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Taxonomy and systematics

New species of *Cosmocerca* (Nematoda: Cosmocercidae), parasite of *Leptodactylus melanonotus* in Western Mexico, and new records for this species and *Incilius marmoreus* (Amphibia)

Especie nueva de Cosmocerca *(Nematoda: Cosmocercidae), parásita de* Leptodactylus melanonotus *y nuevos registros para esta especie y para* Incilius marmoreus *(Amphibia)*

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Abstract

Specimens of the amphibian species *Leptodactylus melanonotus* and *Incilius marmoreus* were collected in several localities in Colima State, Mexico, from July to October 2021. An undescribed species of nematode of the genus *Cosmocerca* was found in the intestine of *L. melanonotus*. The new species differs from every other species assigned to this genus by the combination of the following characters: absence of lateral alae in males, 5 plectanes, spicules size, and gubernaculum size and shape. This is the 39th species included in the genus worldwide and it is the second described in Mexico. Six helminth species were collected during this study: *Rauschiella poncedeleoni*, *Cosmocerca colimense* n. sp., *Oswaldocruzia subauricularis*, and *Rhabdias* sp. of *L. melanonotus*; *Aplectana incerta* and *Oswaldocruzia pipiens* of *I. marmoreus*.

Keywords: Anura; Enteric nematode; Digenea; Sabinal frog; Marbled toad

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Resumen

Se recolectaron ejemplares de las especies de anfibios *Leptodactylus melanonotus* e *Incilius marmoreus* en varias localidades del estado de Colima, México, de julio a octubre de 2021. Durante estos muestreos, se descubrió una especie nueva de nemátodo del género *Cosmocerca* en el intestino de *L. melanonotus*; difiere del resto de las especies del género por la combinación de los siguientes caracteres: ausencia de alas laterales en machos, 5 plectanes, tamaño de las espículas y tamaño y forma del gubernáculo. *Cosmocerca colimense* n. sp. es la 39^{ava} especie asignada a este género en el mundo y la segunda descrita en México. Se recolectaron 6 especies de helmintos en este estudio: *Rauschiella poncedeleoni*, *Cosmocerca colimense* n. sp., *Oswaldocruzia subauricularis* y *Rhabdias* sp. de *L. melanonotus*; *Aplectana incerta* y *Oswaldocruzia pipiens* de *I. marmoreus*.

Palabras clave: Anura; Nemátodo entérico; Digenea; Rana del sabinal; Sapo jaspeado

Introduction

Amphibian diversity in Mexico is one of the highest worldwide (Ramírez-Bautista et al., 2023); 42 species of amphibians have been reported in the State of Colima alone, representing 11% of the total species reported in Mexico (García et al., 2022), although Colima only represents 0.3% of the total country area (INEGI, 2015). Knowledge on the helminth fauna of amphibians and its biology provides valuable information about the conservation state of the ecosystems and can be used as indicators of the health of host populations (Brooks et al., 2014; Marcogliese et al., 2003). In spite of their potential, studies on amphibian helminths in Colima are scarce, and most of them are isolated records. To date, 17 species of helminths (8 digeneans, 1 cestode and 8 nematodes) have been recorded, out of 6 species of anurans (Cabrera-Guzmán et al., 2022). Two of the most common anuran species in Colima are *Leptodactylus melanonotus* (Hallowell, 1861) and *Incilius marmoreus* (Weigman, 1833) (García & Ceballos, 1994), nevertheless, there is only 1 study on the helminth fauna of *L. melanonotus* (Mata-López et al., 2013) and there is nothing for *I. marmoreus* (Table 1).

Leptodactylus Fitzinger, 1826 includes around 84 species, being *Leptodactylus fragilis* (Brocchi, 1877) and *L. melanonotus* the only 2 species distributed in Mexico (Flores-Villela, 1993; Flores-Villela & Canseco-Márquez, 2004). *Leptodactylus melanonotus* (Sabinal frog) is a small frog (svl = 50mm) with indirect development that occurs from Sonora and Tamaulipas in Mexico, throughout Central America, and into South America west of the Andes to Ecuador; inhabit the edges of ponds or flooded pastures, at the base of tufts of grass, or within burrows in the mud through dry and humid premontane habitats (Lee, 1996). To date, 36 helminth taxa had been recorded in *L. melanonotus* from a wide geographical range in México (Table 1), being Guerrero, Oaxaca, Quintana Roo, and Veracruz the states with a larger number of recorded helminth taxa (Goldberg & Bursey, 2002; Mata-López et al., 2013; Terán-Juárez, 2011).

