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PARASITES OF *LITHOBATES MONTEZUMAE* TADPOLES FROM THE "JARDÍN BOTÁNICO EXTERIOR", PEDREGAL DE SAN ÁNGEL ECOLOGICAL RESERVE, NATIONAL UNIVERSITY OF MEXICO

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ABSTRACT

Three parasite taxa, *Trichodina hypsilepis*, *T. koloti* and *Parapharyngodon* sp., were collected from a sample of tadpoles of the species *Lithobates montezumae* from the Pedregal de San Ángel Ecological Reserve, belonging to the National Autonomous University of Mexico. These taxa are new records for the host species and their presence is documented for the first time for anurans in Mexico. This work presents the first morphological characterization for trichodinid ciliates from Mexican amphibians and contributes to the knowledge of parasite fauna of wild species inhabiting in an urban ecological reserve.

Key Words. Amphibia; Ciliophora; Nematoda; *Parapharyngodon*; *Trichodina*.

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The "Jardín Botánico del Instituto de Biología de la Universidad Nacional Autónoma de México" (JB-IB), which forms part of the greater Pedregal de San Ángel Ecological Reserve, is the second oldest botanical gardens in Mexico and is considered to be one of the most important botanical gardens worldwide (Caballero and Balcázar, 2010). The gardens cover almost 13 ha (Vovides et al., 2010), with 3 ha dedicated for public exhibition, where permanent ponds and other water features are maintained to house aquatic plants.

Amongst animal taxa established in these aquatic environments, the Montezuma leopard frog *Lithobates montezumae* Baird, 1854 (Anura: Ranidae) is a native species that was introduced to the Pedregal de San Ángel Ecological Reserve (Méndez de la Cruz et al., 2009; Balderas-Valdivia et al., 2014). The natural distribution of this species occurs from the center of Mexico, throughout the Trans-Mexican Volcanic Belt and the Sierra Madre Occidental mountain range system (Balderas-Valdivia et al., 2014). *Lithobates montezumae* it is one most extensively studied anuran parasite host species in Mexico (Pérez-Ponce de León et al., 2002) however, most of these studies have focused on adult hosts and their helminth parasites, thus no previous attempts to detect other parasitic species, including ectoparasitic protozoans of the tadpole stage has previously been performed.

The only record for parasites of this frog species from Pedregal de San Ángel Ecological Reserve is the nematode *Physaloptera* sp. found in the stomach of adult specimens (Velarde-Aguilar et al., 2014). The present work aims to report the presence of parasites from the tadpoles of *L. montezumae* collected in the JB-IB.

A sample of 15 *L. montezumae* tadpoles were collected from the JB-IB, UNAM in México City, Mexico (19º19'04.8''N, 99º11'38.4''W). The tadpoles were collected using hand nets (collection permit JJBIB/53/2019, Instituto de Biología, UNAM), and then transported to the laboratory in a plastic container with water from the locality. They were kept alive and studied for ectoparasitic ciliates no more than 24 hours after collection. Wet smears of the skin and gills were made to verify the presence of the ciliated ectoparasites, using a light microscope.

Slides with ectoparasites were then air dried and processed as indicated by Islas-Ortega et al. (2018). Most of the samples were impregnated with silver nitrate according to Lom (1958) to observe the adhesive disc and denticular ring; while the remaining samples were stained with Harris's hematoxylin to elucidate the nuclear apparatus. The tadpoles were dissected in search of endo-parasites following the techniques referred in Lamothe-Argumedo (1997).

Several nematodes were recovered from the intestine; worms were relaxed in hot water and preserved in 10% buffered neutral formalin. Nematodes were cleared using a glycerine–water mixture (1:3) for examination, whereafter the mounts were observed, measured, and photographed. After examination, the specimens were stored in vials with 70% ethanol. Diagnostic structures of each parasitic taxon were measured. All measurements are given in micrometers, unless stated otherwise, with minimum and maximum values, followed by the mean and standard deviation in parentheses. Voucher specimens of collected hosts (CRPH Am0019).

Parasites were deposited at the Colección de Referencia Parásito-Huésped Laboratorio de Zoología Acuática (CRPH), Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, Mexico.

