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Cuad. herpetol. 37 (1): 77-83 (*2023)*

# New records of malformations in Amazonian anurans

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Recibida: 09 Agosto 2022
Revisada: 01 Septiembre 2022
Aceptada: 23 Septiembre 2022
Editor Asociado: C. Borteiro

doi: 10.31017/CdH.2023.(2022-020)

ABSTRACT

Reports on morphological malformations in amphibians have been extensively documented worldwide. However, in the Amazon Forest records are recent and still a horizon to be explored, considering that they are potential cues of serious environmental problems. Here, we report five individuals of different species with limb morphological malformations (*Callimedusa tomopterna*, *Trachycephalus cunauaru*, *Dendropsophus marmoratus*, *Ceratophrys cornuta* and *Rhinella proboscidea*), collected between February 2020 and April 2022 during fieldwork expeditions at localities of Brazilian Amazonia, and a rare case of Siamese twins in a specimen of *Synapturanus ajuricaba* previously deposited at the Herpetological Collection of Instituto Nacional de Pesquisas da Amazônia. We provide radiographs and discuss about the affected bone structures. These records provide the first malformation cases known for adult specimens in the abovementioned species, and also in the family Phyllomedusidae.

Key Words: Morphological abnormalities; Radiography.

Records of morphological anomalies in amphibians have appeared in the literature for over 450 years (Gesner, 1554), but significantly increased over the last decades (Ouellet, 2000; Lannoo, 2008; Johnson *et al.*, 2010; Henle *et al.*, 2017). The occurrence of deformations in amphibians can be caused by many factors [*e.g.*, parasitic infestation (Johnson *et al.*, 1999; Kiesecker, 2002), UV-B radiation (Blaustein *et al.*, 1997; Ankley *et al.*, 2000), microbial diseases (Sessions and Ruth, 1990), chemical and agribusiness pollution (Oullet *et al.*, 1997; Taylor *et al.,* 2005; Lannoo, 2008; Koleska and Jablonski, 2016), and other disorders]. Malformations were considered the consequence of serious environmental problems, which highlights the importance of their reports (Johnson *et al.*, 2003), especially when they occur in preserved natural environments (e.g., Mônico *et al.*, 2019).

A variety of reports of morphological malformations in amphibians already documented in the literature involved different families, concerning species with varied habitat use and distributed in all continents (Henle *et al.*, 2017). In the Amazon Forest however, these records are quite recent (Carvalho *et al.*, 2008; Peloso, 2016; Ramalho *et al.*, 2017; Sousa and Costa-Campos, 2016; Santos *et al.*, 2017; Sousa and Costa-Campos, 2017; Oliveira-Souza *et al.*, 2020; Souza *et al.,* 2021; Sodré *et al.*, 2022), and appear as an unexplored topic.

Here, we report six individuals from different families with morphological malformations, five of them collected between February 2020 and April 2022 during fieldwork expeditions in localities of Brazilian Amazonia, and an additional specimen previously deposited at Herpetological Collection of Instituto Nacional Pesquisas da Amazônia (INPA-H), Manaus, Amazonas state, Brazil. The classification of the deformities follows Meteyer (2000) and Lanoo (2008). Collected specimens were also deposited in the INPA-H. Radiographs images were taken on a Nikon XT V 160 Electronics X-ray Inspection.

The first individual was an adult male of *Callimedusa tomopterna* (Cope, 1868) collected on 13 February 2020 at Reserva Florestal Adolpho Ducke (RFAD; 2°55'44.1"S, 59°58'34.2"W; WGS 84; 104 m elevation), Manaus municipality, Amazonas state.

