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First record of *Urotrema scabridum* (Platyhelminthes), and new records of helminths of *Tadarida brasiliensis* from Mexican Plateau

Elizabeth A. Martínez-Salazar[1]*, Andrea J. Medina-Rodríguez[1], Rogelio Rosas-Valdez[1], Melina Del Real-Monroy[2], and Jorge Falcón-Ordaz[3]

[1] Laboratorio de Colecciones Biológicas y Sistemática Molecular, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas. Av. Preparatoria S/N, Campus Universitario II, Col. Agronómica, C. P. 98066. Zacatecas, México. Email: eamsuaz@gmail.com; eamskandii@gmail.com (EAM-S), lunita325@hotmail.com (AJM-R), rogrosas@gmail.com (RR-V).

[2] Laboratorio de Genómica Evolutiva, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas. Av. Preparatoria S/N, Campus Universitario II, Col. Agronómica, C. P. 98066. Zacatecas, México. Email: melir17@hotmail.com (MDR-M).

[3] Universidad Autónoma del Estado de Hidalgo, Centro de Investigaciones Biológicas. Apartado Postal 1-10, C. P. 42001, Pachuca de Soto. Hidalgo, México. Email: jfalcon.ordaz@gmail.com (JF-O).

*Corresponding author

The Mexican free-tailed bat (*Tadarida brasiliensis*) is an abundant, widely distributed species in Mexico, except for most of the Yucatán peninsula. We studied the helminth fauna of *T. b. mexicana* at seven localities in the State of Zacatecas in order to advance the knowledge of helminth parasites of wild vertebrates in northern-central Mexico. Eighty-four bat specimens were examined for the presence of helminth parasites following standard procedures; helminths found were identified and infections were characterized. Of the specimens examined, 65.47 % were parasitized. The helminth fauna comprises five taxa: three digeneans (*Dicrocoelium rileyi*, *Ochoterenatrema labda*, and *Urotrema scabridum*); one cestode (*Vampirolepis* sp.); and one nematode (*Tadaridanema delicatus*). We present a brief morphological description of *Urotrema scabridum*. *D. rileyi* was the most prevalent and abundant helminth species. The intestine was the habitat most parasitized, with four species. Indirect life cycles predominate, and are related to the insectivorous habits of this host. Further studies on this host-parasite system are necessary to contribute to population monitoring and conservation; biogeographic patterns of helminth parasites of bats should also be studied to explore their origins and evolution in the region. *U. scabridum* is reported for the first time from Zacatecas. All species are new locality records.

El murciélago de cola libre mexicano (*Tadarida brasiliensis*), es una especie abundante y ampliamente distribuida en México, excepto en la mayor parte de la península de Yucatán. Para contribuir al conocimiento de los helmintos parásitos de vertebrados de vida silvestre en el centro-norte de México, la fauna helmíntica de *T. b. mexicana* se estudió en siete localidades del estado de Zacatecas. Se analizaron ochenta y cuatro especímenes de murciélagos de siete localidades dentro de Zacatecas. Los huéspedes fueron examinados para detectar helmintos parásitos siguiendo los procedimientos estándar de revisión, identificación taxonómica y se carcaterizaron las infecciones. El 65.47 % de los huéspedes examinados fueron parasitados. La fauna de helmintos consistió en cinco taxones: tres digéneos (*Dicrocoelium rileyi*, *Ochoterenatrema labda,* y *Urotrema scabridum*); un céstodo (*Vampirolepis* sp.) y un nemátodo (*Tadaridanema delicatus*). Se presenta una breve descripción morfológica de *Urotrema scabridum. D. rileyi* es la especie de helminto más prevalente y abundante. El intestino es el hábitat más parasitado con cuatro especies. El ciclo de vida indirecto es predominante y está relacionado con los hábitos alimentarios insectívoros de este huésped. Es necesario aumentar el número de estudios sobre este sistema huésped-parásito para contribuir al monitoreo de las poblaciones y la conservación, así como realizar estudios sobre patrones biogeográficos de helmintos parásitos de murciélagos que permitan explorar su origen y evolución en la región. *U. scabridum* se reporta por primera vez de Zacatecas, México. Todas las especies son registros nuevos de localidad.

Key words: Cestoda; Helminth parasites; Mexican free-tailed bat; Mexican Plateau; Nematoda; Trematoda.

Introduction

Chiroptera is the second most diverse and abundant order of mammals, it is widely distributed worldwide and is most abundant in tropical regions (Hutson *et al.* 2001). The Mexican free-tailed bat, *Tadarida brasiliensis* (Molossidae), is abundant in the Western Hemisphere, from the United States of America and southward into Central and South America (Russell *et al.* 2005). Its distribution range encompasses most of Mexico, except for most of the Yucatan peninsula (Wilkins 1989; Schmidly 1991; Álvarez-Castañeda *et al.* 2015). This bat species comprises nine valid subspecies (Simmons 2005), one of them is *T. b. mexicana*. This insectivorous species may occupy different habitats, including urban zones, deserts, and pine-oak forests, where it can often be found in caves, under bridges, abandoned mines, tunnels, tree holes and wall cracks (Tuttle 1994).

Although the Mexican free-tailed bat is a widely distributed species, its helminth fauna has been seldom studied in Mexico (reported in all cases as *T. brasiliensis*); studies have been carried out in Mexico City and the states of Mexico, Durango, Morelos, Nuevo Leon, Puebla, and Zacatecas (Tuttle 1994, 1942, 1943; Caballero y Caballero and Caballero-Rodríguez 1969; Guzmán-Cornejo *et al.* 2003; Falcón-Ordaz *et al.* 2006; García-Prieto *et al.* 2012; Caspeta-Mandujano *et al.* 2017; Jiménez *et al.* 2017; Falcón-Ordaz *et al.* 2019; Table 1). Sixteen helminth taxa have been recorded parasitizing this bat species in Mexico (Table 1): nine trematodes, two cestodes, four nematodes, and two nematodes. To the best of our knowledge, only four helminth species had been previously recorded in Zacatecas: three platyhelminth species [two digeneans (*D. rileyi* and *O. labda*) and one cestode (*Vampirolepsis* sp.), and one nematode species (*T. delicatus*).

As part of a research program aimed to compile an inventory of invertebrate parasites of wildlife in Northern-Central Mexico, with emphasis in the Mexican Plateau, the helminth fauna of *T. brasiliensis mexicana* was studied. The trematode species *Urotrema scabridum* was recorded for the first time. The aim of this paper is to report on the presence of this trematode in Mexico and expand the known distribution range of the helminth parasite fauna of *T. b. mexicana*.

