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HIGH ECTOPARASITIC LEECH LOAD IN THE MOUNTAIN TREE FROG *HYLA EXIMIA* (HYLIDAE) CAUSED BY ANTHROPIZATION

ELEVADA CARGA PARASITARIA DE SANGUIJUELAS EN LA RANA ARBORÍCOLA DE MONTAÑA *HYLA EXIMIA* (HYLIDAE) CAUSADA POR LA ANTROPIZACIÓN

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Resumen.— En Junio de 2021, observamos por vez primera el parasitismo por sanguijuelas del género *Helobdella* en *Hyla eximia* durante el monitoreo de canales de riego. Este evento ocurrió poco después de que los canales fueran abiertos para proveer agua al agroecosistema en el Cerrillo, Piedras Blancas, Toluca de Lerdo, Estado de México, México. La rana estaba atrapada en uno de los registros del canal, mismo que estaba completamente cubierto por lenteja de agua (*Lemna minor*). Analizamos la carga y la distribución de las sanguijuelas parásitas en el cuerpo de la rana. El individuo presentaba una evidente dificultad en su locomoción. La carga parasitaria observada es una de las más elevadas registradas en anfibios con 65 sanguijuelas. Nuestro descubrimiento demuestra la importancia de la carga parasitaria en la adecuación del hospedero y sugiere una potencial relación entre la antropización y la magnitud en que ocurren estas interacciones.

Palabras clave.— Hábitat antropizado, *Helobdella*, parasitismo, carga de sanguijuelas, rana arborícola.

Abstract.— In June 2021, parasitism by leeches of the genus *Helobdella* was observed for the first time in *Hyla eximia* during an irrigation canal monitoring. This event occurred shortly after the canals were opened to supply an agroecosystem in The Cerrillo, Piedras Blancas, Toluca de Lerdo, State of Mexico, Mexico. The frog was trapped in one of the canal beds, which had an abundant presence of duckweed (*Lemna minor*). We analyzed the load and distribution of the parasitic leeches on the frog's body. An obvious difficulty in individual locomotion was observed. The observed parasite load of 65 leeches is one of the highest ever recorded in anurans. Our findings highlight the importance of parasitic load in the host fitness and suggest a potential relationship between anthropization and the magnitude of these interactions.

Keywords.— Anthropized landscape, *Helobdella*, parasitism, leech load, tree frog.

Amphibians are among the most vulnerable vertebrate groups, with approximately 435 species considered threatened (Stuart et al., 2004). This group is threatened by multiple factors factors, including habitat fragmentation (Becker et al., 2007), pollution (Blaustein et al., 2011), interaction with invasive species (Falaschi et al., 2020), climate change with direct and indirect effects (Carey & Alexander, 2003), pathogens and parasites (Carey, 2000), as well as the different interaction levels of all these factors (Blaustein et al., 2011). Although fungus (e.g., *Batrachochytrium dendrobatidis*) and trematodes (e.g.,

Ribeiroia ondatrae and *Echinostoma* spp.) are the main parasites studied as a cause of amphibian decline, macroparasites species could also affect their survival (Koprivnikar et al., 2012). However, macroparasites have received less attention. Among macroparasites, leeches (Hirudinea) have been identified as possible contributors to amphibians decline (Stead & Pope, 2010). Leeches can affect amphibians at all developmental stages, directly killing adults (Merilä & Sterner, 2002), reducing growth rates in tadpoles (Schalk et al. 2002), or feeding on their eggs (Romano & Cerbo, 2007). Additionally, leeches could



transmit the unicellular parasite *Ichthyophonus* sp. (Raffel et al., 2006). Despite numerous documented cases of leech parasitism in amphibians, little is known about its effects on fitness. In this study, we report a sighting of several leeches attached to an individual of *Hyla eximia*, revealing a parasitic relationship, affection to its locomotor performance, and the relationship of this interaction with anthropization.

On June 3, 2021, we conducted samplings in El Cerrillo, Piedras Blancas, Toluca de Lerdo, State of Mexico, in an anthropized habitat which is primarily used for agriculture. The herbaceous strata vegetation in the area consists mainly of grasslands and

encompasses up to 118 angiosperm species (Álvarez-Lopezello et al., 2016). The study area is heavily impacted by anthropogenic activities, including not just agriculture but cattle raising, and permanent human presence (Fig. 1). During the sample collection of the local fauna, we monitored concrete irrigation structures, which are known to cause mortality among the local fauna when species get trapped in them, through desiccation or drowning. We observed several trapped species, including *Barisia imbricata*, *Thamnophis eques*, *T. scalaris*, *T. melanogaster*, *Crotalus triseriatus*, *Rana montezumae*, *Spea multiplicata*, *Ambystoma* sp., *Peromyscus* sp., *Cryptotis* sp., and *Anas platyrhynchos diazi*. Among the rescued organisms, only two presented leeches attached to their