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The individual (INPA-H 42556; SVL = 43.3 mm) presented ectromelia with ectrodactyly in the left hindlimb (Fig. 1A), characterized by shortening of the tibiale-fibulare, metatarsal bones fused, and absent phalanges (Figs. 1B, 1C). This nocturnal anuran is arboreal, and belongs to the Phyllomedusidae family, being widely distributed in the Amazon Forest (Frost, 2022). Its reproduction takes place in forest environments, the clutches are deposited in leaf nests over ponds, and when hatching, tadpoles fall into the water where they develop until metamorphosis (Lima *et al.*, 2012). We found this individual in the vegetation about 4 m above the ground level. Despite it lacks toes and adhesive discs, an important structure for treefrogs (Smith *et al.*, 2006), the malformation didn't seem to have hindered its natural growth and development. When captured and handled, the treefrog did not demonstrate any unusual behavior resulting from its malformation.

The second specimen was an adult male of *Trachycephalus cunauaru* Gordo *et al.*, 2013. We collected the specimen (INPA-H 43890; SVL = 65.3 mm) on 15 December 2020 in Iranduba municipality, Amazonas state (3°08'49.7"S, 60°13'40.3"W; WGS 84; 82 m elevation). This individual showed ectrodactyly in the left forelimb (Fig. 2A), characterized by the absence of fingers II, III and IV (Figs. 2B, 2C). *Trachycephalus cunauaru* is nocturnal, inhabits the canopy of "*terra firme*" forests and seasonally flooded forests and lay floating egg clutches in tree hollows filled with water, where tadpoles complete their development (Gordo *et al.*, 2013). We found

