

Anomalies in amphibians from the eastern Amazon region

FILLIPE PEDROSO-SANTOS^{1*}, PATRICK R. SANCHES², JACKSON CLEITON SOUSA³
& CARLOS EDUARDO COSTA-CAMPOS¹

¹Universidade Federal do Amapá, Departamento de Ciências Biológicas e da Saúde, Laboratório de Herpetologia, Macapá, AP, Brazil, CEP: 68.903-419

²Programa de Pós-graduação em Biologia (Ecologia) - INPA, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil, CEP: 69.060-001

³Programa de Pós-graduação em Biodiversidade Tropical - PPGBio, Universidade Federal do Amapá, Macapá, AP, Brazil, CEP: 68.903-419

*Corresponding author e-mail: fillipepedrosodossantos@gmail.com

INTRODUCTION

Over the years, a broad range of morphological abnormalities (anomalies) have been documented from the anurans of both wild and urban populations (Ouellet, 2000; Blaustein & Johnson, 2003; Bionda et al., 2012). These anomalies have been recorded as a consequence of factors related to changes in the environment, such as exposure to UV-radiation and environmental pollutants (Cohen, 2001; Lannoo, 2009; Lunde & Johnson, 2012; Agostini et al., 2013) and parasitic infection (Kiesecker, 2002). Anomalies include incomplete or missing digits and limbs, additional digits and limbs, and body deformations, in which limb malformations seems to be the most prevalent (Gardiner & Hoppe, 1999; Blaustein & Johnson, 2003). Records have come from isolated individuals and from populations, and in the latter case they normally occur in about only 5 % of the population (Read, 1997). Thus the rate of occurrence may be considered abnormally high when the value in a population exceeds this mark (Piha et al., 2006; Peltzer et al., 2011; Bionda et al., 2012).

In this study we report anomalies in several anuran species from Amapá state, eastern Amazonia, Brazil, consider an endemic zone for the herpetofauna in the Guiana Shield (Silva et al., 2005), and reinforce the importance of the monitoring and conservation of the habitats for anuran populations in these areas.

MATERIALS AND METHODS

We sampled anurans during fieldwork conducted in the municipalities of Macapá (0° 00'29.9" S, 51° 05'43.1" W), Santana (0° 02'09.2" N, 51° 09'38.8" W) and Serra do Navio (0° 54'50.0" N, 52° 59'59.2" W), all in the State of Amapá, Brazil. Samples were collected between July 2017 to June 2018, with active visual and auditory searches along transects (Crump & Scott Jr., 1994; Heyer et al., 1994) during the night (from 17:00 h to 21:00 h), in temporary lentic water bodies surrounded by arboreal vegetation, wetlands and urban areas. The total search effort amounted to 900 person hours.

Abnormal individuals were examined for malformations which were then identified with reference to a recent

publications on the subject (Lannoo, 2009; Peltzer et al., 2011; Henle et al., 2017; Meteyer, 2000), although for terminology and traits we have followed Henle et al. (2017) (see Table 1).

Specimens with anomalies were collected under permit

Table 1. Terminology of anuran anomalies and traits adopted in our study, according to Henle et al. (2017)

Type of anomaly	Traits
Amely	Missing limb
Anophthalmy	Missing eye
Brachydactyly	Presence of short toe, but the metatarsal bones remain present, with the number phalanges reduced
Ectrodactyly	Completely missing digit, including metatarsal bone
Ectromely	Missing limb segments
Ocular anomaly of the eye	Anomalous pigmentation of iris
Polydactyly	Extra digit, including metatarsal bone
Polymely	Duplication of a complete limb or parts thereof
Syndactyly	Fused digits
Schizodactyly	Forked digits

(authorisation #48102-2) from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), killed with 5 % lidocaine, fixed in 10 % formalin and transferred to 70 % ethanol (Heyer et al., 1994). They have been deposited in the Herpetological Collection of the Universidade Federal do Amapá (CECC).

RESULTS

A total of 255 adult anurans were observed, of which 20 had one or more morphological abnormality. These are listed in Table 2 and illustrated in Figure 1. Between habitat types, 97 individuals were recorded in primary forest, of which 6 (6.2 %) had anomalies; 77 individuals were recorded in urban areas, of which 9 (11.7 %) had anomalies; and, 81 individuals were reported in floodplain areas, of which

Table 2. Anuran species reported in our study with anomalies, Amapá State, Brazil