Incilius Cope, 1863 comprehends 39 species (Mendelson III et al., 2011), 23 of which are native to Mexico and 10 (or 43% of Mexican species) are endemic (Ramírez-Bautista et al., 2023). *Incilius marmoreus* (Marbled toad) is a small toad (svl = 76 mm) with indirect development, and an endemic species inhabiting tropical areas along the Mexican Pacific coast from northern Sinaloa throughout Chiapas, where it is found in the litter of forest floor (García & Ceballos, 1994; Rueda-Hernández, 2023); some populations also occur in the Balsas basin and coast of the state of Veracruz. The only previous helminth records for *I. marmoreus* are from the states of Jalisco and Oaxaca (Galicia-Guerrero et al., 2000; Trejo-Meléndez et al., 2019), both in the Pacific coast of Mexico, including 15 taxa (Table 1).

The aim of this study was to describe a new species of nematode parasite in the intestine of *L. melanonotus* and contribute to the knowledge of the helminth fauna of *L. melanonotus* and *I. marmoreus* in Colima.

Materials and methods

Specimens of *L. melanonotus* (n = 137) and *I. marmoreus* (n = 31) were collected from July to October 2021, in several sites in the state of Colima, Mexico, distributed along the riparian zones of 3 rivers (Comala, Colima, and Manríquez) and in flooded crop fields and suburban areas in Coquimatlán and Colima municipalities. Specimens were collected under the scientific collection permit FAUT0056 issued to VLR by Secretaría del Medio Ambiente y Recursos Naturales (Semarnat). Amphibians were captured by hand and euthanized by an overdose of sodium pentobarbital, frozen and preserved in 96% ethanol until helminthological examination. Amphibians were dissected and examined under stereomicroscope; worms were recovered and preserved in 70% ethanol for morphological study.

Table 1

Helminth records of *Leptodactylus melanonotus* and *Incilius marmoreus* in Mexico. A = Adult, L = larvae, Ch = Chiapas, C = Colima, G = Guerrero, M = Michoacán, N = Nayarit, O = Oaxaca, QR = Quintana Roo, S = Sonora, T = Tabasco, Ta = Tamaulipas.

Helminth species	Host species	Site of infection	Reference, locality
Acanthocephala			
Acanthocephala gen. sp. [L]	*I. marmoreus*	Mesentery	Trejo-Meléndez et al. (2019), O
Centrorhynchus sp. [L]	*I. marmoreus*	Body cavity	Galicia-Guerrero et al. (2000), J
	L. melanonotus	Intestine	Terán-Juárez (2011), QR
		Mesentery	Goldberg et al. (2002), V
Cestoda			
Cyclophyllidae gen. sp. [L]	*L. melanonotus*	Body cavity	Terán-Juárez (2011), QR
Cylindrotaenia americana [A]	*L. melanonotus*	Intestine	Goldberg and Bursey (2002), S
Nematotaeniidae gen. sp. [A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
Digenea			
Catadiscus propinquus [A]	*L. melanonotus*	Intestine	Brooks et al. (2006), V; Mata-López et al. (2013), G, J, N, O, T
Choledocystus hepaticum [A]	*I. marmoreus*	Liver	Trejo-Meléndez et al. (2019), O
Clinostomum tataxumui [A]	*I. marmoreus*	Liver	Trejo-Meléndez et al. (2019), O
Clinostomum sp. [L]	*L. melanonotus*	Mesentery	Mata-López et al. (2013), C
Glypthelmins facioi [A]	*L. melanonotus*	Intestine	Goldberg et al. (2002), V
Gorgoderina attenuata [A]	*I. marmoreus*	Urinary bladder	Trejo-Meléndez et al. (2019), O
	L. melanonotus	Urinary bladder	Mata-López et al. (2013), Ch, C; Goldberg and Bursey (2002), S; Goldberg et al. (2002), V
Gorgoderina festoni [A]	*L. melanonotus*	Urinary bladder	Mata-López and León-Règagnon (2005), C
			Mata-López and León-Règagnon (2005); Mata-López et al. (2013), G
Haematoloechus longiplexus [A]	*L. melanonotus*	Lungs	Goldberg and Bursey (2002), S
Megalodiscus temperatus [A]	*L. melanonotus*	Intestine	Goldberg and Bursey (2002), S
Megalodiscus sp. [A]	*L. melanonotus*	Intestine	Goldberg et al. (2002), V
Mesocoelium americanum [A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
Rauschiella poncedeleoni [A]	*L. melanonotus*	Intestine	Razo-Mendívil and León-Règagnon (2001), T, V; Razo-Mendívil et al. (2006), G; Mata-López et al. (2013), C, G, J, O; Terán-Juárez (2011), QR
R. tineri [A]	*L. melanonotus*	Intestine	Mata-López et al. (2013), G, T
Strigeidae gen. sp. [L]	*L. melanonotus*	Intestine	Mata-López et al. (2013), C
		Mesentery	Mata-López et al. (2013), O
Nematoda			
Aplectana incerta [A]	*I. marmoreus*	Intestine	This study, C
			Galicia-Guerrero et al. (2000), J
			Mata-López et al. (2013), T

