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MANTER: Journal of Parasite Biodiversity (ISSN 2470-8224)
Occasional Papers, Number 32, May 9, 2023
doi: 10.32873/unl.dc.manter32

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Helminth Parasites of the Giant Toad, *Rhinella horribilis* (Wiegmann, 1833) (Anura: Bufonidae) from Central Mexico

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Abstract

As part of an ongoing project to inventory the helminth parasites of amphibians from the state of Hidalgo, Central Mexico, specimens of *Rhinella horribilis* were collected from three municipalities: Eloxochitlán, Huehuetla, and San Felipe Orizatlán. A total of eight taxa of helminths were found: three digeneans (*Haematoloechus* sp., *Langeronia macrocirra*, and *Mesocoelium danforthi*) and five nematodes (*Cosmocerca* sp., *Cruzia morleyi*, *Ochoterenella chiapensis*, *Oswaldocruzia subauricularis*, and *Rhabdias* sp.). The highest species richness was recorded in Huehuetla. All reports of these species of helminths represent new locality records for Mexico.

Keywords: digeneans, nematodes, Hidalgo, new locality

In terms of amphibian diversity, Mexico is among the richest countries around the globe, with 424 recorded species and a high endemic rate (> 50%) (González-Hernández et al., 2021). Nevertheless, the information on the richness of helminth parasites of amphibians is scarce, with a record of 106 species from 113 hosts studied under this perspective (García-Prieto, García-Varela, et al., 2014; García-Prieto, Mendoza-Garfias, et al., 2014; García-Prieto, Osorio-Sarabina, et al., 2014). Particularly, *Rhinella horribilis* (Wiegmann, 1833) [formerly *Rhinella marina* L., see Acevedo et al. (2016)] is the most studied species of anuran in Mexico from a helminthological perspective; its geographic range

in this country spans from Sinaloa to Chiapas on the Pacific Slope, and from Tamaulipas to Yucatan Peninsula in the Gulf of Mexico (López et al., 2009; Acevedo et al., 2016). In the state of Hidalgo, *R. horribilis* occurs in the municipalities of Calnali, Eloxochitlán, Huazalingo, Huejutla, Metztlán, Molango, Pisaflores, Tenango de Doria, San Bartolo Tutotepec, Tepehuacan de Guerrero, Xochicoatlán, and Yahualica (Lemos-Espinal and Dixon, 2013; Ramírez-Bautista et al., 2014). The helminthological record of this anuran species in Mexico consists of 53 taxa; however, such records are scattered in few states (Chiapas, Colima, Guerrero, Jalisco, Michoacán, Nuevo León, Oaxaca, Veracruz, and Yucatán),

leaving gaps in the natural distribution of the toad without knowledge of the helminth fauna they could be housing (Paredes-León et al., 2008; Ruiz-Torres et al., 2017). The wide distribution, high population numbers, resilience to human activities, and generalist habits make this species an ideal model to carry out helminthological surveys. The objective of this work is to present for the first time the record of the helminth parasites of *R. horribilis* from some localities in Central Mexico.

We studied 14 toads that were collected in April 2011 (7 specimens) and May 2015 (7 specimens) in three municipalities of the state of Hidalgo: Eloxochitlán (San Juan Amajac [SJA], 20°42'54.4"N, 98°56'53.1"W), Huehuetla (Río Blanco [RB], 20°27'11.9"N, 98°04'34.7"W), and San Felipe Orizatlán (El Naranjal [NR], 21°11'23.3"N, 98°34'42.0"W). The collection was made under the collecting permit FAUT-0217 issued to Scott Monks by the Secretariat of Environment and Natural Resources of Mexico (SEMARNAT). The toads were killed with an overdose of intraperitoneal sodium pentobarbital. The body cavity was opened by a longitudinal incision and the gastrointestinal tract was removed by cutting across the esophagus and rectum. The internal organs were extracted and dissected under microscope for helminths. Helminths were removed from the viscera, counted in situ, placed in a saline solution (0.65%), fixed by sudden immersion in hot 4% formaldehyde or 70% ethanol, and preserved in 70% ethanol. Platyhelminths were stained with Mayer's paracarmine, dehydrated, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were cleared with Amman's lactophenol and temporarily mounted for morphological study. Ecological terminology follows Bush et al. (1997). Specimens of all helminth species were deposited at Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City.

