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## Correspondence

# Leeches (Hirudinea: Glossiphoniidae: *Maiabdella batracophila* and *Helobdella* sp.) associated with Andean water frogs (Anura: Telmatobiidae: *Telmatobius*) in southern Peru

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Amphibians are the most threatened vertebrates globally, with 41% of all species categorized as threatened (Stuart et al. 2004, IUCN 2023). Among the causes contributing to the decline of amphibian population are habitat loss, climate change, exotic species, pollution, infectious diseases, and illegal trade (de Sá 2005, Aguilar et al. 2010). Infectious diseases caused by microorganisms, such as ranaviruses, affect amphibians, fishes, and reptiles (Bosch 2003). Additionally, bacteria such as *Aeromonas hydrophila* (Ghirardi 2011) and fungi such as *Batrachochytrium dendrobatidis* affect the skin of amphibians (Berger et al. 1998). However, our knowledge of the prevalence and impact of other organisms infecting the skin of amphibians is more limited.

Frogs of the genus *Telmatobius* (Anura: Telmatobiidae) are among the most threatened amphibians (Catenazzi et al. 2010). Of the 63 species distributed along the Andes mountains from Ecuador to Argentina, 37% are categorized as critically endangered (CR), 33% as endangered (EN), and 14% as vulnerable (VU) (IUCN, 2023). In southern Peru, several species of *Telmatobius* have been reported to be infected by pathogens and parasites. The presence of the chytrid fungus has been reported in *T. culeus* (Berenguel et al. 2016), *T. jelskii* (Catenazzi et al. 2013), *T. marmoratus* (Seimon et al. 2005, Seimon et al. 2007, Catenazzi et al. 2010, Kosch et al. 2012), and *T. ventriflavum* (Catenazzi et al. 2015). Studies of gastrointestinal parasites have found high parasite prevalence in frogs used for human consumption, including *T. intermedius* (Rubio et al. 2018), *T. jelskii, T. marmoratus*, and *T. peruvianus* (Chero et al. 2014, Serrano-Martínez et al. 2017) from markets in Lima, Peru. Studying the prevalence of these organisms on their amphibian hosts is essential for improving our understanding of potential causes of amphibian population declines (Stead & Pope 2010, Catenazzi & von May 2014).

Ectoparasite blood-sucking organisms including species of leeches, midges, mosquitoes, and ticks may transmit pathogens to anurans (Cotes-Perdomo et al. 2018). Leeches can parasitize a wide range of vertebrates, and some are even host specialists. In Europe, North and Central America, leeches in the genus *Placobdella* specialize in amphibians and aquatic reptiles and transmit blood parasites such as *Haemogregarina* (a protist parasite of red blood cells found in cold-blooded vertebrates), *Ichthyophonus*, and *Trypanosoma* (Siddall & Desser 1990, Raffel et al. 2006, Siddall & Bowerman 2006, de Carle et al. 2017). The trophic interactions between amphibians and leeches are complex, as leeches can parasitize amphibian eggs, larvae, and adults (Ayres & Comesaña 2010).

Even though the presence of leeches attached to the skin of their host strongly suggests parasitism, it has been determined that some leeches, in particular the species of the genus *Helobdella*, might only be phoretic, with no evidence that these leeches actually feed on the host's blood (Reyes-Prieto et al. 2013, Starzecka et al. 2020, Lyngaard et al. 2022). However, some authors have argued that species of *Helobdella* might be parasitic (Platt et al. 1993), an issue that requires further attention.

Previous records of leech parasitism in ampibians include the observation of Billet in 1904 of an unidentified annelid on the frog *Pelophylax saharicus* (Merabet et al. 2017). Subsequent observations of leeches attached in both anurans (Anura) and salamanders (Caudata) were reported from different parts of the world, including several reports from Peru. Recharte (1995) reported leeches *Helobdella* sp., along with other organisms on the skin of captive *T. jelskii* in Cusco. Additionally, researchers reported leeches on captive *T. culeus* in Puno region and *T. macrostomus* in Junín region (Calmet et al. 2002).

Here, we report new observations of leeches in three species of *Telmatobius* in the southern Peruvian Andes. The records presented are based on field surveys conducted at high elevations (> 3000 m a.s.l.).

