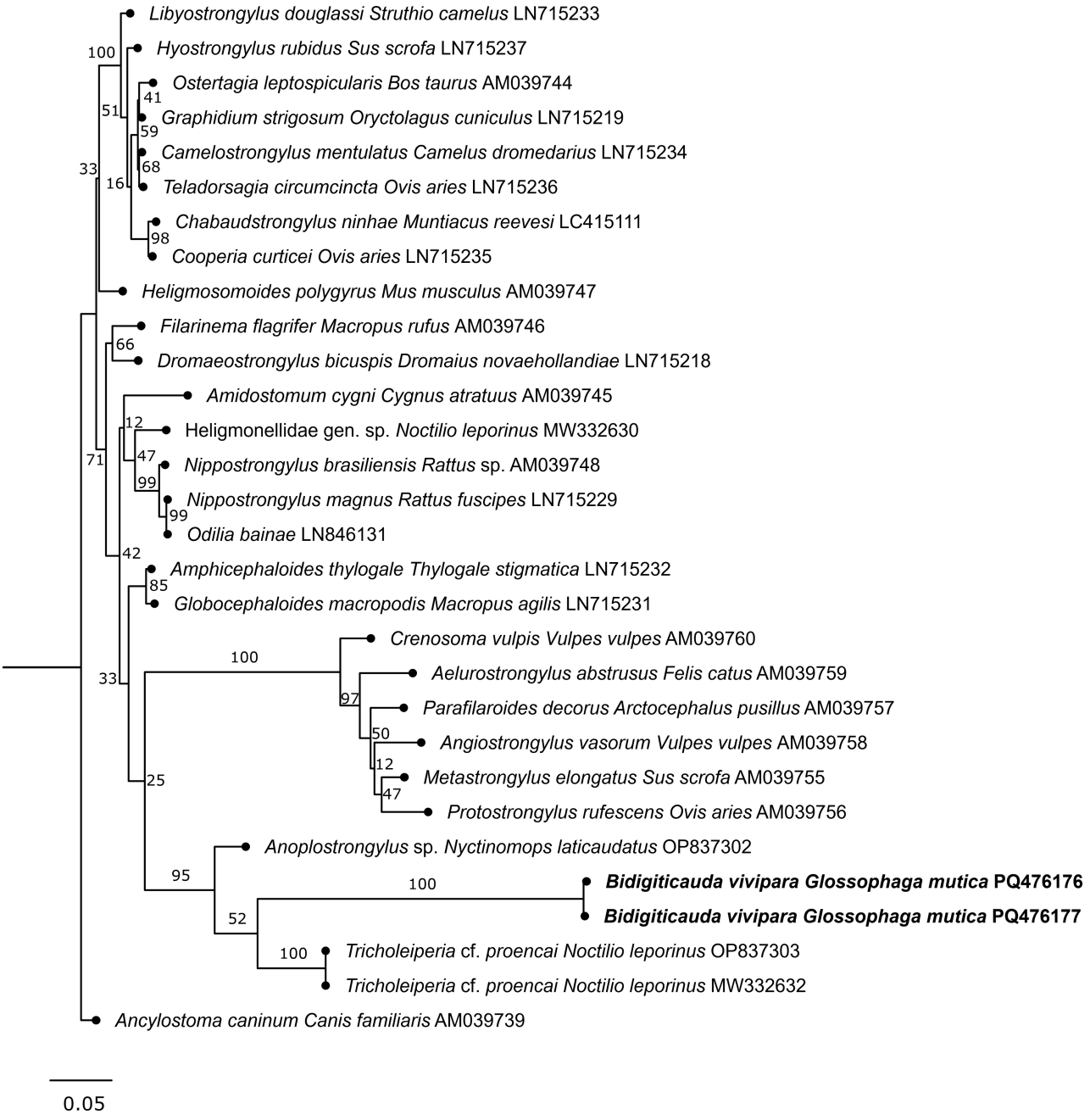

FIGURE 6. Phylogenetic tree based on the maximum likelihood analysis constructed on partial 28S ribosomal RNA gene of the subfamily Trichostrongylina. GenBank accession number precedes nematode species, followed by the host species. Nodal numbers indicate bootstrap support values. The scale indicates the number of mutations per unit of time.