Platyhelminthes: Digenea: Plagiorchiida

Haematoloechidae

Haematoloechus sp.

Prevalence, intensity, and range: 1/14 (7.14%), 11, 1–11

Site of infection: Lungs

Temporal distribution: Río Blanco, Huehuetla, Hidalgo

Specimens deposited: CNHE 10190

Type host and locality: Members of the genus *Haematoloechus* inhabiting the lungs of the different species of amphibians of the genus *Rana* in México [following the proposal of Yuan et al., 2016] (Paredes-León et al., 2008; Cabrera-Guzmán et al., 2010; Velazquez-Urrieta and León-Règagnon, 2018).

Additional Mexican records: Chiapas (Velazquez-Urrieta and León-Règagnon, 2018), Colima (Cabrera-Guzmán et al., 2010; León-Règagnon, 2010), Jalisco (Pérez-Ponce de León et al., 2000; León-Règagnon, 2010; Romereo-Mayén et al., 2016), Oaxaca (León-Règagnon, 2010).

Other reported hosts: Fowler's Toad, *Anaxyrus fowleri* (Muzzall and Andrus, 2014); Bolivian Swamp Frog, *Pseudopaludicola boliviana* (Magalhães-Campião et al., 2014); *Lithobates* sp. (Cabrera-Guzmán et al., 2010; León-Règagnon, 2010); *L. brownorum* (Velazquez-Urrieta and León-Règagnon, 2018); Bullfrog, *L. catesbeiana* (Marcogliese et al., 2009; Moy, 2013; Takaki et al., 2013; Frantz, 2019); Forrer's Grass Frog, *L. forreri* (Pérez-Ponce de León et al., 2000); *L. cf. forreri* (León-Règagnon, 2010; Velazquez-Urrieta and León-Règagnon, 2018); Northern Leopard Frog, *L. pipiens* (Hsu et al., 2004; Dare and Forbes, 2009); *L. psilonota* (León-Règagnon, 2010; Romereo-Mayén et al., 2016); Wood Frog, *Rana sylvatica* (Dare and Forbes, 2009); Dorbigny's Toad, *Rhinella dorbignyi* (Devantier-Henzel et al., 2020).

Geographic range: Argentina (Magalhães-Campião et al., 2014); Brazil (Devantier-Henzel et al., 2020); Canada (Dare and Forbes, 2009); Japan (Takaki et al., 2013); United States (Hsu, et al., 2004; Marcogliese et al., 2009; Moy, 2013; Muzzall and Andrus, 2014; Frantz, 2019).

Remarks: On the other hand, specimens assigned to *Haematoloechus*, a highly morphologically uniform group, were not identified to species level by two reasons: (1) the poor condition of the specimens and (2) the lack of molecular data, which is strongly recommended to support the determination based on morphological characteristics (see León-Règagnon et al., 1999).

Lecithodendriidae

Langeronia macrocirra

Caballero y Caballero y Bravo-Hollis, 1949

Prevalence, intensity, and range: 1/14 (7.14%), 1, 1

Site of infection: Intestine

Temporal distribution: Río Blanco, Huehuetla, Hidalgo

Specimens deposited: CNHE 10189

Type host and locality: *Lithobates* sp., Mexico (Caballero y Caballero and Bravo-Hollis, 1949).

Additional Mexican records: Colima (Cabrera-Guzmán et al., 2010), Nuevo León, Sonora, Veracruz (Paredes-León et al., 2008; Jacinto-Maldonado et al., 2022), Yucatán (Espínola-Novelo and Guillén-Hernández, 2008; Yáñez-Arenas and Guillén-Hernández, 2010).

