

First report of the interaction between the toad *Melanophryniscus nigricans* and the leech *Helobdella cordobensis* in the Tandilia Mountains

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ABSTRACT

Melanophryniscus nigricans is an endangered endemic anuran species restricted to the Tandilia Mountains in Argentina. We report for the first time the interaction between this toad species and the leech *Helobdella cordobensis*. We observed the interaction in a temporary pond at the "Sierra del Tigre" Natural Reserve during a breeding event of *M. nigricans* on February 7th, 2024. A leech was found attached to the belly of a toad and removed manually with no evidence of injury in the amphibian, suggesting a case of phoresis instead of parasitism. This report is also the first documented phoretic interaction between *H. cordobensis* and anurans from Argentina.

Key Words: Anuran; Argentina; Endemic species; Phoresis; Temporary pond.

Melanophryniscus nigricans is a recently described anuran species endemic from the highland grasslands of the Tandilia Mountains, a conservation priority area within the Pampas ecoregion in Argentina (Bilenca & Miñarro, 2004; Martínez-Aguirre *et al.*, 2021). This species is considered as endangered due to its restricted distribution, habitat loss and fragmentation, modification of natural grasslands, climate change, and woody invasive species (Cairo & Zalba, 2007; Martínez-Aguirre *et al.*, 2021).

Under climate change and habitat fragmentation scenarios, infectious diseases and macroparasites could pose additional threats to *Melanophryniscus nigricans* populations, that would merit future monitoring due to its high conservation priority (Soler *et al.*, 2014; Agostini *et al.*, 2015). For example, the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has contributed to amphibian population declines worldwide and has also been found infecting some

M. nigricans specimens in the Tandilia Mountains (Blaustein *et al.*, 2011; Agostini *et al.*, 2015). Macroparasites, such as leeches (Hirudinea), could affect amphibian survival and were reported to interact with a great variety of anuran species (Kutschera *et al.*, 2010; Stead & Pope, 2010; Canazas-Teran *et al.*, 2024). The ecological relationships of leeches with amphibians are complex, either being considered as predators or parasites for all life stages of the latter (Romano & Di Cerbo, 2007; Soler *et al.*, 2014).

Helobdella cordobensis (Ringuelet, 1942) is a freshwater leech endemic to the Neotropical region, with records in Argentina and Chile, generally associated with montane environments (Ringuelet, 1985; Siddall & Borda, 2004; Christoffersen, 2009). It was first reported in Argentina for the Province of Córdoba, and later for Buenos Aires (Gullo, 2014; Gullo, 2015; Cortelezzi *et al.*, 2018), Tucumán and Jujuy (Romero, 2023).

Species in the genus *Helobdella* are small, dorsoventrally flattened, predators of aquatic invertebrates, derived from putatively blood-feeding ancestors (Siddall & Borda, 2003; Ocegüera-Figueroa, 2007). However, some authors argued that species of *Helobdella* might be parasitic (Platt *et al.* 1993).

Leeches attach to other animal species to feed (Govedich *et al.*, 2010), and also move to different places at their expense (phoresis), which allows them to colonize new habitats (Govedich & Mosser, 2015). Predatory species are known to have several phoretic hosts, including birds and amphibians (Platt *et al.*, 1993, Khan & Frick, 1997; Maia-Carneiro *et al.*, 2012).

Only two interactions between *Helobdella* and South American anurans were reported, both from Perú (Canazas-Teran *et al.*, 2024). Recharte (1995) recorded *Helobdella* sp., parasitizing *Telmatobius jelskii* in Cusco, and Canazas-Teran *et al.* (2024), who conducted the most recent review of amphibian-leech associations, confirmed the occurrence of *Helobdella* sp. attached to *Telmatobius arequipensis* in Arequipa and Apurímac. The main goal of this work is to report for the first time the interaction

between *Melanophryniscus nigricans* and the leech *H. cordobensis* in the Tandilia Mountains, Argentina.

As part of the “Darwin’s Blackish Toad conservation project,” we conducted field surveys in the “Sierra del Tigre” Natural Reserve within the Tandilia Mountains between August 2023 and March 2024. This Reserve protects 140 ha of natural grasslands with high conservation priority, and although being part of a suburban landscape it holds several breeding sites of *Melanophryniscus nigricans* (Fig. 1; Bilenca & Miñarro, 2004; Cortelezzi *et al.*, 2015).

