Herpetology Notes, volume 15: 229-232 (2022) (published online on 26 April 2022)

# New records of limb malformations in the endemic Palm Rocket Frog, *Rheobates palmatus* (Anura: Aromobatidae), from Colombia

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Considering the multiple functions of amphibians in natural systems and their vulnerability to habitat changes, these animals are considered ideal bioindicators of environmental quality (Carr and Fahrig, 2001; Blaustein et al., 2003; Smith and Sutherland, 2014). The early detection of malformations in this taxonomic group can raise warnings about the health of a site and quantifying abnormalities and their causal factors is highly significant when it comes to planning and taking actions to control the sources of deformations. At the same time, conservation strategies can be generated to help preserve amphibian species, guarantee the ecological services provided by these organisms, and maintain overall ecosystem balance (Meteyer, 2000; Lannoo, 2008; Whittaker et al., 2013). However, some authors have proposed that up to 5% of individuals in a stable natural population can display body deviations (Blaustein and Johnson, 2003; Lunde and Johnson, 2012), and studies exist where even slightly higher percentages are reported (Vershinin, 1989; Severtsova et al., 2012). Although cases of malformations in amphibians have been reported worldwide, the number of reports in South America is scarce compared to Europe or North America (Lannoo, 2008). In particular, in Colombia there are few studies to date, and none allow accurate identification of possible causes (Rojas-Morales and Escobar-Lasso, 2013; Cruz-Esquivel et al., 2017; Zuluaga-Isaza et al., 2017; Marín-Martínez and Serna-Botero, 2019).

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Several factors are known to cause malformations in amphibians, including parasites, such as the trematode *Ribeiroia ondatrae* (Johnson et al., 2001, 2002; Blaustein and Johnson, 2003; Ankley et al., 2004; Johnson and Lunde, 2005; Rajakaruna et al., 2008), selective predation (Gray and Lethaby, 2010), inbreeding (Williams et al., 2008), high levels of ultraviolet radiation (Blaustein and Johnson, 2003; Ankley et al., 2004, Barragán-Ramírez and Navarrete-Heredia, 2011), and various environmental contaminants, such as agrochemicals (Ouellet et al., 1997; Blaustein and Johnson, 2003; Ankley et al., 2004; Piha et al., 2006; Robles-Mendoza et al., 2009).

The Palm Rocket Frog, *Rheobates palmatus* (Werner, 1899), is endemic to the Colombian Andes and is found at elevations between 300 and 2400 m in different habitat types with certain levels of disturbance (Lüddecke, 2003). It is a cryptic, rheophilic frog species found in humid environments with rocky slow-current streams, and it breeds in shallow lotic currents (Acosta-Galvis, 2012). The IUCN categorizes this species as Least Concern (LC) with stable populations and no known specific threats. However, its conservation could be compromised by deforestation related to agricultural activities and by pollution from crop fumigation (Ramírez et al., 2010). Recently, a case of hemimelia and brachydactyly was reported in an individual of *R. palmatus* from near the *vereda* Montebello, Norcasia Municipality, Caldas Department, Colombia (Zuluaga-Isaza et al., 2017), a distance of approximately 5 km from the nearest site visited in this study.

We here describe limb abnormalities in three individuals of *R. palmatus* observed during herpetological monitoring in the northeastern region of Caldas Department, Colombia, specifically in the buffer zone of the the Manso River's interbasin transfer in the municipalities of Norcasia and Samaná. The animals were collected, radiographed, and deposited in the vertebrate and invertebrate collection of the Museo de Historia Natural de la Universidad de Caldas (MHNUCa).

The first individual captured was a juvenile (MHNUCa-0841; snout–vent length, SVL = 14.7 mm; body weight, BW = 0.4 g) that was swimming in Agüetarro Stream, *vereda* Berlín, Samaná Municipality (5.5603°N, 74.9596°W; elevation 792 m) on 6 December 2017 at 10:21 h. We observed ectromelia in the left forelimb (Fig. A1–A3).

The second individual (MHNUCa-0840; SVL = 31.5 mm; BW = 3.6 g) was found moving on rocks in the water current of Bolloliso Stream, *vereda* Berlín, Samaná Municipality (5.5898°N, 74.9456°W; elevation 807 m) on 27 September 2017 at 11:04 h. This individual showed radioulnar ectromelia (complete absence of the anterior segment of the limb; Meteyer, 2000; Lannoo, 2008) in the right forelimb, as well as ectrodactyly in the left hindlimb (Fig. B1–B3).

The last individual captured (MHNUCa-0839; SVL = 30 mm; BW = 2.9 g) was observed on 24 September 2017 at 11:08 h in La Piscina stream, *vereda* Berlin, Samaná Municipality (5.5854°N, 74.9433°W; elevation 831 m). It was found immobile on leaf litter at the edge of the stream. The individual showed brachydactyly in the right forelimb, showing all hand bones, except for the last two phalanges of the first digit (Meteyer, 2000; Lannoo, 2008). It also displayed ectrodactyly, a complete absence of the metatarsal bones and phalanges (Meteyer, 2000; Lannoo, 2008), in the left hindlimb (Fig. C1–C3). Overall, this study provides the first report of ectrodactyly and ectromelia for this species in Colombia.