Three parasite species were found, the ectoparasitic ciliates *Trichodina hypsilepis* Wellborn, 1967 and *Trichodina koloti* Jager, Basson & van Marwijk, 2019 (Ciliophora: Trichodinidae) (Figs 1a and 1b respectively), and the nematode *Parapharyngodon* sp. (Nematoda: Pharyngodonidae) (Fig. 2).

Trichodina hypsilepis (prevalence 60%, CRPH C0029) presents a bell-shaped body, adhesive disc diameter between 50.17-61.92 (54.93 ± 4.03), a well-developed border membrane with a width of 3.11-5.22 (4.45 ± 0.72); the denticle ring diameter varies from 29.76-38.46 (34.44 ± 2.41), consisting of 20-25 (22) denticles and 10-13 (11) radial pins per denticle; the blade is strongly developed, with the distal and posterior surfaces curve, mostly sickle-shaped; blade with apophysis present, blade wide 4.26-6.82 (5.62 ± 0.77); the connection with the central part is wide and short, central part well developed, wide, with triangular shape and round tip, part fitting into the denticle on the side; the top and bottom center sections of the X axis are equal; connection of the central part with the ray is little or no developed.; ray almost parallel with the Y axis, length 6.74-8.65 (7.84 ± 0.58); (ray) wide and long, triangular and with pointed end; ray apophysis present; span of denticle 14.39-17.86 (16.21 ± 1.18), length of denticle 7.17-11.83 (9.26 ± 1.55).

Trichodina koloti (prevalence 100%, CRPH C0028) is a medium-size organism with adhesive disc diameter varying from 42.97-62.30 (52.7 ± 5.1); the diameter of the denticle ring is 27.18- 40.54 (33.1 ± 3.3), consisting of 20-26 (23) denticles, and 5-11 radial pins per denticle; the distal blade surface is slightly rounded, with a curved anterior margin, which does not extend beyond Y axes, and a posterior margin of blade curved; the blade have an apophysis, which however, is not visible in all of the denticles; blade wide 4.30-7.21 (5.6 ±. 6.6); connection of the blade with the central part is wide and short; central part well developed, wide and triangular with a round tip fitting into the next denticle, the upper and lower central sections to the X axis are equal. Connection of the central part to the ray little or not developed. Ray almost parallel to the Y axis, length 2.73-9.04 (7.03 ± 1.5); ray long and thin with rounded termination, ray apophysis present; span of denticle 9.45-17.86 (15 ± 1.8), length of denticle 5.77-11.09 (7.8 ± 1.2).

Specimens of *Parapharyngodon* sp. (prevalence 80%, CRPH N0027) have conspicuous cuticular annulation along the entire body; sexual dimorphism in size and cuticular ornamentation consisting of lateral alae present in males, females with conical tail terminated in a short stout spike. Males (based on two specimens). Body length 1214-1313, maximum width 179-210; lateral alae start at the end of the first third or the body, ends near tail; stoma funnel shaped 9-10.4; total length of esophagus 277-283, corpus 204-216 in length, 27-30 width, esophageal bulb 72-69 long by 75-76 wide; nerve ring located at 95-97 from anterior end; spicule 48-59, gubernaculum present; tail projected dorsally, total length 266-274; cloaca opens terminally with 1 pair of subventral precloacal papillae, a medial postcloacal papillae, and 1 pair of lateral postcloacal papillae.

Females (based on 12 specimens). Body length 2980-3760 (3380 ± 0.31), maximum width 320-520 (440 ± 0.4); stoma straight 12-20 (15.5 ± 2.4), total length of esophagus 514-610 (582 ± 37.62); corpus length 390-470 (442 ± 27.85), width 81-90 (86.66 ± 3.44), esophageal bulb 120-160 (147 ± 12.64) in length, 150-172 (162 ± 7.48) width; nerve ring located at 290-348 (311 ± 20.96) from anterior end; vulva opening at the second third of the body, located 1180-2060 (1194 ± 0.21) from anterior end; eggs oval, with subterminal operculum and textured thick shell, 109-117 (113 ± 3.12) long, 69-75 (72.66 ± 2.5) wide.

