



A new gorgoderid species of the urinary bladder of *Rana zweifeli* from Michoacán, Mexico

Una nueva especie de gorgodérido de la vejiga urinaria de *Rana zweifeli* de Michoacán, México

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Abstract. *Gorgoderina tarascae* n. sp. is described from the urinary bladder of *Rana zweifeli*, from Cutzaróndiro, Michoacán, Mexico. *Gorgoderina tarascae* differs from the other species of the genus by possessing the following combination of characters: follicular vitelline glands, arranged in two clusters of 4-7 follicles dorsal to the ovary, compact and smooth reproductive organs situated far from each other and from the acetabulum, suckers close to each other, and a slender, spindle shaped body with a flexed position due to the situation of the acetabulum.

Key words: *Gorgoderina tarascae* n. sp., Digenea, Amphibia, Cutzaróndiro.

Resumen. *Gorgoderina tarascae* n. sp. se describe como parásita de la vejiga urinaria de *Rana zweifeli* de Cutzaróndiro, Michoacán, México. *Gorgoderina tarascae* difiere del resto de las especies del género por la siguiente combinación de características: posee glándulas vitelinas arregladas en dos racimos de 4-7 folículos situados dorsalmente al ovario, órganos reproductivos compactos y de bordes lisos, muy separados entre si y con respecto del acetáculo, ventosas cercanas una de la otra y cuerpo delgado, en forma de huso y flexionado debido a la posición del acetáculo.

Palabras clave: *Gorgoderina tarascae* n. sp., Digenea, Amphibia, Cutzaróndiro.

Introduction

The genus *Gorgoderina* Looss, 1902, currently comprising 53 species, has a worldwide distribution (Mata-López et al., 2005; Mata-López and León-Règagnon, 2005). Six species of this genus have been reported in Mexico (Table 1). *Rana zweifeli* Hillis, Frost and Webb is a native Mexican amphibian that inhabits tropical seasonal forest at low elevations, such as the Pacific coast of Mexico, the Balsas River basin and central depression of Chiapas. In addition, it has also been collected from highland localities of the Transverse Volcanic Axis, the Sierra Madre del Sur and the highlands of northern Oaxaca by Flores-Villela (1993). It is highly associated with water such as streams, rivers and permanent and temporary ponds where it also breeds, and is an abundant species throughout most of its range (InfoNatura, 2005).

As part of an inventory of amphibian helminths in Mexico, a new gorgoderid species was collected from

the urinary bladder of *Rana zweifeli* in Cutzaróndiro, Michoacán. The goal of this paper is to describe the new species based on morphological data.

Materials and Methods

Four specimens of *Rana zweifeli* were collected in September 2003 in Cutzaróndiro, Michoacán, Mexico and examined for helminth parasites. Worms were removed from hosts and placed in 0.6% saline solution, whereas others were fixed in hot 4% buffered formaldehyde and preserved in 70% ethanol for light microscopy. Specimens for light microscopy were stained in Mayer's paracarmine, dehydrated in a graded series of ethanol, cleared in methyl salicylate, and whole mounted in Canada balsam. Three of the collected specimens were saved for scanning electron microscopy (SEM). They were fixed as described above, dehydrated in a graded series of ethanol, critical point dried, sputter coated with a gold-palladium mixture, and examined using a Hitachi S2460N scanning electron

Table 1. Records of *Gorgoderina* Looss, 1902 in Mexico.