Between 17 August and 8 November 2019, we conduced field surveys in various locations in the regions of Apurimac, Arequipa, Cusco and Tacna in southern Peru. The surveys focused on aquatic habitats used by species of *Telmatobius*, including freshwater streams and wetlands. When frogs were found infected by leeches, we recorded the number of specimens on each individual and then proceeded to measure the snout–vent length (SVL) and mass of each amphibian. Additionally, we obtained photographs to document which parts of the body leeches were attached to. Leech specimens were collected and subsequently deposited in the collection of the Museum of Natural History, National University of San Agustín de Arequipa (MUSA), Peru. Leech specimens were immersed in a 1.5 mL cryovial with 70% ethanol, and the total length (TL) of preserved leeches was measured in the museum.


Figure 1. *Helobdella* sp. (MUSA-MI-001; TL: 20.02 mm) in dorsal (A) and ventral (B) views.

Additionally, we reviewed the literature to compile a list of amphibian–leech associations to provide updated information on the diversity of leeches attached to their amphibian hosts. Using georeferenced data from previous studies and the present study, we mapped the known distribution of leeches associated with *Telmatobius* species in Peru and South America. We examined the relationship between the number of leeches and the body size and body mass of *Telmatobius* hosts to test if parasite load is related to body size or condition, and calculated the corresponding Spearman correlation coefficients. We used R (R Core Team 2021) to carry out this analysis and we used the R package maptools (Bivand & Lewin-Koh 2014) to produce a map depicting the known distribution of leeches associated with *Telmatobius* spp.

We observed two species of leeches attached to the skin of three species of aquatic frogs of the genus *Telmatobius* in southern Peru: *Helobdella* sp. attached to *T. arequipensis*, and *Maiabdella batracophila* attached to *T. jelskii* and *T. marmoratus* (Table 1). Both leech taxa were identified through close inspection of specimens collected from three localities: Cotahuasi, Arequipa region (MUSA-MI-001; two specimens), Cancahuane, Cusco region (MUSA-MI-002; two specimens) and Mara, Apurimac region (MUSA-MI-003; eight specimens). The taxonomic determination of the genus *Helobdella* was based on Siddall & Borda (2003) and Marchese et al. (2020), who indicated that members of this genus are generally small and dorsoventrally flattened, and have an evers-

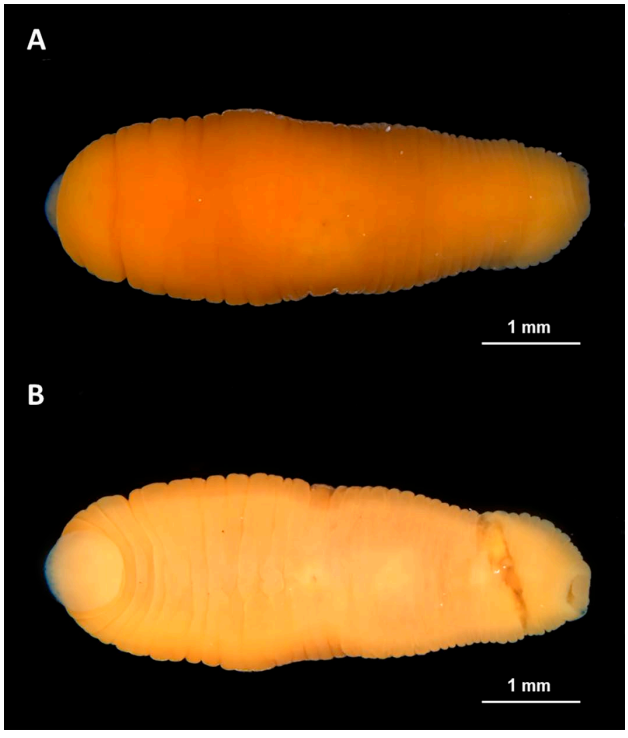


Figure 2. *Maiabdella batracophila* (MUSA-MI-003; TL: 5.52 mm) in dorsal (A) and ventral (B) views.