Table 1. Continued

Helminth species	Host species	Site of infection	Reference, locality
A. itzocanensis [A]	*L. melanonotus*	Intestine	Goldberg and Bursey (2002), S
			Mata-López et al. (2013), G, N, O, V
Aplectana sp.[A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
	L. melanonotus	Intestine	Mata-López et al. (2013), Ch, G, O, T
Ascarididae gen. sp.[L]	*L. melanonotus*	Mesentery	Mata-López et al. (2013), C, G, O, T
Ascarops sp. [L]	*L. melanonotus*	Stomach	Goldberg et al. (2002), V
Contracaecum sp. [L]	*L. melanonotus*	Stomach	Terán-Juárez (2011), QR
Cosmocerca colimense n. sp. [A]	*L. melanonotus*	Intestine	This study, C
C. parva [A]	*L. melanonotus*	Intestine	Mata-López et al. (2013), O
C. podicipinus [A]	*L. melanonotus*	Intestine	Mata-López et al. (2013); Ch, C, G, J, M, O, T, Ta; Goldberg et al. (2002), V, Terán-Juárez (2011), QR; Goldberg and Bursey (2002), S
Cosmocerca sp. [A]	*L. melanonotus*	Stomach	Terán-Juárez (2011), QR
Eustrongylides sp. [L]	*L. melanonotus*	Mesentery	Mata-López et al. (2013), Ch
Kalicephalus sp. [A]	*L. melanonotus*	Intestine	Mata-López et al. (2013), G
Oswaldocruzia lescurei [A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
O. pipiens [A]	*L. melanonotus*	Intestine	Goldberg and Bursey (2002), S
O. cf *pipiens* [A]	*I. marmoreus*	Intestine	This study, C
O. subauricularis [A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
	L. melanonotus	Intestine	This study, C; Mata-López et al. (2013), C, G, J, O, T, Ta
Oswaldocruzia sp. [A]	*I. marmoreus*	Intestine	Trejo-Meléndez et al. (2019), O
	L. melanonotus	Intestine	Mata-López et al. (2013), Ch, G; Terán-Juárez (2011), QR; Goldberg et al. (2002), V
Physocephalus sp.	*I. marmoreus*	Body cavity	Galicia-Guerrero et al. (2000), J
		Stomach	Trejo-Meléndez et al. (2019), O
Porrocaecum sp. [L]	*L. melanonotus*	Mesentery	Goldberg et al. (2002), V
Rhabdias americanus [A]	*L. melanonotus*	Lungs	Goldberg et al. (2002), V
R. elegans [A]	*L. melanonotus*	Lungs	Goldberg et al. (2002), V; Terán-Juárez (2011), QR
R. fuellernorni [A]	*I. marmoreus*	Lungs	Galicia-Guerrero et al. (2000), J
R. pseudosphaerocephala [A]	*I. marmoreus*	Lungs	Trejo-Meléndez et al. (2019), O
Rhabdias ranae [A]	*L. melanonotus*	Lungs	Goldberg and Bursey (2002), S
Rhabdias sp. [A]	*L. melanonotus*	Lungs	This study, C; Mata-López et al. (2013), G, O, T, Ta, V
Schrankiana sp. [A]	*L. melanonotus*	Intestine	Terán-Juárez (2011), QR,
Spiroxys sp. [L]	*L. melanonotus*	Intestine	Terán-Juárez (2011), QR
		Mesentery	Goldberg and Bursey (2002), S; Mata-López et al. (2013), T, Ta
		Stomach	Goldberg et al. (2002), V
Subulascaris falcaustriformis [A]	*L. melanonotus*	Intestine	Goldberg et al. (2002), V

Platyhelminths were stained with Mayer´s paracarmine and Delafield´s hematoxylin, and permanently mounted in Canada balsam. Nematodes were cleared with a mixture of glycerine and ethanol solution 1:1, for 24 h and mounted between coverslips. In the case of the new species description, measurements are given in millimetres (unless otherwise indicated), with minimum and maximum, mean and standard deviation in parentheses. Morphometric data were obtained with an Olympus CX40 optical microscope; micrographs were taken with a Leica DM750 optical microscope equipped with a digital camera Leica ICC50 E. Line drawings were made on the basis of digital micrographs using a Wacom One drawing tablet with Adobe Illustrator CS6 and Adobe Photoshop CS6. Scale bars of all figures are given in micrometers. Specimens were deposited in the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (CNHE).