During an explosive breeding event of *Melanophryniscus nigricans* in temporary ponds on February 7th, we observed a male carrying a leech specimen (37°22’40.4” S 59°08’00.4” W). The leech was attached to the belly of the toad (Fig. 2), which was collected and transported to the laboratory. The leech was cleared in glycerine and preserved in 70% alcohol, measurements and observations were performed using a stereomicroscope, and identification was based on Ringuelet (1985), Siddall & Borda (2004) and Marchese *et al.* (2020).

The leech was removed manually, with no evidence of injury, or further bleeding in the toad;

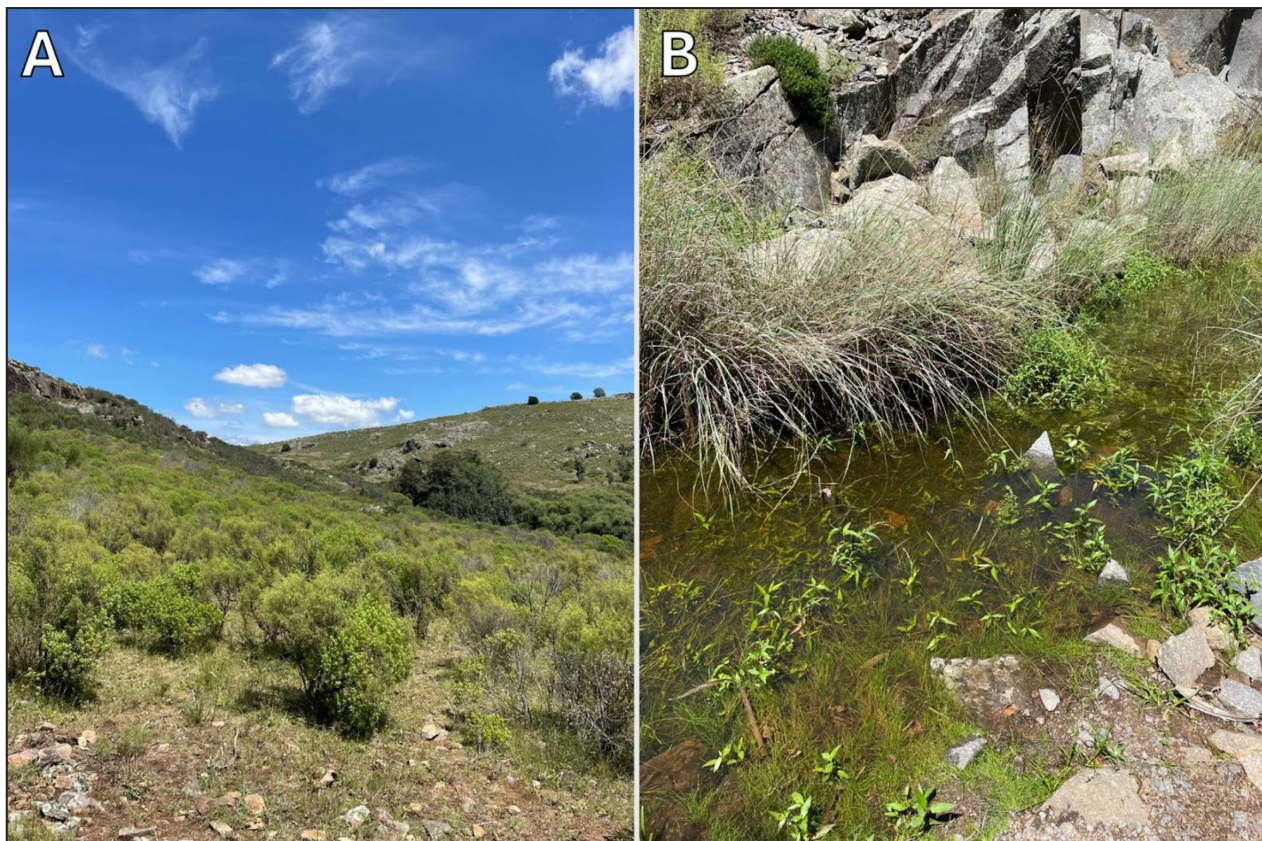


Figure 1. Highland Grasslands of Tandilia Mountains. Habitat of *Melanophryniscus nigricans* (A), and collection site at a breeding pond (B). Photo: Clara Trofino-Falasco.

