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A NEW SPECIES OF NEMATODE (MOLINEIDAE) FROM *RHINELLA MARINA* (AMPHIBIA: BUFONIDAE) IN GUERRERO, MÉXICO

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ABSTRACT: *Oswaldocruzia lamotheargumedoi* n. sp., inhabiting the intestine of the cane toad, *Rhinella marina* (L.), in Laguna de Coyuca, Guerrero, México, is described here. The new species differs from 10 congeners infecting bufonid hosts because it has a type I bursa. In contrast, 7 of these species have type II bursa and 3 more a type III bursa. The species most similar to the species described herein is *Oswaldocruzia pipiens* Walton, 1929. These 2 species share traits such as body size, bursa type, presence of cervical alae, and dorsal ray morphology. Nevertheless, both species can be distinguished based on the number of synlophe ridges at mid-body (54–56 for *O. lamotheargumedoi* vs. 45–48 for *O. pipiens*) and by the presence of a chitinous support in the long, and well developed, cervical alae of *O. pipiens*. In the new species, these structures are short, poorly developed, and lack chitinous support. Previous records of species of *Oswaldocruzia* in México include *Oswaldocruzia subauricularis* (Rudolphi, 1819) Travassos, 1917 in the Neotropical Realm and *O. pipiens* in the Nearctic.

To date, 50 taxa of helminths (32 nominal species and 18 undetermined) have been recorded parasitizing the cane toad, *Rhinella marina* (L.), in México (Pérez-Ponce de León et al., 2011). Twenty-nine of these taxa are nematodes, with 11 trematodes, 3 cestodes, 5 acanthocephalans, 1 monogenean, and 1 hirudinean. This information is based on 39 localities situated in 11 Mexican states, 7 in the Neotropical Realm, and 4 in the Nearctic (Paredes-Calderon et al., 2008). These records establish *R. marina* as the most species-rich amphibian host and the most widely sampled in México (see Pérez-Ponce de León et al., 2011). However, it has been noted that the helminthological record for this host species could increase even further inventory work in poorly sampled regions (Espinoza-Jiménez et al., 2007). In this context, the main goal of the present work is to describe a new species of *Oswaldocruzia* Travassos, 1917, collected in cane toads from a previously unsampled site. The new species is compared with the 34 congeners distributed in the Neotropical Realm and with *Oswaldocruzia pipiens* Walton, 1929, a Nearctic species collected in central and northern México (see Paredes-León et al., 2008).

MATERIALS AND METHODS

In August 2010, 68 specimens of *R. marina* were collected in Los Mogotes (16°52′59′′N, 100°04′31′′W), a locality near Laguna de Coyuca, Guerrero, México, with permit FAUT 0084. Hosts were killed with an overdose of intraperitoneal sodium pentobarbital, necropsied within the following 4 hr, and examined for helminth parasites. Nematodes were removed from the intestine and placed in saline solution (0.65%), fixed in hot 4% formaldehyde, and preserved in 70% ethanol. Specimens were cleared with Amman's lactophenol and temporarily mounted for morphological study. Measurements (expressed in millimeters unless otherwise stated) are given as the range, with means, standard deviation, and sample size in parentheses, followed by measurements of holotype and allotype, also in parentheses. Figures were drawn with the aid of a drawing tube. Specimens for scanning electron microscopy were dehydrated with a graded ethanol series, critical-point dried with CO_2, and then coated with gold–palladium mixture. Specimens were examined with an S2460N electron microscope (Hitachi, Tokyo, Japan). Prevalence and mean intensity were used following Bush et al. (1997). Type specimens were deposited the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, México City, México.

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DESCRIPTION

Oswaldocruzia lamotheargumedoi n. sp.

(Figs. 1, 2)

Diagnosis: Small, slender nematodes. Evident sexual dimorphism; males approximately three-fourths length of females. Anterior region with simple cephalic cuticular vesicle, transversally striated. Short cervical alae. Synlophe: cuticle bears uninterrupted longitudinal crests, without reinforcement. Mouth with 3 simple, inconspicuous lips; dorsal lip with 2 sessile papillae; ventrolateral lips with 1 sessile papillae each, and lateral amphid. Excretory gland well developed; excretory pore without circular cuticular disc around it, situated on posterior third of esophagus.