Other reported hosts: *Lithobates* sp. (Cabrera-Guzmán et al., 2010; Paredes-León et al., 2008); Rio Grande Leopard Frog, *L. berlandieri* (Paredes-León et al., 2008); *L. brownorum* (Yáñez-Arenas and Guillén-Hernández,

2010); *L. palmipes* (Magalhães-Campião et al., 2014); Tarahumara Frog, *L. tarahumarae* (Paredes-León et al., 2008); Vallant's Frog, *L. vaillanti* (Paredes-León et al., 2008); Warszewitsch's Frog, *L. warszewitschii* (Brenes and Arroyo, 1959); Mexican Treefrog, *Smilisca baudinii* (Paredes-León et al., 2008); Cane Toad, Marine Toad, Giant Toad, *Rhinella marina* (Paredes-León et al., 2008); reported as *Bufo marinus marinus* Brenes and Arroyo, 1959; reported as *Chaunus marinus* Espínola-Novelo and Guillén-Hernández, 2008).

Geographic range: Costa Rica (Brenes et al., 1959); Ecuador (Magalhães-Campião et al., 2014).

Remarks: *Langeronia macrocirra* was identified based on its diagnostic traits according to Caballero y Caballero and Bravo-Hollis (1949), Brenes et al. (1959), and Martínez-Salazar and León-Règagnon (2010). Our material differs on *Langeronia jimenezi* Iruegas and Salinas, 1989, the other species of the genus distributed in Mexico, by lack of a prepharynx (present in *L. macrocirra*), by the testes and ovary lobulations present in *L. jimenezi* (while they are smooth in *L. macrocirra*), and by the position of the oral sucker (terminal in *L. jimenezi* and subterminal in *L. macrocirra*) (see Martínez-Salazar and León-Règagnon, 2010).

Mesocoeliidae

Mesocoelium danforthi

Hoffman, 1935

Prevalence, intensity, and range: 2/14 (14.28%), 15, 1–30

Site of infection: Intestine

Temporal distribution: Río Blanco, Huehuetla, Hidalgo

Specimens deposited: CNHE 10188

Type host and locality: *Diploglossus (Celestus) pleii*, Puerto Rico (Hoffman, 1935).

Additional Mexican records: Veracruz (López-García and García-Prieto, 2017).

Other reported hosts: Cane Toad, Marine Toad, Giant Toad, *Rhinella marina* (reported as *Bufo marinus* Cofresi-Sala and Rodríguez de Vega, 1963; Mettrick and Dunkley, 1968); Flat-backed Toad, *Sclerophrys maculata* (Soubeiga et al., 2020).

Geographic range: Burkina Faso (Soubeiga et al., 2020); Jamaica (Mettrick and Dunkley, 1968); Puerto Rico (Cofresi-Sala and Rodríguez de Vega, 1963).

Remarks: The presence of *M. danforthi* in Mexico is ratified in our study; the previous record of this species in *Rhinella horribilis* of Veracruz State (López-García and García-Prieto, 2017) was published as *Mesocoelium* cf. *danforthi* because of the presence of a tripartite seminal vesicle and differences in some measurements respect to the redescription made by Dronen et al. (2012).

The morphometry of our specimens clearly fits with the redescription.

Nematoda: Secernentea: Ascaridida: Cosmoceroidea

Cosmocercidae

Cosmocerca sp.

Prevalence, intensity, and range: 8/14 (57.14%), 21.5, 1–172

Site of infection: Intestine

Temporal distribution: Río Blanco, Huehuetla; San Juan Amajac, Eloxochitlán and El Naranjal, San Felipe Orizatlán

Specimens deposited: CNHE 10252, 10245, 20248

Type host and locality: Members of the genus *Cosmocerca* inhabiting the intestine of the different species of amphibians in México (Paredes-León et al., 2008).

Additional Mexican records: Chiapas (Velazquez-Urrieta and León-Règagnon, 2018), Oaxaca, Veracruz (Paredes-León et al., 2008).