Material and Methods

Eighty-four bat specimens were collected from seven localities in the State of Zacatecas between November 2011 and September 2013 (Table 2, Figure 1). The specimens were euthanized with an intraperitoneal overdose of sodic pentobarbital, following the guidelines of the American Society of Mammologists (Sikes *et al.* 2016; scientific collecting license FAUT-268 to EAMS), and examined for the presence of helminth parasites. The body cavity was exposed by means of

Table 1. Summary of helminth fauna of *Tadarida brasiliensis* in Mexico. G = gall bladder and hepatic conduits; I = Intestine; S = Stomach.

Helminths	Locality/State	Reference
	Platyhelminthes	
	Trematoda	
	Dicrocoeliidae	
Dicrocoelium sp.[G]	Cueva San Bartolo (Santa Catarina), Nuevo León.	García-Prieto *et al*. 2002
Dicrocoelium rileyi [G]	Campus Chamilpa (UAEM), Morelos; Concepción del Oro, Zacatecas; Cueva de La Boca (Santiago), Nuevo León; Cueva del Guano (Santa Catarina), Nuevo León; Cueva San Bartolo (Santa Catarina), Nuevo León; Nombre de Dios, Durango; Río Salado (Zapotitlán), Puebla; Tequixquiac, State of Mexico.	Caballero y Caballero, and Caballero-Rodríguez 1969; García-Prieto *et al*. 2002; Guzmán-Cornejo *et al*. 2003; Caspeta-Mandujano *et al.* 2017; Falcón-Órdaz *et al.* 2019
Platynosomum beltrani [I]	Monterrey, Nuevo León.	Caballero y Caballero and Caballero-Rodríguez 1969
	Lecithodendriidae	
Limatulum limatulum [I]	Campus Chamilpa (UAEM), Morelos; Cuicatlán, Morelos.	Caballero y Caballero and Bravo 1950; Caspeta-Mandujano *et al.* 2017
Limatulum oklahomense [I]	Zoológico de Chapultepec, México City.	García-Prieto et al. 2012
Ochoterenatrema labda [I]	Bosque de Chapultepec, México City; Concepción del Oro, Zacatecas; Cueva de La Boca (Santiago), Nuevo León; Cueva San Bartolo (Santa Catarina), Nuevo León; Nombre de Dios, Durango; Río Salado (Zapotitlán), Puebla; State of México; Campus Chamilpa Campus Chamilpa (UAEM), Morelos.	Caballero y Caballero 1940; Caballero y Caballero 1943; García-Prieto *et al*. 2002; Guzmán-Cornejo *et al*. 2003; Caspeta-Mandujano *et al.* 2017
Paralecithodendrium naviculum [I]	Azcapotzalco, México City.	Caballero y Caballero, 1940
	Plagiorchiidae	
Plagiorchis vespertilionis [I]	Azcapotzalco, México City; Bosque de Chapultepec, México City; Locality unknown, Morelos.	Caballero y Caballero 1940; Caballero y Caballero 1943; Caspeta-Mandujano *et al.* 2017
	Pleurogenidae	
Urotrema scabridum [I]	Acolman, State of México; Cueva de La Boca (Santiago), Nuevo León; Cueva San Bartolo (Santa Catarina), Nuevo León; Nombre de Dios, Durango; Río Salado (Zapotitlán), Puebla; Locality unknown, Morelos.	Caballero y Caballero 1942; García-Prieto *et al.* 2002; Guzmán-Cornejo *et al*. 2003; Caspeta-Mandujano *et al.* 2017
	Cestoda	
Vampirolepis decipiens [I]	Campus Chamilpa (UAEM), Morelos.	Jiménez *et al*. 2017; Caspeta-Mandujano *et al.* 2017
Vampirolepsis sp.[I]	Concepción del Oro, Zacatecas; Cueva de La Boca (Santiago), Nuevo León; Río Salado (Zapotitlán), Puebla.	Guzmán-Cornejo *et al.* 2003
	Nematoda	
	Acuaridae	
Acuaridae gen. sp.[S]	Campus Chamilpa (UAEM), Morelos.	Jiménez *et al.* 2017; Caspeta-Mandujano *et al.* 2017
	Capillaridae	
Aonchotheca speciosa [S]	Campus Chamilpa (UAEM), Morelos.	Jiménez *et al*. 2017; Caspeta-Mandujano *et al.* 2017
Capillaria palmata [S]	Campus Chamilpa (UAEM), Morelos.	Jiménez *et al.* 2017; Caspeta-Mandujano *et al.* 2017
	Ornithostrongylidae	
Allintoshius tadaridae [I]	Alcoman, State of México; Bosque de Chapultepec, México City; Cueva El Salitre, Morelos.	Caballero y Caballero 1942; Caspeta-Mandujano *et al.* 2017
	Molineidae	
Tadaridanema delicatus [I]	Campus Chamilpa (UAEM), Morelos; Concepción del Oro, Zacatecas; Cueva de La Boca (Santiago), Nuevo León; Nombre de Dios, Durango; Río Salado (Zapotitlán), Puebla	Guzmán-Cornejo *et al.* 2003; Falcón-Ordaz *et al.* 2006; data in Jiménez *et al.* 2017; Caspeta-Mandujano *et al.* 2017
Nematoda gen. sp.[I]	Campus Chamilpa (UAEM), Morelos.	Jiménez *et al.* 2017; Caspeta-Mandujano *et al.* 2017

Table 2. Localities surveyed in Zacatecas, Mexico for helminth parasites. Locality codes are used in Figure 1 and Table 3.

Code	Locality, municipality	Geographic coordinates (elevation)	Sample size (number of host infected)	Collection date (month/ year)
1	Tepechitlán, Tepechitlán	21° 40' 13.4" N; -103° 19' 42" W (1,788 masl)	*n* = 18 (8)	5/2012 10/2012
2	Guadalupe, Zacatecas	22° 44' 38.1" N; -102° 30' 53.6" W (2,479 masl)	*n* = 35 (27)	11/2011 05/2012 06/2012
3	Zacatecas, Zacatecas	22°46'28.63" N; -102° 32' 39.66" W 2,379 masl)	*n* = 1 (1)	06/2013
4	Sombrerete, Sombrerete	23° 38' 4.47" N; -103° 38' 29.51" W (2,307 masl)	*n* = 7 (5)	08/2012
5	San José de la Parada, Sombrerete	23° 31' 52.7" N; -103° 36' 16.6" W (2,163 masl)	*n* = 1 (0)	08/2013
6	Susticacán Dam, Susticacán	22° 36' 38.42" N; -103° 8' 40.01" W (2,081 masl)	*n* = 1 (0)	09/2013
7	San Felipe Nuevo Mercurio, Mazapil	24° 13' 35.08" N; -102° 9' 16.48" W (1,731 masl)	*n* = 21 (12)	04/2012

a longitudinal incision; all the organs were removed and examined separately under a dissecting stereoscope. Parasites found were first placed in a 0.85 % w/v saline solution, then fixed by sudden immersion in hot (steaming) 70 % ethanol and stored in 70 % ethanol to preserve morphological traits for identification. Nematodes were cleared for morphological study with Ammans's lactophenol and with a 2:8 ethanol–glycerin mixture. Cestodes were stained with Mayer's hydrochloric carmine, and whole-mounted in Canada balsam (Hycel de México, Jalisco). Techniques used are fully described elsewhere (Lamothe-Argumedo 1997).