Results

Six helminth species were collected during this study, 1 digenean, *Rauschiella poncedeleoni* Razo-Mendívil & León-Règagnon, 2001 and 5 nematodes, *Aplectana incerta* Bravo-Hollis, 1943, *Cosmocerca colimense* n. sp., *Oswaldocruzia subauricularis* (Rudolphi, 1819), *Oswaldocruzia pipiens* Walton, 1929, and *Rhabdias* sp. *Rauschiella poncedeleoni* (prevalence 5.8%), *C. colimense* n. sp. (31.4%), *O. subauricularis* (2.2%), and *Rhabdias* sp. (1.45%) were collected from *L. melanonotus*, while *A. incerta* (38.7%) and *O. pipiens* (6.4%) were found in *I. marmoreus*.

Description

Family Cosmocercidae Railliet, 1916 (Travassos, 1925)
Genus *Cosmocerca* Diesing, 1861
Cosmocerca colimense n. sp. (Figs. 1, 2)
http://zoobank.org/urn:lsid:zoobank.org:act:C8ADE45C-E6FF-4380-8B90-6854F9DF6EBE

Diagnosis. Robust, small and fusiform nematodes, males smaller than females (Fig. 1A, B). Cuticle with transverse striations along the entire body. Oesophageal bulb well developed, with sclerotized valves (Fig. 1A, B). Protruding excretory pore, anterior to oesophageal bulb (Fig. 1A, B). Females with lateral alae (Fig. 1B), absent in males. Caudal alae absent. Spicules equal in length, with a conical, sclerotized gubernaculum (Figs. 1C, 2). Females with a monodelphic-prodelphic reproductive system (Fig. 1B), without larvated nor undeveloped eggs, only with free larvae.

Male (based on 3 mature specimens). Fusiform nematodes with robust, small and cylindrical body, ventrally curved, anteriorly truncated by the obtuse oral region and acuminated at posterior end. Finely transversely striated cuticle along the entire body, without caudal alae. Total body length 1.872-2.043 (1.944 ± 0.088, n = 3), maximum width 0.135-0.198 (0.171 ± 0.032, n = 3) at middle region. Lateral alae absent. Oesophagus divided into an anterior and comparatively short pharynx, measuring 0.022-0.028 (0.025 ± 0.003, n = 3) in length, a sub-cylindrical corpus 0.179-0.222 (0.198 ± 0.021, n = 3) long, a well-defined isthmus 0.033-0.042 (0.039 ± 0.005, n = 3) long and a sub-pyriform oesophageal bulb with sclerotized valves measuring 0.076-0.058 (0.067 ± 0.009, n = 3) wide and 0.057-0.075 (0.066 ± 0.008, n = 3) long. Nerve ring located at 0.144-0.155 (0.151 ± 0.005, n = 3) from anterior end. Evident excretory pore, slightly protruding, located near to the anterior margin of oesophageal bulb, at 0.255 (0.243 ± 0.023, n = 3) from anterior end. Cloacal opening at 0.147-0.162 (0.159 ± 0.011, n = 3) from posterior end; this region is markedly muscular and ventrally curved, with 5 pairs of pre-cloacal plectanes each one forming 2 rows of 16-18 cuticular tubercles (1 in the anterior side and 1 in the posterior side) with 1 central papilla rounded by 8-10 internal punctuations and 9-10 external punctuations. Three pairs of rosettes (1 pre-cloacal, 1 ad-cloacal and 1 post-cloacal), and several pairs of small sub ventral simple papillae at surface of cloacal region: 17-18 ventral, 4 lateral and 9 dorsal pairs. Both spicules 0.071-0.091 (0.082 ± 0.010, n = 3) in length, slightly sclerotized and tapering in point, with a well sclerotized gubernaculum, conical in shape, measuring 0.097-0.124 (0.117 ± 0.010, n = 3) in length.