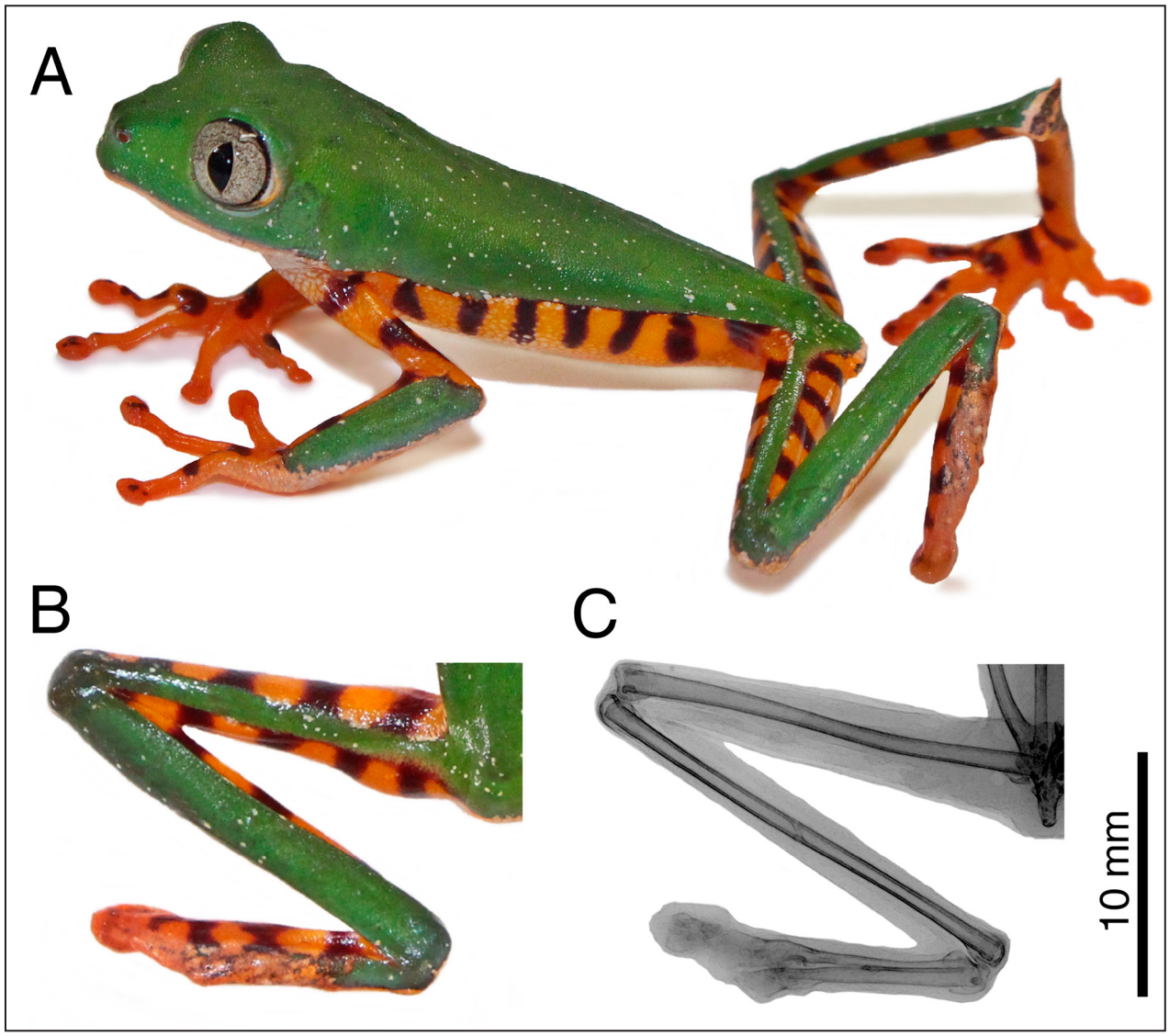


**Figure 1.** Adult male of *Callimedusa tomopterna* with ectromelia and ectrodactyly in the left hindlimb. (A) Natural posture of specimen alive; (B) Left hindlimb malformed; (C) Radiography.

the individual in a cavity with water, 70 cm above the ground, calling along with another adult male. When captured and handled, the frog did not show any unusual behavior generated by the malformation, despite the absence of three toes and the respective adhesive discs.

The third individual was an adult male of *Dendropsophus marmoratus* (Laurenti, 1768) (INPA-H 43886; SVL= 31.4 mm) collected on 17 December 2020 in Iranduba municipality, Amazonas state (3°08'49.7"S, 60°13'40.3"W; WGS 84; 77 m elevation). The individual showed syndactyly in the left


**Figure 2.** Adult male of *Trachycephalus cunauaru* with ectrodactyly in the left forelimb. (A) Natural posture of specimen alive; (B) Left forelimb malformed; (C) Radiography.

forelimb (Fig. 3A), a malformation characterized by partial or complete fusion of one or more fingers. The radiography showed in this case indicated that all the fingers and phalanges were fused and united by the skin, without adhesive discs on fingers II, III and IV (Figs. 3B, 3C). We found the individual on the ground, calling next to a water pond. This species is nocturnal and inhabits the vegetation of humid areas on "*terra firme*" forests and primary alluvial forests (Morato *et al.*, 2014). We could not observe whether the malformation was causing any unusual behavior, as we were aware of the malformation after the specimen was euthanized.

The fourth individual was a juvenile of *Ceratophrys cornuta* (Linnaeus, 1758) collected on 07 December 2021 in Vitória do Xingu municipality, Pará state (3°30'18.2"S, 51°53'07.4"W; WGS 84; 129 m elevation). The individual (INPA-H 44019, SVL= 56.5 mm) has ectrodactyly with brachydactyly in the right hindlimb (Figs. 4A, 4B), malformations characterized by absence of toes and phalanges, respectively (Figs. 4C, 4D). Even with the radiography, it was not possible to determine which fingers are absent or which are shortened (phalanges absent), and is only evident the absence of two fingers. The species is nocturnal, terrestrial and its reproduction occurs in '*várzea*' wetlands forest ponds (Lima *et al.*, 2012). We observed the individual on the forest floor, and certainly the absence of spade like interdigital membranes must affect the burrowing capacity (Fabrezi *et al.*, 2019). The same malformations (i.e. ectrodactyly and brachydactyly) were already reported on its congener *C. cranwelli* (Medina *et al.*, 2013).

