





COMUNICACIONES BREVES

## LIMB ANOMALIES OF *Scinax Nasicus* (COPE, 1862) (Anura: Hylidae) TADPOLES, FROM CORRIENTES, ARGENTINA.

*Anomalías de los miembros de larvas de Scinax nasicus (Cope, 1862) (Anura: Hylidae), de Corrientes, Argentina.*

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**ABSTRACT:** This paper presents a description of limb anomalies observed on *Scinax nasicus* tadpoles. The most frequent skeletal malformations were detected on the autopodium of the hindlimb. Apody and ectrodactyly, and aphalangy were identified but we cannot establish a cause-effect relationship of the observed anomalies. This report highlights the importance of monitoring morphological anomalies of anurans at larval stages as a possible indicator of environmental disturbance e.g. the presence of contaminants in the water.

**KEYWORDS:** Amphibians, larvae, leg, skeletal malformations.

**RESUMEN:** En este artículo se describen anomalías observadas en las extremidades de renacuajos de *Scinax nasicus*. Las malformaciones esqueléticas más frecuentes se registraron en el autopodio del miembro posterior. Se identificó apodia, ectrodactilia, y afalangia, sin embargo no fue posible establecer una relación causa-efecto. Este trabajo destaca la importancia de monitorear las anomalías morfológicas de los anuros en estadios larvarios, como posible indicador de perturbaciones ambientales, por ejemplo, la presencia de contaminantes en el agua.

**PALABRAS CLAVES:** Anfibios, larvas, extremidades, malformaciones esqueléticas.

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Como citar este artículo: Vaca, A. D., Kassor, R.G., Curi, L. M. & Sandoval, M. T. (2023). Limb anomalies of *Scinax Nasicus* (COPE, 1862) (Anura: Hylidae) tadpoles, from Corrientes, Argentina. Revista FACENA 33, 1-8 p. Doi: <https://doi.org/10.30972/fac.3307005>

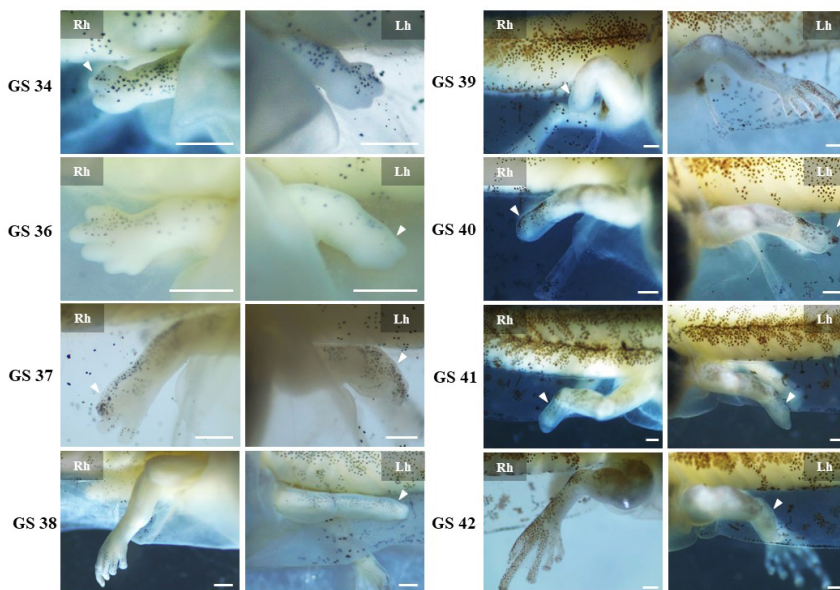
Recibido/Received: 30/08/2023. Revisión: 14/09/2023. Aceptado/Accepted: 31/10/2023.

Editor asociado: Federico Marangoni .