Female (based on 5 gravid specimens). Fusiform nematodes with robust and cylindrical body, usually ventrally curved at middle level, anteriorly truncated by the obtuse oral region and subulated at posterior end. Total body length 2.678-3.53 (3.185 ± 0.383, n = 5), maximum width 0.277-0.495 (0.353 ±0.098, n = 5) at vulva level. Finely transversely striated cuticle along the whole body, with lateral alae beginning at isthmus level, at 0.199-0.277 (0.234 ± 0.039, n = 4) from the anterior end, and ending at the posterior region at 0.459-0.577 (0.523 ± 0.059, n = 4) from de posterior end, slightly anterior to the anus. Oesophagus is divided into an anterior and short pharynx, measuring 0.024-0.039 (0.031 ± 0.006, n = 5) in length, a sub-cylindrical corpus 0.235-0.277 (0.256 ± 0.019, n = 5) long, a well-defined isthmus 0.044-0.055 (0.047 ± 0.005, n = 5) long and a sub-pyriform oesophageal bulb with sclerotized valves, measuring 0.055-0.077 (0.064 ± 0.010, n = 5) wide, and 0.075-0.099

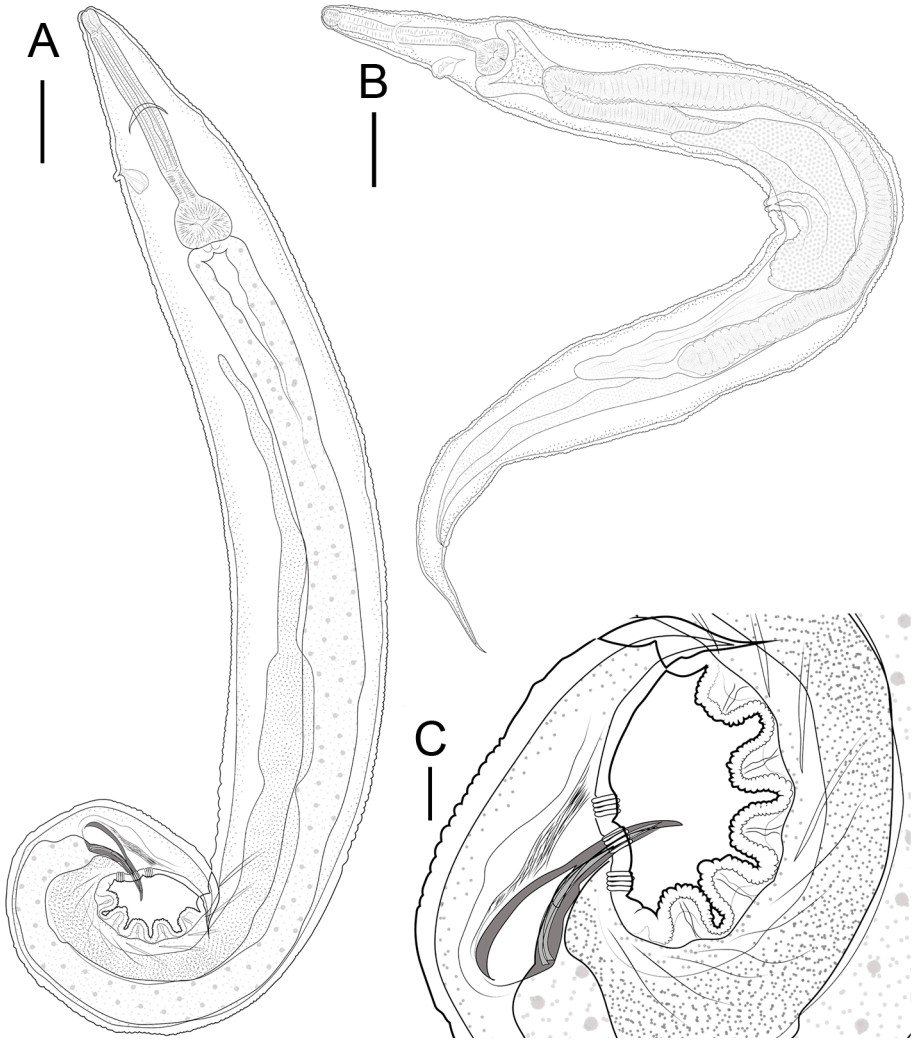

Figure 1. Drawings of *Cosmocerca colimense* n. sp. A, Male, entire, lateral view; B, female, entire, lateral view; C; male, detail of the cloacal region, lateral view. Scale bars: A = 100 µm, B = 150 µm, C = 25 µm.

(0.086 ± 0.009, n = 5) long. Nerve ring located at 0.155-0.261 (0.187 ± 0.049, n = 5) from anterior end. Evident excretory pore, noticeably protruding from, located about the level of corpus basis, between the nerve ring and oesophageal bulb, at 0.207-0.360 (0.281 ± 0.062, n = 5) from anterior end. Vulva located at the middle region, at 1.008-1.395 (1.196 ± 0.169, n = 5) from anterior end, with slightly dilated lips. Short and muscular vagina, with a monodelphic-prodelphic reproductive system, without larvated nor undeveloped eggs, only with numerous free larvae.