The fifth individual was a *Rhinella proboscidea* juvenile (Spix, 1824) (unvouchered; SVL= 29.8 mm) collected on 03 May 2022 at Cachoeira da Suçuarana, Balbina Village, Presidente Figueiredo municipality, Amazonas state (1°54'29.5"S, 59°24'23.2"W). The individual has syndactyly with brachydactyly in the right forelimb (Figs. 5A, 5B), with fingers II, III and IV completely fused and shortened (absence of phalanges; Fig. 5C). The species is diurnal, inhabits

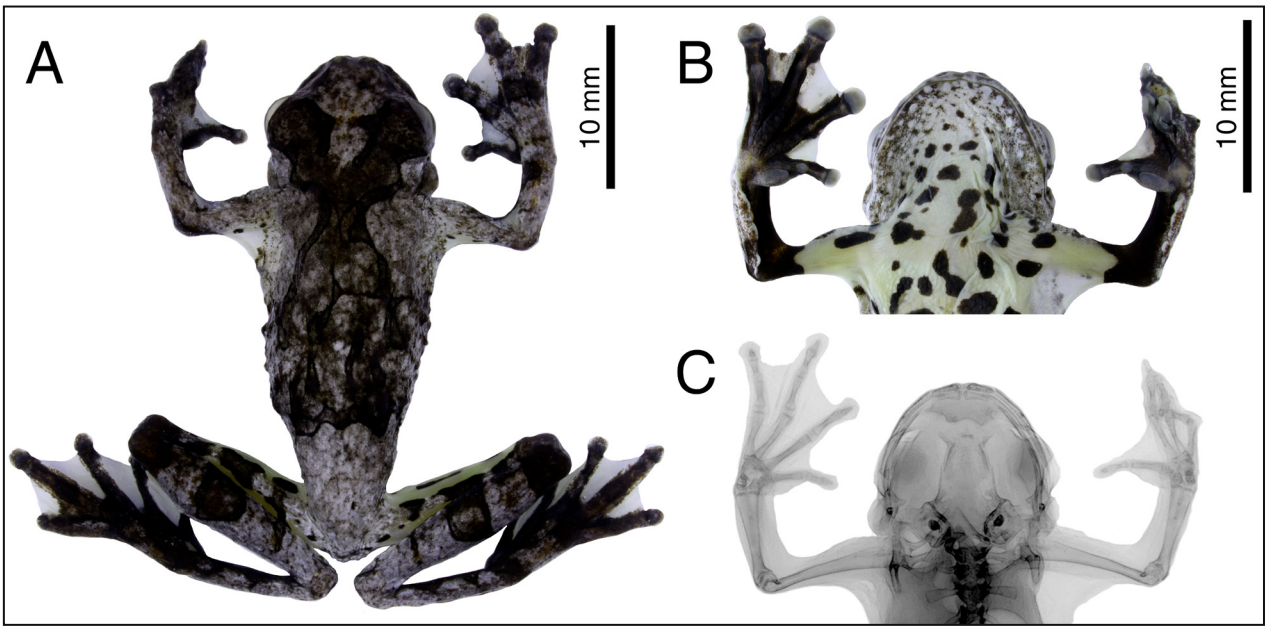


**Figure 3.** Adult male of *Dendropsophus marmoratus* with syndactyly in the left forelimb. (A) Dorsal view of specimen; (B) Forelimbs in ventral view; (C) Radiography.