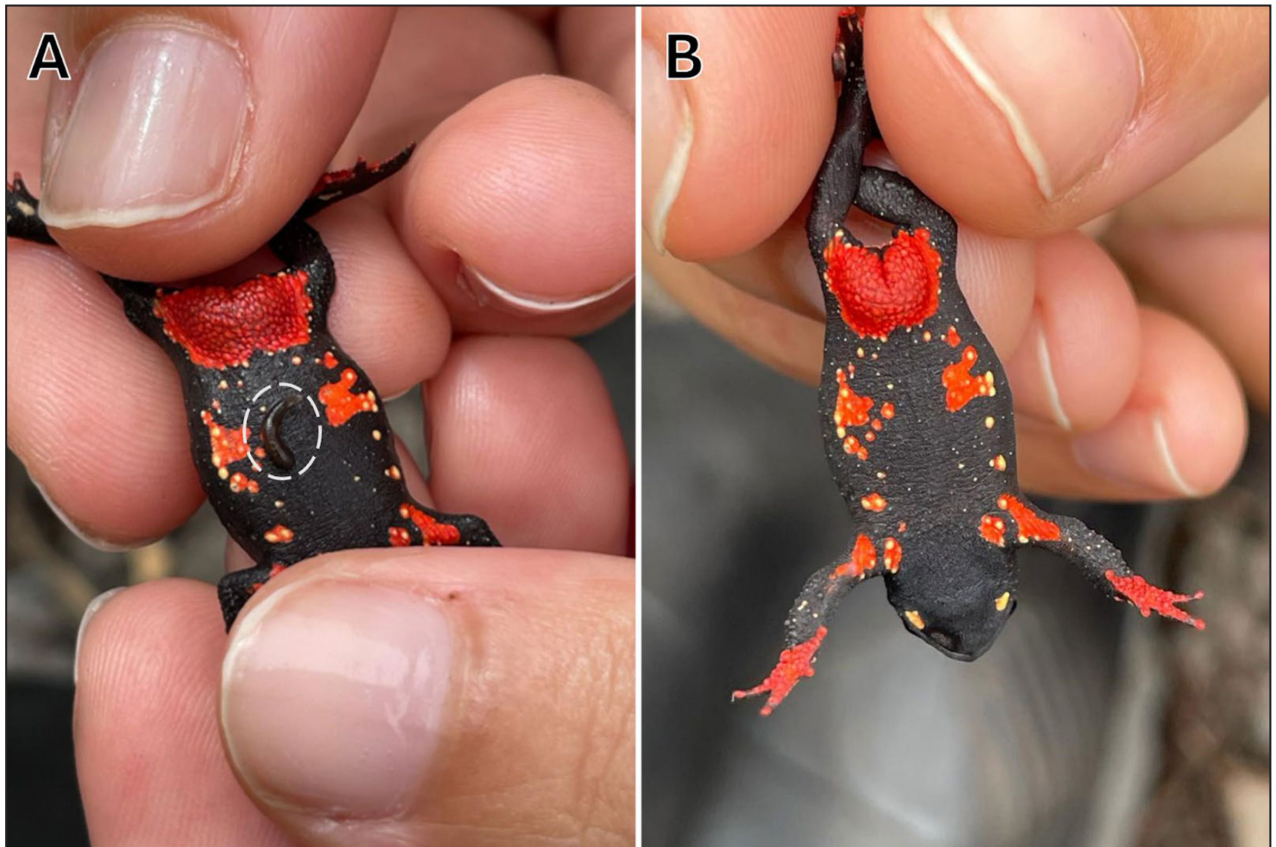


Figure 2. Phoresis of the leech *Helobdella cordobensis* by the toad *Melanophryniscus nigricans*, Tandilia, Argentina. Leech attached to the toad belly (A), ventral view of the skin once removed (B). Photo: Juan Boeris.

besides, we did not observe wounds or scars in satellite skin areas. We identified the leech specimen as *Helobdella cordobensis*. Adults of this species typically measure approximately 12 mm length and 6 mm width, with coloration that can vary from brown to light brown (Gullo, 2015). The specimen measured 4.5 mm in total length and 1.7 mm in maximum width. It had a pair of eyes located in somite IV, gonopores in XII separated by one annulus, simple annuli without gland or nuchal plate, with longitudinal lines of pigments on the dorsal surface. A straight pharynx extended to somite XIII with thick salivary ducts at its base and diffuse salivary glands, five pairs of gastric caeca of increasing size plus the pair of postcaeca in somite XIX with a descending path (Fig. 3). The reproductive system organs could not be observed since the specimen presented an immature state of development.

