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Case Report

Absence of the right forelimb in *Corythomantis greeningi* Boulenger, 1896: case report

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Description of absence of the distal part of the right thoracic limb in an individual of the species *Corythomantis greeningi*. Thereunto, it was performed radiographic evaluations in ventrodorsal projection followed by dissection of the piece and scanning electron microscopy. It was possible to verify in the radiograph that the affected limb still had the humerus. Scanning electron microscopy revealed irregularities, grooves, and fissures, typical of clipping fractures, which led to limb amputation. Considering the scarce literature, the importance of studies to distinguish malformations from those lesions that lead to the amputation of body segments is noted.

INTRODUCTION

Corythomantis greeningi (*C. greeningi*) is an anuran amphibian belonging to the family Hylidae. It presents a restricted distribution to the xeric and sub-humid regions of Brazil, being widely distributed in the Caatinga Biome (RODRIGUES, 2003). It has a morphologically differentiated head, being flat, very rustic and presenting co-ossification with the skin. These are characteristics usually associated with a supposed role in saving water and protecting against predators. It inhabits or takes refuge in bromeliads, tree holes and cracks in rocks, reproducing during the rainy season (JARED et al., 1999).

Predation and congenital malformations are the leading causes of morphological changes in amphibians, dating to their descriptions of more than 200 years (SILVA-SOARES; MÔNICO, 2017). However, there has been a significant increase worldwide (OUELLET et al., 1997) with reports in several families and animal genera and different habitats (MEDINA et al., 2013; WAGNER et al., 2014). The broad geographic distribution of toads, frogs and tree-frogs species with deformations and the variety of malformations are a concern for biologists, environmentalists, and scientists because the potential of these abnormalities are indicative of a disturbance of the ecosystem and the effect of this interference falls on other organisms that share the same environment. Among the possible causes are trematode parasitism (BOSCH, 2003; KIESECKER, 2004), high concentrations of heavy metals in the environment (HUANG; DUAN; JI, 2004; JOHNSON et al., 2003), exposure to ultraviolet radiation (BLAUSTEIN et al., 1997), pesticides and other chemical agents used in agriculture (KOLESKA; JABLONSKI, 2016), among other congenital or infectious factors. However, there is no description in the literature of suspected occurrence of ectromelia or complete

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congenital absence of the thoracic limb in *Corythomantis greeningi*.

Given the above, the present study had as objective to report the importance of the use of radiology and scanning electron microscopy to differentiate ectromelia from the amputation of body segments after traumatic injury in *C. greeningi* collected in Cabrobó, PE.

CASUISTRY

An amphibian of the species *C. greeningi* (BOULENGER, 1896), collected in Cabrobó (PE) on July 9, 2008 and included in the Herpetology Collection of the Center for Wildlife Conservation and Management (MFCH 1069), was diagnosed clinically with Complete ectromelia of the right thoracic limb. The animal was submitted to x-rays in ventrodorsal projection to confirm the clinical diagnosis. Then, the affected leg was dissected, and the humerus was removed and macerated in water for two days for morphological evaluation by scanning electron microscopy (SEM), Hitachi Table Microscope TM1000, in increments of 100 to 500 times. In the micrographs, surface characteristics of the bone epiphyses and diaphysis were evaluated.

Initially, it was evidenced by radiographic images that the right thoracic limb presented only the scapula and the humerus (Figure 1). At necropsy, it was confirmed that the humerus was the only distal bone structure of the thoracic limb (Figure 2). However, macroscopically, it was not possible to verify if this condition was a consequence of fracture with limb amputation or agenesis.

Figure 1 – Radiograph of *C. greeningi* demonstrating the presence of the right humerus (arrow) on the abscent limb – Petrolina, 2016.

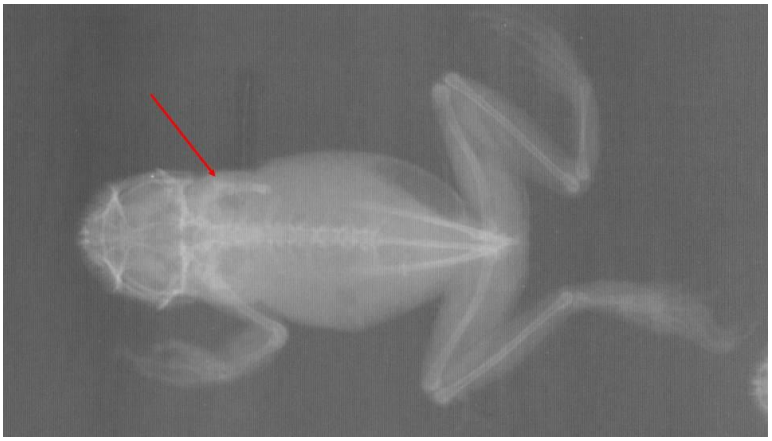


Figure 2 – Photos of *C. greeningi*, before and after dissection. It is noted in (A), the abscense of the right toracic limb; in (B), the presence of the humerus after dissection; and in (C), the humerus after extirpation.