Male (based on 1 holotype and 3 paratypes): Total length 10–11.7 (10.84 ± 0.57, n = 12) (11.01); width at spicular level 0.13–0.21 (0.17 ± 0.2, n = 6) (0.14). Cuticular inflation 0.036–0.045 (0.042 ± 0.003, n = 5) (0.045) length by 0.041–0.047 (0.044 ± 0.002, n = 4) (0.45) width, rising 0.0043–0.006 (0.0048 ± 0.001, n = 4) (0.006) above body wall. Synlophe: crests starting behind cephalic inflation and ending slightly anterior to bursa; 35 crests at esophageo-intestinal junction; 54–56 (55, n = 2) at mid-body region and 50 at pre-spicule level. Claviform esophagus 0.41–0.55 (0.49 ± 0.05, n = 10) (0.47) length, 0.06–0.075 (0.06 ± 0.006, n = 10) (0.06) width posteriorly. Nerve ring and excretory pore 0.22–0.32 (0.25 ± 0.02, n = 10) (0.26) and 0.38–0.48 (0.43 ± 0.03, n = 10) (0.29) from anterior end, respectively. Excretory pore-esophagous ratio: 0.68. Deirids not observed. Bursa 3-lobed, type I (sensu Ben Slimane et al., 1996), 2-1-2 pattern; rays 6 and 8 arise independently of dorsal ray; origin of rays 8 on and perpendicular to dorsal ray, not touching the edge of bursal membrane. Rays 8 turning caudal near its distal end, separate from rays 6 along entire length. Rays 2 and 3 connected throughout, without touching the margin of the bursal membrane. Rays 4, 5, and 6 with common origin, 4 separated from 5 and 6, not reaching bursal margin; rays 5 and 6 running together, turning caudal, without touching the edge of bursal membrane. Dorsal ray conical 0.065–0.088 (0.080 ± 0.008, n = 6) (.09) in length, originating from a common base with rays 4–6. Rays 9 arising distally on trunk of dorsal ray, before division of the latter in 2 branches at each side. Prominent genital cone [0.023 (0.028) length, 0.034 (0.03) width at its base], bearing a large papilla "0" on anterior lip, and 2 minute papillae 7 on posterior lip. Spicules 0.19–0.23 (0.214 ± 0.012, n = 10) (0.219) long, divided proximally in 3 main branches: shoe, blade (distally divided into 12 unequal processes), and fork. Fork bifurcated (at 32.2% [32.8%] of whole length of spicule), right branch further divided into 2 branches, 1 of which also bifurcates.

Female (based on 1 allotype and 9 paratypes): Total length 12.18–16.80 (15.16 ± 1.58, n = 6) (17.72); width at vulva level: 0.043–0.054 (0.048 ± 0.003; n = 10) (0.25). Cuticular inflation 0.041–0.047 (0.044 ± 0.002; n = 5) (0.47) length by 0.043–0.054 (0.048 ± 0.003, n = 10) (0.04) width, rising 0.004–0.006 (0.005 ± 0.001, n = 10) (0.006) above body wall. Synlophe: crests starting behind cephalic inflation and terminating on tail; 38 crests