Other reported hosts: *Adenomera diptyx* (reported as *Lepidodactylus diptyx* (Zaracho et al., 2012)); Silverstone's Poison Frog, *Ameerega pulchripecta* (Tavares-Costa et al., 2019); *Arthroleptis poecilnotus* (Edo-Taiwo and Aisien, 2020); *Boana albopunctata* (reported as *Hypsi-boas albopunctatus* (Magalhães-Campião et al., 2014; Martins-Sobrinho et al., 2017)); Fire-Bellied Toad, *Bombina bombina* (Yildirimhan and Birlik, 2013) *Bufo* sp., *Bufo viridis* (Barus and Tenora, 1976; Myers et al., 1962; the host is registered in Europe and Asia, currently *Bufo viridis* to Europe and *Bufo pseudoraddei* to Asia (AmphibiaWeb, 2019); *Chiasmocleis carvalhoi* (Aguiar et al., 2014); *Dendropsophus branneri*, *D. elegans*, *D. haddadi*, Lesser Treefrog, *D. minutus* (Martins-Sobrinho et al., 2017); Hourglass Treefrog, *Dendropsophus ebraccatus* (Paredes-León et al., 2008); Indus Valley Toad, *Duttaphrynus stomaticus* (reported as *Bufo stomaticus* (Khan et al., 2021)); Common Skittering Frog, *Euphlyctis cyanophlyctis* (Bursey et al., 2015); Rosy Ground Frog, *Eupsophus roseus* (Magalhães-Campião et al., 2014); African Reed Frogs, *Hyperolius* sp. (Edo-Taiwo and Aisien, 2021); Gulf Coast Toad, *Incilius valli-ceps* (Paredes-León et al., 2008); Kuhl's Wart Frog, *Limnonectes kuhlii* (reported as *Rana kuhlii* (Wongsawad et al., 2004)); *L. gyldenstolpei* (reported as *Limnonectes pileata* (Wongsawad et al., 2004)); Klappenbach's Red-Bellied Frog, *Melanophryniscus klappenbachi* (Hamann et al., 2014); Marsh Frog (Amin et al., 2012; Yildirimhan and Birlik, 2013); Weeping Frog, *Physalaemus bilingonigerus*; Bolivian Swamp Frog, *Pseudopaludicola boliviana* (Magalhães-Campião et al., 2014); Dwarf Frog, *P. cuvieri* (Bocchiglieri et al., 2008); Rhacophorid frog,

Polypedates braueri (Hasegawa and Ota, 2021); Mascarene Ridged Frog, *Ptychadena mascareniensis* (Abdel-Gaber et al., 2017; reported as *Rana mascareniensis* (Myers et al., 1962)); *L. brownorum* (Velazquez-Urrieta and León-Règagnon, 2018); Cope's Toad, *Rhinella ditycha* (Sampaio et al., 2022); Iranian Long-Legged Frog, *Rana macrocnemis* (Yildirimhan and Birlik, 2013); South American Common Toad, *Rhinella margaritifera*; Pepper Treefrog, *Trachycephalus typhonius* (Magalhães-Campião et al., 2014); Cane Toad, Marine Toad, Giant Toad, *Rh. marina*; Rio Grande Leopard Frog, *L. berlandieri*, (Paredes-León et al., 2008); Vallant's Frog, *L. vailanti* (Paredes-León et al., 2008); Tree Frog, *Scinax auratus* (Martins-Sobrinho et al., 2017); Tree Frog, *Scinax hayii* (Aguiar et al., 2014); Venezuelan Snouted Treefrog, *Scinax x-signatus* (Sampaio et al., 2022); Common African Toad, *Sclerophrys regularis* (reported as *Bufo regularis* (Myers et al., 1962); reported as *Amietophrynus regularis* (Okeagu et al., 2022)).