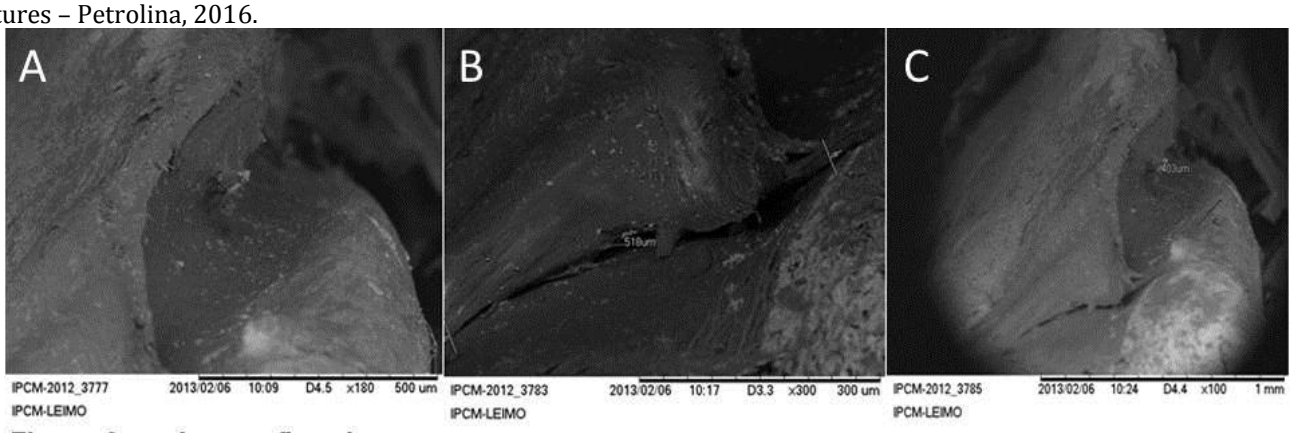
Scanning electron microscopy revealed that the surface of the distal epiphysis of the humerus showed furrows, irregularities, and bone protrusions (Fig. 3), typical of fractures.

DISCUSSION

The literature on amphibian fractures is scarce and, as a result, the debate was promoted with works described from reports of ectromelia (agenesis).

According to Stebbins; Cohen (1995), anurans are fundamental elements in ecological chains, acting as prey or predators. In addition, they are biological and environmental indicators, since they need a balanced ecosystem for their survival. Among the environmental imbalance indicators that fall on amphibians, there are increasing cases of malformations. These generate great interest on the part of the scientific community, in search of its etiology (BARRAGÁN-RAMÍREZ; NAVARRETE-HEREDIA, 2011).

Figure 3 – Scanning electron photomicrography of the distal epiphysis of the *C. greeningi* humerus at 500 (A), 300 (B) and 100 (C) fold increases. It is possible to notice the presence of furrows, irregularities, and bone protrusions, typical of fractures – Petrolina, 2016.



This was the first report of suspicion of right thoracic ectromelia in *C. greeningi*. Voitena et al. (2013) reported a case of polymelia in *Rhinella paracnemis*, known as the Marine or Cane Toad. Meteyer et al. (2000) described that in 1995, students found numerous frogs with malformations during field trips to the Minnesota pond and, since then, reports of abnormalities have been reported on 38 frog species and 19 tree-frog species in 44 states.

Barragán-Ramírez; Navarrete-Heredia (2011) reported anomalies in two of the four members of *Lithobates neovolcanicus*. The left anterior limb had features suggesting the duplication of these structures (polymelia) and the right posterior leg had no structures at the bottom, which terminated in the mid-distal region of the femur, leading the authors to conclude that it was not due to trauma but due to a malformation (ectromelia).

Peltzer et al. (2011), found in the central-eastern region of Argentina 16 types of abnormalities in 15 different species of anurans. Among the anomalies, ectromelia was the most frequent, corresponding to 54.76% of the cases. Moreira et al. (2012), in a study on the contamination of surface water and rainwater by pesticides, used 16 species of anuran amphibians as bio-indicators of environmental pollution. During collection, appendicular malformations such as ectromelia and syndactyly were detected using external visual examination and radiological examination, corroborating with the method adopted in this study. Garin et al. (2009) performed radiographs on an amphibian of the species *Epidalea calamita*, which showed the absence of metatarsals and phalanges of the left pelvic limb, proving the presence of ectromelia.

Silva-Soares; Mônico (2017) collected an adult male *Corythomantis greeningi* Boulenger, 1896 with a malformation of the left hind limb. Also using the radiography the authors diagnosed that it was brachydactyly. The radiological characteristics were bones of the non-fully developed phalanges and fused metatarsals. Although they detected anthropogenic interference at the site where the specimen was captured and the radiographic findings, they were not able to determine whether the deformation was naturally developed or whether it was derived from some physicochemical agent or stress.

The application of the X-ray is a diagnostic method for the bone tissue (FIGUEIREDO et al., 2004; OLIVEIRA et al., 2006), providing important information regarding fractures, tumors, degenerative conditions and osteomyelitis, also providing valuable information about the ossification process (CRUZ et al., 2007). In our study the radiographic characteristics were indicative of the presence of humerus, with an irregular but well-calcified morphology.

Although conventional radiographs, because they are two-dimensional, do not allow the qualitative visualization of images with high magnification (up to 300,000 X) and resolution, nor the observation and recording of three-dimensional images such as scanning electron microscopy - SEM (PICON et al., 2006). Therefore, studies using SEM in bone tissues allow the analysis of the bone architecture, bone neoformation and, in cases of bone repair, its maturation (CRUZ et al., 2007; SCHIAPPARELLI; ZEFIRO; TACCINI, 2009). Given the circumstances of the environment where the specimen was found (Cabrobó, Pernambuco, Brazil, 08°27'00.1"S, 39°24'28.1"W), in a region of works of transposition of the São Francisco River and thereby, the presence of furrows, irregularities and bone protrusions on the surface of the distal epiphysis of the humerus can be observed through SEM, confirming that the case involved thoracic limb amputation after traumatic injury and not ectromelia.

CONCLUSION

The present report shows that the diagnosis of fractures in amphibian limbs can be performed using radiographs,

but the information can only be confirmed by scanning electron microscopy, after that eliminating the possibility of occurrence of ectromelia.

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