

Description of Morphological Abnormalities in *Rhinella arenarum* (ANURA: BUFONIDAE)

P.D. Chilote¹ L.E. Bozzolo² F.R. Gutierrez¹ L.E. Moreno^{1,2,*}

¹Zoology Department, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis – UNSL, Chacabuco y Pedernera, San Luis, Argentina

²Vertebrate Functional Morphology, Zoology Department, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis – UNSL, Chacabuco y Pedernera, San Luis, Argentina

Address for correspondence L.E. Moreno, National University of San Luis – UNSL, Chacabuco y Pedernera, San Luis, 5700, Argentina (e-mail: lilianaemoreno@gmail.com).

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Abstract

The rise of anatomical abnormalities in anurans over recent years has increased the interest in producing more and better information about the abnormalities observed in specimens within natural environments that are currently being altered. The aim of this work was to identify and describe the osseous abnormalities found in *Rhinella arenarum*. Four samplings were conducted between March 2009 and February 2010 in the Claro river, located in San Francisco in the Ayacucho Department in the province of San Luis. Specimens of *R. arenarum* with visible abnormalities were observed and collected manually. They were euthanized in the laboratory, preserved in 10% formaldehyde, and stored in the Herpetological Collection Unit of the National University of San Luis (CH-UNSL). The Alizarin-Alzian technique was also applied to the specimens. Photographs of the abnormalities were taken, and observations were made with a stereoscopic microscope. The following abnormalities were identified and described: femur ectromelia, tibial and fibular ectromelia, ectrodactylia and brachydactyly. Further work could focus on determining the causes of the abnormalities observed in this species, conducting an integrated study, and attempting to link this with the worldwide phenomenon of amphibious declination.

Keywords

- ▶ abnormalities
- ▶ *Rhinella arenarum*
- ▶ brachydactyly
- ▶ ectrodactylia
- ▶ ectromelia

Introduction

Amphibians are considered good indicators of healthy environments.^{1–3} Reports on amphibian deformations have increased dramatically in recent years.⁴ The causes and implications of these abnormalities remain poorly explained, but several researchers have suggested they may indicate an emerging threat for populations of amphibians and other forms of life.^{5–7}

Several causes or groups of causes are proposed as originators of abnormalities in amphibians, including UV-B radiation,^{8–10} trematodes,^{11–14} retinoids,⁶ pesticides,^{15–17} other chemical pollutants^{18–21} and predation.^{22,23} However, knowledge about the abnormalities resulting from heredity,

development, traumatic factors, and particularly, knowledge regarding the variation in abnormality rates across species, life stages, types of habitat, and geographical areas remain unexplained, thus making the identification of differences between natural and artificial abnormalities problematic.¹¹

There is evidence of anuran specimens with supernumerary extremities in Argentina.²⁴ The interest in dealing with this issue has grown in recent years in Argentina due to the increasing number of anurans with abnormalities.^{20,25–30}

These findings have also occurred in the province of San Luis.^{31–33} Consequently, the objective of this work was to identify and describe the abnormalities found in *Rhinella arenarum* from a natural environment in the province of San Luis.

Materials and Methods

Description of the Site and Natural History

The work was conducted in the Claro river, one of the main tributaries of the San Francisco river, in the town of San Francisco (S 32° 36.7771 - WO 66° 08.0357), Ayacucho Department, province of San Luis. San Francisco del Monte de Oro is located to the North of the province, in a valley framed by the Sierras Centrales and the Socoscora hills. One of the characteristics of this area is the presence of Caranday palms (*Trithrinax campestris*), surviving in the Southern most habitat for this species.

The climate is dynamic, with strong daily and seasonal temperature fluctuations. During summer, the temperature ranges between 17°C and 38°C. Nights are often cold in winter, with minimal temperatures below 0°C. However, daytime temperatures are temperate, ranging between 15°C and 20°C.

Sampling Method

Four nocturnal samplings were made between March 2009 and February 2010, and six abnormal specimens of *Rhinella arenarum* (2 juveniles and 4 adult individuals) were observed and collected. Specimens were collected using the Collection by Visual Encounters method (CVE), described by Crump and Scott Junior.³⁴

Laboratory

Specimens were taken to the laboratory, euthanized with an MS-222 solution, fixed with 10% formaldehyde, and stored in the Herpetological Collection Unit of the National University of San Luis (CH-UNSL: 0431; 0432; 0433; 0434; 0435 and 0436). Differential staining of cartilage, bone and subsequent diafanization these animals was apply according to the technique developed by Wassersug.³⁵ All observations were made using a stereoscopic microscope (Olympus SZ51). Photographs of the abnormalities found were taken with a digital camera (Canon EOS Rebel T5).