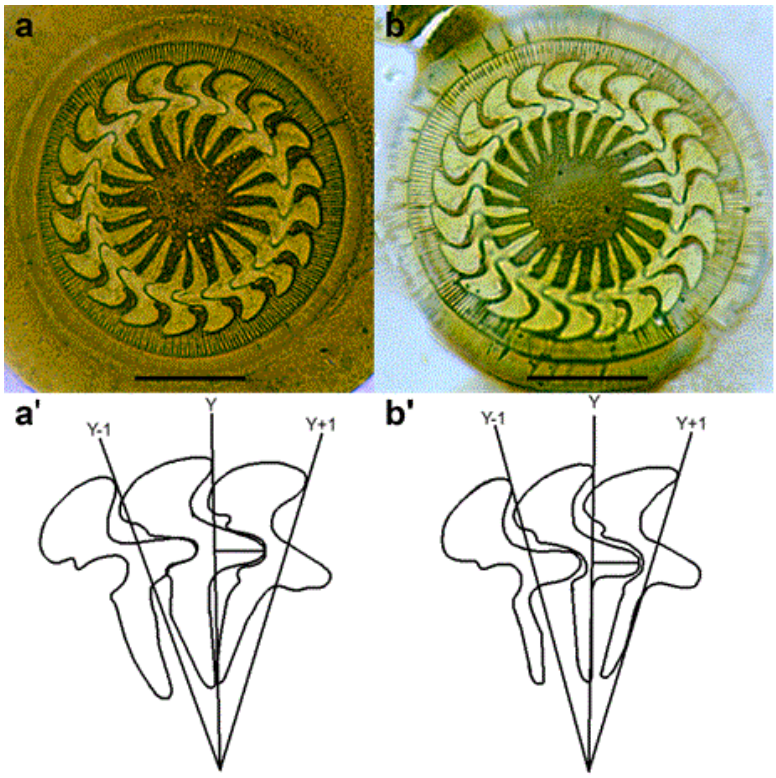

Fig. 1. Microphotograph of the adhesive disc of *Trichodina hypsilepis* from the tadpole's skin, scale-bar 20 µm (a). Microphotograph of the adhesive disc of *Trichodina koloti* from the tadpole's skin scale-bar 20 µm (b). Diagrammatic drawing of the denticles of *Trichodina hypsilepis* (a'). Diagrammatic drawing of the denticles of *Trichodina koloti* (b').

This work represents the first report of parasites of tadpoles of *Lithobates montezumae* inhabiting the JB-IB, in Mexico City. All parasitic taxa are new records for *L. montezumae*, being also the first to report trichodinid ciliates infesting anurans in Mexico. Ciliates of the genus *Trichodina* are protozoan symbionts frequently found on different organisms, in both fresh and marine environments (Zanolo and Yamamura, 2006). These protozoans, commonly found as ectoparasites on the skin and gills of fishes (Lom and Dyková, 1992; de Jager et al., 2019), have also been described from the skin and gills of anuran tadpoles (Lom, 1961; Chen, 1963; Arthur and Lom, 1984; Kazubski, 1988; de Jager et al., 2019).

Trichodina hypsilepis was described from the body and fins of the cyprinid fish *Notropis hypsilepis* from Alabama (Wellborn, 1967), and later redescribed by Arthur and Lom (1984) from tadpoles. There are several morphological descriptions for this species both in fish and amphibian hosts, from which an observable morphological variability is discerned. In the case of the current study's population, the morphology, morphometrics of the denticular ring and the measurements fits well with those characterizing *T. hypsilepis* described by Kruger et al. (1993). Although there are morphological similarities between these two trichodinid species found in our study (*T. hypsilepis* and *T. koloti*), *T. hypsilepis* is more closely related with fish hosts, while the *T. koloti* is associated with anurans (de Jager et al., 2019), and the presence of *T. hypsilepis* on tadpoles of *L. montezumae* may be attributed to the co-existence with fishes, mainly poeciliids and cyprinids, found in the same pond.