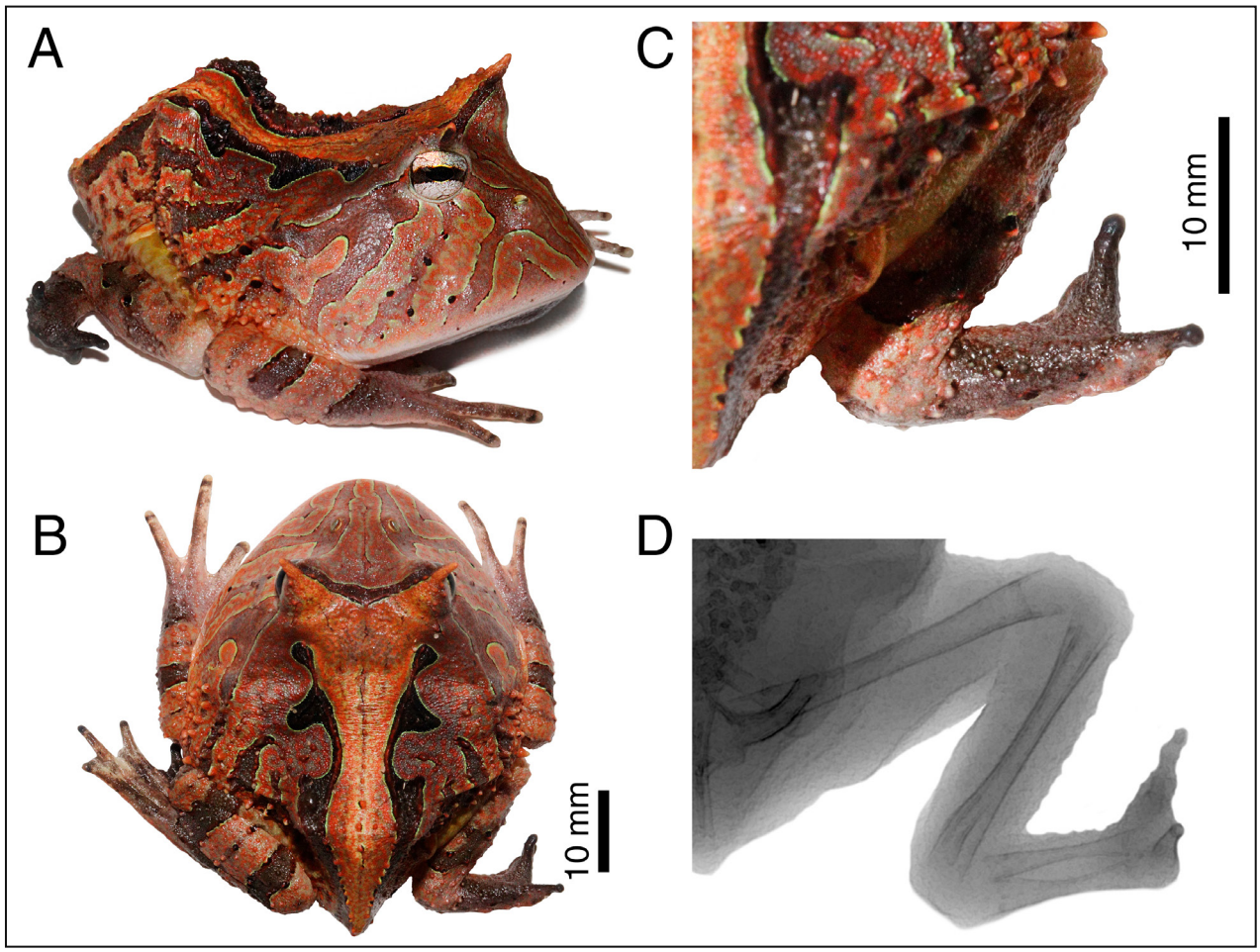


**Figure 4.** Juvenile male *Ceratophrys cornuta* with ectrodactyly and brachydactyly in the right hindlimb. (A) Natural posture of specimen alive; (B) Dorsal view of specimen; (C) Right hindlimb malformed; (D) Radiography.

the forest floor and breeds in ponds or seeps near the headwaters of streams (Lima *et al.*, 2012). Souza *et al.* (2021) reported one case of anophthalmy (absence of an eye) for this species in Manaus region.