Table 1. Localities and number of leeches associated with *Telmatobius* spp. observed in this study.

| N° | Frogs | Leeches | Regions | Localities | Coordinates | | Altitude (m a.s.l) | search effort | n | Adults with leeches | n | Tadpoles with leeches |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Latitude | Longitude | | | | | | |
| 1 | *T. arequipensis* | *Helobdella* sp. | Arequipa | Cotahuasi | -14.704336° | -72.754257° | 4,638 | 1 h/h | 1 | 1 | 20 | 0 |
| | | | Apurimac | Mara | -14.082621° | -72.132105° | 3,892 | 1 h/h | 1 | 1 | 15 | 0 |
| 2 | *T. jelskii* | *M. batracophila* | Cusco | Cancahuane | -14.072752° | -71.981657° | 4,244 | 1 h/h | 3 | 2 | 3 | 0 |
| | | | | Mamuta | -17.307688° | -69.797443° | 4,290 | 1 h/h | 4 | 3 | 6 | 1 |
| 3 | *T. marmoratus* | *M. batracophila* | Tacna | Sicullani | -17.360643° | -69.667209° | 4,348 | 1 h/h | 6 | 3 | 14 | 2 |

ible proboscis, gonopores separated by single annuli, and a pair of eyespots (Fig. 1). The taxonomic determination of *Maiabdella batracophila* was based on Ringuelet (1980) and Marchese et al. (2020), who indicated this species has biannulated somites, a pair of eyespots and a brood pouch or marsupium in the ventral surface (Figs 2–3).

To date, about 14 species of *Helobdella* have been reported from Peru, one of them was reported as parasitic (Ringuelet 1985). Recharte (1995) recorded *Helobdella* sp. parasitizing *Telmatobius jelskii* in Cusco, Peru (Ttito, 2021), whilst Torres-Carrera et al. (2023) only found *Maiabdella batracophila* attached to *T. jelskii* in Cusco. Unfortunately, Recharte (1995) did not deposit specimens in any scientific collection, limiting a confirmation or reassignment of the taxonomic identity of her specimens. However, based on the results presented here, it is more likely that leech specimens observed by Recharte (1995) belong to *M. batracophila*. Additionally, we confirm the presence of *Helobdella* sp. attached to *T. arequipensis,* representing a new record of this association (Fig. 1).

Our review of data on amphibian-parasitizing leeches and their amphibian hosts revealed that two species of *Helobdella*, *H. stagnalis* in Europe and *H. modesta* in the New World, have been reported attached to different species of Anura and Caudata (Table 2).

On the other hand, *Maiabdella batracophila* is a monotypic species, which provides parental care through a brood pouch or marsupium, and has been reported parasitizing *Telmatobius* in northern Argentina in the high Andes from Jujuy (Christoffersen 2009) (Ringuelet 1980) and *T. jelskii* in Cusco, Peru (Torres-Carrera et al. 2023). Only two species of leeches present this type of parental care, *M. batracophila* (South America) and *Marsupiobdella africana* (Africa). However, molecular studies would indicate that this behavior is convergent in the evolutionary line and that *Maiabdella* would be more related to *Helobdella* than to *Marsupiobdella* (Torres-Carrera et al. 2023). We report the second record of *Maiabdella batracophila* for *T. jelskii* in Peru, we confirm two more localities for this association, and we also report the first record of *M. batracophila* for *T. marmoratus* (Fig. 3C).

Both species of leeches, *Helobdella* sp. and *M. batracophila* belong to the family Glossiphoniidae, including nearly half of the 139 species of leeches known to parasitize amphibians. However, little is known about other species of Glossiphoniidae infecting anurans in South America (Christoffersen 2009).