Species	Host	Locality	Author
<i>G. attenuata</i> (Stafford, 1902)	<i>Rana montezumae</i> <i>R. dunni</i>	Mexico City Zacapu, Michoacán	Sokoloff and Caballero, 1932 Lamothe et al., 1997
Stafford, 1905	<i>R. vaillanti</i> <i>R. megapoda</i> <i>R. neovolcanica</i> <i>Ambystoma tigrinum</i> <i>A. lermaensis</i> <i>A. andersoni</i> <i>Leptodactylus melanotus</i>	Laguna Escondida, Veracruz Cointzio, Michoacán Lerma, Mexico State Sonora	Pérez-Ponce de León et al., 2000 Goldberg et al., 2002 Goldberg and Bursey, 2002 Mata-López et al., 2005
<i>G. parvicava</i> Travassos, 1922	<i>R. berlandieri</i> <i>R. vaillanti</i>	Los Tuxtlas, Veracruz	Lamothe et al., 1997 Guillén-Hernández et al., 2000 Paredes-Calderón et al., 2004
<i>G. megalorchis</i> Bravo, 1948	<i>Bufo marinus</i> <i>Hyla miotympanum</i>	Tuxtepec, Oaxaca Villa Santiago, Nuevo León	Bravo, 1948 Lamothe et al., 1997
<i>G. rhyacosiredonis</i> (Bravo, 1943) Prudhoe and Bray, 1982	<i>A. altamirani</i>	Mexico City	Bravo, 1943
<i>G. skarvilovitschi</i> Pigulevsky, 1953	<i>R. montezumae</i>	Unspecified locality in Central Mexico	Pigulevsky, 1953
<i>G. festoni</i> Mata- López and León- Règagnon, 2005	<i>Gastrophryne usta</i> <i>L. melanotus</i>	Los Tuxtlas, Veracruz; Arcelia and San Vicente, Guerrero; Río Armeria tributary, Colima; Paso Canoa, Oaxaca.	Mata-López and León- Règagnon, 2005

microscope. Measurements are given as ranges with means in parentheses expressed in millimeters. Specimens used for both light and scanning electron microscopy were deposited in the Colección Nacional de Helmintos (CNHE) at the Instituto de Biología of the Universidad Nacional Autónoma de México; accession numbers follow description. The following abbreviations are used: CNHE, Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México; CHIOC, Coleção Helmitológica do Instituto Oswaldo Cruz,

Fundaçao Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; HWML, Harold W. Manter Laboratory of Parasitology, Lincoln, Nebraska; NBM, New Brunswick Museum, New Brunswick, Canada; USNPC, United States National Parasite Collection, Beltsville, Maryland, USA; PCDRB: personal collection of Daniel R. Brooks. The following specimens were examined for comparison: *Gorgoderina attenuata*: CNHE: 1178- 1180, 1182, 1446, 1544-1548, 2416, 3401-3405, 3412, 3413, 3793, NBM: 3542, 10 not catalogued, HWML: 740, 17079, 20121-20126, 20888,

20955, 21344, 21949, 24898, 31259, 33206, USNPC: 542-14/18, M1288-16, M1546-17, CHIOC: 10563, CPDRB: 1 specimen; *G. bilobata*: USNPC: M182-14/16 (type), HWLM: 15239; *G. bufonis*: HWML: 21309-21315; *G. carioca*: CHIOC: 22017, 22018a-d; *G. diaster*: NHM: 1980. 11. 12. 7-8 (type), HWLM: 20250, CHIOC: 17426, 25255, 25256, PCDRB: 1 specimen; *G. festoni*: CNHE: 3426 (type), 3427, 5100-5102; *G. megalorchis*: CNHE: 810 (type), 1447; *G. multilobata*: USNPC: 350-12 (type), HWLM: 23427 (paratype), 23428; *G. rochalimai*: CHIOC: 22019a-m, 22020, 22021; *G. simplex*: USNPC: M1288-17, NBM: 784, 788, 789, 790, 2 not catalogued, HWLM: 20112-20120, PCDRB: 1 specimen; *G. translucida*: HWLM: 20111, PCDRB: 1 specimen.

Description.