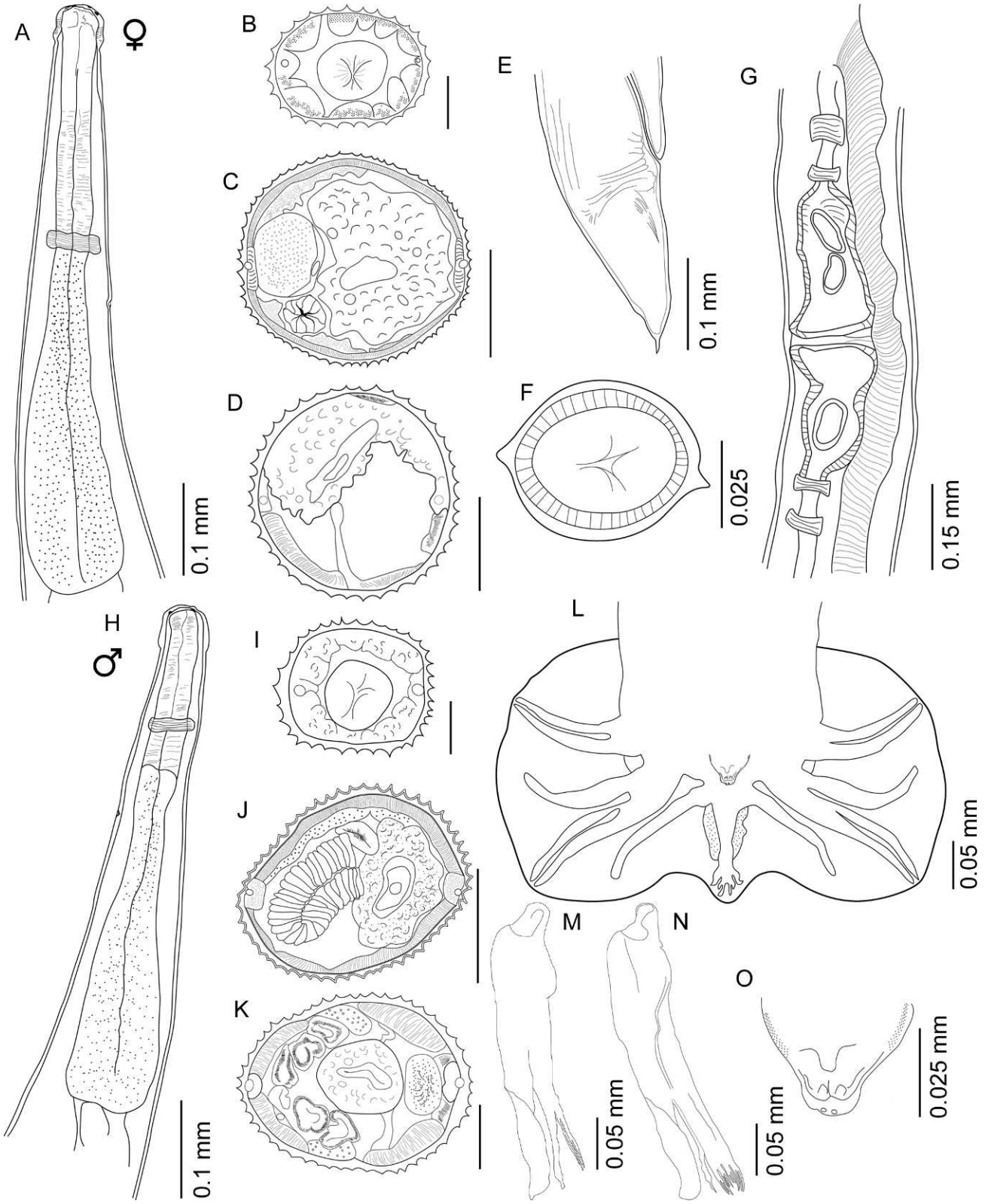

FIGURE 1. *Oswaldocruzia lamotheargumedoi* n. sp., a parasite of *Rhinella marina* from Laguna de Coyuca, Guerrero, México. (**A**) Female, anterior end, lateral view. (**B**) Synlophe at esophageo-intestinal junction. Bar = 0.025 mm. (**C**) Synlophe at mid-body region. Bar = 0.05 mm. (**D**) Synlophe at anus level. Bar = 0.05 mm. (**E**) Caudal region, lateral view. (**F**) Synlophe at cervical alae level. (**G**) Vulva region, showing ovojector. (**H**) Male, anterior end, lateral view. (**I**) Synlophe at esophageo-intestinal junction. Bar = 0.025 mm. (**J**) Synlophe at mid-body region. Bar = 0.05 mm. (**K**) Synlophe at pre-spicule level. Bar = 0.05 mm. (**L**) Caudal bursa, ventral view. (**M**) Left spicule, shoe and fork, latero-ventral view. (**N**) Left spicule, shoe and blade, dorsal view. (**O**) Genital cone.