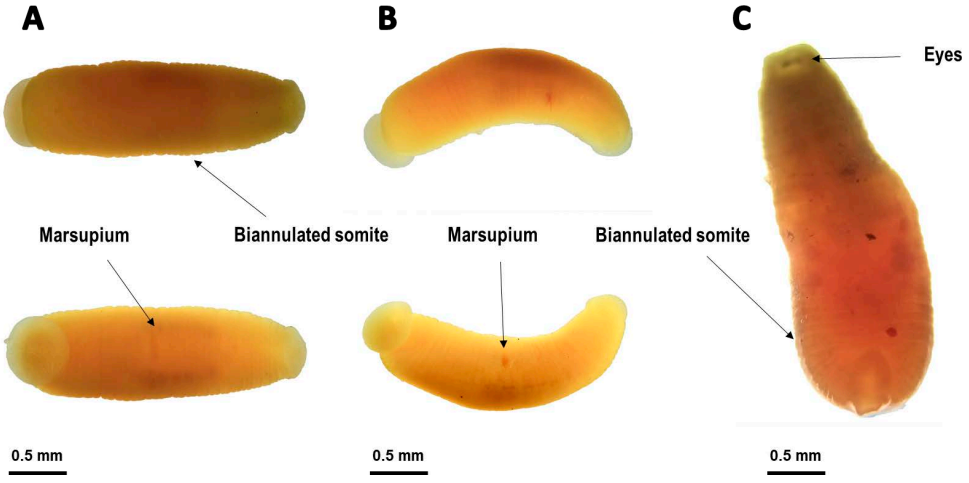


Figure 3. Diagnostic features of preserved *Maiabdella batracophila* (MUSA-MI-003) associated to *T. jelskii* (A, B) and associated to *T. marmoratus* (C).

Table 2. A synopsis of the literature on leeches associated with amphibians. We followed Frost (2022) for amphibian taxonomy.

| Amphibians | | Leeches | Leech Family | Literature |
|---|---|---|---|---|
| *Anaxyrus americanus* | Anura | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001<br>Bolek & Janovy 2005 |
| *Anaxyrus boreas* | Anura | *Placobdella sophieae* | Glossiphoniidae | Moser et al. 2014 |
| *Anaxyrus woodhousii* | Anura | *Placobdella picta* | Glossiphoniidae | Bolek & Janovy 2005 |
| *Babina subaspera* | Anura | *Orobdella dolichopharynx* | Orobdellidae | Nishi & Nakano 2020 |
| *Boana pulchellus* | Anura | *Oxyptychus* sp. | Macrobdellidae | Soler et al. 2014 |
| *Bombina variegata* | Anura | *Helobdella stagnalis* | Glossiphoniidae | Zimić 2015 |
| *Boophis madagascariensis* | Anura | *Malagabdella niarchosorum* | Haemadipsidae | Rocha et al. 2012 |
| *Boophis roseipalmatus* | Anura | *Malagabdella fallax* | Haemadipsidae | Rocha et al. 2012 |
| *Bufo bufo* | Anura | *Helobdella stagnalis* | Glossiphoniidae | Starzecka et al. 2020 |
| *Ceratophrys ornata* | Anura | *Oxyptychus ornatus* | Macrobdellidae | Ringuelet 1943 |
| *Dryophytes chrysoscelis* | Anura | *Placobdella picta* | Glossiphoniidae | Bolek & Janovy 2005 |
| *Dryophytes versicolor* | Anura | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001 |
| *Gephyromantis luteus* | Anura | *Malagabdella* sp. | Haemadipsidae | Rocha et al. 2012 |
| *Leptodactylus gr. latrans* | Anura | *Oxyptychus ornatus* | Macrobdellidae | Ringuelet 1943 |
| *Leptodactylus macrosternum* | Anura | Hirudinea sp. | Hirudinea sp. | Loebmann et al. 2008 |
| *Lithobates blairi* | Anura | *Placobdella picta* | Glossiphoniidae | Bolek & Janovy 2005 |
| *Lithobates catesbeianus* | Anura | *Placobdella picta* | Glossiphoniidae | Barta & Desser 1984<br>Barta & Sawyer 1990<br>Briggler et al. 2001<br>Bolek & Janovy 2005 |
| *Lithobates clamitans* | Anura | *Placobdella picta* | Glossiphoniidae | Barta & Desser 1984 |
| *Lithobates pipiens* | Anura | *Placobdella picta* | Glossiphoniidae | Bolek & Janovy 2005 |
| *Lithobates septentrionalis* | Anura | *Placobdella picta* | Glossiphoniidae | Barta & Desser 1984 |
| *Lithobates sphenocephalus* | Anura | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001 |
| *Lithobates sylvaticus* | Anura | *Placobdella picta* | Glossiphoniidae | McAllister et al. 1995 |
| *Limnodynastes peronii* | Anura | *Bassianobdella fusca* | Richardsonianidae | Burgin & Schell 2005 |
| *Limnodynastes tasmaniensis* | Anura | *Bassianobdella fusca* | Richardsonianidae | Burgin & Schell 2005 |
| *Mantidactylus sp. aff. grandidieri* | Anura | *Malagabdella vagans* | Haemadipsidae | Rocha et al. 2012 |
| *Odontophrynus americanus* | Anura | Hirudinea sp. | Hirudinea sp. | Loebmann et al. 2008 |
| *Pelophylax saharicus* | Anura | *Batracobdella algira* | Glossiphoniidae | Boulenger 1913 |
| *Pleurodema somuncurense* | Anura | *Oxyptychus inexpectatus* | Macrobdellidae | Velasco et al. 2016 |
| *Pseudacris crucifer* | Anura | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001 |
| *Rana arvalis* | Anura | *Hirudo medicinalis* | Hirudinidae | Merilä & Sterner 2002 |
| *Rana cascadae* | Anura | *Haemopis marmorata* | Haemopidae | Stead & Pope 2010 |
| *Rana iberica* | Anura | *Batracobdella algira* | Glossiphoniidae | Ayres & Comesaña 2010 |
| | | *Hirudo medicinalis* | Hirudinidae | Ayres & Comesaña 2010 |
| *Rana pretiosa* | Anura | *Placobdella sophieae* | Glossiphoniidae | Moser et al. 2014 |
| *Rana temporaria* | Anura | *Hirudo medicinalis* | Hirudinidae | Merilä & Sterner 2002 |
| | | *Helobdella stagnalis* | Glossiphoniidae | Tiberti & Gentilli 2010 |
| *Rhinella arenarum* | Anura | *Oxyptychus ornatus* | Macrobdellidae | Ringuelet 1943 |
| | | *Oxyptychus striatus* | Macrobdellidae | Masetti et al. 2015 |
| | | *Oxyptychus inexpectatus* | Macrobdellidae | Velasco et al. 2016 |
| *Rhinella dorbignyi* | Anura | Hirudinea sp. | Hirudinea sp. | Loebmann et al. 2008 |
| | | *Oxyptychus* sp. | Macrobdellidae | Soler et al. 2014 |
| *Rhinella marina* | Anura | *Haementeria lopezi* | Glossiphoniidae | Oceguera-Figueroa 2006 |
| *Smilisca baudinii* | Anura | *Haementeria lopezi* | Glossiphoniidae | Oceguera-Figueroa 2006 |
| *Sclerophrys mauritanica* | Anura | *Batracobdella algira* | Glossiphoniidae | Beukema & de Pous 2010 |
| *Telmatobius arequipensis* | Anura | *Helobdella* sp. | Glossiphoniidae | This study |
| *Telmatobius culeus* | Anura | Hirudinea sp. | Hirudinea sp. | Calmet et al. 2002 |