Geographic range: Afghanistan (Barus and Tenora, 1976); Argentina (Zaracho et al., 2012; Hamann et al., 2014; Magalhães-Campião et al., 2014); Brazil (Bocchiglieri et al., 2008; Vrcibradic et al., 2008; Aguiar et al., 2014; Magalhães-Campião et al., 2014; Martins-Sobrinho et al., 2017; Tavares-Costa et al., 2019; Sampaio et al., 2022); Chile (Magalhães-Campião et al., 2014); Egypt (Myers et al., 1962; Abdel-Gaber et al., 2017); India (Burse et al., 2015); Nigeria (Edo-Taiwo and Aisien, 2020, 2021; Okeagu et al., 2022); Pakistan (Khan et al., 2021); Taiwan (Hasegawa and Ota, 2021); Thailand (Wongsawad et al., 2004); Turkey (Yildirimhan and Birlik, 2013).

Remarks: The collected specimens of *Cosmocerca* sp. contain exclusively females, and thus their specific identification was not possible because males bear the diagnostic characteristics at this level (Baker, 1980). The absence of males in the sample could be attributed to a phenomenon similar to that observed by Kirillov and Kirillova (2016) when studying the reproductive structure of *Cosmocerca ornata* in marsh frogs; these authors reported that the reproductive structure of this nematode is characterized by the permanent domination of females (present in frogs all year round); instead, males occurs rarely, being represented generally only by one specimen. Our specimens were assigned to the genus *Cosmocerca* from the following characters: presence of well-developed lateral alae, starting at the level of the nerve ring and reaching the anal region; three labia present in the oral region; poorly developed pharynx; esophagus swollen at mid-body region; and, finally, the equatorial position of the vulva (Yamaguti, 1961; Falcón-Ordaz et al., 2007). Three species of this genus have

been recorded in amphibians from Mexico: *Cosmocerca acanthurum*, Falcón-Ordaz, Winfield, Mendoza-Garfias, Parra-Olea, and Pérez Ponce de León, 2007; *Cosmocerca parva* Travassos, 1925; and *Cosmocerca podicipinus* Baker and Vaucher, 1984, but none have been collected in bufonids of the country nor in the state of Hidalgo (see Paredes-León et al., 2008).

Kathlanidae

Cruzia morleyi

Pearse, 1936

Prevalence, intensity, and range: 1/14 (7.14%), 0.5, 1–2

Site of infection: Intestine

Temporal distribution: El Naranjal, San Felipe Orizatlán

Specimens deposited: CNHE 10246

Type host and locality: *Rh. horribilis* (= *Bufo marinus*), Yucatan (Pearse, 1936)

Additional Mexican records: Oaxaca, Veracruz, Yucatán.

Other reported hosts: All records in Mexico are parasitizing Cane Toad, Marine Toad, Giant Toad, *Rh. horribilis* (Paredes-León et al., 2008).

Geographic range: Mexico

Spirurida: Filarioidea

Onchocercidae

Ochoterenella chiapensis

Esslinger, 1988

Prevalence, intensity, and range: 5/14 (35.71%), 6.4, 1–32

Site of infection: Corporal cavity

Temporal distribution: Río Blanco, Huehuetla; San Juan Amajac, Eloxochitlán and El Naranjal, San Felipe Orizatlán

Specimens deposited: CNHE 10250, 10243, 10249

Type host and locality: *Rh. horribilis* (= *Bufo marinus*), Chiapas (Esslinger, 1988)

Other reported hosts: The records in Mexico are parasitizing Cane Toad, Marine Toad, Giant Toad, *Rh. horribilis* (Paredes-León et al., 2008).

Geographic range: Chiapas in Mexico; Guatemala.

Remarks: The filarial nematodes collected from the body cavity of the studied toads were identified as *O. chiapensis* Esslinger, 1988 based on the comparison with the females of this species described by Esslinger (1988): vulva slightly prominent, number of cuticular bosses at mid (10–12 vs. 6–10, respectively) and posterior (2–4 vs. 4–5) body regions, and the distance between cuticular bosses at the mid-body region (29–40 μm vs. 19–30 μm). *Ochoterenella*, one of the genera most found in the body cavity of Mexican anurans, is represented by five additional species parasitizing *R. horribilis* in Mexico: *O. caballeroi* Esslinger, 1987; *O. digiticauda* Esslinger,