Gorgoderina tarascae n. sp. (Figs. 1-3)

Description based on 6 specimens, 3 examined using light microscopy and 3 with SEM. Body slender, spindle shaped, anterior end blunt, posterior end pointed; body length (BL) 8.66-9.6 (9.23). Forebody 0.97-1.19 (1.06) long (10.3-12.41% [11.52%] of BL), 0.52 wide at level of cecal bifurcation. Hindbody 7.38-8.41 (7.84) long (81.71-87.59% [84.86%] of BL), 0.88 of maximum width at level of vitelline glands; width at level of acetabulum 0.71 (Fig. 1A). Tegumental smooth. Oral sucker surface with papillae, two pairs of papillae flanking the stylet pit on apical region of body, 1 dorsal (Figs. 2B and 3, SP I) and 1 on the ventral base of stylet pit (Figs. 2B and 3, SP II). Three lateral pairs of papillae between stylet pit and oral sucker (Figs. 2B and 3, SP III-SP V). Penetration gland openings situated around the stylet pit (Fig. 2C). Oral sucker subterminal, spherical, 0.37-0.44 (0.40) long, 0.42 wide; oral opening ventral (Figs. 1A and 2A); 10 papillae surrounding oral opening, fourth and seventh double (Figs. 2A and 3, OS I-X). One additional pair of papillae on anterior lip and 1 pair on lateral borders of the oral opening (Figs. 2A and 3, OS XI, OS XII). Pharynx absent. Esophagus thick walled, 0.24-0.28 (0.26) long (2.59-2.97% [2.84%] of BL), 0.04-0.06 (0.05) wide, esophageal glands present. Intestinal bifurcation at 0.54-0.65 (0.61) (6.33-6.97% [6.57%] of BL) from anterior end of body. Ceca simple, smooth walled; right ceca ending at 0.31-0.51 (0.44) (3.57-5.37% [4.74%] of BL) and left ceca ending at 0.12-0.86 (0.58) (1.45-8.98% [6.14%] of BL) from posterior end of body. Ventral sucker 0.7-0.72 (0.72) long, 0.7 wide, located at 0.53-0.78 (0.67) (5.66-8.13% [7.23%] of BL) from oral sucker. Six papillae on external margin of acetabulum (Figs. 2F and 3, VS I-VI). Sucker ratio 1: 1.65-1.96 (1.79).

Testes 2, oval, compact, with smooth borders, situated

in tandem, intercecal at the end of the second third of the body; anterior testis larger than posterior testis, dorsally overlapping right cecum, at 3.15-3.79 (3.43) (33.38-39.51% [37.11%] of BL) from posterior margin of ventral sucker; 0.44-0.57 (0.50) long (4.64-5.99% [5.44%] of BL), 0.33 wide; posterior testis medial at 4.38-4.94 (4.59) (46.44-51.5% [49.79%] of BL) from posterior margin of ventral sucker; 0.42-0.6 (0.53) long (4.50-6.33% [5.70%] of BL), 0.22 wide. Sperm duct junction 1.49-2.16 (1.90) from posterior border of acetabulum. Seminal vesicle oval, 0.15-0.34 (0.27) long (1.62-3.92% [2.93%] of BL), 0.11 wide, partially overlapping anterior region of acetabulum (Fig. 1D). Distal end of seminal vesicle surrounded by prostatic cells, opening into genital atrium; ejaculatory duct 0.18-0.21 (0.20) long, 0.11 wide. Genital pore medial anterior to ventral sucker, at 0.72-0.83 (0.77) (7.98-8.38% [8.35%] of BL) from anterior end of body.