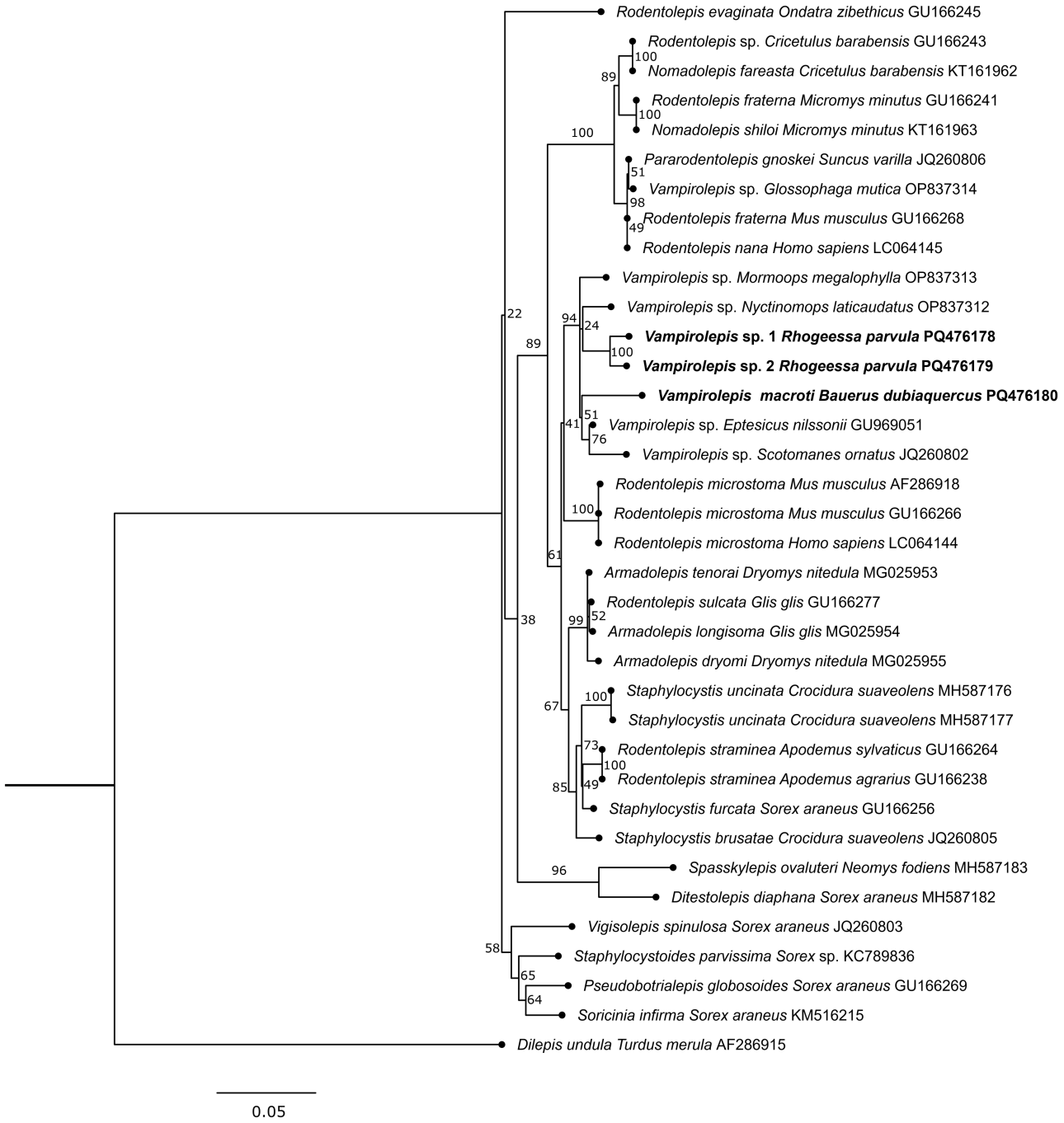

FIGURE 7. Phylogenetic tree based on the maximum likelihood analysis constructed on partial 28S ribosomal RNA gene of the family Hymenolepididae. GenBank accession number precedes nematode species, followed by the host species. Nodal numbers indicate bootstrap support values. The scale indicates the number of mutations per unit of time.

Bidigiticauda vivipara was described by Chitwood in 1938 and redescribed by Caballero Deloya in 1971. Subsequently, Ubelaker *et al*. (1977) provided SEM micrographs of the female *B*. *vivipara*. Since the study of Caballero Deloya (1971), which included only morphometric data, no additional complementary morphological and morphometric data had been obtained before this study. We provided a comprehensive morphological and morphometric data, including the description of the L3 larva, SEM micrographs of the male and partial sequences of the 28S rRNA gene.

The number of studies combining traditional morphological descriptions and modern genetic data to describe the helminth fauna of wild mammals have been increased in last decades (Pérez-Ponce de León & Poulin 2018; Poulin *et al.* 2019). Although the use of molecular genetic markers has become part of the accepted best practice for

parasite identification, the availability of parasite DNA sequences is not uniform across taxa, regions (Poulin *et al.* 2019), and most hosts. In most cases, helminths of bats from Mexico have been identified only with morphological methods (Moguel-Chin *et al.* 2023). Few helminthological surveys on bats have generated DNA sequences from helminths (Jiménez *et al.* 2014; Caspeta-Mandujano *et al.* 2015; Rendón-Franco *et al.* 2019; Panti-May *et al.* 2021; Moguel-Chin *et al.* 2023, 2024). The present study provided 28S rRNA sequences of *B. vivipara*, *V. macroti*, *Vampirolepis* sp. (1), and *Vampirolepis* sp. (2). Considering that the *Vampirolepis* specimens were immature or incomplete, the phylogenetic analysis was useful as it indicated that *Vampirolepis* sp. (1) and *Vampirolepis* sp. (2) are different species.