1986; *O. figueroai* Esslinger, 1988; *O. lamothei* Esslinger, 1988; and *O. nanolarvata* Esslinger, 1987. Our material can be differentiated from *O. caballeroi*, *O. lamothei*, and *O. nanolarvata* because these species have a non-prominent vulva and a greater distance between cuticular bands in the mid-body region (44–69, 48–50, and 33–44 μm vs. 21–30 μm , respectively). In addition, our specimens can be separated from *O. figueroai* based on the higher number of cuticular bosses at the mid-body region of this species (15–18), the distance between cuticular bosses (37–44 μm), and cuticular bands (58–67 μm vs. 21–30 μm in the material of this study). Finally, according to Esslinger (1988), the main trait that allows distinguishing *O. chiapensis* from *O. digiticauda* is the distance between cuticular bands (21–30 in our specimens, 34–51 μm in the original description vs. 62–92 μm in *O. digiticauda*). We consider that the great morphological similarity between the species of the genus, particularly of the species distributed in Mexico, makes its revision necessary, using molecular tools.

Strongylida: Trichostrongyloidea

Molineidae

Oswaldocruzia subauricularis

(Rudolphi, 1819)

Prevalence, intensity, and range: 3/14 (21.42%), 6, 1–18

Site of infection: Intestine

Temporal distribution: Río Blanco, Huehuetla

Specimens deposited: CNHE 10253

Type host and locality: “*Rana muscae*,” Brazil. The determination of the type species is erroneous; the current nomenclature the species corresponds to *Anaxyrus terrestris* (= *Bufo terrestris*), which is distributed in United States.

Additional Mexican records: Oaxaca (Trejo-Meléndez et al., 2019).

Other reported hosts: Blacksmith Treefrog, *Boana faber* (reported as *Hypsiboas faber* (Magalhães-Campião et al., 2014)); Surinam Horned Frog, *Ceratophrys cornuta*; Polymorphic Robber Frog, *Craugastor rhodopis* (Magalhães-Campião et al., 2014); Clay Robber Frog, *Haddadus binotatus* (Aguiar et al., 2020); Wiegmann’s Toad, *Incilius marmoratus* (Trejo-Meléndez et al., 2019); Gulf Coast Toad, *I. valliceps*, *Leptodactylus melanonotus*; Criolla Frog, *Le. latrans* (Magalhães-Campião et al., 2014; Toledo et al., 2015); Northeastern Pepper Frog, *Le. vastus*; Burmeister’s Frog, *Phyllomedusa burmeisteri* (Magalhães-Campião et al., 2014); Barker Frog, *Physalaemus cuvieri* (Aguiar et al., 2015); Rio Grande Leopard Frog, *L. berlandieri* (Paredes-León et al., 2008); Forrer’s

Grass Frog, *L. cf. forreri* (Paredes-León et al., 2008); Vallant’s Frog, *L. vaillanti* (Paredes-León et al., 2008); Striped Toad, *Rhinella crucifer*; Yellow Cururu Toad, *Rh. icterica* (Magalhães-Campião et al., 2014; Moretti et al., 2017); Cope’s Toad, *Rh. diptycha* (González et al., 2021); *Rh. diptycha* (= *Rh. jimí*) (Campião et al., 2014); *Rh. major* (Hamann et al., 2013); Cane Toad, Marine Toad, Giant Toad, *Rh. marina* (Magalhães-Campião et al., 2014; Paredes-León et al., 2008); Cururu Toad, Rococo Toad, *Rh. schneideri* (Magalhães-Campião et al., 2014); Mexican Treefrog, *Smilisca baudinii* (Paredes-León et al., 2008); Blue-Spotted, *S. cyanosticta*; and Porto Alegre Golden, *Trachycephalus mesophaeus* (Magalhães-Campião et al., 2014).

Geographic range: Argentina (Hamann et al., 2013; González et al., 2021); Brazil (Magalhães-Campião et al., 2014; Aguiar et al., 2015, 2020; Toledo et al., 2015; Moretti et al., 2017); Chiapas, Guerrero, Veracruz, and Yucatán in México (Cabrera-Guzmán et al., 2007; Paredes-León et al., 2008).