Some of the specimens were photographed with a Leica ICC50HD camera fitted to a Leica DM750 microscope (Leica Microsystems, Wetzlar, Germany). For the new record specimens, morphometric characteristics were measured in micrometers (µm) unless otherwise stated (total body length and width in millimeters, mm). Minimum and maximum values are reported, along with the mean and standard deviation (SD) in parentheses.

Two specimens of the species newly recorded in Zacatecas and one of the dicrocoeliid species were further examined with scanning electron microscopy (SEM) and environmental scanning electron microscope (ESEM), respectively. The former were stored in 4 % formalin, dehydrated through a series of baths of gradually increasing ethanol concentrations, and critical-point dried. Specimens were coated with a gold–palladium mixture and examined under a Hitachi S-2460N (Hitachi, Tokyo, Japan) SEM at 15 kV. The second specimen was observed directly under an ESEM Quanta 250 FEG (Thermo Fisher Scientific Inc., Brno, Czech Republic) operating in low vacuum mode (p between 100 and 130 Pa).

Taxonomic identification was carried out by examining morphological traits and comparing them with taxonomic keys and descriptions in the specialized literature (Macy 1931;Macy 1931 Caballero y Caballero 1942, 1943; Travassos 1944; Bray *et al.* 1999; Anderson 2000; Lunaschi 2002; Falcón-Ordaz *et al.* 2006; Lunaschi and Notarnicola 2010). Helminth parasites were identified, counted and their sites of infection recorded. Infection parameters of prevalence (P), mean abundance (MA), mean intensity (MI) and intensity range (IR), were calculated according to Bush *et al.* (1997; Table 3).

Voucher specimens were deposited into the reference collection of the Colección de Invertebrados no Artrópodos (CINZ) and Colección de Vertebrados (CVZM), Laboratorio de Colecciones Biológicas y Sistemática Molecular, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas. Additional vouchers specimens were entered into the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico.

Results

Eighty-four bat specimens were collected from seven localities in the State of Zacatecas and examined for the presence

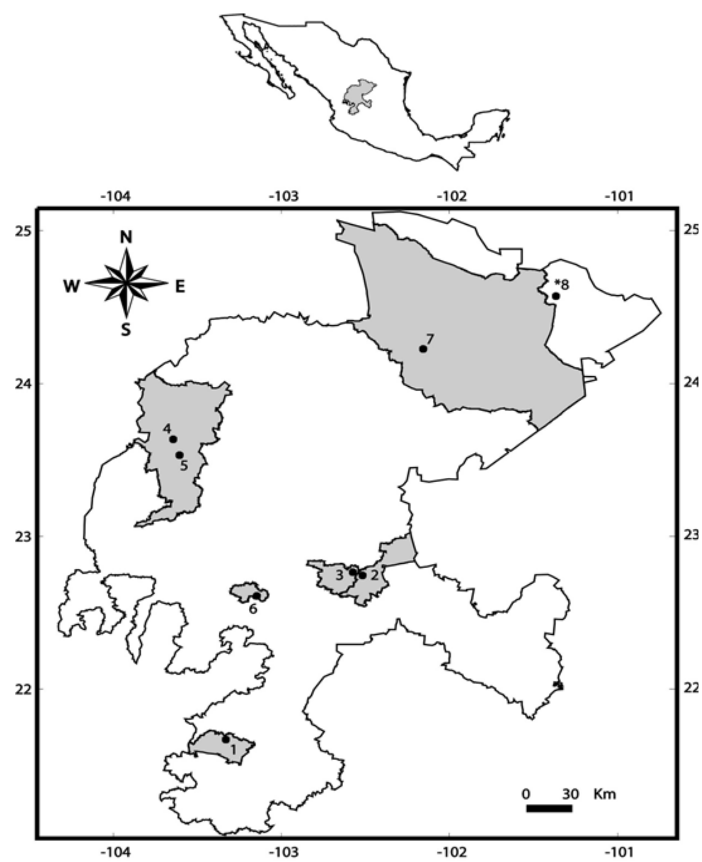

Figure 1. Sampling localities in Zacatecas, Mexico. Municipalities: 1= Tepechitlán; 2 and 3 = Zacatecas; 4 and 5 = Sombrerete; 6 = Susticacán; 7= Nuevo Mercurio. *8 = Concepción del Oro. Previous records are shown in Table I. Codes for localities are shown in Table 2.

of parasites; 65.47 % of the specimens were parasitized. The helminth fauna comprises five species in five different families (Figure 2, 3) the first group, Platyhelminthes, the trematodes *Dicrocoelium rileyi* (Dicrocoeliidae), *Ochoterenatrema labda* (Lecithodendriidae), and *Urotrema scabridum* (Pleurogenidae), and one cestode, *Vampirolepis* sp. (Hymenolepididae; the second group, Nematoda, *Tadaridanema delicatus* (Molineidae). Most of the helminth parasites had been previously recorded in Zacatecas, except for the trematode *Urotrema scabridum* (Table 1; Figure 2) which inhabits the gall bladder and hepatic conduits. A brief morphological description follows.

Class Trematoda

Orden Strigeidida

Family Pleurogenidae (Syn. Urotrematidae Ponche, 1926 according to Tkach *et al.* 2019).

Urotrema scabridum

Characterization (based on 14 specimens, 12 measured and 2 SEM, Figure 2 and Table 4): body elongated, posterior end truncated, yellow color *in vivo* and whitish post fixing, total body length (TBL) 2.012 - 3.580 mm (2.944 ± 0.498) by 0.339 - 0.497 mm (0.433 ± 0.049) wide (W). Tegument covered with small spines (a SEM microphotograph shows collapse of the tegument and spines shape). Oral sucker subterminal, 78 - 132 (109 ± 18) long (OSL) by 75 - 146 (119 ± 24) wide (OSW). Ratio of oral sucker width to ventral sucker width 1:1.21 - 1.49. Pre-pharynx short. Pharynx 33 - 58 (49 ± 8) long by 37 - 61 (51 ± 8) wide. Esophagus 176 - 397 (230 ± 60) long by 17 - 29 (23 ± 4) wide. Ventral sucker on the first third of the body, 107 - 178 (143 ± 21) long (VSL) by 112 - 178 (144 ± 19) wide (VSW). Intestine bifurcation preacetabular and narrow, extending to the posterior region of the body to the posterior testis. Ovary intercecal, anterior to testes, 115 - 210 (155 ± 29) long (OL) by 127 - 184 (153 ± 21) wide (OW). Testes tandem; anterior testis 181-334 (247 ± 41) long (ATL) by 160 - 390 (262 ± 60) wide (ATW), posterior testis 207 - 332 (265 ± 41) long (PTL) by 156 - 339 (253 ± 53) wide (PTW). Cirrus sac fusiform, located in the posterior end of the body, 164 - 291 (221 ± 40) long. Genital pore ventral. Vitelline follicles in two lateral fields wrapped around the ceca, both extracecal, several clusters of vitelline glands dorsally situated between ventral sucker and ovary, extending anteriorly to ventral sucker from the anterior testis. Uterus mainly intercecal, the uterine loops descending to posterior end of the body. Eggs 18 - 24 (24 ± 2, *n* = 60) long (EL) by 10 - 12 (11 ± 1, *n* = 60) wide (EW).