Taxonomic summary

Type host: *Leptodactylus melanonotus* (Hallowell, 1861). Representative specimens deposited at Colección Nacional de Anfibios y Reptiles, Instituto de Biología, Universidad Nacional Autónoma de México (CNAR)

Site of infection: posterior intestine.

Type locality: Coquimatlán-Pueblo Juárez Road, Coquimatlán, in Colima, Mexico (19°9'39.43" N, 103°53'0.51" W).

Other localities: Río Colima, Arroyo Pereyra, Río Manríquez, Colima, Río Comala, Comala, in Colima, Mexico.

Prevalence of infection: 43 of 137 examined (31.4%).

Type specimens deposited: CNHE 12079, holotype; CNHE 12080, paratypes.

Etymology: the name of the new species refers to the Mexican state of Colima, where the specimens were collected.

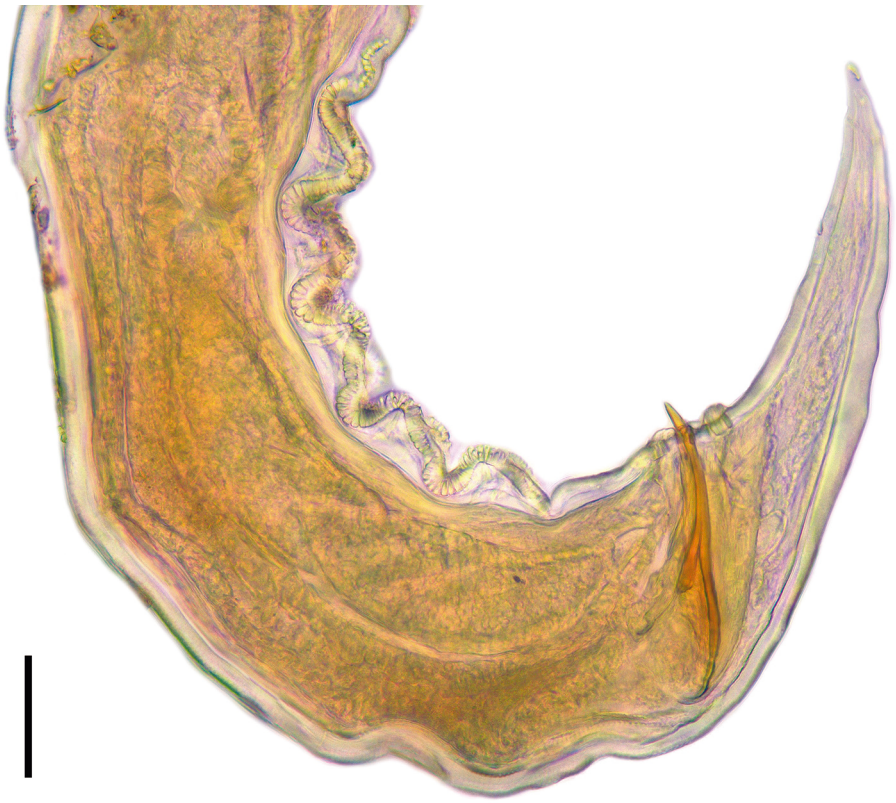

Figure 2. Digital photography of *Cosmocerca colimense* n. sp. Male. Detail of the cloacal region, lateral view. Scale bar = 50 µm.

Remarks

Species of *Cosmocerca* are common intestinal parasites associated with amphibians and reptiles worldwide. To date, 38 nominal species have been assigned to this genus (Alcántara et al., 2022); with this study, this number increases to 39 species. Of these, 35 are associated with amphibians, and 4 with reptiles (Table 2). The biogeographical realm with the highest number of described species is the Neotropical realm, with 13 species, followed by the Oriental and the Palearctic realms with 7 and 5 species, respectively (Table 2).

