NEW FOSSIL RECORD OF BUFONIDAE (AMPHIBIA, ANURA) IN THE LATE PLEISTOCENE-EARLY HOLOCENE OF NORTHEASTERN BRAZIL AND ITS PALEOENVIRONMENTAL SIGNIFICANCE

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Abstract
We report the first occurrence of anurans in the Quaternary fossil record of Rio Grande do Norte State. The specimen UERN PV-50 was assigned to the bufonid Rhinella jimii. This finding extends the geographic distribution of this species in the northeastern Brazil during the Late Pleistocene-early Holocene. In paleoenvironmental terms, the occurrence of R. jimii in Riacho Verde paleontological site suggests an arid climate with marked seasonality in that area during the Late Pleistocene-early Holocene and that the paleobiodiversity in that region was higher than the observed in the fossil record. Furthermore, the tanks seem to have been areas of aggregating anurans during raining periods. Finally, based on sedimentological and biostratinomic data, we interpret that Riacho Verde paleontological site preserved a peripheral assemblage.

Keywords: Anura. Quaternary. Tank deposit. Paleoenvironment.

1. Introduction

The anuran fossil record is known to be one of the most incomplete among terrestrial vertebrates. The fragility of the anuran bones and a geographic distribution limited mainly to moist environments are the main reasons for this incompleteness (Sanchiz and Roček, 1996; Pauly et al., 2004). Consequently, such poor fossil record makes any fossil finding essential to the knowledge of the evolution and paleoecology of the order Anura.

Not surprisingly, the anuran fossil record of South America is also scarce and underexplored. Most of the findings comes from Argentina, and it is a reflex of the relatively prolific fossil record and noteworthy amount of researchers interested in anurans there (see Cione and Báez, 2007). In Brazil, studies on fossil anurans have been sporadic and mostly related to Cretaceous specimens of the Araripe and Bauru basins (Báez and Peri, 1989; Leal and Brito, 2006; Báez et al., 2009, 2012).
The Cenozoic record of anurans is scarcer and little attention has been given to it (Estes, 1975; Bedani and Hadad, 2002; Araújo-Júnior and Moura, 2014). Recently, Araújo-Júnior and Moura (2014) reported the first occurrence of toads (Bufonidae) in Late Pleistocene deposits of Brazil and, thus, they opened a new window into the knowledge of paleoecology and taphonomy of anurans from the Quaternary of Brazil.

An excavation carried out in 2014 in a tank deposit of Rio Grande do Norte State, in northeastern Brazil, allowed recovering a new material of Anura. Although the material consists of a sole specimen, it was found associated to megafaunal remains and, besides being a key for understanding the Quaternary fossil record of Anura, it can be a diagnostic tool to interpret paleoenvironmental contexts in the Quaternary of Brazil, as anurans are better environmental indicators than megamammals or large-sized mammals. Thus, this work aims to describe this new specimen of Anura recovered from a Quaternary tank deposit and provide paleoenvironmental insights into the Late Pleistocene-early Holocene of northeastern Brazil.

2. Study area

The specimen comes from a tank deposit at Riacho Verde site (6°43’33.73”S; 36°50’57.88”W), located at 11 km southwest of Ouro Branco city, Rio Grande do Norte State, Brazil (Fig. 1). The depression of the tank was formed in Paleoproterozoic gneisses belonging to the Várzea Grande Suit (~2.1 Ba; U-Pb dating) (CPRM, 2005). The tank has elliptical outline with orientation N-S and measures 52 m in maximum length, 11 m in maximum width and 2.15 m in maximum depth. Its sedimentary infilling has about 1.56 m in maximum thickness.

This paleontological site was originally excavated in 1956 by Mr. Celso de Afonso, the owner of the area. His aim was to drain out the deposit to facilitate the storage of rainfall waters. During this early excavation, several fossils were found; however, their current location is unknown. Only part of the deposit was excavated in 1956 (see Fig. 1) and a huge amount of dragged fossiliferous sediments was thrown on the non-excavated portion and on the borders of the tank.

During 2014, three new excavations conducted in the non-excavated portion of the tank deposit provided new fossils recovered from both the non-perturbed strata of the tank deposit – that is, they were collected in situ – and the tailing sediments of the 1956 excavation, as well as the collection of detailed sedimentological, stratigraphic and taphonomic data.

The anuran fossil was collected in the tailing sediments of the 1956 excavation. Two independent clues support the conclusion that the specimen analyzed herein comes from the fossiliferous layer: (i) it was found associated with elements of the Pleistocene Megafauna (e.g. Notiomastodon, Eremotherium, Toxodon, Palaeolama and Panochthus); and (ii) both megafaunal and anuran remains are stained by iron oxide, suggesting that they were buried and stained at the same time. Considering the small area of the deposit, the existence of a single fossiliferous layer inside the tank (see stratigraphic log in Fig. 2 and its description below) and the stratigraphic continuity between the areas excavated in 1956 and 2014, we believe that all fossils collected belong to the same fossiliferous layer (including the anuran).

