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First Report of Limb Anomalies in the Mashpi Stream Frog, *Hyloscirtus mashpi* (Anura: Hylidae)

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Researchers have recently documented a growing incidence of anatomical malformations in amphibians in the Americas (Jorgewich-Cohen et al. 2019; Mônico et al. 2022; Peñaherrera-R. and Triana 2022; Olivera et al. 2024). One hypothesis posits the elevated use of pesticides in agricultural practices as a contributing factor (Datta et al. 1998; Olvera-Mendoza et al. 2023). Other in-situ or ex-situ factors influencing developmental abnormalities include parasitism, predation, and UVB light (Johnson et al. 2001; Christin et al. 2003; Taylor et al. 2005). Among Neotropical hylids, malformations of various nature affecting the animals' limbs have been documented (Appendix I). Herein, we present the first report of two hindlimb malformations from an individual Mashpi Stream Frog (*Hyloscirtus mashpi*) in Mashpi, Pichincha Province, Ecuador.

The nocturnally active Neotropical hylid, *Hyloscirtus mashpi*, inhabits vegetation near riverbanks in the evergreen forests of northwestern Ecuador, specifically on the slopes of the Cordillera Occidental, within the Chocó Andino Biosphere (Guayasamin et al. 2015). The species occurs in non-protected areas as well as in reserves, ranging from low montane evergreen to montane evergreen forests at elevations of 548–1,279 m asl (Guayasamin et al. 2015; Mattea et al. 2020), and was listed as endangered (EN) primarily due to the limited extent of occurrence (708 km^2) in two-to-three threat-defined locations and a continuing decline in the extent and quality of its habitat (IUCN SSC Amphibian Specialist Group 2022). No previous records document malformations in any species of the genus *Hyloscirtus*.

During a nocturnal survey at 1800 h on 16 January 2023, we found a juvenile *H. mashpi* (Fig. 1) perched on a leaf approximately 1 m above the ground along a small stream in the "Sacha Guatusa" Reserve in Mashpi, Metropolitan District of Quito, Ecuador (0.1864, -78.8572, elev. 994 m asl). This location, although protected, faces increasing human pressures from the surrounding populations and non-local mining companies. When we noticed the malformation on its right hindleg, we documented the observation with photographs and released the frog where initially encountered. Of the 21 *Hyloscirtus mashpi* in collections and photographic repositories (five individuals in Bioweb, four in ZSFQ, and twelve in iNaturalist), this individual is the only one with malformations.

Figure 1. A Mashpi Stream Frog (*Hyloscirtus mashpi*) metamorph in the "Sacha Guatusa" Reserve, Mashpi, Metropolitan District of Quito, Ecuador, with an abnormal orientation of the right hindlimb. Photograph by Elias Figueroa-Coronel.

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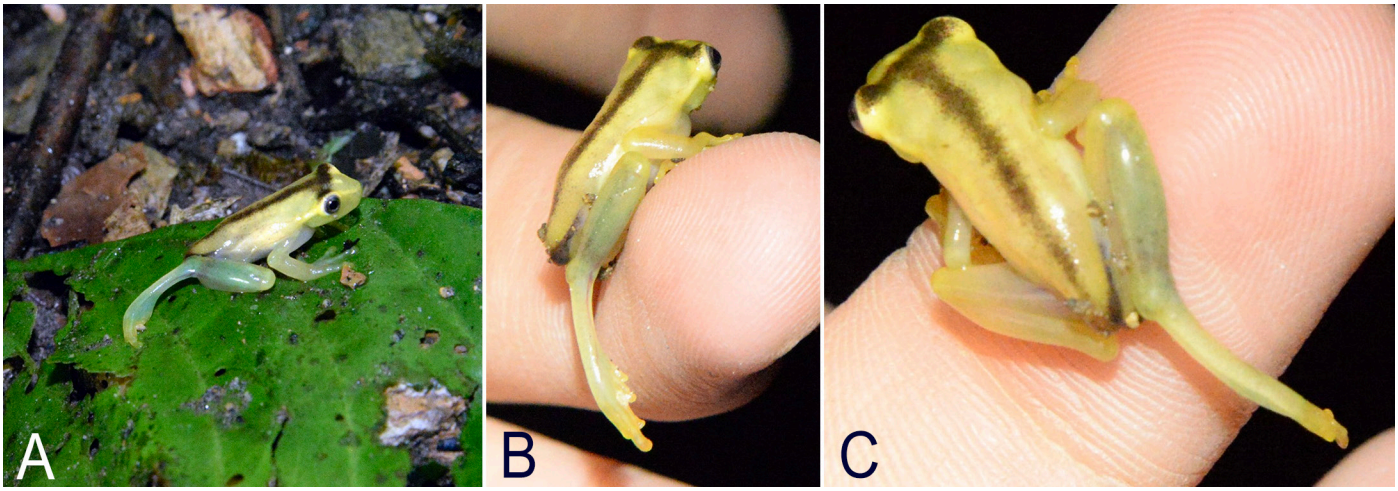