We analyzed helminths from bats in two understudied regions of Mexico through a collaborative network of mammalogists and parasitologists to collect and use bat tissues using an integrated approach for sampling bats. Although we examined few bat specimens, the results increase the number of helminth taxa recorded Mexican bats from 78 to 79. At the state level, the number of helminth taxa increases from 8 to 11 in Veracruz and from 1 to 5 in Nayarit. In addition, the number of bat species with helminthological records increases from 35 to 37, which corresponds to 25.7% of Mexican bat species.

Studies on the helminth fauna of wildlife are important to describe the full scope of life on Earth and to provide baseline datasets for insights into ecosystem change (Bennett *et al.* 2021). In this study, we provided morphological descriptions of each helminth species found in three bat species from poorly studied areas of Mexico. Additionally, we generated 28S rRNA gene sequences of four helminth taxa, which will provide useful information for comparison between congeners in further phylogenetic studies. Based on previous helminthological surveys (Jiménez *et al.* 2017; Salinas-Ramos *et al.* 2017; Luviano-Hernández *et al.* 2018; Martínez-Salazar *et al.* 2020; Moguel-Chin *et al.* 2023, 2024), is evident that the inventory of helminths of Mexican bats is incomplete. Much more effort will be required to describe and record the entire helminth fauna of bats in this country.

Acknowledgements

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper:

TABLE S1. Description of each data set used for the phylogenetic analyses.

TABLE S2. Percentage of uncorrected “p” distance matrix among anoplostrongyline nematodes in bats based on 28S rRNA sequences.

TABLE S3. Percentage of uncorrected “p” distances matrix among *Vampirolepis* species.