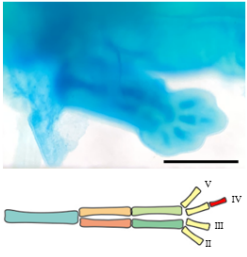
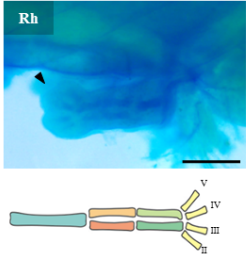
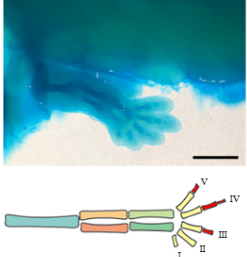
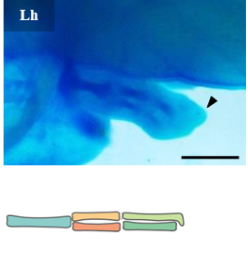
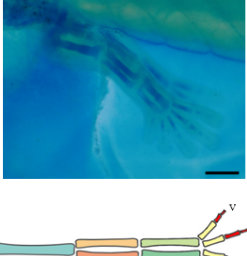
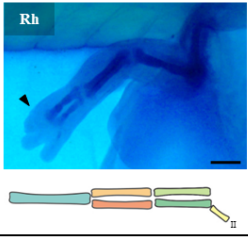
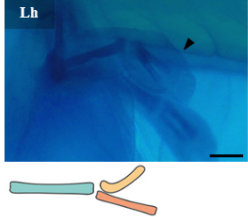
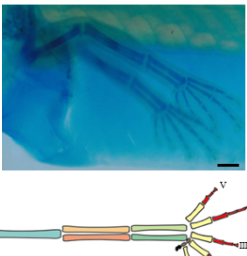
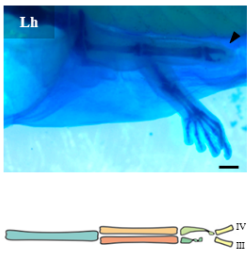
Publicado en línea: 12/12/2023. ISSN 1851-507X en línea.

The increasing incidence and severity of reported anuran malformations have generated scientific warning, especially regarding its global decline (Green *et al.*, 2020; Toledo *et al.*, 2023). In amphibian populations, less than 5 % of morphological anomalies due to mutation, developmental errors, or trauma can be expected (Ouellet, 2000). But, in some cases, this frequency is higher (15-90 %) and may be related to human-induced environmental damage such as increased ultraviolet (UV) radiation, chemical contamination, predation injury or parasite infection, among others (Ballengee & Sessions, 2009; Blaustein *et al.*, 2011; Peltzer *et al.*, 2011, 2021). In this study, limb anomalies of *Scinax nasicus* tadpoles were described. This species is widely distributed in Argentina and is common in urban and periurban environments. As part of a student fieldwork corresponding to a graduation project, premetamorphic tadpoles (Gosner Stage 25) were collected on 1st December 2021 in an artificial pool currently used for fish farming belonging to the Instituto de Ictiología (27° 27' 25.60" S, 58° 49' 23" W), Facultad Ciencias Veterinarias, Corrientes, Argentina. In the laboratory, they were kept in plastic containers with dechlorinated tap water and fed with boiled lettuce ad libitum, free of predators. Every five days, groups of 10 tadpoles were euthanized with 5 % ethyl aminobenzoate solution Muelita® added to the rearing water, according to the American Veterinary Medical Association (Leary *et al.*, 2013). This protocol was approved by the Ethics Committee of the Facultad de Ciencias Exactas y Naturales y Agrimensura-UNNE (RES. 0756/18 CD). After that, tadpoles were fixed and conserved in a 10 % formalin solution. The developmental stages were determined according to Gosner (1960). Tadpoles with limb anomalies and normal tadpoles at equivalent Gosner Stage (GS) were double staining and diaphanized following the Wassersug (1976) procedure. Limb components were recognized according to Fabrezi *et al.* (2017), and the terminology and description of anomalies were based on Henle *et al.* (2017). Of a total of 640 tadpoles, 1.25 % (N = 8) presented anomalous hindlimbs, whereas the forelimbs presented a normal morphology. Anomalies were detected in either one or two hindlimbs of the same tadpole, even with different types of anomalies on the right and left limb (Fig. 1). The main anomalies were observed in the autopodium, whereas

the stylopodium and zeugopodium were well-developed. Missing elements of mesopodium, metapodium, and/or phalanges, apody, ectrodactyly and aphyalangy were recorded (Fig. 2 and 3). The analyzed tadpoles did not show signs of trauma or predator (cannibalism) injuries as facial/cranial, tail, or limb damage. Anuran limb anomalies have been widely described for adults (Bionda *et al.*, 2012, Shin *et al.*, 2020; Souza *et al.*, 2021; Goodman *et al.*, 2022; Mônico *et al.*, 2019; 2023), but descriptions of larval stages are scarce (Ballengee & Sessions, 2009). The most frequently reported limb anomalies include missing and/or partially missing limbs, multiple extra limbs and/or extra digits, incomplete limb formation, and body deformities (Pedroso-Santos *et al.*, 2020). However, assigning the origin of these malformations is difficult because their origin may be multifactorial (Pommer-Barbosa *et al.* 2022). We cannot establish a cause-effect relationship of the observed limb anomalies on *Scinax nasicus* tadpoles. This report highlights the importance of monitoring morphological anomalies of anurans at larval stages as a possible indicator of environmental disturbance e.g. the presence of contaminants in the water.