Figure 2. Dorsolateral (A), dorsal (B), and lateral (C) views of a right hindlimb malformation involving antoversion and digital fusion in a Mashpi Stream Frog (*Hyloscirtus mashpi*) metamorph from the "Sacha Guatusa" Reserve, Mashpi, Metropolitan District of Quito, Ecuador. Photographs by Elias Figueroa-Coronel.

We identified two types of malformations on the frog's right foot; the tibiofibular region was completely reversed, resulting in an arcuate shape of the limb when fully extended (Fig. 2A) and projected upward when flexed (Fig. 2B), and digits III–V fused along the entire length of all phalanges (Fig. 2C). Presumably, discs were partially free. Following the terminology of Henle et al. (2017), the twisted tibiofibular condition was an anteversion of the tibiofibular region as the tibia and digits remained in their natural orientation (i.e., unrotated). The fused digits III–V were a partial syndactyly at the soft tissue level; because we could not rigorously examine the limb (i.e., X-ray images), we were unable to fully evaluate the extent of fusion to determine if the fusion extended to the bones. Hindlimb shape somewhat resembled the limb malformation reported in *Boana cinerascens* (Pommer-Barbosa et al. 2022). Although that malformation was not identified, we suggest that both records could be the result of a size reduction or atrophy of the hindlimb muscles or tendons, given the thin spoon-shaped appearance of the foot and the fusion of digits III–V, although we cannot rule out that a combination of different muscular or tendonous malformations were responsible. In either case, additional studies should analyze the muscular morphology of individuals with similar malformations to determine the responsible anomaly.

We are unable to identify the presumably environmental agent that caused this malformation. However, factors including chemical water contamination should be considered, as this is known to affect the amphibian community in Mashpi (Burkhart et al. 1998; Fort et al. 2001). Also, ecosystems surrounding Mashpi are undergoing substantial transformations caused by human-mediated deforestation, mining, and agrochemical usage (Morabowen et al. 2019). Globally, widely employed agricultural practices characterized by extensive deforestation and the use of pesticides like organophosphates (OP) and organochlorines (OC) are known to negatively affect amphibian life expectancy and development (Duellman and Trueb 1994; Burkhart et al. 1998; Fort et al. 2001). Additionally, such factors increase susceptibility of amphibians to other stressors like infectious diseases and parasites as well as weakening the immune system (Christin et al. 2003; Taylor et al. 2005; Johnson et al. 2008). The effects of parasitic trematode infestations, particularly those of the genus *Ribeiroia*, are well-documented on amphibian limb development (Johnson et al. 2001, 2002; Johnson and Sutherland 2003).

We cannot confirm any direct contamination in the area where we found the malformed individual. Further research is needed, and we recommend conducting additional studies on the prevalence of limb abnormalities in the Mashpi amphibian community to determine whether this was an isolated event or the issue is widespread.

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Appendix I. Documented cases of Neotropical hylids with limb malformations. Data are provided for each species by type of malformation, description of the malformation, locality, and source.