Finally, a juvenile specimen of *Synapturanus ajuricaba* Fouquet *et al.*, 2021 (INPA-H 15837; SVL= 6.8 mm of left and 6.9 mm of right) collected in April 2004 at Reserva Florestal Adolpho Ducke by Marcelo Menin. The species is fossorial, with diurnal and nocturnal calling activity, and its reproduction occurs in burrows out of the water (Lima *et al.*, 2012). The specimen is, an extremely rare case of Siamese

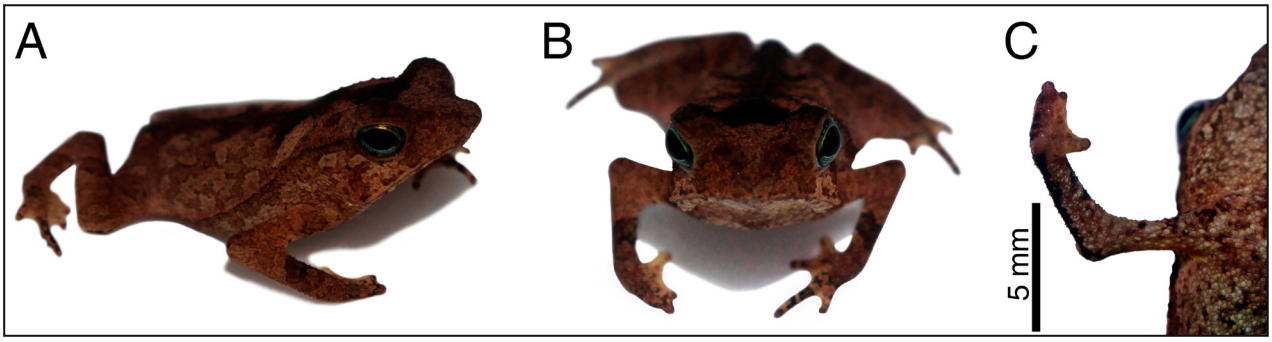


**Figure 5.** Juvenile of *Rhinella proboscidea* with syndactyly and brachydactyly in the right forelimb. (A) Dorsal view of individual alive; (B) Frontal view; (C) Right forelimb in ventral view.

twins (Figs. 6A, 6B), that has two heads, two vertebral columns, two pairs of forelimbs, but only one pair of hindlimbs. Among the few cases that have been recorded for the world, most of them derived from individuals born in captivity (Henle *et al.,* 2017), which makes this record even more relevant.

The most common reports of the malformation in anuran species are from taxa that mostly depend on water for their development. The aquatic environments are an important vehicle for many of the main factors that can cause these anomalies (Henle *et al.*, 2017). In our study, the development of five out of the six species is related to this environment, but the most impressive and severe case occurred in *S. ajuricaba*, a species that does not rely at all on water bodies for reproduction. This finding suggests that among our records different causes should have taken place for the induction of malformations.

Reports of anuran malformations in the Amazon Forest are alarming growing. Even when mining activities in the Amazon is associated to the release of many potentially teratogenic substances (Britson and Threlkeld, 1998; Prati *et al.*, 2002), none of our records were close to mining areas. The use of mercury is common in the Brazilian for gold mining (Biller, 1994; Wasserman *et al.,* 2007; Ramos *et al.,* 2020), and its eventual link with anuran malformations as of other mining by-products should be carefully studied.

Our records represent the first known malformation cases on adults of the six studied species, and also for the family Phyllomedusidae and the genus *Synapturanus*.

## Acknowledgments

ATM and EDK thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for scholarships (process n° 142153/2019-2 and 132131/2020-0, respectively). IYF thanks Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM, Brazil) for scholarship. We acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). K.C. Torralvo and L. Wronski helped during fieldwork and Marcelo Menin (*in memoriam*) collected the specimens of *S. ajuricaba*, also guided research on the Amazonian herpetofauna and on anuran malformations; we also thanks to Laboratório de Confiabilidade (Isaias A.L. Saraiva, Paulo Gonçalves and Veridiana L. Ferreira) of Instituto Senai de Inovação em Microeletrônica for the radiographs of the specimens; to Fernanda P. Werneck and Ariane Silva for access to the INPA-H collection; to Claudio Borteiro and an anonymous reviewer for their philosophical and grammatical contributions to the manuscript; to Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade (Sisbio; Process n° 70834-3 and 73647-3) and Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA; Process 983/2018) for sampling permits.