**Figure 1. Hindlimb anomalies of *Scinax nasicus* tadpoles.** Photographs show the right hindlimb (Rh) and left hindlimb (Lh) of the same specimen. The white arrowhead indicates the abnormal hindlimb. Scale bar: 0.5 mm.

GS	NORMAL HINDLIMB	ABNORMAL HINDLIMB	ANOMALY
34			<b>Aphalangy</b> Missing phalange of digit IV.
36			<b>Ectrodactyly</b> Abnormal fibulare. Missing metatarsals of digits I-V, and phalanges of digits III-V.
37			<b>Ectrodactyly</b> Missing metatarsal I, III-V, and phalanges of digits II-V.
			<b>Apody</b> Abnormal fibulare. Missing fibulare, tibiale, metatarsals, and phalanges of digits I-V.
38			<b>Ectrodactyly</b> Abnormal fibulare and tibiale. Missing distal tarsals and prehallux. Missing metatarsals and phalanges of digits I, II, and V. Missing phalanges of digits III and IV.

**Figure 2. Normal and abnormal morphology of hindlimbs of *Scinax nasicus* tadpoles.** Photographs show the details of diaphanized hindlimbs of tadpoles at GS 34-38. The black arrowhead indicates the abnormal limb. Illustrations depict the proximal-distal elements of hindlimbs (see color references in Fig. 3). Scale bar: 0.5 mm. Illustrations: Ayelen Vaca.

GS	NORMAL HINDLIMB	ABNORMAL HINDLIMB	ANOMALY										
39			<p><b>Apody</b></p> <p>Missing fibulare, tibiale, distal tarsals, metatarsals, and phalanges of digits I-V.</p>										
40			<p><b>Ectrodactyly</b></p> <p>Incomplete fibulare and tibiale. Missing distal tarsals, metatarsals and phalanges of digits I-V.</p>										
41			<p><b>Ectrodactyly</b></p> <p>Incomplete fibulare and tibiale. Missing distal tarsals, metatarsals and phalanges of digits I-V.</p>										
			<p><b>Ectrodactyly</b></p> <p>Abnormal tibiale. Missing distal tarsals, metatarsals and phalanges of digits I-V.</p>										
42			<p><b>Ectrodactyly</b></p> <p>Incomplete fibulare and tibiale. Missing distal tarsals, metatarsals and phalanges of digits I-V.</p>										
<table border="0"> <tr> <td> Femur</td> <td> Tibia</td> <td> Tibiale</td> <td> Distal tarsals</td> <td> Metatarsals</td> </tr> <tr> <td> Fibula</td> <td> Tibiofibula</td> <td> Fibulare</td> <td> Prehalux</td> <td> Phalanges</td> </tr> </table>				Femur	Tibia	Tibiale	Distal tarsals	Metatarsals	Fibula	Tibiofibula	Fibulare	Prehalux	Phalanges
Femur	Tibia	Tibiale	Distal tarsals	Metatarsals									
Fibula	Tibiofibula	Fibulare	Prehalux	Phalanges									

**Figure 3. Normal and abnormal morphology of hindlimbs of *Scinax nasicus* tadpoles.** Photographs show the details of diaphanized hindlimbs of tadpoles at GS 39-42. The black arrowhead indicates the abnormal limb. Illustrations depict the proximal-distal elements of hindlimbs. Scale bar: 0.5 mm. Illustrations: Ayelen Vaca.

## ACKNOWLEDGMENTS

PI: 20F 002. SGCyT-UNNE.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Vaca A.D.: execution, analysis, interpretation, illustration, and writing; Kassor R.G.: analysis and interpretation. Curi L.M.: material collection, interpretation and writing; Sandoval M.T.: design, execution, analysis and writing. All authors read and approved the final manuscript

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