Table 3. Infection parameters of helminth parasites of the free-tailed bat, *Tadarida brasiliensis mexicana* in seven localities from Zacatecas, Mexico. L = Locality (codes as in Figure 1 and Table 2). Abbreviations: 1) Infection sites: I = Intestine, G = gall bladder and hepatic conduits. 2) Life cycle: CD = Direct, CI= Indirect. 3) Ecological parameters: *n* = Total number of parasites collected, HIP = Host infected by parasite, P (%) = Prevalence, MA = Mean abundance, MI = Mean intensity, IR = Intensity range. * = Accession numbers in the CINZ and CNHE collections.

Helminth species	L	n	P (%) (HIP)	MA	MI	IR	Accession Number*
Platyhelminthes:							
Trematoda							
Dicrocoeliidae							
Dicrocoelium riley[iG, CI]	1	60	44.44 (8)	3.33	7.50	1 - 23	CINZ158; CNHE 11103
	2	95	42.86 (15)	2.71	6.33	1 - 40	CINZ150; CINZ155; CNHE 11104
	4	85	57.14 (4)	12.14	21.25	2 - 48	CINZ09; CNHE 11105
	7	101	33.33 (7)	4.81	14.43	1 - 52	CINZ 10; CNHE 11106
Lecithodendriidae							
Ochoterenatrema labda[I, CI]	2	22	11.43 (4)	0.63	5.50	1 -15	CINZ151; CINZ154; CNHE 11107
	4	1	14.29 (1)	0.14	1	1	CINZ 11
	7	3	4.76 (1)	0.14	3	3	CINZ 12
Pleurogenidae							
Urotrema scabridum[I, CI]	2	40	11.43 (4)	1.14	10	4 - 27	CINZ156; CNHE 11108
Cestoda							
Hymenolepididae							
Vampirolepis sp.[I, CI]	2	6	14.29 (5)	0.17	1.20	1 - 2	CINZ148; CINZ153; CNHE 11109
	4	1	14.29 (1)	0.14	1	1	CINZ 13
Nematoda							
Molineidae							
Tadaridanema delicatus[I, CD]	1	2	11.11 (2)	0.11	1	2	CINZ159
	2	67	40.00 (27)	1.91	4.79	1 - 12	CINZ149; CINZ152; CINZ157 CNHE 11110
	3	3	100 (1)	3.00	3	3	CINZ 14; CNHE 11111
	4	9	28.57 (9)	1.29	4.50	3 - 6	CINZ 15; CNHE 11112
	7	18	33.33 (7)	0.86	2.57	1 - 6	CINZ 16; CNHE 11113

Taxonomic remarks: The genus *Urotrema* was first described from specimens collected in the intestine of the greater bulldog bat *Noctilio leporinus* (originally recorded as *Noctilio macropus*) and included in *Urotrema scabridum* as the type species. This species mainly parasitizes insectivorous bats and is widely distributed from North to South America and Africa (Zamparo *et al.* 2005). It has also been reported in polychrotid lizards (anoles) from Florida and Louisiana (Sellers and Graham 1987; Goldberg *et al.* 1994), the Caribbean and Central America (Goldberg *et al.* 1994, Goldberg *et al.* 1996; Goldberg *et al.* 1998).