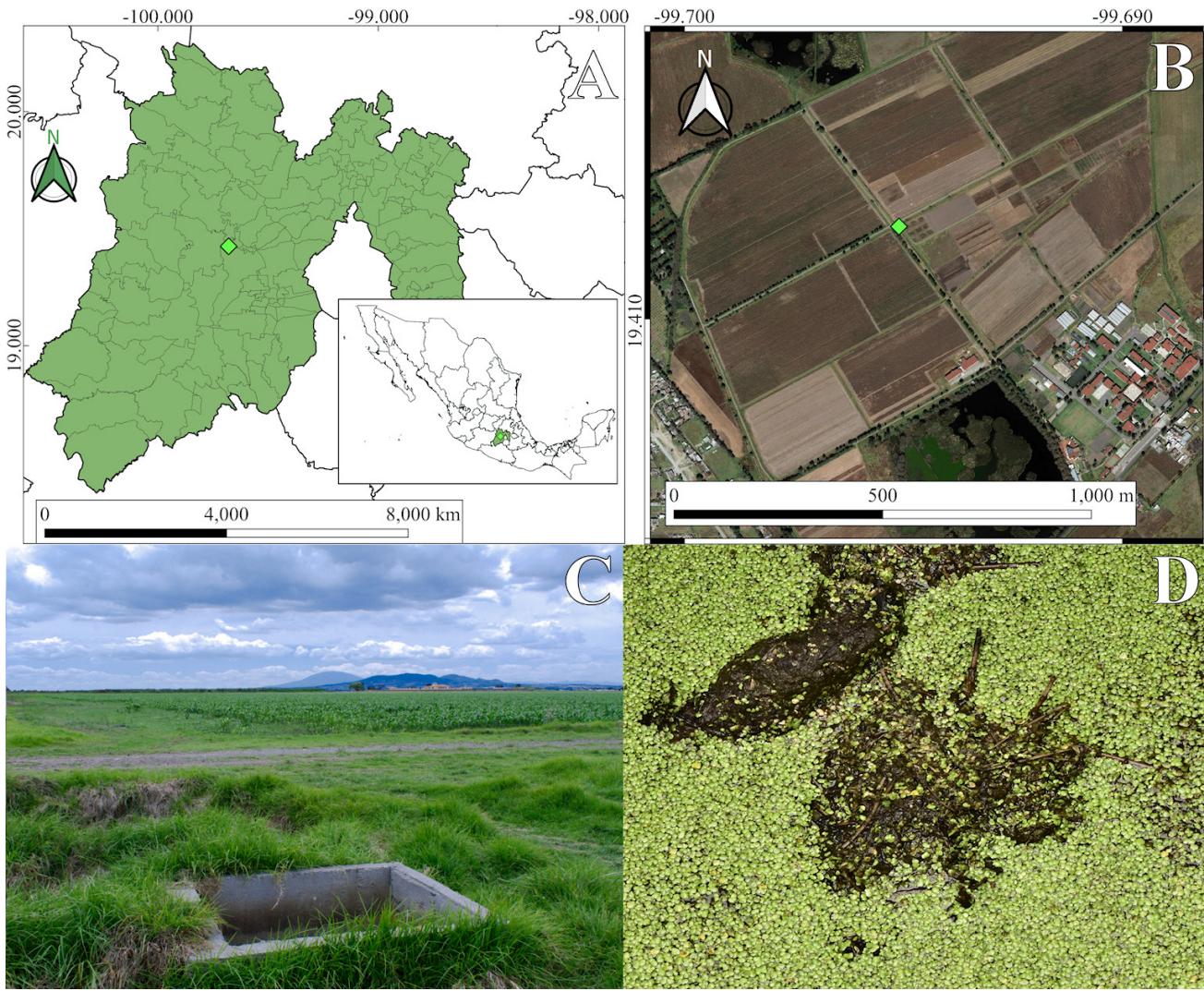


Figura 1. A) Ubicación del área de estudio en el Estado de México. B) La agricultura es el principal uso de suelo en el sitio, C) exterior del canal de riego, cultivo de maíz y casa de los pueblos aledaños, y D) roca en la que se encontró la rana dentro del canal, nótense que está rodeada por abundante Lenteja de agua.

Figure 1. A) Study area location in the State of Mexico. B) agriculture is the main land use, C) exterior of the canal bed, corn crop, and a house from the surrounding towns, and D) the rock on which the frog was found inside the canal, note that it is surrounded by abundant duckweed.



body, one living individual of *T. scalaris* (which was rescued in a good health status) and one deceased individual of *B. imbricata*. However, the leech load was low, with only three and five leeches attached respectively.