Table 2 continued

| Amphibians | | Leeches | Leech Family | Literature |
|---|---|---|---|---|
| *Telmatobius jelskii* | Anura | *Maiabdella batracophila* | Glossiphoniidae | Torres-Carrera et al. 2023<br>This study |
| *Telmatobius marmoratus* | Anura | *Maiabdella batracophila* | Glossiphoniidae | This study |
| *Telmatobius macrostomus* | Anura | Hirudinea sp. | Hirudinea sp. | Calmet et al. 2002 |
| *Telmatobius* sp. | Anura | *Maiabdella batracophila* | Glossiphoniidae | Ringuelet 1980 |
| | | *Macrobdella decora* | Macrobdellidae | Cargo 1960 |
| *Ambystoma maculatum* | Caudata | *Erpobdella punctata* | Erpobdellidae | Khan & Frick 1997 |
| | | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001 |
| *Ambystoma mavortium* | Caudata | *Placobdella picta* | Glossiphoniidae | Rhoden & Bolek 2012 |
| *Ambystoma tigrinum* | Caudata | *Helobdella modesta* | Glossiphoniidae | Platt et al. 1993,<br>Beresic-Perrins et al. 2017 |
| | | *Placobdella picta* | | Bolek & Janovy 2005 |
| *Hydromantes flavus* | Caudata | *Batracobdella algira* | Glossiphoniidae | Lunghi et al. 2018) |
| *Hydromantes imperialis* | Caudata | *Batracobdella algira* | Glossiphoniidae | Lunghi et al. 2018 |
| *Hydromantes sarrabusensis* | Caudata | *Batracobdella algira* | Glossiphoniidae | Lunghi et al. 2018 |
| *Hydromantes supramontis* | Caudata | *Batracobdella algira* | Glossiphoniidae | Lunghi et al. 2018 |
| *Ichthyosaura alpestris* | Caudata | *Helobdella stagnalis* | Glossiphoniidae | Starzecka et al. 2020 |
| *Lissotriton helveticus* | Caudata | *Helobdella stagnalis* | Glossiphoniidae | Stark et al. 2017 |
| *Lissotriton vulgaris* | Caudata | *Helobdella stagnalis* | Glossiphoniidae | Gandola & Hendry 2014<br>Starzecka et al. 2020 |
| *Notophthalmus viridescen* | Caudata | *Placobdella picta* | Glossiphoniidae | Briggler et al. 2001 |
| *Pleurodeles nebulosus* | Caudata | *Hirudo troctina* | Hirudinidae | Merabet et al. 2017 |
| *Siren intermedia* | Caudata | *Macrobdella ditetra* | Macrobdellidae | Graham & Borda 2010 |
| *Taricha granulosa* | Caudata | *Placobdella sophieae* | Glossiphoniidae | Moser et al. 2014 |
| *Triturus cristatus* | Caudata | *Helobdella stagnalis* | Glossiphoniidae | Starzecka et al. 2020 |