Our observations suggest that the relationship between *Melanophryniscus nigricans* and *Helobdella cordobensis* is a case of phoresis and not parasitism. Leech attachment to amphibian skin is favored in habitats where water level is low, as indicated for

populations of *Rana iberica* in northern Spain (Ayres & Comesaña, 2010). Aquatic habitat reduction caused by usual hydrological cycles, droughts, or the pressure exerted by human activities could favor these interactions.

Species of the genus *Helobdella* are assumed to be predators, but some authors recognize the potential occurrence of ectoparasitism on amphibians, especially anurans. Tiberti & Gentilli (2010) reported parasitism of *Helobdella stagnalis* on *Rana temporaria*. On the other hand, Zimić (2015) and Gómez-Benitez *et al.* (2023) documented interactions between leeches and anurans, quoting both the possibility of parasitism and phoresy in their studies.

Our observation is the first case of phoresy between an anuran species and the genus *Helobdella* in Argentina. Little is known about other species of Glossiphoniidae leeches infesting anurans in South America (Christoffersen, 2009) and our work indicates that these ecological interactions may be more extended between *Helobdella* and Neotropical anurans. Further research may attend the possible impact of leeches on this endangered anuran, in the

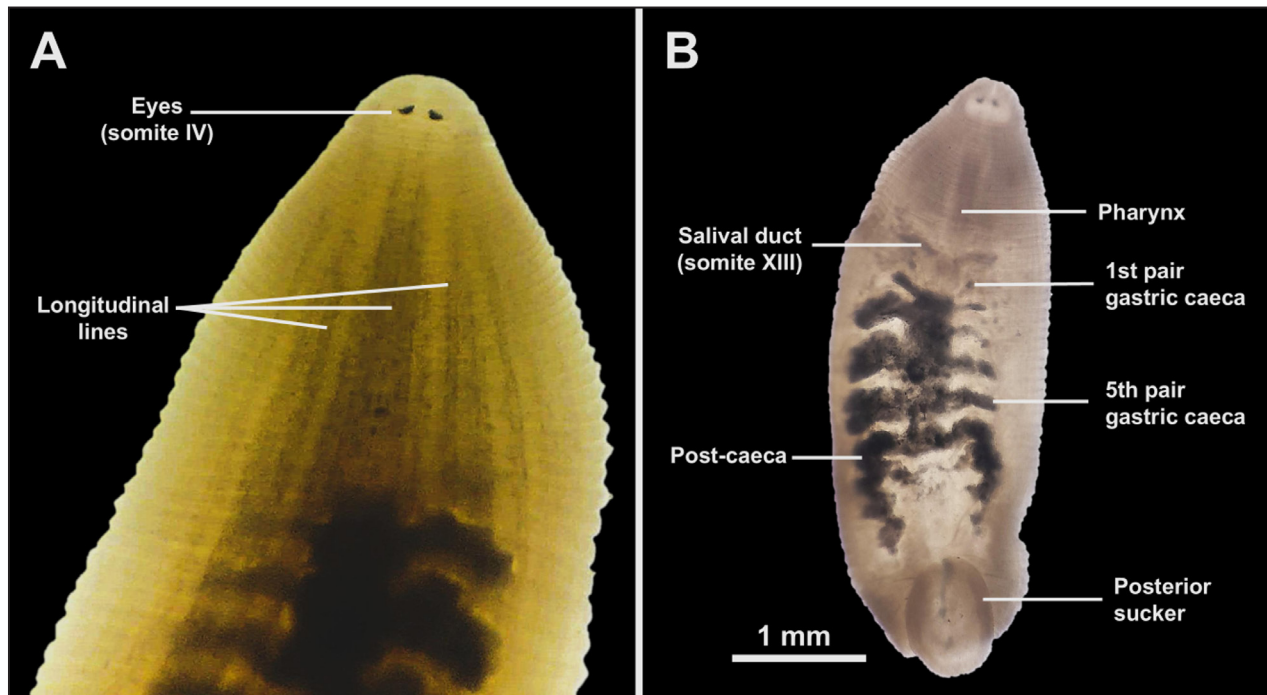


Figure 3. *Helobdella cordobensis* removed from *Melanophryniscus nigricans*. Dorsal view (A) and ventral view of clarified specimen (B). Photo: Facundo Tejedor.

context of current habitat loss and fragmentation of the natural grasslands it inhabits.

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