We captured a total of 158 individuals during the monitoring study conducted between October 2016 and September 2018. The three frogs with abnormalities represent 2% of this population and this percentage is found within the expected range of malformations resulting from genetic and epigenetic processes and alterations in a natural population (Stocum, 2000; Blaustein and Johnson, 2003; Lunde and Johnson, 2012). However, an increase in the appearance of deformations can reflect environmental alterations, as suggested by various studies (e.g., Ouellet et al., 1997; Sparling et al., 2001; Lannoo, 2008; Peltzer et al., 2011; Bacon et al., 2013). Ectrodactyly, brachydactyly, and ectromelia are associated with chemical contaminants (e.g., pesticides used in the agricultural industry), minerals, UV radiation, or damage caused by predation (Ouellet et al., 1997; Gardiner and Hoppe, 1999; Lannoo, 2008; Bacon et al., 2013). Although the latter is among the most common causes (Gardiner and Hoppe, 1999), in our case, it is unlikely that the observed deformations resulted from amputation trauma during a predator attack. In particular, two of the individuals displayed malformations in different limbs and it is questionable that these individuals escaped twice from a predator and that each attack resulted in a deformation. On the other hand, a history of malformations, including brachydactyly and hemimelia (Zuluaga-Isaza et al., 2017) and taumelia (Marín-Martínez and Serna-Botero, 2019) in different streams of the same zone suggests that these malformations could be induced by causes that include radiation, toxic substances, or parasites. This fact justifies conducting studies in this area to identify the real causes of these deformations, especially considering that the zone is under different anthropic pressures such as deforestation, agriculture, livestock farming, and artisanal mining.

**Acknowledgments.** We thank ISAGEN S.A E.S.P. (contract 47/623) for funding the research. We are grateful to Beatriz Toro Restrepo, Viviana Andrea Ramirez Castaño, and Sergio Escobar Lasso for their useful comments that improved the manuscript.

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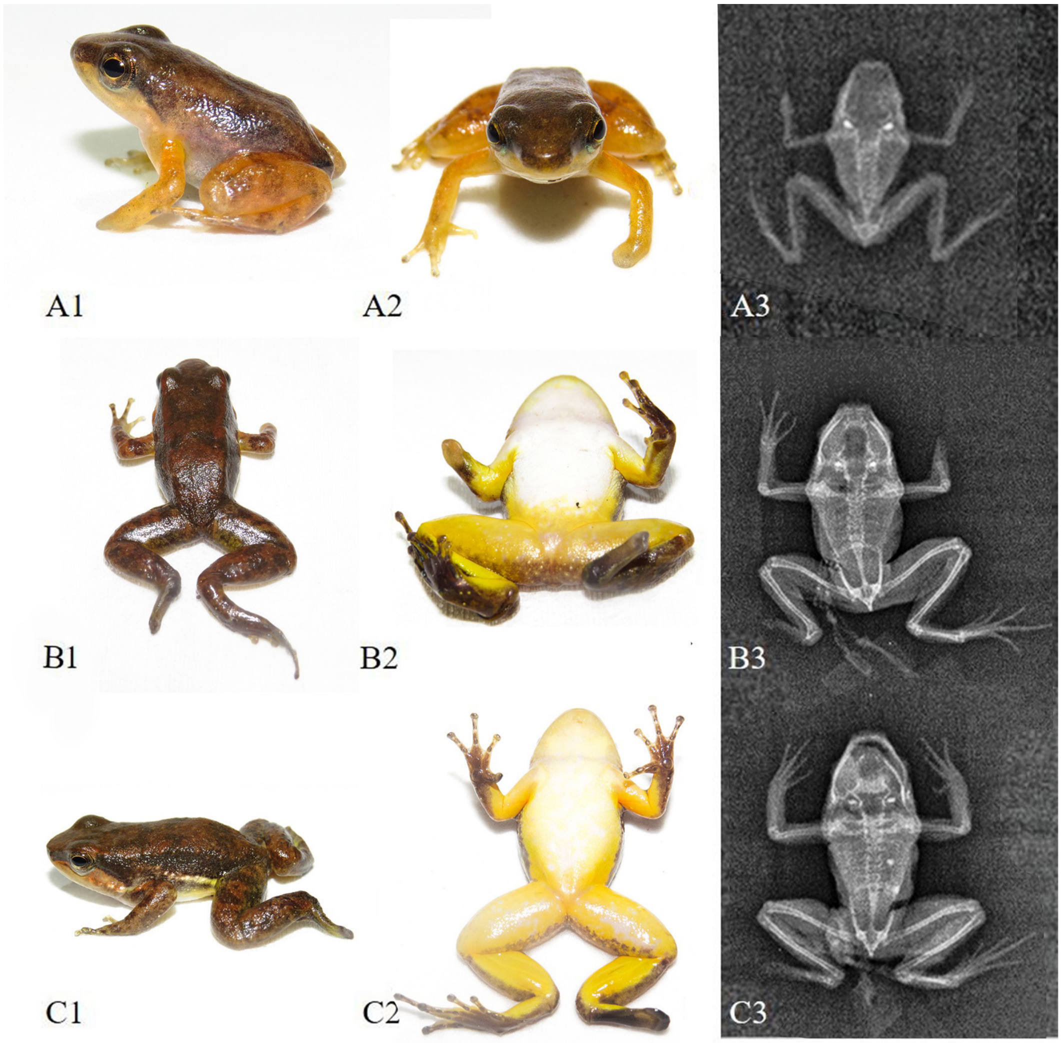


**Figure 1.** External views and x-rays of three *Rheobates palmatus* (A: MHNUCa-0841; B: MHNUCa-0840; C: MHNUCa-0839) of unknown sex from the buffer zone of the interbasin transfer of the Rio Manso, Caldas, Colombia, displaying limb malformations. (A1–3) Lateral, frontal, and x-ray views, respectively, of a juvenile with ectromelia in the left forelimb. (B1–3) Dorsal, ventral, and x-ray views, respectively, of an adult with ectromelia in the right forelimb and ectrodactyly in the left hindlimb. (C1–3) Lateral, ventral, and x-ray views of an adult with brachydactyly in the right forelimb and ectromelia in the left hindlimb. Photographs by Mateo Marín-Martínez.

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*Accepted by Hinrich Kaiser*