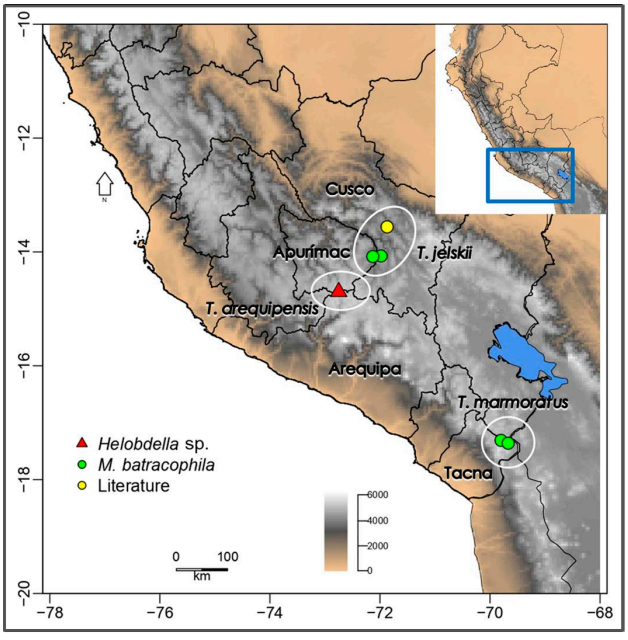


Figure 4. Report of leeches associated to *Telmatobius* spp. from Peru: red triangle: *Helobdella* sp. (this study), green circles: *Maiabdella batracophila* (this study), yellow circle: literature record, white circles with a cross: regions.

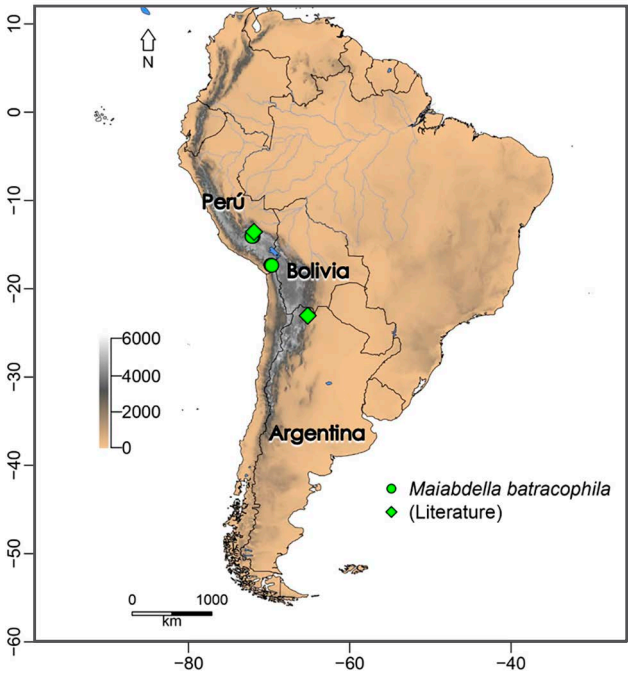


Figure 5. Known distribution of leeches associated in *Telmatobius* spp. showing new records of *Maibdella batracophila.*