Ovary oval, compact, with smooth edges, on midline of the body, overlapping vitellaria ventrally, at 2.16-2.87 (2.49) (22.93-29.96% [27.02%] of BL) from posterior end of acetabulum, 0.26-0.33 (0.29) long (2.93-3.56% [3.16%] of BL), 0.25 wide. Vitellarium in two clusters of 4-7 follicles, at 1.89-2.57 (2.18) (20.03-26.82% [23.58%] of BL) from posterior margin of acetabulum on middle line of body; right vitelline cluster with 4-7 follicles; left vitelline cluster with 4 follicles. Mehlis's gland at level of vitelline glands; Laurer's canal opening dorsally at level of ovary. Uterine loops filling postacetabular region and overlapping dorsally and ventrally with testes, partially overlapping ovary and forming intercecal loops between vitelline glands and acetabulum, opening to genital atrium. Eggs thin-shelled, embryonated, 0.025-0.029 (0.027) x 0.017-0.022 (0.019). Bifurcation of excretory vesicle at 3.26-3.83 (3.64) (37.65-40.64% [39.41%] of BL) from posterior end of body. Excretory pore terminal (Fig. 2H).

Taxonomic summary

Type-host: *Rana zweifeli* Hillis, Frost and Webb, 1984 (Ranidae).

Site of infection: Urinary bladder.

Type-locality: Cutzarón diro, Michoacán, Mexico ($19^{\circ} 10' 59''$ N, $101^{\circ} 30' 31''$ W).

Prevalence and intensity: 17 digenetic specimens in 4 examined hosts.

Type-specimens: Holotype: CNHE 5334; paratypes: CNHE 5335.

Etymology: The specific epithet refers to the Tarasca people of Michoacán and neighboring regions of Mexico.