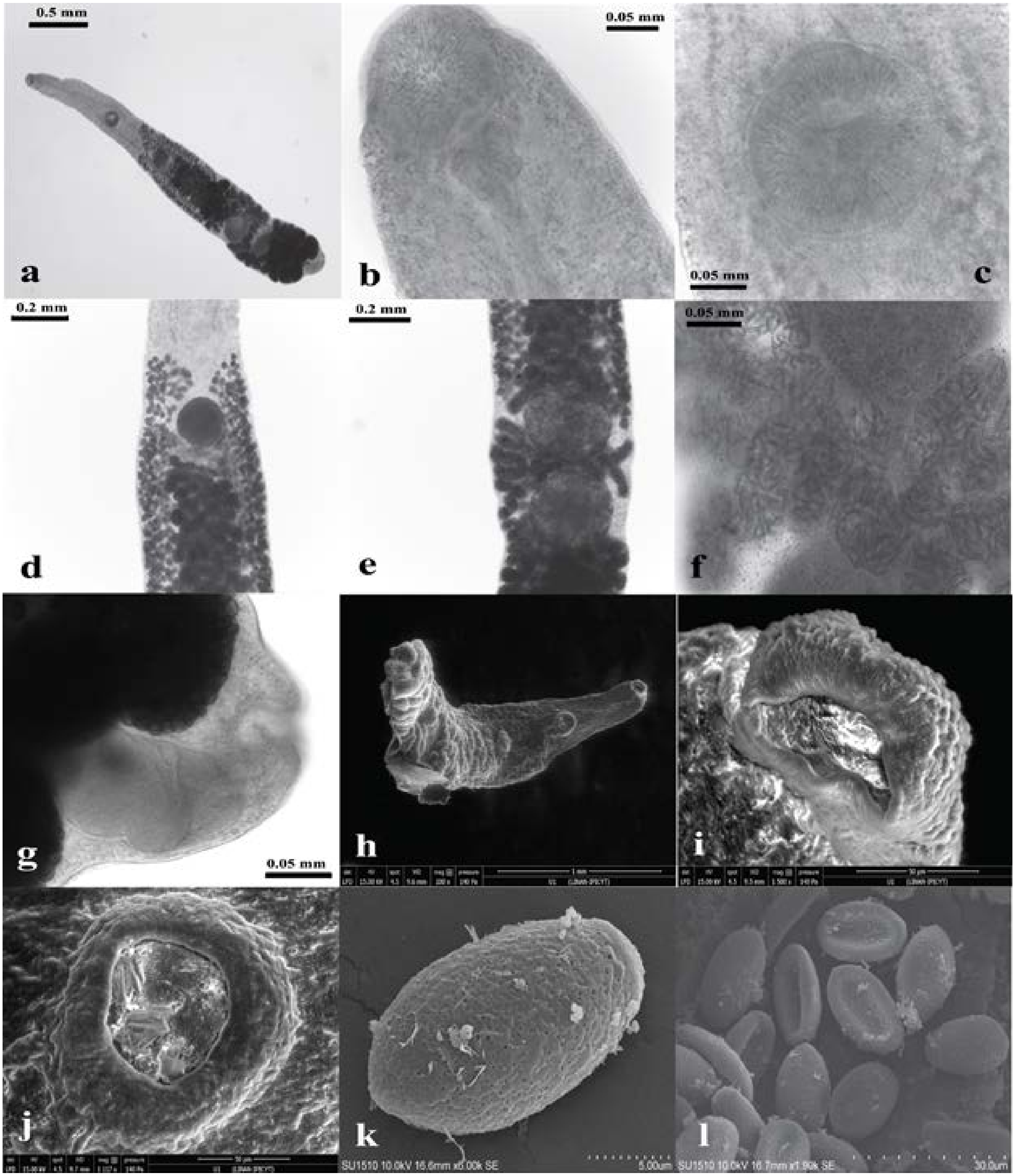

Figure 2. General morphology and ESEM-SEM photomicrographs of *Urotrema scabridum* from Zacatecas, Mexico. Optical microscopy: a) Whole body, ventral view; b) Oral sucker, ventral view; c) Ventral sucker, ventral view; d) Ovary, vitelline follicles arranged in two lateral fields and anterior end of the uterus, ventral view; e) Testis in tandem, ventral view; f) Uterine loops with eggs between testes, ventral view; g) Cirrus sac located in the posterior end of the body, ventral view. ESEM: h) Whole body, ventral view; i) Oral sucker, ventral view; j) Ventral sucker, ventral view. SEM: k and l) Eggs, ventral view. Scale bars are shown in each microphotograph.

Through the taxonomic history of the genus *Urotrema*, eight nominal species have been described parasitizing various mammal and lizard species (Zamparo *et al.* 2005): *U. scabridum*, *U. shillingeri*, *U. lasiurensis*, *U. minuta*, *U. wardi*, *U. aelleni* Baer, 1957, *U. macrotestis*, and *U. shirleyae*. However, the validity of some of the species has been questioned (Caballero y Caballero 1942; Caballero and Grocott 1960; Yamaguti 1971; Bray *et al.* 1999; Tkach *et al.* 2019). Caballero y Caballero (1942) considered *U. scabridum* and *U. wardi* as valid species, and synonymized *U. lasiurensis*, *U. minuta* and *U. shillingeri*. Later on, Caballero and Grocott (1960) synonymized *U. aelleni* with *U. scabridum*. Bray *et al.* (1999) suggested that *U. scabridum* is a species complex showing intraspecific variation (= *U. aelleni, U. lasiurensis*, *U. macrotestis,* and *U. shillingeri*). At least three species are currently considered valid: "*U. scabridum* complex", *U. minuta* and *U. shirleyae.* Recently, the phylogenetic position of the genus *Urotrema* within Digenea was clarified by Tkach *et al.* (2019) based on the partial sequence of 28S rDNA; they transferred the genus to the Pleurogenidae and showed that the clade of *U. scabridum* + *U minuta* + *U. shirleyae* is phylogenetically closest to *Parabascus* spp. Our specimens are smaller than those described by Caballero y Caballero (1942) but bigger than the specimens measured by Lunaschi and Notarnicola (2010). Morphological characteristics of the specimens analyzed are shown in Table 4.

Infection parameters: The prevalence, mean abundance, mean intensity, intensity range, site of infection, hosts and localities are shown in Table 3. No helminth parasites were found in the San José de la Parada (Sombrerete) and Susticacán Dam (Susticacán) localities. Platyhelminthes (Trematoda) were the species-richest group. Four of the five helminth taxa inhabit the intestine (*O. labda, U. scabridum, Vampirolepis* sp. and *T. delicatus*), with a general intensity from 1 to 101 helminths per infected free-tailed bat. The highest number of parasite species was recorded in the Guadalupe locality, with four helminth species, three trematodes (*D. rileyi, O. labda,* and *U. scabridum*), one cestode (*Vampirolepis* sp.) and one nematode (*T. delicatus*). Indirect life cycles are predominant in the helminth fauna found. *Dricocoelium rileyi* was the most prevalent helminth in four localities (Table 3): Guadalupe (42.86 %), Nuevo Mercurio (33.33 %), Sombrerete (57.14 %), and Tepechtitlán (44.44%). *Tadaridanema delicatus* was the only parasite found in the Zacatecas locality, with 100 % prevalence in three individuals examined. The species *D. rileyi* and *T. delicatus* from Nuevo Mercurio showed the same prevalence, mean intensity, and intensity range (Table 3).

Discussion

The helminth fauna of the order Chiroptera in Mexico, has been poorly studied compared to the diversity of Mexican bats (144 spp.; helminths: *e. g.* García-Prieto *et al.* 2012; Caspeta-Mandujano *et al.* 2017; Clarke-Crespo *et al.* 2017; Jiménez *et al.* 2017; Salinas-Ramos *et al.* 2017; Luviano-Hernández *et al.* 2018; Falcón-Ordaz *et al.* 2019; bats: Sánchez-Cordero *et al.* 2014). To the best of our knowledge, only 28 bats species in the families Emballonuridae (one species), Molossidae (two species), Mormoopidae (four species), Natalidae (one species), Phyllostomidae (15 species) and Vespertilionidae (five species) have been examined for helminths (see García-Prieto *et al.* 2012; Clarke-Crespo *et al.* 2017; Caspeta-Mandujano *et al.* 2017; Jiménez *et al.* 2017; Salinas-Ramos *et al.* 2017; Luviano-Hernández *et al.* 2018; Falcón-Ordaz *et al.* 2019), only 19.44 % of the bat species occurring in Mexico. Mexican insectivorous bats are one of the groups bearing the highest diversity of parasitic helminths. The species bearing the highest richness is the ghost-face bat, *Mormoops megalophylla*, with 23 helminth species; followed by the Davy´s naked-backed bat, *Pteronotus davyi*, with 15 helminth species; the Mexican greater funnel-eared bat, *Natalus stramineus,* with 14 species; and the Mexican free-tailed bat, *T. brasiliensis* with 17 species (Guzmán-Cornejo *et al.* 2003; Falcón-Ordaz *et al.* 2006; García-Prieto *et al.* 2012; Caspeta-Mandujano *et al.* 2017; Jiménez *et al.* 2017; Table 1).

Previous studies had reported four species parasitizing the Mexican free-tailed bat in Concepción del Oro, Zacatecas: Two digeneans (*D. rileyi* and *O. labda*), one cestode (*Vampirolepis* sp.) and one nematode (*T. delicatus*) (Guzmán-Cornejo *et al.* 2003; Falcón-Ordaz *et al.* 2006). The trematodes were recovered in their adult form; this is the group with the highest number of species parasitizing *T. brasiliensis mexicana* from Zacatecas, compared to cestodes and nematodes which together account for 40 % of the species diversity.

Table 4. Morphometric comparison of *Urotrema scabridum* with some previous records.