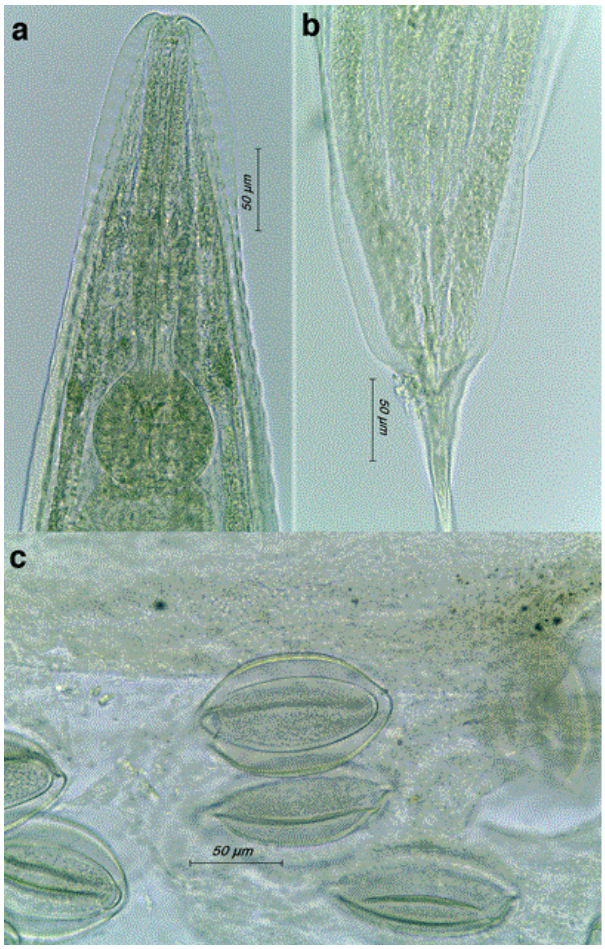

Fig. 2. Microphotographs of adults of *Parapharyngodon* sp. Anterior end of male, scale-bar 50 µm (a). Posterior end of male, frontal view, scale-bar 50 µm (b). Eggs, scale-bar 50 µm (c).

The morphological similarities between the species *T. hypsilepis* and *T. koloti*, which were analyzed by de Jager et al. (2019). These authors studied numerous records on fish and amphibians hosts across the world detecting a great similarity between *T. hypsilepis* and *T. heterodentata* Duncan, 1977. After meticulous morphological and molecular analyzes, they considered that *T. heterodentata* was actually composed of a mixture of morphologically similar individuals, proving to be *T. hypsilepis* when found on fishes, while those associated with amphibians belong to a separate species, *T. koloti* (de Jager et al., 2019). In the present study, we recorded a high number of individuals of *T. koloti* in all the 15 hosts specimens, reinforcing the suggestion that this species is a characteristic taxon found on tadpoles; the morphometric data and the shape of the denticles in our population are strongly consistent with the description of Arthur and Lom (1984) and de Jager et al. (2019).

The present records of *T. hypsilepis* and *T. koloti* represents the first ones of trichodinids on anurans, and the second ones on amphibians in Mexico, since the only previous report was provided by Ramírez-Hernández et al. (2019), who recorded *Trichodina* sp. parasitizing the Mexican stream salamander *Ambystoma ordinarium*.

Nematodes of the genus *Parapharyngodon* are typical lizard parasites (Ramallo et al., 2002; Araujo Filho et al., 2015), however, about 10 species have been described for anurans (Araujo Filho et al., 2015; Velarde-Aguilar et al., 2015).

Consequently, most of the recorded species of *Parapharyngodon* in Mexico have been made for squamate reptiles (Paredes-León et al., 2008), except for the species *P. chamelensis*, described from the hylidid frog *Diaglena spatulata* from Chamela, near the Pacific coast in the state of Jalisco, and *P. hylidae*, found in another hylidid frog, *Triprion petasatus*, from the Yucatán Peninsula. Certain factors, such as the great distance from the above-mentioned localities to Mexico City, and differences in host families (Hylidae for *P. chamelensis* and *P. hylidae* vs. Ranidae for hosts sampled in this study), along with the low number of male nematodes analyzed, made species identification challenging. Further collections are necessary to obtain a more extensive series of morphological and molecular data and focus on a larger population of male specimens.

Although there are published reports for eight nematode species found as parasites in the Montezuma leopard frog, *Lithobates montezumae* (Paredes-León et al., 2008; Velarde-Aguilar et al., 2014), the present finding confirms the first record of the genus *Parapharyngodon* and the second nematode taxon recorded from *L. montezumae* at the JB-IB, where the presence of larvae of the genus *Physaloptera* were previously recorded (Velarde-Aguilar et al., 2014).

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