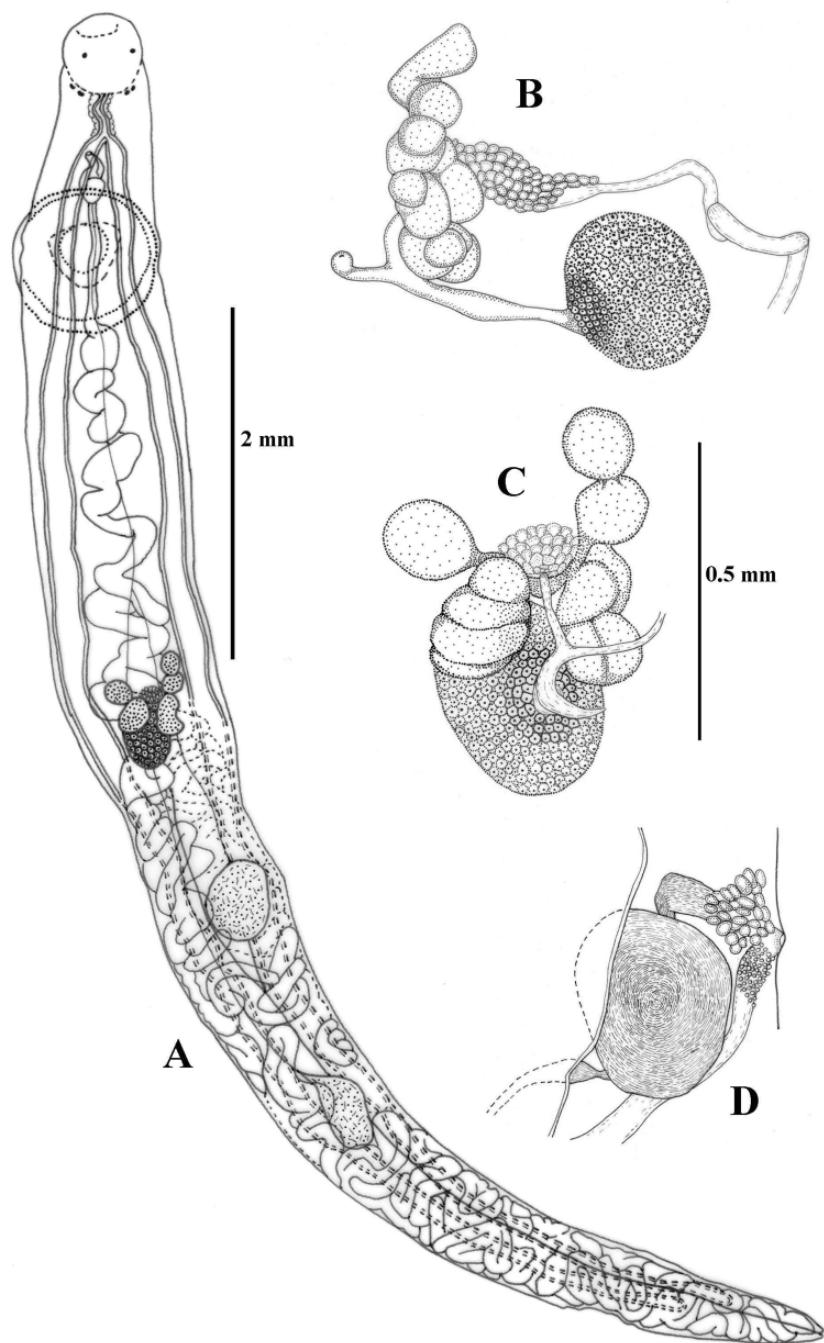


Figure 1. *Gorgoderina tarascae* n. sp. **A**, whole specimen, dorsal view. **B-C**, vitelline glands, ovary and Mehlis' gland, lateral and dorsal view, respectively. **D**, seminal vesicle, ejaculatory duct, metraterm and genital pore, lateral view.