Characteristic[1]	Present work	Caballero y Caballero (1942)[2]	Caballero y Caballero and Grocott (1960)[3]	Lunaschi and Notarnicola (2010)[4]
Distribution	Mexico	Mexico	Panama	Argentina
TBL (mm)	2.01 - 3.58	4.53 - 5.29	2.08 - 2.30	1.70 - 2.50
OSL	78 - 132	102 - 143	91 - 137	133 - 142
OSW	75 - 146	123 - 163	108 - 125	123 - 142
VSL	107 - 178	175 - 245	121 - 125	128 - 144
VSW	112 - 1782	135 - 233	125 - 133	134 - 152
OL	115 - 210	208 - 237	166 - 175	154 - 218
OW	127 - 184	155 - 163	158 - 187	112 - 209
ATL	181 - 334	300 - 350	133 - 141	128 - 323
ATW	160 - 390	200	146 - 162	134 - 285
PTL	207 - 332	310 - 380	133 - 187	112 -351
PTW	156 - 339	200 - 220	154 - 183	122 - 323
EL	18 - 24	22 - 24	21	21 - 22
EW	10 - 12	12 - 14	12	10 - 13

[1] Acronyms as listed in the description of *U. scabridum* in the main text.
[2] Host: *Natalus stramineus* and *T. brasiliensis*.
[3] Host: *Phyllostomus hastatus panamensis* Allen 1904
[4] Hosts: *Myotis levis*; *Molossops temminckii*; *Eumops bonariensis*; and *T. brasiliensis.*

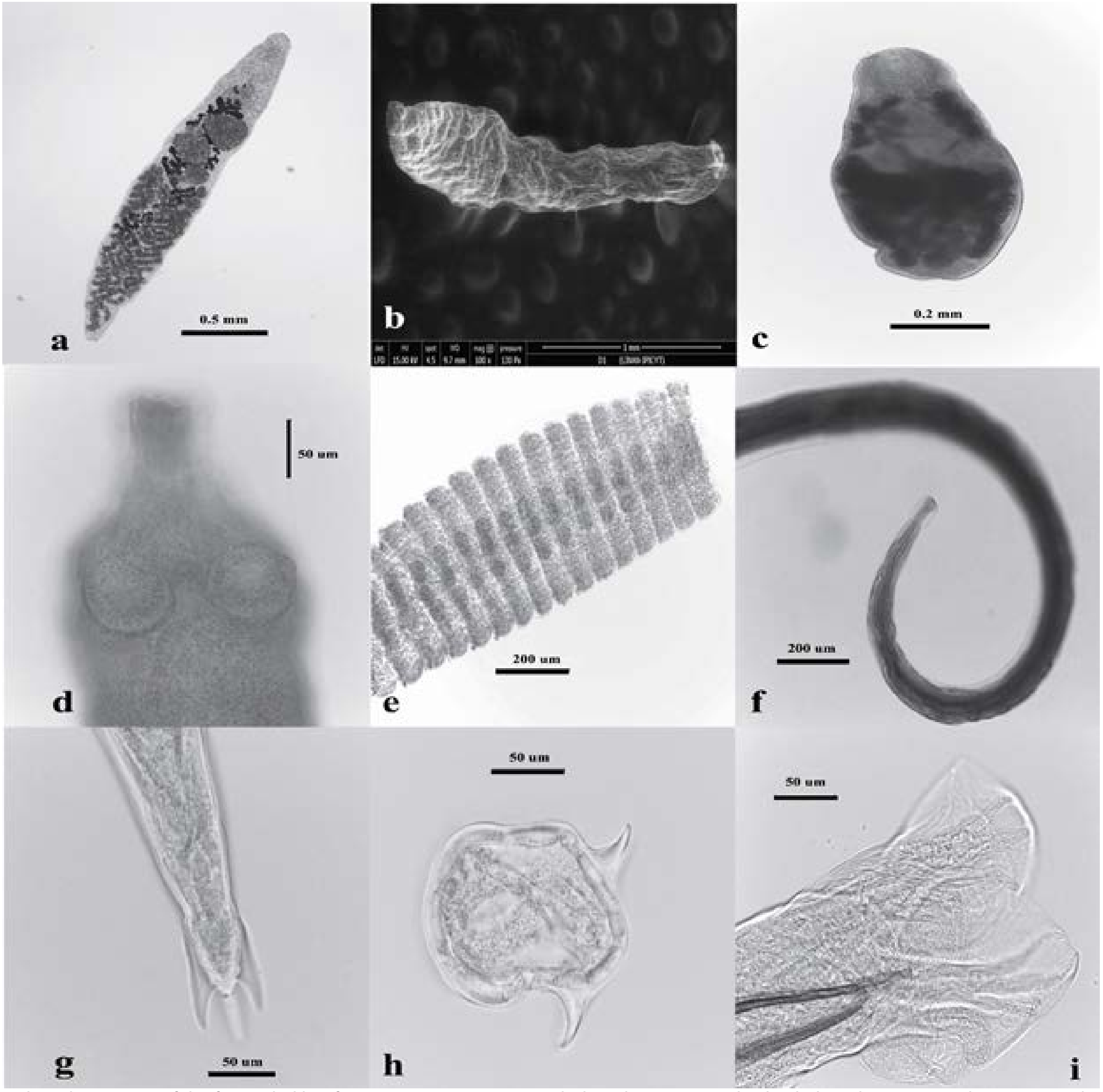

Figure 3. Helminth parasites of the free-tailed bat from Zacatecas, Mexico (excluding the new record). Platyhelminthes: Trematoda: a) *Dicrocoelium rileyi*, full body, ventral view; b) ESEM of *Dicrocoelium rileyi*, full body, ventral view; c) *Ochoterenatrema labda*, full body, ventral view. Cestoda: *Vampirolepis* sp.: d) Anterior end, ventral view; e) Immature proglottids, ventral view. Nematoda: *Tadaridanema delicatus:* f) Female anterior end, ventral view; g) Female posterior end, ventral view; h) Synlophe at midbody level, apical view; i) Male, posterior end, ventral view. Scale bars are shown in each microphotograph.

The trematode *D. rileyi* was the most important helminth in terms of infection parameters, showing the highest prevalence, mean abundance, mean intensity and intensity range in five localities: Tepechitlán (examined in October 2012, not found in May 2011; Medina-Rodríguez pers. obs.), Guadalupe (Zacatecas, October 2011, May and June 2012), Sombrerete (August 2012), San Felipe Nuevo Mercurio (Mazapil, April 2012; see Table 3; Martínez-Salazar, *Pers. Obs.*). In addition, Guzmán-Cornejo *et al.* (2003) also reported infections by this species in Concepción del Oro, Zacatecas (n = 3 of the 28 hosts examined in October 1997 and April 1988).