Here we present a sighting of *H. eximia*, identified by its dorsal coloration and length of hind limb webbing, following Méndez de la Cruz et al. (2018). The frog was discovered trapped in one of the canal beds (2.64 m^3), completely covered by an abundant population of the common duckweed (*Lemna minor*). We observed a high ectoparasitic load (with respect to what was observed in *T. scalaris* and *B. imbricata*) on the individual, due to several leeches attached to its body.

After rescuing the *Hyla eximia* individual, we observed that it was an adult female with a snout-vent length of 30 mm. We noted a high number of leeches attached to its ventral side, specifically its belly and extremities (65 leeches), with a greater concentration on the left side (41 leeches; see Fig. 2A, B). Upon release, the frog exhibited an obvious difficulty in locomotion, moving in short, paused jumps (see Fig. 2C, D). Some leeches fell off the frog during handling and forced displacement. The leeches were identified by taking samples of the Hirudinea to the Instituto de Biología, Universidad Nacional Autónoma de México for its identification, belonging to the *Helobdella* genus (Oceguera-Figueroa pers. comm.). In Mexico, there are seven recorded species of *Helobdella*, namely *H. atli*, *H. elongata*, *H. modesta*, *H. octatestisaca*, *H. socimulcensis*, *H. temiscoensis*, and *H. virginiae* (Oceguera-Figueroa & León Règagnon, 2014; Salas-



Figura 2. A y B) Sanguíjuelas depredando a *Hyla eximia*, la mayor carga parasitaria se observa del lado izquierdo de la rana, C y D) rana liberada del canal aún con sanguíjuelas adheridas, como el organismo no podía escapar, permaneció inmóvil por un largo período de tiempo.

Figure 2. A and B) Leeches predating *Hyla eximia*, the highest parasitic load is observed at the left side of the frog, C and D) frog released out of the canal still presenting leeches, as the organism could not escape, it remained motionless for a long time period.

Montiel et al., 2014). Based on the distribution of these species and the location of our observation in Toluca, we suggest that the leeches on the *H. eximia* individual are from the genus *Helobdella*, either *H. socimulcensis* or *H. octatestisaca*, since these species were already observed in the State of Mexico (Salas-Montiel et al., 2014) but further investigation is necessary to confirm the species identity.

In El Cerrillo, Piedras Blancas, our observations revealed the presence of four anuran species, including *H. eximia*, *H. plicata*, *R. montezumae*, and *S. multiplicata*. However, we did not find any other of these species with leeches attached in the area, even in water tanks that supply facilities, ponds, agricultural plots, and irrigation canals. This observation could suggest a potential relationship between the observed biological interaction and the anthropogenic conditions of the specific canal bed in which the affected *H. eximia* was observed. The stagnation of water in anthropogenic structures may have facilitated the presence of *Lemna minor* and made the canal bed suitable for *Helobdella* sp. to inhabit, since leeches on this genus prefers habitats in which aquatic vegetation is abundant (Mann, 1955). The arrival of *H. eximia* to the irrigation structure in which the frog got trapped could represent the unique source of food for leeches in a long time, due to the low abundance of invertebrates in the canal bed, leading to the leeches that commonly feeds on invertebrates to prey on the frog.

The *Helobdella* species are known to feed on a variety of organisms such as snails, crustaceans, dipterans, dragonflies, oligochaetes, planarians, plecopterans, and trichopterans (Young, 1980; Bradley & Reynolds, 1987; Lynggaard et al., 2021), but there is only one previous report on anurans (Tiberti & Gentilli, 2010). While the possibility of the observed interaction being phoresy cannot be ruled out since it has been recorded in the genus *Helobdella* before (Stark et al., 2017), the reddish coloration of the leeches stomachal content and abrasions in the frog's skin suggest parasitism (Tiberti & Gentilli, 2010). Nevertheless, if the observed event was indeed phoresy, it would challenge the current definition of phoresy from commensalism to an antagonistic interaction under certain circumstances, such as those resulting from anthropogenic pressures.

Phlegmatic movements and apathetic behavior, like to those observed in *H. eximia*, or even death due to leech parasitism, have been previously recorded in frogs when the parasitic load is high (Merilä & Sterner, 2002; Ayres & Comesaña Iglesias, 2008). On the other hand, low parasitic loads do not appear to affect amphibians (Rocha et al., 2012; Lunghi et al., 2018). Thus, in addition to the parasitic species, it is essential to focus on

the observations of leeches' parasitic load and possibly fitness affection in further studies related to ectoparasitism.

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