Remarks: The cosmopolitan genus *Oswaldocruzia* Travassos, 1917 comprises 94 nominal species, all parasites from amphibians and reptiles (Guerrero, 2013; Ruiz-Torres et al., 2013; González and Hamann, 2016; Svitin, 2017). Our nematodes were assigned to *Oswaldocruzia* by having the anterior end divided by the buccal capsule but with cephalic vesicle present; synlophe with cuticular ridges oriented perpendicular to the wall. Caudal bursa of male type 2-3 tending to type 2-1-2, gubernaculum absent. Didelphic females with posterior vulva and caudal spine present (Ben-Slimane et al., 1996). In Mexico, three species of *Oswaldocruzia* are recorded as parasites of *R. horribilis*: *O. lamotheargumedoi* Ruiz-Torres, García-Prieto, Osorio-Sarabia, and Violante-González, 2013; *O. pipiens* Walton, 1929; and *O. subauricularis* (Rudolphi, 1819) (Paredes-León et al., 2008; Ruiz-Torres et al., 2013); our specimens were identified as *O. subauricularis* by having caudal bursal type II (sensu Ben-Slimane et al., 1996), 44 synlophe ridges with reinforcement at mid-body level and alae present but reduced; in the same way, measurements such as body length, esophagus length, and the distance of excretory pore and deirids to anterior end are similar to that of *O. subauricularis* (Ben-Slimane and Durette-Desset, 1995). The nematode registered in Hidalgo can be distinguished from *O. lamotheargumedoi* and *O. pipiens* because these species have the caudal bursa type I and the synlophe ridges without reinforcement; in addition, *O. lamotheargumedoi* lack this structure (Baker, 1978; Espinoza-Jiménez, 2007; Ruiz-Torres et al., 2013).

Rhabditida: Rhabditoidea

Rhabdiasidae

Rhabdias sp.

Prevalence, intensity, and range: 8/14 (57.14%), 13.2, 1–106

Site of infection: Lungs

Temporal distribution: Río Blanco, Huehuetla; San Juan Amajac, Eloxochitlán and El Naranjal, San Felipe Orizatlán

Specimens deposited: CNHE 10251, 10244, 10247

Type host and locality: Members of the genus *Rhabdias* inhabiting the intestine of the different species of amphibians and reptiles in México (Paredes-León et al., 2008).

Additional Mexican records: None

Other reported hosts: See Romero-Mayén et al. (2016) to Mexico; Silverstone's Poison Frog, *Ameerega pulchripecta* (Tavares-Costa et al., 2019); *Arthroleptis poecilnotus* (Edo-Taiwo and Aisien, 2020); *Boana albopunctata* (reported as *Hypsiboas albopunctatus*, Magalhães-Campião et al., 2014); Green Toad, *Bufo viridis* (reported as *Pseudepidalea viridis* and *Bufo viridis*, Yildirimhan and Birlik, 2013; the host is registered in Europe and others (AmphibiaWeb, 2019); Clay Robber Frog, *Haddadus binotatus* (Aguiar et al., 2020); Vizcacheras' White-Lipped Frog, *Le. Bufonius* (González et al., 2021); *Le. chaquensis*, Criolla Frog, *Le. latrans*, *Le. macrosternum*, *Le. podicipinus* (Alves et al., 2018; Freire-Vieira et al., 2021; Magalhães-Campião et al., 2014); Barker Frog, *Physalaemus cuvieri* (Aguiar et al., 2015); Paradoxical Frog, *Pseudis paradoxa*, *Rh. bergi*, *Rh. crucifer*; Cane Toad, Marine Toad, Giant Toad, *Rh. marina* and Yellow Cururu Toad, *Rh. icterica* (Alves et al., 2018; Magalhães-Campião et al., 2014); Cope's Toad, *Rh. diptycha* (González et al., 2021); *Rh. jimi* (Amorim et al., 2019); Stauffer's Treefrog, *Scinax staufferi* (Martínez-Salazar et al., 2009).