Until the present study, only 3 species of *Cosmocerca* had been recorded in Mexico (*C. acanthurum* Falcón-Ordaz, Winfield-Pérez, Mendoza-Garfias, Parra-Olea & Pérez-Ponce de León, 2007, *C. parva* Mordeglia & Digiani, 1998, and *C. podicipinus* Baker & Vaucher, 1984), being the new species the second described in the country. *Cosmocerca colimense* n. sp. shares the number of plectane pairs and the absence of lateral alae with *C. travassosi* Rodrigues & Fabio, 1970 and *C. cruzi* Rodrigues & Fabio, 1970 from the Neotropical realm, and with *C. lymnodynastes* Johnston & Simpson, 1942, from the Australian realm (Table 2). The new species differs from *C. travassosi* in the body length, being smaller in the new species (1.944 vs. 3.66). Regarding the male reproductive system, the spicules and gubernaculum are larger in *C. travassosi* than in *C. colimense* n. sp. (0.167 and 0.130 vs. 0.082 and 0.117, respectively). They also differ in the geographical distribution and the host group: *C. travassosi* was described in Brazil (Rio de Janeiro) as a parasite of *Hyla faber* (Rodrigues & Fabio, 1970). On the other hand, *C. colimense* n. sp. can be clearly distinguished from *C. cruzi* by the size of the spicules, which are larger in *C. colimense* n. sp. (0.082 vs. 0.074). Another differential character is the gubernaculum size, which is smaller in *C. cruzi* (0.08 vs. 0.117). Finally, one additional character that differentiates the 2 species is the position of the excretory pore; it opens at the level of the nerve ring in *C. cruzi* (Rodrigues & Fabio, 1970), but it is located near to the anterior margin of the oesophageal bulb in *C. colimense* n. sp. Regarding the Australian species, the new species can be differentiated from *C. lymnodynastes* on the basis of the body length, because this species is smaller than *C. colimense* n. sp. (1.6 vs. 1.94). Also, the spicules are slightly smaller in the Australian species than in *C. colimense* n. sp. (50-75 vs. 71-91). However, it should be

Table 2
Geographical, biological and morphometric characteristics relevant to the taxonomy of nominal species in *Cosmocerca.*

Geographical realm Species	Type host group	Body length mm	Spicules µm	Gubernaculum µm	Plectane pairs	Lateral alae	Reference (description)
Afrotropical							
C. daly	Anura	1-2.7	61-102	60-89	5	Present	Harnoster et al. (2022)
C. makhadooensis	Anura	1.50-2.29	38-103	98-110	5	Present	Harnoster et al. (2022)
C. monicae	Anura	1.77-2.13	87-107	100-108	5	Present	Harnoster et al. (2022)
Australian							
C. archeyi	Anura	1.77	95	87	4	Present	Baker and Green (1988)
C. lymnodynastes	Anura	1.6	50-75	110	5	Absent	Johnston and Simpson (1942)
C. multipapillata	Anura	3.10-3.55	169-219	125-146	10-12	Present	Ni et al. (2020)
C. australis	Anura	1.28-1.78	99-123	82-88	4	Present	Baker and Green (1988)
Nearctic							
C. acanthurum	Caudata	2.18-2.38	51-66	75-90	5	Present	Falcón-Ordaz et al. (2007)
Neotropical							
C. colimense n. sp.	Anura	1.87-2.04	71-91	97-124	5	Absent	Present study
C. paraguayensis	Anura	1.33-1.79	83-108	106-122	5	Present	Baker and Vaucher (1984)
C. rara	Anura	2.8	200	206	6	Present	Baker and Vaucher (1984)
C. albopunctata	Anura	6.25	89.6-99.8	119.7-157.8	7	Present	Alcántara et al. (2022)
C. gymnophthalmicola	Squamata	2.2	266	108	6	Absent	Ávila and Da Silva (2019)
C. podicipinus	Anura	2.97	94	134	5	Present	Baker and Vaucher (1984)
C. brasiliensie	Anura	2.24-3.99	158-185	140-156	8	Absent	Dyer and Altig (1976)
C. cruzi	Anura	2.99	74	85	5	Absent	Rodrigues and Fabio (1970)
C. parva	Anura	1.43-2.01	90-110	85-108	5-7	Present	Mordeglia and Digiani (1998)
C. travassosi	Anura	3.66	167	130	5	Absent	Rodrigues and Fabio (1970)
C. vrcibradici	Squamata	1.62-1.86	171-183	92-98	7	Present	Bursey and Goldberg (2004)
C. chilensis	Anura	1.4	80	84	6	Absent	Lent and Freitas (1948)
C. uruguayensis	Anura	2.42	155	133	7	Absent	Lent and Freitas (1948)
Oceanian							