Although the life cycles of the majority of the helminth fauna of bats are still unknown, we can infer some information from their occurrence and their host species; most of the information available in the literature is for the supraspecific level (Yamaguti 1975; Schmidt 1986; Olsen 1986; Anderson 2000). The life cycle of *D. rileyi* is completely unknown; however, the life cycle of *Dicrocoelium dendriticum* has been described as infecting wild and domestic mammals (typically ruminants, not reported in bats) and, very occasionally, humans; its intermediate hosts are terrestrial snails (*i.e. Zebrina* sp., and *Cionella* sp.) and ants (*i.e. Formica* sp.). Pojmańska (2008) suggests that, in general, the Dicrocoeliid-type life cycle involves two intermediate hosts (a terrestrial snail and an arthropod): The xiphidiocercaria leaves the first intermediate host (snail) and becomes encysted in the second intermediate host (an arthropod), which is finally ingested by the definitive host. In Mexico, *D. rileyi* exclusively parasitizes *T. brasiliensis* as definitive host (Caballero y Caballero-Rodríguez 1969; Guzmán-Cornejo *et al.* 2003; García-Prieto *et al.* 2012; Jiménez *et al.* 2017; Falcón-Ordaz *et al.* 2019), but it has also been reported in *T. brasiliensis cyanocephala* and *Vespertilio humeralis* from the United

States (Macy 1931). There are published reports on the presence of *D. rileyi* in four species of terrestrial snails from Zacatecas (Naranjo-García 2014) including *Holospira* (*Holospira*) *temeroso* and *Humboldtiana* (*Humboldtiana*) *chrysogona* in Concepción del Oro; *Humboldtiana* (*Humboldtiana*) *bicincta* in Sierra de Mascarón, Mazapil; and *Humboldtiana* (*Humboldtiana*) *tescola* in San Tiburcio, Mazapil (Thompson 2011). The presence of *D. rileyi* in these five localities in Zacatecas suggests that its intermediate host on which the Mexican free-tailed bat feeds could be present at least from May to October. It is necessary to further examine the genera *Holospira* and *Humboldtiana* in Concepción del Oro; as well as *Humboldtiana* in Mazapil to confirm their role as intermediate hosts of this trematode species in Zacatecas. On the other hand, some 14 Formicidae species have been recorded in Zacatecas (Vásquez-Bolaños 2011) but their helminth fauna has not yet been examined as a possible intermediate host. Further studies on the diversity of terrestrial snails and arthropods (ants) in Guadalupe, Mazapil, Sombrerete and Tepechitlán would help to understand the life cycle of *D. rileyi* in Zacatecas, as well as those of other helminth parasites of wild vertebrates.

Ochoterenatrema labda is an intestinal parasite that has bats as the only known definitive host; it parasitizes bats from United States, Mexico, Panama, Chile and Argentina. Originally described from *T. brasiliensis* from Bosque de Chapultepec, Mexico City and *Natalus stramineus* from Tlalpan, Mexico City (Caballero y Caballero 1943; Caballero y Caballero 1964; Cain 1966; Guzmán-Cornejo *et al.* 2003; Lunaschi and Notarnicola 2010; García-Prieto *et al.* 2012; Jiménez *et al.* 2017), it is the only species that has been reported parasitizing several bat species in Mexico (*Balantiopteryx plicata, Mormoops megalophylla*, *Myotis velifer*, *Natalus mexicanus*, *Pteronotus davyi*, and *T. brasiliensis*; García-Prieto *et al.* 2012; Caspeta-Mandujano *et al.* 2017; Jiménez *et al.* 2017). Guzmán-Cornejo *et al.* (2003) reported this species of digenetic trematode as the most prevalent and abundant helminth in *T. brasiliensis* from Concepción del Oro, Zacatecas. By contrast, our data showed *D. rileyi* as the species with highest infection parameters (Table 3). The taxonomic status of this genus has been questioned; Yamaguti (1958) suggested that it is a synonym of *Prosthodendrium*. However, Cain (1966) and later Yamaguti (1971) considered the genus as valid. Lunashi (2002) provided a redescription of *O. labda* and considered both genera as valid, as *Ochoterenatrema* can be distinguished mainly by the presence of a pseudogonotyl to the left of the ventral sucker. A taxonomic revision of this genus was published by Tkach *et al.* (2003), but a molecular analysis of *Ochoterenatrema* and *Prosthodendrium* is necessary to clarify this issue. The life cycle of this intestinal trematode has not been elucidated and little is known of its intermediate hosts; however, the family Lecithodendriidae requires three hosts: a mollusk, an arthropod, and a vertebrate (Bray *et al.* 2008; Lord *et al.* 2012). In general, the xiphidiocercaria (the first developmental stage) occurs in prosobranch mollusks; larvae of aquatic insects are the intermediate hosts for metacercariae (encysted stage; Yamaguti 1971); chiropterans become infected when they consume infected adult insects, and the adult parasite develops (Lord *et al.* 2012).

In our study we found the trematode *U. scabridum*, which had not been previously recorded in Zacatecas, even though this species ranges from North America to South America (Font and Lotz 2008). To the best of our knowledge, only one species of the genus *Urotrema* has been reported parasitizing Mexican bats: *U. scabridum*. This is a parasite of the Mexican free-tailed bat, and has been recorded in several localities in Mexico, including Acolman, State of Mexico; Cueva de la Boca (Santiago) and Cueva San Bartolo (Santa Catarina), Nuevo León; Nombre de Dios, Durango; and Río Salado (Zapotitlán), Puebla (Caballero y Caballero 1942; García-Prieto *et al.* 2002; Guzmán-Cornejo *et al.* 2003), and an unspecified locality in the State of Morelos (Caspeta-Mandujano *et al.* 2017). It has also been found in *N. stramineus* from Tlalpan, Mexico City (Caballero y Caballero 1942). This is the first time this species has been recorded in the State of Zacatecas; the nearest locality where this species had been previously recorded is in the State of Nuevo León (Guzmán-Cornejo *et al.* 2003). A study on the morphological and molecular variation of this species throughout its distribution range would help to confirm whether or not this is a species complex, as its taxonomic history suggests (*e. g.*, Caballero y Caballero 1942; Caballero and Grocott 1960; Bray *et al.* 1999; Table 4). Life cycles and larval morphology of the Urotrematidae are still completely unknown. Bats acquire this urotrematid parasite by ingesting insects that act as intermediate hosts and harbor encysted metacercariae (Bray *et al.* 2008).