Geographic range: Baja California Sur, Chiapas, Jalisco, Veracruz in México (Romero-Mayén et al., 2016); Argentina (Magalhães-Campião et al., 2014; González et al., 2021); Brazil (Aguiar et al., 2015, 2020; Alves et al., 2018; Freire-Vieira et al., 2021; Magalhães-Campião et al., 2014; Tavares-Costa et al., 2019); Panama (Kelehear et al., 2019); Turkey (Yildirimhan and Birlik, 2013); Nigeria (Edo-Taiwo and Aisien, 2020).

Remarks: In Mexico, three species of the genus *Rhabdias* have been recorded as parasites of *R. horribilis*: *R. americanus* Baker, 1978; *R. fülleborni* Travassos, 1926; and *R. pseudosphaerocephala* Kuzmin, Tkach, and Brooks, 2007 (Paredes-León et al., 2008). Our specimens showed similar body length to those 3 species; however, specimens from Hidalgo differ from *R. americanus* and *R.*

fülleborni because they present an inflated esophageal corpus (absent in our material). Our material and *R. pseudosphaerocephala* share several traits, such as body length (5–11 mm vs. 6.1–9.6 mm), depth (10–30 µm vs. 7–12 µm), and width (10–15 µm vs. 15–17 µm) of buccal capsule and esophagus length (310–540 µm vs. 400–460 µm) as well as the lack of inflated corpus. According to Martínez-Salazar (2008), the structural uniformity between the species of the genus is very high, which makes its specific identification difficult and the use of molecular characters recommended to perform it with precision. For this reason, our material was identified as *Rhabdias* cf. *pseudosphaerocephala*, until performing a DNA analysis of it.

Three hundred seventy-two helminth individuals were collected in the samplings made during April 2011 and May 2015; they represent three species of digenetic trematodes (37.5%) and five nematodes (62.5%). The locality with the major helminth richness and abundance was RB, where the three species of trematodes (*Haematoleuchus* sp.; *Langeronia macrocirra* Caballero and Bravo Hollis, 1949; and *Mesocoelium danforthi* Hofmann, 1935) and four of the five species of nematodes (*Cosmocerca* sp.; *Ochoterenella chiapensis* Esslinger, 1988; *Oswaldocruzia subauricularis* Rudolphi, 1819; and *Rhabdias* cf. *pseudosphaerocephala* Kuzmin, Tkach, and Brooks, 2007) were recorded, with a total of 218 specimens. On the other hand, giant toads of NR housed 59 specimens belonging to four species of nematodes (*Cosmocerca* sp.; *Cruzia morleyi* Pearse, 1936; *O. chiapensis*; and *R. cf. pseudosphaerocephala*), and the SJA samplings contained three nematodes (*Cosmocerca* sp., *O. chiapensis*, and *R. cf. pseudosphaerocephala*) and one trematode (*M. danforthi*), totaling 75 specimens.

In spite of the reduced number of giant toads sampled in our study, we could detect some shared traits with the helminth fauna of other bufonids studied in the Americas, according to Ruiz-Torres et al. (2013): the helminthological composition of this host shows (1) a clear dominance of nematodes with direct life cycle (*Cosmocerca* sp., *C. morleyi*, *O. subauricularis*, and *Rhabdias* sp.); (2) the predominance of generalist helminth species in the giant toads sampled (75%); (3) the influence of food-web dynamic in the helminth richness (50% of the species enter by ingestion); and (4) the role of percutaneous infection in determining the abundance of recruited individuals (328 of 372 helminths collected).

Acknowledgments – JFO thanks Programa de Desarrollo Profesional y Docente (PRODEP) for the founding of the project "Biodiversity of helminth parasites of amphibians

as biological indicators of two biogeographic regions in the state of Hidalgo," No. UAEH-PTC-649. Thanks to Daniel Lara-Tufiño for collecting hosts during 2011.

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