Table 2. Continued

Geographical realm Species	Type host group	Body length mm	Spicules µm	Gubernaculum µm	Plectane pairs	Lateral alae	Reference (description)
C. novaeguineae	Anura	1.90	69	111	5	Present	Moravec and Sey (1990)
C. oroensis	Anura	0.77-0.99	37-52	24-29	4	Present	Bursey et al. (2013)
C. tyleri	Anura	1.02-1.43	37-43	58-61	9	Present	Bursey et al. (2006)
C. zugi	Squamata	1.63-1.84	79-116	92-122	4	Present	Bursey et al. (2005)
Oriental							
C. asansolensis	Anura	2.09-3.89	120-150	100-102	5	Present	Banerjee and Sou (2020)
C. bengalensis	Anura	1.90-2.69	100-130	Absent	5	Present	Sou et al. (2018)
C. ishaqui	Anura	2.92	100	Absent	11	Absent	Islam et al. (1981)
C. simile	Anura	.93-2.71	79-99	50-69	5-6	Present	Chen et al. (2020)
C. *kalesari*	Anura	1.11-1.92	82-90	85-90	5	Present	Rizvi et al. (2011)
C. microhylae	Anura	0.74-0.79	100-112	65-68	5	Present	Sou and Nandi (2015)
C. *leytensis*	Squamata	1.91	110	79	4	Present	Bursey et al. (2015)
Palearctic							
C. banyulensis	Anura	0.97	10	80	5-6	Present	Chabaud and Campana (1955)
C. commutate	Anura	4.03-4.39	180	186-213	7	Present	Moravec and Vojykova (1974)
C. longicauda	Caudata	2.90	92	190	6	Present	Skrjabin et al. (1961)
C. ornata	Anura	1.1-2.8	Rudimentary	100-120	5	Present	Skrjabin et al. (1961)
C. sardiniae	Caudata	3.41-4.18	375	260-303	4	Absent	Ricci (1987)
Panamanian							
C. longispicula	Anura	1.52-2.38	294-300	138	7	Present	Moravec and Kaiser (1994)
Sino-Japanese							
C. japonica	Anura	1.8	104	84	5	Present	Yamaguti (1938)

noted that Johnston and Simpson (1942) indicated that the length of the spicules was not determined with certainty in *C. lymnodynastes*. Therefore, this character should be considered with caution. More evident is the difference in the gubernaculum shape, which is spicule-shaped in *C. lymnodynastes*, while it shows a conical shape in *C. colimense* n. sp.

Mata-López et al. (2013) reported *C. podicipinus* in *L. melanonotus* of Colima, but after examining the material deposited at the CNHE (4608-10) from that study, we observed that those specimens correspond to *C. colimense* n. sp., because males lack the lateral alae that is characteristic in *C. podicipinus*, and also differ from that species and are similar to *C. colimense* in the body, spicules and gubernaculum length, which are shorter in the new species (1.87-2.04 vs. 2.97; 71-91 vs. 94 µm; 97-124 vs. 134 µm, respectively). The absence of lateral alae in males also distinguishes the new species from *C. acanthurum* and *C. parva*, the other 2 species of *Cosmocerca* that have been recorded in Mexico. Body length is another characteristic that differentiates *C. colimense* n. sp. from *C. acanthurum*, being shorter in the new species (1.87-2.04 vs. 2.18-2.38); also, the spicules and gubernaculum are shorter in *C. acanthurum* (51-66 vs. 71-91 µm, and 75-90 *v*s 97-124 µm, respectively) (Table 2). On the other hand, *C. colimense* n. sp. differs from *C. parva* mainly

in the length of the spicules, which is shorter in the new species (71-91 vs. 90 -100 µm) (Table 2); also, they differ in the shape of the gubernaculum, which is y-shaped in *C. parva*, and conical in *C. colimense* n. sp. (Mordeglia & Digiani, 1998).

Discussion

It is interesting to note that the helminth species richness found in this study was low compared with previous studies on these host species. Mata-López et al. (2013) recorded 8 helminth species (3 adult and 2 larval digeneans, 2 adult and 1 larval nematodes) in *L. melanonotus* in several localities of Colima, while we only found 1 digenean and 3 nematode species. In the case of *I. marmoreus*, previous record in Jalisco (Galicia-Guerrero et al., 2000) was similar in species richness (4 species vs. 3 in this study), but a study in Oaxaca resulted in a 10 taxa record. This could be the result of several factors, including the massive growth of urban and agricultural areas in recent years in Colima, with the consequent pollution of streams and rivers (Arceo et al., 2023; Villaseñor-Cortés et al, 2014). This condition affects mostly those helminths with complex life cycles; this could be the case of digeneans of the genus *Gorgoderina* (reported by Mata-López et al. [2013], and absent in this study) that use aquatic intermediate hosts and present free swimming larval stages (Velázquez-Urrieta & Pérez-Ponce de León, 2021). Also, in the Summer of 2021, Colima registered the highest volumes of precipitation in decades, 2,280 mm, the highest cumulative annual amount of all entities in Mexico during that year (Zavala-Fajardo, 2022), which resulted in high water levels and speed in the streams and rivers, washing away entire populations of amphibians. This also may have affected the intermediate hosts populations, such as molluscs or aquatic insects, and consequently, the recruitment of helminths in that season. On the other hand, nematode species, which have a direct life cycle as *Cosmocerca colimense* n. sp. (Kirillova & Kirillov, 2021), and which larval stages penetrate the amphibians´ skin directly, were widely distributed across the collecting sites.

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