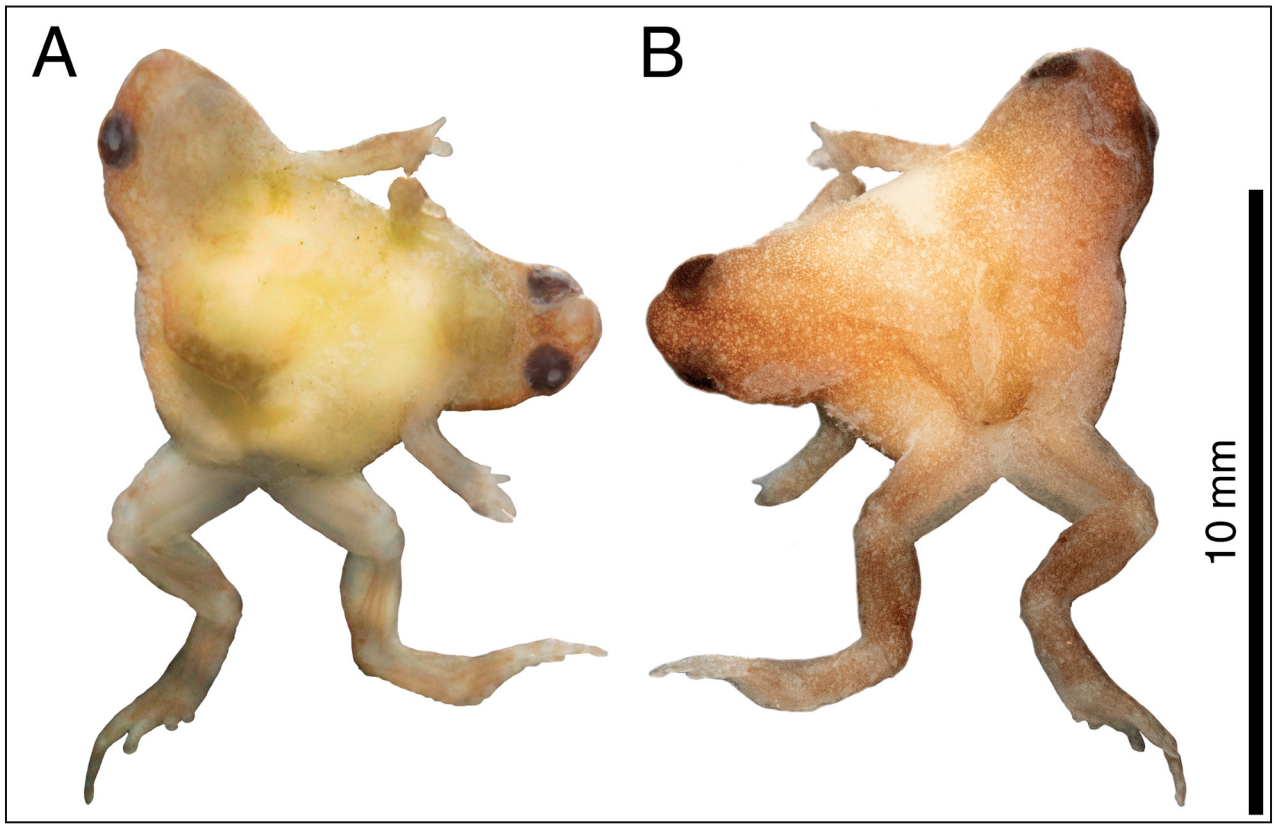


**Figure 6.** Juvenile Siamese twins of *Synapturanus ajuricaba*. (A) Dorsal view; (B) Ventral view.

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