Species	Type of abnormalities	Localities	Voucher specimens (CECC)
Bufonidae			
<i>Rhinella</i> cf. <i>margaritifera</i>	Brachydactyly	0° 54'39.0" N 52° 00'42.1" W	2465
<i>Rhinella</i> cf. <i>margaritifera</i>	Brachydactyly + Syndactyly	0° 54'38.7" N 52° 00'41.9" W	1275
<i>Rhinella</i> cf. <i>margaritifera</i>	Schizodactyly	0° 54'38.8" N 52° 00'41.1" W	1218
<i>Rhinella marina</i>	Ectrodactyly	0° 54'28.9" N 52° 00'51.0" W	1416
<i>Rhinella marina</i>	Ectromely	0° 54'28.8" N 52° 00'50.9" W	1417
<i>Rhinella marina</i>	Brachydactyly + Polydactyly	0° 54'28.5" N 52° 00'51.2" W	1418
<i>Rhinella marina</i>	Polydactyly	0° 54'28.4" N 52° 00'51.1" W	2696
<i>Rhinella marina</i>	Ectrodactyly	0° 54'28.2" N 52° 00'51.2" W	2697
<i>Rhinella marina</i>	Brachydactyly + Ectrodactyly	0° 54'28.0" N 52° 00'51.4" W	2698
Dendrobatidae			
<i>Ranitomeya amazonica</i>	Syndactyly	0° 54'45.9" N 52° 00'32.7" W	2699
Hylidae			
<i>Boana boans</i>	Colour anomaly of the eye	0° 54'36.5" N 52° 00'37.5" W	0836
<i>Lysapsus bolivianus</i>	Ectromely	0° 02'09.0" S 51° 09'33.0" W	0696
<i>Scinax</i> cf. <i>ruber</i>	Colour anomaly of the eye	0° 02'06.8" S 51° 09'31.5" W	2390
<i>Sphaenorhynchus lacteus</i>	Amelia + Colour anomaly of the eye	0° 02'06.8" S 51° 09'31.5" W	0436
Leptodactylidae			
<i>Adenomera hylaedactyla</i>	Ectromely	0° 53'55.1" N 52° 00'05.4" W	0839
<i>Leptodactylus longirostris</i>	Ectromely	0° 54'29.8" N 52° 00'50.1" W	1516
<i>Leptodactylus mystaceus</i>	Anophthalmy	0° 54'23.8" N 52° 00'26.3" W	0368
<i>Leptodactylus</i> cf. <i>podicipinus</i>	Polymely	0° 00'30.8" S 51° 05'43.9" W	2628
<i>Leptodactylus petersii</i>	Brachydactyly	0° 02'08.5" S 51° 09'31.4" W	2403
<i>Leptodactylus</i> sp.	Amely	0° 02'09.4" S 51° 09'32.5" W	2633

with 5 (6.2 %) had anomalies. The species most frequently showing anomalies was *Rhinella marina* (Bufonidae) (n = 6). We identified 10 types of anomalies and their frequencies were as follows: Brachydactyly (25 %; n = 5); Ectromely (20 %; n = 4); Ectrodactyly (15 %; n = 3); Ocular anomaly (15 %; n = 3); Syndactyly (10 %; n = 2); Amely (10 %; n = 2); Polydactyly (10 %; n = 2); Anophthalmy (5 %; n = 1); Polymely (5 %; n = 1); and Schizodactyly (5 %; n = 1). Individuals of the species *Sphaenorhynchus lacteus*, *Rhinella* cf. *margaritifera*, and *R. marina* presented more than one type of abnormality (Table 2).

DISCUSSION

Globally, most accounts of morphological abnormalities in anurans have been reported in species of Bufonidae (e.g., García-Muñoz et al., 2010; Bionda et al., 2012; Correia et



Figure 1. Examples of morphological abnormalities shown by anuran species in this study- Colour anomaly of the eye: **A.** *Boana boans*, **B.** *Scinax* cf. *ruber* and **C.** *Sphaenorhynchus lacteus*; Anophthalmy: **D.** *Leptodactylus mystaceus*; Polymely: **E.** *Leptodactylus* cf. *podicipinus*; Brachydactyly: **F.** *Rhinella* cf. *margaritifera*; Ectromely: **G.** *Rhinella marina*; Polydactyly: **H.** *Rhinella marina*; Ectromely: **I.** *Adenomera hylaedactyla* and **J.** *Lysapsus bolivianus*; Amely: **K.** *Sphaenorhynchus lacteus*

al., 2018) and Ranidae (e.g., Haas et al., 2017). For Brazil, there are also reports mentioning other families but with apparently lower frequencies of anomalies, in particular Leptodactylidae (Sousa & Costa-Campos, 2016; Santos et al., 2017), Hylidae (Sousa & Costa-Campos, 2017; Ramalho et al., 2017; Silva-Soares & Mônico, 2017) and Aromobatidae (Santana et al., 2020).