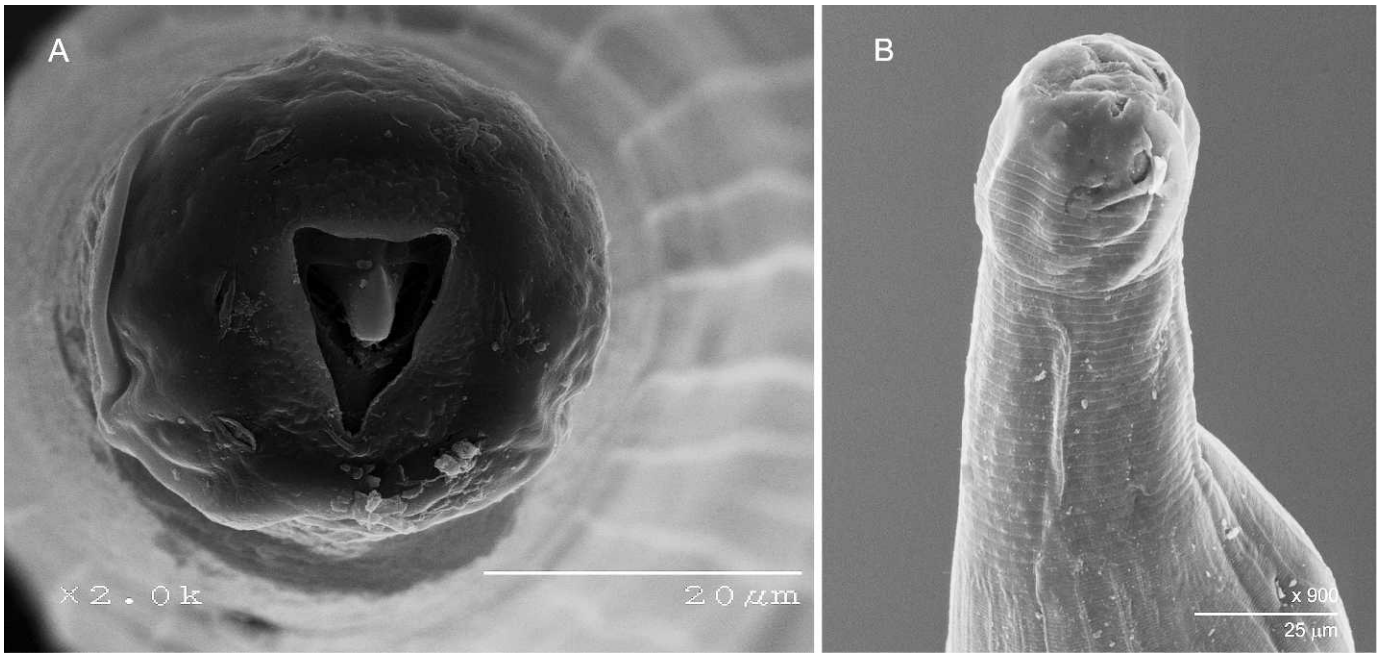

FIGURE 2. *Oswaldocruzia lamotheargumedoi* n. sp., a parasite of *Rhinella marina* from Laguna de Coyuca, Guerrero, Mexico. Scanning electron micrographs: oral opening, frontal view (**A**) and (**B**) cervical alae, lateral view (**B**).

at esophageo-intestinal junction; 74 at mid-body region and 41 at anus level. Claviform esophagus 0.5–0.6 (0.54 ± 0.03, n = 10) (0.57) in length by 0.07–0.08 (0.078 ± 0.006, n = 10) (0.09) in width posteriorly. Nerve ring and excretory pore 0.23–0.35 (0.27 ± 0.03, n = 10) (0.34) and 0.42–0.52 (0.47 ± 0.03, n = 8) (0.37) from anterior end, respectively. Tail 0.13–0.22 (0.17 ± 0.02, n = 10) (0.215) length, ending in flexible filament approximately 0.012–0.016 (0.013 ± 0.001, n = 10) (0.01) in length. Vulva at 3.87–6.22 (5.12 ± 0.79, n = 10) (6.29) from caudal extremity; vulvar opening transverse; lips not prominent. Vagina short 0.16–0.18 (0.17 ± 0.006, n = 4) (0.19) length, entering totally in vestibule, dividing it in 2 equivalent portions, 0.19–0.32 (0.24 ± 0.06, n = 3) (0.20) in length. Sphincter 0.086–0.09 (0.088 ± 0.002, n = 3) (0.08) long by 0.10–0.12 (0.11 ± 0.007, n = 9) (0.11) wide at junction of vestibule and uterus; amphidelphic; eggs in single file 0.07–0.09 (0.08 ± 0.09, n = 16) (0.07) long by 0.04–0.06 (0.05 ± 0.004, n = 16) (0.04) wide; maximum number found in uterus 215 (193).