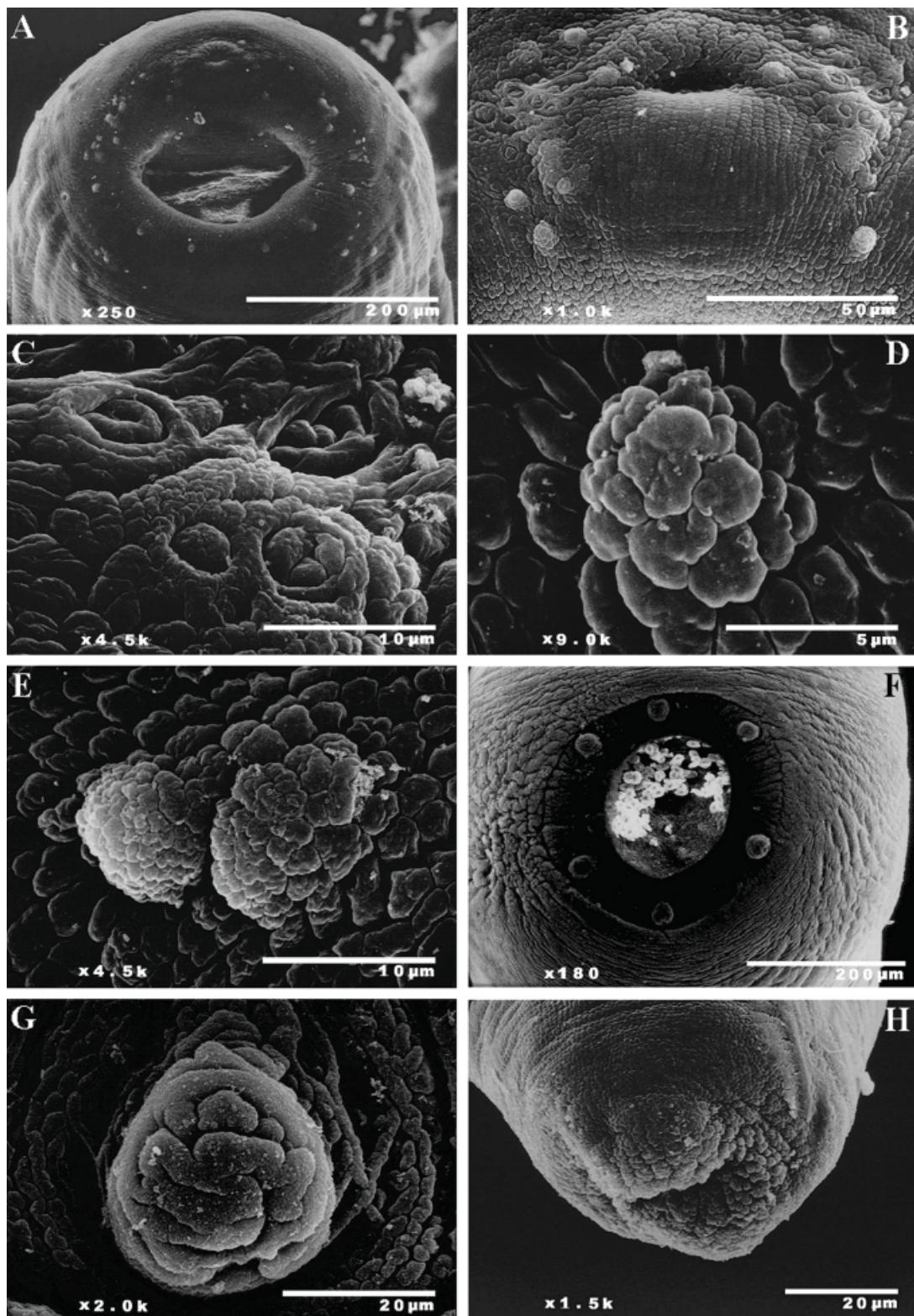


Figure 2. SEM photomicrographs of *Gorgoderina tarascae* n. sp. **A.** Oral sucker. **B.** Stylet pit. **C.** Penetration gland openings. **D.** Single papilla on oral sucker surface. **E.** Double papilla on oral sucker surface. **F.** Ventral sucker. **G.** Papilla on external border of ventral sucker. **H.** Excretory pore.

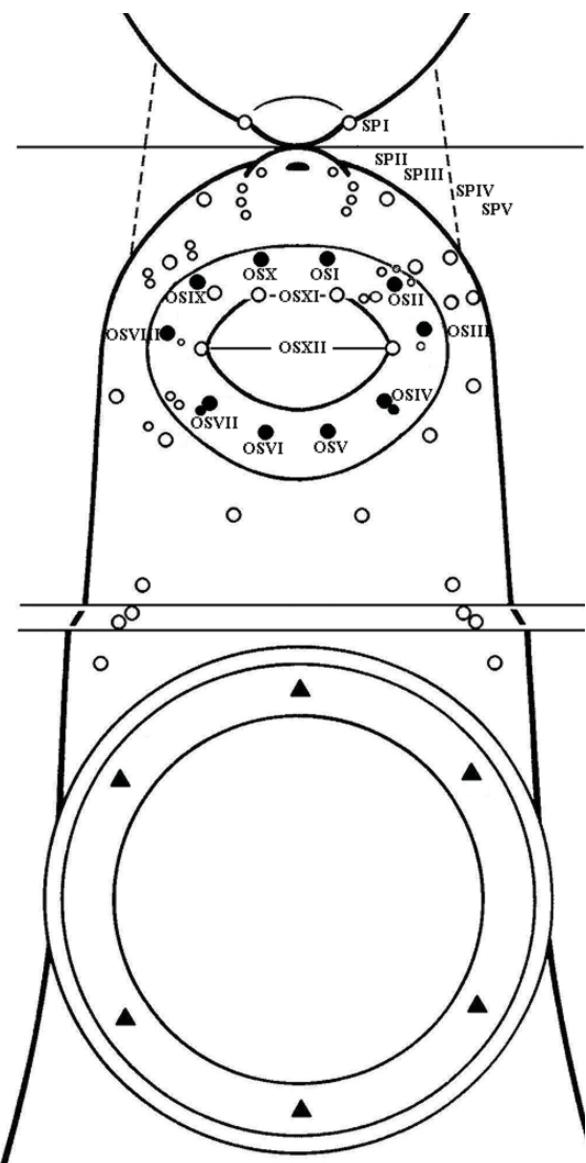


Figure 3. Papillar pattern on the ventral surface of *Gorgoderina tarascae* n. sp. (modified from Bakke and Hoole, 1988).