In the literature, brachydactyly has been cited in several cases (e.g., Bionda et al., 2012; Sousa & Costa-Campos, 2016; Oliveira-Souza et al., 2020), being the most common in semi-aquatic and terrestrial species (Agostini et al., 2013), and in our study was observed in the genera *Leptodactylus* and *Rhinella* (Table 2). In addition, it is of interest to note the anomalies of *Leptodactylus* sp., *L. cf. podicipinus* (Fig. 1E) and *Sphaenorhynchus lacteus* (Fig. 1K). Previously, in these cases duplication of a complete limb or parts thereof (polymely), and missing limb (amely) have been cited as malformations caused by the trematode *Ribeiroia* (Kiesecker, 2002; Stopper et al., 2002; Schotthoefer et al., 2003; Johnson et al., 2012). However, there is no evidence of the presence of this parasite in Amapá state.

During this study, the urban areas provided the greatest number of specimens with anomalies although differences between habitat types were small. The urban areas of Amapá state have a historical record of environmental impacts. For example, in the 1990s an environmental accident in the port area of the municipality of Santana caused the pollution of

the water table and surface waters with arsenic, barium and manganese in several areas of the region, in which heavy metals from mining tailings were responsible for a biological imbalance (Casara, 2003; Muniz & Oliveira-Filho, 2006); this area is located 3.5 km from the floodplain area and 4 km from the urban area sampled in our study. Also, there are reports of environmental impacts of Serra do Navio mining in and around the Parque Natural Municipal do Cancão (protected conservation unit sampled in our study), resulting in contamination with chemical residues, including arsenic, deforestation and pollution of streams (Drummond, 2000; Queiroz et al., 2008), which might be considered exogenous factors affecting the frog populations (Santana et al., 2020). Future ecotoxicological studies of anuran populations are needed to establish the actual causes of morphological anomalies, especially in areas with a history of environmental impacts.

ACKNOWLEDGEMENTS

Thanks to Diego J. Santana for comments on an earlier version of this manuscript. We thank Christopher Jaster (PARNA Montanhas do Tumucumaque) for logistical support during fieldwork.

REFERENCES

- Agostini, M.G., Kacolis, F., Demetrio, P., Natale, G.S., Bonetto, C. & Ronco, A.E. (2013). Abnormalities in amphibian populations inhabiting agroecosystems in northeastern Buenos Aires Province, Argentina. *Diseases of Aquatic Organisms* 104: 163–171.
- Bionda, C., Salas, N., Caraffa, E., Baraquet, M. & Martino, A. (2012). On abnormalities recorded in an urban population of *Rhinella arenarum* from central Argentina. *Herpetology Notes* 5: 237–241.
- Blaustein, A.R. & Johnson, P.T. (2003). The complexity of deformed amphibians. *Frontiers in Ecology and the Environment* 1: 87–94.
- Casara, M. (2003). Predatory Mining in the Brazilian Amazon: Five Decades of Social and Environmental Irresponsibility in Amapá state. The Social Observatory, Florianópolis, Santa Catarina. 48 pp.
- Cohen, M.M. (2001). Frog decline, frog malformations, and a comparison of frog and human health. *American Journal of Medical Genetics* 104: 101–109.
- Correia, L.L., Almeida, J.P.F.A., Lisboa, B.S. & Nascimento, F.A.C. (2018). Brachydactyly in the toad *Rhinella granulosa* (Bufonidae) from the Caatinga of Brazil: a rare case with all limbs affected. *Herpetology Notes* 11: 445–448.
- Crump, M.A. & Scott, Jr N.J. (1994). Visual Encounter Surveys. In *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, 84–92 pp. Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C. & Foster, M.S. (Eds.). Washington, USA, Smithsonian Institution Press.
- Drummond, J.A. (2000). Private investments, environmental impact, and the quality of life at a mining venture in the Amazon – the case of the Serra do Navio manganese mine in Amapá. *História, Ciências, Saúde* 6: 753–792.
- García-Muñoz, E., Jorge, F., Rato, C. & Carretero, M.A. (2010). Four types of malformations in a population of *Bufo boulengeri* (Amphibia, Anura, Bufonidae) from the Jbilet Mountains (Marrakech, Morocco). *Herpetology Notes* 3: 267–270.
- Gardiner, D.M. & Hoppe D.M. (1999). Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). *Journal of Experimental Zoology* 284: 207–216.
- Haas, S.E., Reeves, M. K., Pinkney, A.E. & Johnson, P.T.J. (2017). Continental-extent patterns in amphibian malformations linked to parasites, chemical contaminants, and their interactions. *Global Change Biology* 2017: 1–15.
- Henle, K, Dubois, A. & Vershinin, V. (2017). Commented glossary, terminology and synonymies of anomalies in natural populations of amphibians. *Mertensiella* 25: 9–48.
- Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C. & Foster, M.S. (1994). *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians*. Washington, Smithsonian Institution Press.
- Johnson, P.T.J., Rohr, JR., Hoverman, J.T., Kellermanns, E., Bowerman, J. & Lunde, K.B. (2012). Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecology Letters* 15: 235–242.
- Kiesecker, J.M. (2002). Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences of the United States of America* 99: 9900–9904.
- Lannoo, M.J. (2009). *Malformed Frogs: the Collapse of Aquatic Ecosystems*. University of California Press, Berkeley and Los Angeles, 288 pp.
- Lunde, K.B. & Johnson, P.T.J. (2012). A practical guide for the study of malformed amphibians and their causes. *Journal of Herpetology* 46: 429–441.
- Meteyer, C.U. (2000). *Field Guide to Malformations of Frogs and Toads with Radiographic Interpretations*. Biological Science Report USGS/BRD/BSR–2000–0005, 18 pp.
- Muniz, D.H.F. & Oliveira-Filho, E.C. (2006). Metais pesados provenientes de rejeitos de mineração e seus efeitos sobre a saúde e o meio ambiente. *Universitas: Ciências da Saúde* 4: 83–100.
- Oliveira-Souza, A.E., Lima, P.H.G, Afonso, D.D., Santana, M.M. S., Pinheiro, R.T., Pedroso-Santos, F., Sanches, P.R. & Costa-Campos, C.E. (2020). First record of polymelia in the paradox frog *Pseudis paradoxa* (Linnaeus, 1758) from Northern Brazil. *International of Zoology and Animal Biology* 3: 1–3.
- Ouellet, M. (2000). Amphibian deformities: current state of knowledge. In *Ecotoxicology of Amphibians and Reptiles*, pp. 617–661 pp, Sparling, D.W., Linder, G. & Bishop C.A. (Eds.). Pensacola, Florida: SETAC Press.
- Peltzer, P.M., Lajmanovich, R.C., Sanchez, L.C., Attademo, A.M., Junges, C.M., Bionda, C.L., Martino, A.L. & Bassó, A. (2011). Morphological abnormalities in amphibian populations from the mid-eastern region of Argentina. *Herpetological Conservation and Biology* 6: 432–442.
- Piha, H., Pekkonen, M. & Merilä, J. (2006). Morphological abnormalities in amphibians in agricultural habitats: A case study of the common frog *Rana temporaria*. *Copeia* 4: 810–817.