Taxonomic summary

Type host: Rhinella marina (L.).

Type locality: Laguna de Coyuca (18°39′28″N, 95°40′25″W), Guerrero, México.

Site of infection: Intestine.

Prevalence and mean intensity: Six of 68 analyzed hosts (9.09%); 10.66 nematodes per infected host.

Type specimens: Holotype: CNHE 8260 (male), allotype CNHE 8261 (female); paratypes: CNHE 8262 (14 females, 3 males).

Etymology: The species is named after Dr. Marcos Rafael Lamothe y Argumedo, celebrating 80 yr of his productive life in the academic world.

Remarks

According to Bursey and Goldberg (2011), 86 nominal species of *Oswaldocruzia* have been described worldwide. Of these species, 39 are distributed in the Neotropical Realm, with 24 parasitizing amphibians, 14 reptiles, and 1 (*Oswaldocruzia jeanbarti* Ben Slimane, Durette-Desset and Chabaud, 1995) that infects both host groups. *Oswaldocruzia lamotheargumedoi* n. sp. can be separated from 12 of the 14 Neotropical species parasitizing reptiles on the basis of the bursal type (type I in the new species, types II and III in the other 12 species). The remaining 2 species, *Oswaldocruzia brasiliensis* Lent and Freitas, 1935 and *O. vitti* Bursey and Goldberg, 2004, have the same bursal type as *O. lamotheargumedoi*. However, both species differ from the Mexican species by having smaller spicules (0.112–1.135, 0.120–0.150 vs. 0.190–0.230, respectively) and a distinct spicule blade terminus (spatulate in *O. brasiliensis*, with 3 bifurcating processes in *O. vitti* and 12 processes in the new species) (Lent and Freitas, 1935; Bursey and Goldberg, 2004).

Ten of the 24 species of *Oswaldocruzia* that parasitize amphibians have been described from bufonid host species, including the new species; this information notwithstanding, *O. lamotheargumedoi* can be distinguished from all of these other species because the new species has a type I bursa, whereas 7 of these species (*O. barusi* Ben Slimane and Durette-Desett, 1995; *O. belenensis* Santos, Maldonado and Lanfredi, 2008; *O. dlouhyi* Ben Slimane and Durette-Desset, 1995; *O. lescurei* Ben Slimane and Durette-Desset, 1996; *O. mazzai* Travassos, 1935; *O. proencai* Ben Slimane and Durette-Desset, 1995; and *O. venezuelensis* Ben Slimane, Guerrero and Durette-Desset, 1996) have a type II bursa and 3 more species (*O. chambrieri* Ben Slimane and Durette-Desset, 1993; *O. subauricularis* (Rudolphi, 1819) Travassos, 1917; and *O. taranchoni* Ben Slimane and Durette-Desset, 1995) have a type III bursa. Regardless of bursal type, 5 of these species (*O. barusi*, *O. belenensis*, *O. chambrieri*, *O. dlouhyi*, and *O. venezuelensis*) differ from the Mexican species because the number of synlophe ridges at mid-body region is <50, whereas in the new species this number is 55. From *O. proencai* and *O. taranchoni*, for which no ridge number has been determined (Ben Slimane and Durette-Desset, 1995), *O. lamotheargumedoi* is separated by having cervical alae (absent in the other 2 species) and larger spicules (0.190–0.230 in *O. lamotheargumedoi* vs. 0.150–0.200 in *O. proencai* and 0.175 in *O. taranchoni*). Finally, the 3 remaining species (*O. lescurei*, *O. mazzai*, and *O. subauricularis*) can be distinguished from the new species by the number of processes at the spicule blade (6, 10, and 8 vs. 12, respectively).