Table 3. Number of leeches per frog individual according to their developmental stage.

| N° | Species | Stage | Mass (g) | SVL (mm) | Tail (mm) | N° Leeches |
|---|---|---|---|---|---|---|
| 1 | *T. marmoratus* | Adult | 69.18 | 86.60 | – | 3 |
| 2 | *T. marmoratus* | Adult | 39.54 | 74.60 | – | 2 |
| 3 | *T. marmoratus* | Adult | 31.93 | 65.70 | – | 2 |
| 4 | *T. marmoratus* | Adult | 26.46 | 65.30 | – | 4 |
| 5 | *T. marmoratus* | Juvenile | 17.00 | 53.10 | – | 7 |
| 6 | *T. marmoratus* | Juvenile | 4.20 | 35.90 | – | 1 |
| 7 | *T. marmoratus* | Tadpoles | 14.20 | 47.50 | 53.10 | 1 |
| 8 | *T. jelskii* | Adult | 25.35 | 63.20 | – | 8 |
| 9 | *T. arequipensis* | Adult | 16.63 | 52.90 | – | 2 |

Our findings extend the known distribution of leech association in *T. arequipensis*, *T. jelskii,* and *T. marmoratus* in southern Peru (Figs 4–5), and include five new localities with streams and wetlands between 3,892–4,638 m a.s.l. (Table 3, Fig. 6). The maximum number of leeches on a single frog was eight, and were observed on an adult individual of *T. jelskii* with SVL = 63.5 mm and mass = 27.5 g. Leeches preferred to attach to specific body regions including the throat, flanks, and limbs (Figs 7–8). Additionally, the number of leeches in *T. marmoratus* was not correlated with either SVL or mass (P = 0.92 and P = 0.80, respectively; r = 0.58 in both).

We surveyed frogs during the dry season, when water level is low, and when leeches may have greater opportunities to attach to frogs, as shown in populations of *Rana iberica* in northern Spain (Ayres & Comesaña 2010). Additional studies will increase the number of records and molecular studies will allow researchers obtain species-level data to refine our knowledge of the group.

According to the IUCN Red List of Threatened Species, *T. marmoratus* is categorized as (EN) Endangered, *T. arequipensis* and *T. jelskii* as (NT) Near Threatened (IUCN, 2023). However, ectoparasites are not mentioned