Remarks

The combination of characters distinguishing *Gorgoderina tarascae* n. sp. from the other *Gorgoderina* species is: vitelline glands arranged in two clusters of 4-7 follicles dorsal to the ovary and far posterior to the acetabulum; compact and smooth gonads situated far from each other, suckers close to each other, and the shape and size of the body.

Gorgoderina tarascae n. sp. most closely resembles the following species in possessing vitelline glands arranged in

clusters of follicles (Mata López et al., 2005): *G. carioca* Fernandes, 1958 (right cluster= 7, left cluster= 12-13), *G. diaster* Lutz, 1926 (right cluster= 12, left cluster= 7-8), *G. multilobata* Ingles and Langstone, 1933 (right cluster= 7-9, left cluster= 7-9), *G. pigulevskyi* Fernandes, 1958 (right cluster= 5-6, left cluster= 5-6), *G. rochalimai* (right cluster= 7-9, left cluster= 12), and *G. skrjabini* Pigulevsky, 1953 (right cluster= 3, left cluster= 3). The species described here closely resembles *G. pigulevskyi* and *G. skrjabini* in number of follicles. However, these species differ from the new species by having a smaller acetabulum and vitelline glands that are immediately posterior to this organ, whereas in the new species the vitelline glands are situated far from the acetabulum. *Gorgoderina carioca* and *G. diaster* also differ from the new species in having testes immediately posterior to vitelline glands, while in *G. tarascae* testes are located far posterior from the vitelline glands and ovary. *Gorgoderina tarascae* additionally differs from *G. multilobata* in the position of the ovary, which is posterior to the vitelline glands in that species, and position of seminal vesicle, being anterior to the acetabulum in that species.

Discussion

Members of the genus *Rana* in Mexico belong to three groups: “*pipiens*”, “*palmipes*” and “*tarahumare*” (sensu Hillis and Wilcox, 2005). Species of *Gorgoderina* have been recorded in Mexico only in the two first groups: *G. attenuata* in both groups, *G. skarvilovitschi* only in the “*pipiens*” group, and *G. parvicava* in the “*palmipes*” group (see Table 1). The other 3 species known from Mexico, *G. megalorchis*, *G. rhyacosiredonis*, and *G. festoni* have been found as parasites of other amphibian groups (Mata-López et al., 2005; Mata-López and León-Règagnon, 2005). *Gorgoderina attenuata* is the most widely distributed species of this genus in Mexico, and there are also records of this species in *Ambystoma* and *Leptodactylus* (Lamothe et al., 1997; Goldberg and Bursey, 2002).

Although the “*tarahumare*” group is represented by 4 species in Mexico, namely *R. zweifeli*, *R. psilonota* Webb, *R. pustulosa* Boulenger and *R. tarahumare* Boulenger, there are no records of *Gorgoderina* parasitizing them. According to Hillis and Wilcox (2005), *R. zweifeli* and *R. pustulosa* may be the only remaining species of this group with relatively widespread and healthy populations in Mexico. However, *Gorgoderina tarascae* n. sp. has not been found in other localities in Mexico where *R. zweifeli* has been registered, despite our collecting efforts. It is possible that the distribution of *G. tarascae* n. sp. is limited by the combination of historical geographic events in the

region and the distribution of intermediate host(s), rather than the distribution of its definitive host species.

On the other hand, the morphology of *G. tarascae* is a mixture of features characterizing other North American species of this genus, which might indicate a close relationship with species distributed in the Nearctic region, in other groups of frogs. These might suggest colonization to *R. zweifeli*, and a subsequent speciation event in the new host. Future parasitological examination of frogs, particularly of those species belonging to the *R. tarahumare* group, in the central plateau and Northern Mexico, as well as phylogenetic analyses of *Gorgoderina* species may provide some answers to these interesting questions.

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