Ten additional species of *Oswaldocruzia* recorded as parasites of several families of Neotropical amphibians differ from the new species by having type II caudal bursa (*Oswaldocruzia albarerti* Ben Slimane and Durette-Desset, 1996; *Oswaldocruzia costaricensis* Bursey and Goldberg, 2005; *Oswaldocruzia moraveci* Ben Slimane and Durette-Desset, 1995; *Oswaldocruzia touzeti* Ben Slimane and Durette-Desset, 1993) or type III (*Oswaldocruzia brevispicula* Moravec and Kaiser, 1995; *Oswaldocruzia cassonei* Ben Slimane and Durette-Desset, 1996; *Oswaldocruzia chabaudi*

Ben Slimane and Durette-Desset, 1996; *Oswaldocruzia lenteixeirai* Pérez-Vigueras, 1938; *Oswaldocruzia petterae* Ben Slimane and Durette-Desset, 1996; and *Oswaldocruzia tcheprakovae* Ben Slimane and Durette-Desset, 1996). Moreover, 3 of the 4 species with type II bursa can be distinguished from *O. lamotheargumedoi* by having a smaller number of synlophe ridges at mid-body (20–43 vs. 55); the fourth species (*O. moraveci*) is smaller (7.6–8.5 vs. 10–11.7, respectively), parasitizes a different host family (Hylidae vs. Bufonidae), and has smaller spicules (0.144–0.179 vs. 0.190–0.230) (Ben Slimane and Durette-Desset, 1995). Five of the 6 species with the type III bursa lack cervical alae that are present in the Mexican species; the remaining species (*O. brevispicula*) has cervical alae similar to those described for the new species, but its cuticular longitudinal ridges are poorly developed (almost indistinct in lateral view), and their body and spicule sizes are smaller (2.5–2.7, 0.093 vs. 10–11.5 and 0.190–0.230, respectively) (Moravec and Kaiser, 1995).

Oswaldocruzia lamotheargumedoi closely resembles *Oswaldocruzia bonsi* Ben Slimane and Durette-Desset, 1993; *Oswaldocruzia cartagoensis* Bursey and Goldberg, 2011; *Oswaldocruzia lopesi* Freitas and Lent, 1938; and *Oswaldocruzia neghnei* Puga, 1981 by having a type I bursa. However, these 4 species can be easily differentiated by the spicule size (0.110–0.160 vs. 0.190–0.230 in the new species). In addition, *O. bonsi*, *O. cartagoensis*, and *O. neghnei* have a smaller number of synlophe ridges at mid-body (38–50, 20, and smooth, respectively, rather than 55 as in our specimens). Finally, the presence of 12 processes in the spicule blade terminus of *O. lamotheargumedoi* is characteristic of this species and allows us to distinguish it from *O. lopesi* that has 4 processes (Ben Slimane and Durette-Desset, 1993; Bursey and Goldberg, 2011).

The only Neotropical species reported as a parasite of both amphibians and reptiles, *O. jeanbarti*, is separated from the new species based on the bursa type (III vs. I, respectively), smaller size of spicules (0.185 rather than 0.190–0.230), and number of synlophe ridges at mid-body (50 vs. 55) (Ben Slimane et al., 1996).

In addition to *O. lamotheargumedoi*, 2 species of *Oswaldocruzia* have been recorded in México infecting amphibians, i.e., *O. subauricularis* (distributed in the Neotropics) and *O. pipiens* (in the Nearctic). The first species, a common parasite of bufonids, hylids, leptodactylids, and ranids in México (Paredes-León et al., 2008), is differentiated (see above) from the new species in this work. The other species (*O. pipiens*) closely resembles *O. lamotheargumedoi* in several traits: a type I bursa, the presence of cervical alae, and the dorsal ray morphology (Walton, 1929); both species also can be distinguished based on number of synlophe ridges at mid-body (45–48 vs. 54–56, respectively) and by the presence of chitinous support in the long and well-developed cervical alae of *O. pipiens* (Ben Slimane and Durette-Desset, 1997), whereas in our species, these structures are short, poorly developed, and lack chitinous support. Other characteristics that allow us to differentiate *O. lamotheargumedoi* from *O. pipiens* are the size (averaging 0.215 vs. 0.175, respectively) and morphology (with blade terminus divided in 12 processes in *O. lamotheargumedoi* and spatulate in *O. pipiens*) of the spicule (Baker, 1976).

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