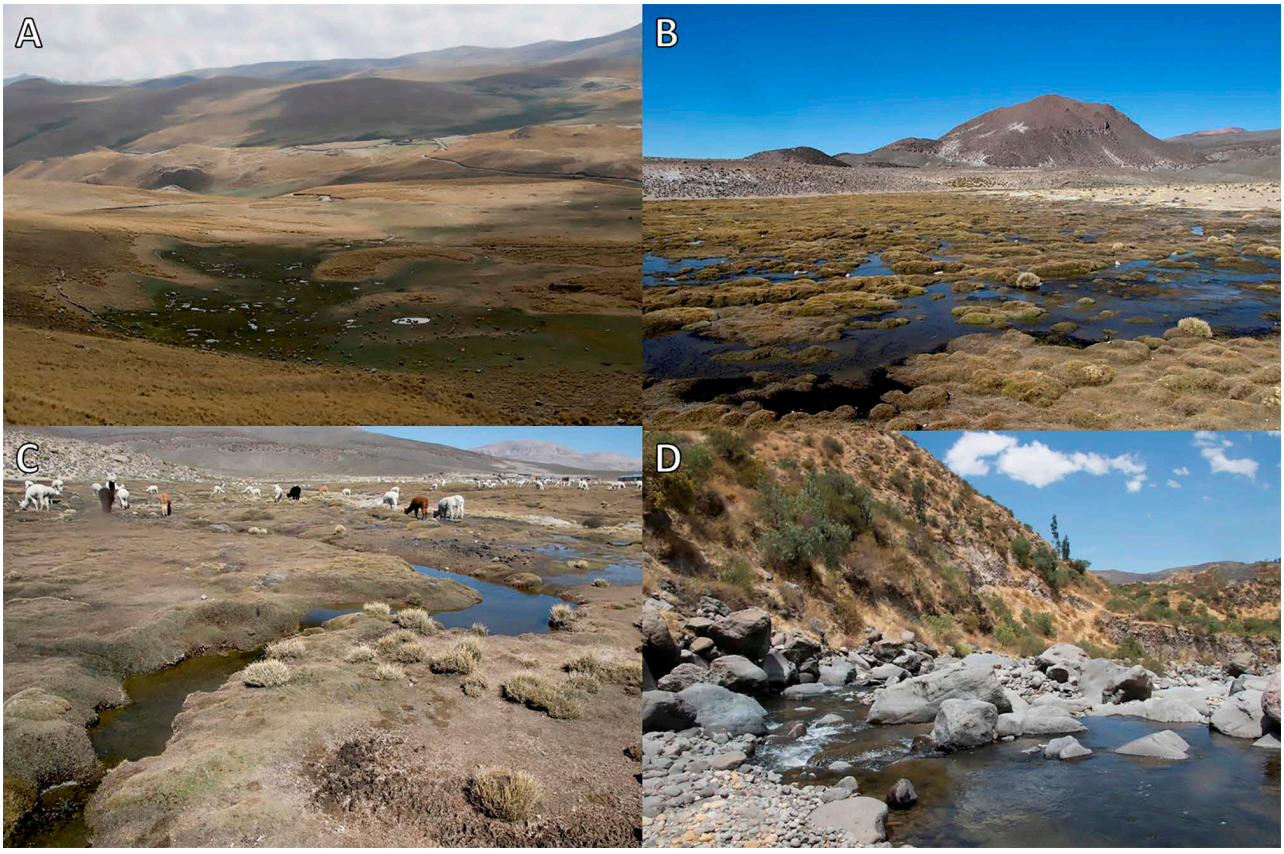


Figure 6. Habitat of *Telmatobius* spp. with records of leeches on the western slopes of the Andes in southern Peru.

as threats for any of these species. Although our data do not imply that leeches are causing declines in the three species of *Telmatobius* reported here, their widespread occurrence highlights the need of further studies to understand the effect of these ectoparasites on frog populations in the high Andes.


Figure 7. Location of leeches on the body of adults of *T. marmoratus* (A–D) and *T. jelskii* (E, F). In *T. marmoratus*, leeches were found on the flank and arm (A), throat (B), head (C), and thigh (D). In *T. jelskii*, leeches were found on the flank (E) and arm (F).

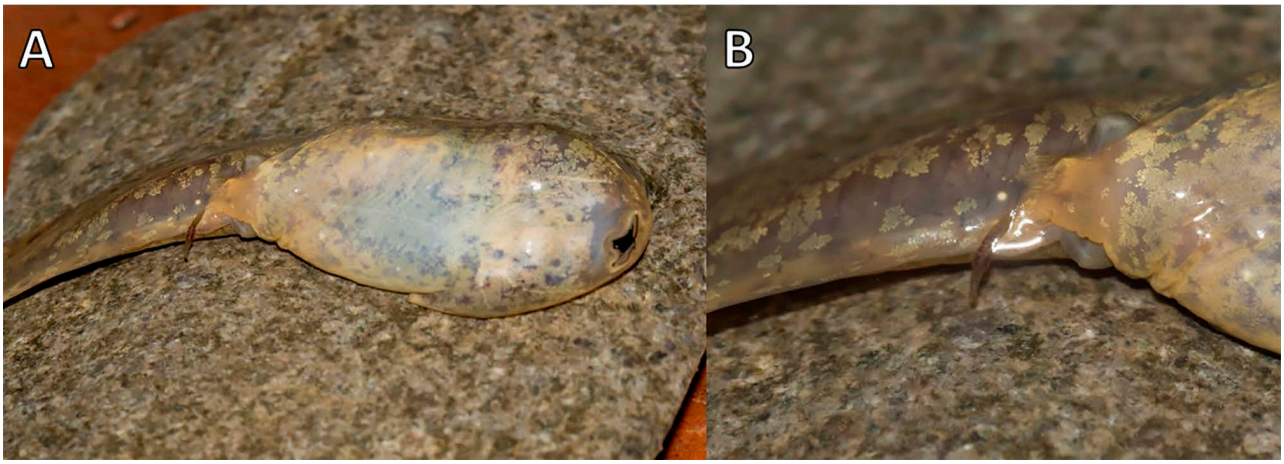


Figure 8. Location of leeches in tadpoles of *T. marmoratus* tail (A), tail (close-up view, B).

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