The taxonomic history of the genera *Vampirolepis* and *Rodentolepis* has been controversial. Both genera parasitize chiropterans and share several morphological characteristics, particularly in their body structure. However, the distinguishing difference between these two genera is the number of ovaries, two in *Rodentolepis* and one in *Vampirolepis* (Khalil *et al.* 1994), a characteristic that we confirmed in our study. We were not able to identify all of our specimens to the species level because some of them could not be mounted adequately for morphological study. Nevertheless, we were able to observe some diagnostic characteristics (*e. g.*, presence of an armed rosette endowed with 32 hooks; Medina-Rodríguez, *pers. obs.*) that allowed identification at the genus level as *Vampirolepis* sp. Distinctive features of this genus include between 18 and 50 hooks; linear disposition of the testicles, divided into two groups by the ovary; and transversely elongated proglottids. Only *V. decipiens* has been reported parasitizing *T. brasiliensis* in Mexico (García-Prieto *et al.* 2012); collecting more specimens of *Vampirolepis* would help to clarify the identity of the species present in Zacatecas. Hymenolepididae generally exhibit an indirect life cycle (insects are the intermediate host that is ingested by the vertebrate host) and parasitize insectivorous bats (Bray *et al.* 2008; Roberts and Janovy 2009).

Tadaridanema delicatus is widely distributed across North and South America (Falcón-Ordaz *et al.* 2006; Cheuiche *et al.* 2015); in the State of Zacatecas it is the species with the widest known geographic distribution, having been recorded in six localities (Table 2, Figure 1): Tepechitlán (Tepechitlán), Guadalupe (Zacatecas), Zacatecas (Zacatecas), Sombrerete (Sombrerete), and San Felipe Nuevo Mercurio (Mazapil); it was also previously reported from Concepción del Oro, Zacatecas by Guzmán-Cornejo *et al.* (2003) and Falcón-Ordaz *et al.* (2006). It occurs in simpatry with *D. rileyi* in four localities (Table 2, 3), with exception of Zacatecas (Zacatecas), where we examined only one bat and found three individuals of *T. delicatus*. Additionally, *T. delicatus* has been recorded parasitizing *T. brasiliensis mexicana* from the States of Durango, Nuevo León, and Puebla (Falcón-Ordaz *et al.* 2006), as well as *Molossus ater* from the State of Sinaloa (unspecified locality; Cain and Studier 1974).

The life cycle of *Tadaridanema* spp. is unknown. However, it has been suggested that members of the family Molineidae have a direct life cycle in which the females release numerous eggs into the intestine of the vertebrate definitive host. The eggs are afterwards released to the external medium through the feces, where the first-stage starts developing; later on, they develop into infective third-stage larvae (Roberts and Janovy 2009). It has also been suggested that Molineidae can utilize insects as paratenic hosts (Bush *et al.* 2001), which could increase the possibility of bats being parasitized by nematodes. However, this hypothesis must be tested in *T. delicatus*.

The parasites' infection mechanism can be related to the host's life style and habitat use (Kunz *et al.* 1995; McWilliams 2005). This bat species lives in colonies numbering up to millions of individuals (McCracken *et al.* 1994); this favours infection through their grooming habits and the accumulation of guano in humid environments that harbour a unique ecosystem, including parasites and hosts (Kerth 2008; Altringham 2011). That bats usually host only one parasite taxon is to be expected since some bats are dietary specialists. Parasites are obtained from what bats eat and, being dietary specialists, bats may be predisposed to become parasitized only by the helminth that uses its prey items as intermediate host. Although many species of insectivorous bats have a diversified diet including insects from different orders and families, some species selectively feed on only one type of prey and show foraging habitat specificity. Thus, mayfly and beetle specialists such as *Myotis lucifugus*, and moth specialists such as *Lasiurus borealis* and *T. brasiliensis* are less likely to have multiparasitism than species showing generalist feeding habits (Hilton and Best 2000). These bats roost in a variety of places, including caves, rock crevices, bridges, culverts, highway overpasses and other structures in urban areas (Scales and Wilkins 2007). Infection of insectivorous bats by indirect life cycle parasites occurs outside urban areas as insects are attracted by the city lights (Rydell 2006), and bats prey on them. On the other hand, when bats return to overnight in the colony, the high humidity and feces accumulation in the place, together with the bats' self-cleaning or cleaning between colony members habits, allow the bats to acquire direct life cycle parasites either by autoinfection, by contact between the colony members or by the site's humidity that favours the development of direct life cycle helminths (Bray *et al.* 2008; Bush *et al.* 2001; Roberts and Janovy 2009; Lord *et al.* 2012).

No easily discernable pattern could be found in the diversity of helminths recorded in this bat species in urban areas (localities as Tepechitán, Guadalupe, Zacatecas or Sombrerete) versus those recorded in natural environments (as San Felipe Nuevo Mercurio). Five helminth species were recorded in Guadalupe, four in Sombrerete and three in San Felipe Nuevo Mercurio, and most of them were Platyhelminthes (indirect life cycle). These results might be related to the asymmetric sampling effort (Table 2, 3).

Trematodes showed the highest species richness, and some six helminth species have been reported parasitizing *T. brasiliensis* in Mexico. This pattern had been previously observed in insectivorous bats in North America (Table 1): *D. rileyi*, *P. beltrani*, *O. labda*, *P. vespertilionis*, and *U. scabridum* (Guzmán-Cornejo *et al.* 2003; in García-Prieto *et al.* 2012; Jiménez *et al.* 2017). Two of these indirect life cycle species, *D. rileyi* and *O. labda,* and the nematode *T. delicatus* (considered as a direct life cycle parasite) have been reported as typical helminth fauna of *T. brasiliensis* in Zacatecas and occur in sympatry in the localities of Guadalupe, Sombrerete and San Felipe Nuevo Mercurio. These species had been previously reported in the same condition in Nuevo León and Puebla (Table 1; Guzmán-Cornejo *et al.* 2003).

Tadarida brasiliensis is an opportunistic, generalist, insectivorous bat species (Kunz *et al.* 1995). Food items forming its diet have been generally identified to the order level and, in some cases, to the family level. This bat mainly consumes insects in the orders Lepidoptera (moths), Coleoptera (beetles) and, occasionally, Diptera (true flies), Hemiptera (stinkbugs), Homoptera (leafhoppers), Hymenoptera (wasps), Neuroptera (lacewings) and Odonata (dragon flies, damselflies; Fabián *et al.* 1990; Kunz *et al.* 1995; Whitaker *et al.* 1996; McWilliams 2005; Armstrong 2008). In the United States and Brazil, Coleoptera and Lepidoptera are consumed with higher frequency and volume during the summertime (Fabián *et al.* 1990; McWilliams 2005). Studies on the bat's diet and monitoring the populations in those localities could help to elucidate the indirect life cycles of the helminth fauna of the Mexican free-tailed bat.

The diversity of invertebrates in Zacatecas is almost unknown; studies on the diversity of terrestrial snails and arthropods are necessary to better understand parasite life cycles in wild vertebrates. Monitoring wildlife and their parasites is crucial for conservation and for understanding biogeographic patterns and the evolution of host-parasite interactions. It is, therefore, necessary to further study the helminth parasites of bats to explore their origin and evolution in this region. In this paper, we present the first record of *U. scabridum* in Zacatecas and new locality records for all the helminth taxa recorded.

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