Boana cinerascens. Unspecified malformation. Porto Velho Municipality, Rondônia, Brazil, in the southwestern part of the Brazilian Amazon (Pommer-Barbosa 2022).

Boana leptolineata. Ectrodactyly; missing adhesive disc, fused fingers, and absence of adhesive discs on the limb. Sertão Municipality, Rio Grande do Sul, Brazil (Ascoli-Morrete et al. 2019).

Boana pulchella. Ectrodactyly; fused fingers. Sertão Municipality, Rio Grande do Sul, Brazil (Ascoli-Morrete et al. 2019).

Callimedusa tomopterna. Ectrodactyly, ectromely; fused fingers and reduction or absence of limbs. Reserva Florestal Adolpho Ducke, Manaus Municipality, Amazonas, Brazil (Mônico et al. 2022).

Corythomantis greeningi. Brachydactyly; shortened fingers. Cafarnaum Municipality, northwestern Bahia, northeastern Brazil (Silva-Soares and Mônico 2017).

Dendropsophus marmoratus. Syndactyly; fusion of fingers in left forelimb. Iranduba Municipality, Amazonas, Brazil (Mônico et al. 2022).

Dendropsophus minutus. Brachydactyly, syndactyly, ectrodactyly; shortened and fused fingers in multiple individuals. Sertão Municipality, Rio Grande do Sul, Brazil (Ascoli-Morrete et al. 2019).

Dryophytes eximius. Ectromely; substantial reduction or absence of limbs. Canta Ranas, Santiago Tulantepec Municipality, Hidalgo, Mexico (Olvera-Mendoza et al. 2023).

Hyla arenicolor. Polyphalangy, additional phalanges. Sierra Nanchititla Natural Reserve, Sierra Madre del Sur Province, central Mexico. Monroy-Vilchis et al. 2015).

Isthmohyla rivularis. Brachydactyly, syndactyly; shortened and fused fingers. Monteverde Cloud Forest Preserve (MCFP), Atlantic Slope of the Cordillera Tilarán, Costa Rica (Hedrick and Cossel 2014).

Lysapsus bolivianus. Ectromely; substantial reduction or absence of a limb. Santana Municipality, Amapá, Brazil (Pedroso-Santos 2020).

Nyctimantis brunoi. Various malformations; extranumerary tubercles, absence of subarticular tubercles, abnormal adhesive discs, brachydactyly, microdactyly, ectrodactyly, polyphalangia, phalanx rotation, syndactyly, scoliosis, abnormal tarsalia, and urostyle torsion. Parque Nacional da Restinga de Jurubatiba, Rio de Janeiro, southeastern Brazil (Carmo et al. 2021).

Pseudis paradoxa. Ectrodactyly, polymelia; fused fingers and presence of additional limbs. Coastal zone of Amapá, Brazil (Oliveira-Souza et al. 2020).

Scinax aromothyella. Brachydactyly, ectrodactyly; shortened and fused fingers. At the edge of a forest fragment in Sertão Municipality, Rio Grande do Sul, Brazil (Ascoli-Morrete et al. 2019).

Scinax fuscovarius. Ectromely; substantial reduction or absence of limbs. Paraná, Brazil (de Souza et al. 2021).

Scinax quinquefasciatus. Polyphalangy; presence of additional phalanges. Northwestern Ecuador (Ramírez-Jaramillo 2019).

Scinax squalirostris. Schizodactyly; abnormal separation between fingers. Botucatu Municipality, São Paulo, southeastern Brazil (Jorgewich-Cohen et al. 2019).

Smilisca phaeota. Schizodactyly, syndactyly; abnormal separation and fusion of fingers. Wilson Botanical Garden at Las Cruces Research Station and Wilson Botanical Garden, southern Pacific highlands of Costa Rica (Peñaherrera-R. and Triana 2022).

Sphaenorhynchus lacteus, Amely; missing limb. Amapá, Brazil (Pedroso-Santos 2020).

Trachycephalus cunauaru. Ectrodactyly; fused fingers. Iranduba Municipality, Amazonas, Brazil (Mônico et al. 2022).