Morphological Analysis and Terminology

The abnormalities observed were described according to the terminology proposed by Meteyer.³⁶ We adhered to Fabrezi and Goldberg³⁷ for the description of skeletal structures.

Results and Discussion

CH-UNSL0431 Juvenile specimen: Ectromelia of the right hindlimb (► Fig. 1).

CH-UNSL0432: Juvenile specimen: Ectrodactylia of the left hindlimb. Absent metatarsal bone in digit V, curved digit III and digit II, and merged cartilages. It is not possible to differentiate digit I from digit II, although associated with these there are two phalanges and an ossified distal phalanx with a more robust appearance than that observed in the right limb with normal development. Absence of prehallux (► Fig. 2).

CH-UNSL0433 Adult specimen: Ectrodactylia of the right back limb. Complete absence of metatarsal bones and digits, except for the prehallux. Undetermined ossified



Fig. 1 Juvenile specimen with ectromelia.

traces were also observed. The fibula presents a slight curvature in relation to the left limb (► Fig. 3).

CH-UNSL0434 Adult specimen: Brachydactyly and ectrodactylia of the right hindlimb. Digit I presents one phalanx, and digit IV presents 2 phalanges. Complete absence of digit V. The prehallux is cartilaginous in both limbs (► Fig. 4).

CH-UNSL0435 Adult specimen: Brachydactyly of the right hindlimb. Digit III has 2 phalanges, the last of which is

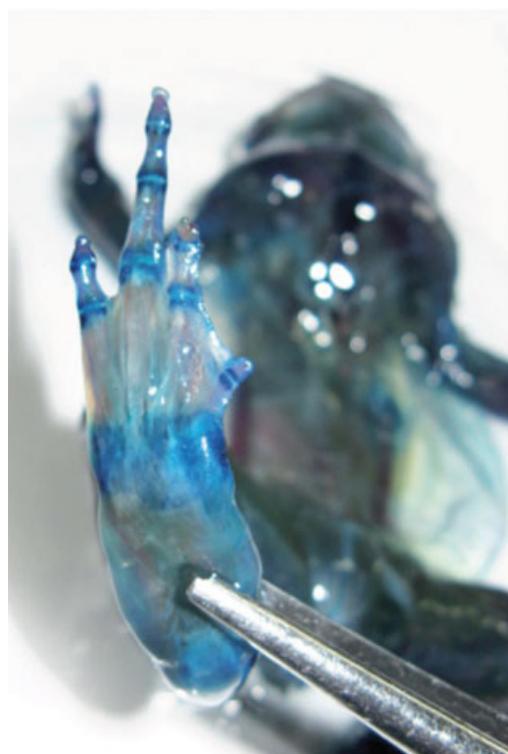


Fig. 2 Young specimen with ectrodactylia.



Fig. 3 Ectrodactyly of the right back limb.



Fig. 4 Detail of the right back limb with brachydactyly and ectrodactyly.



Fig. 5 Adult specimen with brachydactyly.

forked; digit IV has 2 phalanges, and the last one is forked, swollen and cartilaginous (► **Fig. 5**).

CH-UNSL0436 Adult specimen: Ectromelia in the tibiofibula of the left hindlimb, where only the proximal portion of the ossified and strong tibial and fibular traces are observable. The basal portion of the tibiofibula in the lateral view is swollen and shorter than the normal tibiofibula (► **Fig. 6**).

All the abnormalities observed were located in the hindlimbs. The most common abnormality was ectrodactyly, followed by brachydactyly and ectromelia. Multiple abnormalities in a single limb were observed in one specimen. The cases recorded present different forms in a single abnormality.

The abnormalities in the hindlimbs of the captured specimens are the most common abnormalities observed in



Fig. 6 with ectromelia in the tibiafibula of the left back limb.

natural areas.^{6,7,12} However, the causes of these morphological abnormalities have not yet been identified. This makes it difficult to determine the difference between natural abnormalities and anthropogenic abnormalities.¹¹

We have examined unilateral abnormalities in the hind extremities in this study, which coincides with findings of studies conducted by Ouellet et al.⁷ and Johnson et al.¹² However, while the most frequent abnormalities reported in the literature are additional extremities (polymelia),^{6,7,38} or reduced limbs (hemimelia),¹¹ we did not observe these abnormalities. The variation of abnormalities in the limbs recorded in this study suggest etiological heterogeneity.

Conclusion

These cases may represent the first documented report of morphological abnormalities in an anuran population of San Luis North area, in Argentina. We believe it is important to establish precedents for abnormalities in different anuran populations to document the increase of occurrence rates and abnormality recurrence.

Collection Permission

The collection of the *Rhinella arenarum* specimens that were used to conduct this study was authorized by Resolution N° 2525 of the Biodiversity Program of San Luis Province.

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