- Ramalho, W.P., Maffei, F., Guerra, V., Da Silva, D.P., Matos, L.R.A. & Vieira, L.J.S. (2017). Anophthalmia in adults of two Amazonian treefrogs (Anura: Hylidae). *The Herpetological Bulletin* 139: 43–44.
- Read, J.L. (1997). Comparative abnormality rates of the trilling frog at Olympic Dam mine. *Herpetofauna* 27: 23–27.
- Santos, F.P., Sanches, P.R., Sousa, J.C. & Costa-Campos, C.E. (2017). Morphological abnormality in *Leptodactylus podicipinus* (Cope, 1862) (Anura: Leptodactylidae) in an urban area of north Brazil, Eastern Amazon. *Boletín de la Asociación Herpetológica Española* 28: 70–72.
- Santana, M.M.S., Sanches, P.R., Oliveira-Souza, A.E., Okada-Aguiar, K.M., Almeida-Santos, W. & Costa-Campos, C.E. (2020). Records of limb abnormalities in three anurans from eastern Amazon – *Atelopus hoogmoedi*, *Allobates femoralis* and *Dendropsophus leucophyllatus*. *The Herpetological Bulletin* 151: 37–38.
- Schotthoefer, A.M., Koehler, A.V., Meteyer, C.U. & Cole, R.A. (2003). Influence of *Ribeiroia ondatrae* (Trematoda: Digenea) infection on limb development and survival of Northern Leopard Frogs (*Rana pipiens*): effects of host stage and parasite-exposure level. *Canadian Journal of Zoology* 81: 1144–1153.
- Silva, J.M.C., Rylands, A.B. & Fonseca, G.A.B. (2005). The fate of the Amazonian areas of endemism. *Conservation Biology* 19: 689–694.
- Silva-Soares, T. & Mônico, A.T. (2017). Hind limb malformation in the tree frog *Corythomantis greening* (Anura: Hylidae). *Phyllomedusa* 16: 117–120.
- Sousa, J.C. & Costa-Campos, C.E. (2016). Natural History Notes. *Leptodactylus podicipinus* (Pointedbelly Frog). Malformations. *Herpetological Review* 47: 112–113.
- Sousa, J.C. & Costa-Campos, C.E. (2017). Records of ocular anomaly in two species of anurans in the eastern Amazon region. *Herpetology Notes* 10: 413–415.
- Stopper, G.F., Hecker, L., Franssen, R.A. & Sessions, S.K. (2002). How trematodes cause limb deformities in amphibians. *Journal of Experimental Zoology* 294: 252–263.

Accepted: 25 August 2020