Five layers form the sedimentary infilling of the tank (from the bottom to the top; Fig. 2): (i) 50 cm thick clast-supported conglomerate, with sandy-clayey matrix, cemented by calcium carbonate; the gravels vary in size, including boulders, cobbles, pebbles and granules, though pebbles are dominant; composition consists of lithics and quartz grains; feldspar grains are rare; roundness varies from subangular to well-rounded, with the predominance rounded and well-rounded pebbles; some pebbles are imbricated; fossils come only from this layer, which overlies the saprolite on the tank floor; (ii) 20 cm thick matrix-supported gravel, with sandy-clayey matrix; gravel varies from pebbles to granules the latter being predominant; mineralogical composition includes feldspar and quartz grains; this layer is unfossiliferous; (iii) sandy-clayey sediments 10 cm thick; granulometry of the sands varies from medium to fine; this layer is unfossiliferous; (iv) 30 cm thick layer similar to that described in (ii); and (v) 40 cm thick unfossiliferous layer of sandy sediments, with sparse granules; granules and sands are composed of quartz and feldspar; two muddy lenses with 50 cm in the major width (in section) and 20 cm in major height are embedded in this layer.

Absolute datings for Riacho Verde fossil assemblage are yet absent. Nonetheless, geochronological studies involving comparable megafaunal accumulations have indicated a Late Pleistocene-early Holocene age for tank deposits of Brazil (Kinoshita et al., 2005, 2008; Dantas et al., 2011; Ribeiro et al., 2013, 2014). Herein, we assign this same age to the Riacho Verde taphocoenosis.
3. Material and methods

The specimen analyzed here is housed at the vertebrate paleontology collection of the Laboratório de Sistemática e Ecologia Animal (LABSECO), of the Universidade do Estado do Rio Grande do Norte (UERN), at Mossoró city, state of Rio Grande do Norte, Brazil. It is labeled as UERN PV-50 and consists in the only anuran specimen recovered from the tank deposit of Riacho Verde so far.

Due to the relatively huge size of the specimen, we excluded small-sized anurans (e.g. Hylidae, Leiuperidae, Pipidae) from the morphological comparative study, which
took into account only large-sized anurans typical from the Quaternary of South America. We made direct comparisons with extant specimens of the bufonid species *Rhinella jimi* (Stevaux, 2002), *Rhinella schneideri* (Werner, 1894), *Rhinella icterica* (Spix, 1824) and the leptodactylid *Leptodactylus* Fitzinger, 1826, belonging to the paleontological collection of Faculdade de Geologia of Universidade do Estado do Rio de Janeiro and Comparative Anatomy collection of Departamento de Geologia of Universidade Federal do Rio de Janeiro. Moreover, the specimen was compared to those figured and described in literature (e.g. Tihen, 1962a, 1962b; Martin, 1972; Gandee, 2005; Evans et al., 2014). The osteological nomenclature follows Sanchiz (1988) and the Systematic Paleontology is according to Frost (2013). Measures were taken with digital caliper rule and are expressed in millimeters.

We used the tridimensional technique of graphic computation through the indirect method of photogrammetry (Dardon et al., 2010), where several photographs of the specimen are acquired in different positions. The acquisition of the photographs was performed using a cam Oregon Scientific 5 mega pixels. The tool Python Photogrammetry Toolbox Gui V.1 (developed by Arc-Team) was used to construct the 3D model. Then, noises in the imaging and texture of the model were treated in the software 3Dmax version 2014 (licensed for the fourth author of this paper).


### 4. Systematic paleontology

Class AMPHIBIA Gray, 1825
Order ANURA Fischer von Waldheim, 1813
Family BUFONIDAE Gray, 1825
*Rhinella* Fitzinger, 1826
*Rhinella jimi* (Stevaux, 2002)  
(Figures 3-4)

**Material.** UERN PV-50, right tarsal (= tibiale-fibulare) (Figs. 3-4).

**Geographic provenance and age.** Riacho Verde paleontological site (6°43’33.73’S; 36°50’57.88’’W), Ouro Branco municipality, state of Rio Grande do Norte, Brazil; Late Pleistocene–early Holocene age.

**Description.** The specimen UERN PV-50 consists of an almost complete right tarsal (= tibiale-fibulare), lacking only part of the distal end of the fibulare (= calcaneus) and of the articular surface for distal tarsals. It measures 32 mm length and 14 mm width (in the proximal end).

In dorsal view, the articular surface for the tibial condyle is visible and bears little abrasion. The proximal articular surface of the fibulare is 3 mm higher than the tibiale one.

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*Fig. 2.* Stratigraphic log of the tank deposit of Riacho Verde paleontological site.
In the tibiale, the articular surface for Y element is preserved. The plantar tuberosity – a feature often noticeable only in posterior view in the homologous bone of other anurans – is expanded and medially projected, being partially visible in dorsal view. Abrasion is observed below this tuberosity. A columnar fracture occurs in the distal end of the fibulare. The tibiale is slightly more curved than the fibulare.

In plantar view, the articular surface for tibial condyle is strongly abraded and the tibiale seems to be more curved than the fibulare in contrast to the plantar view. The plantar tuberosity is very prominent and extends conspicuously toward the medial portion of the tibiale. Fragmentation precludes the morphological description of the distal end of the fibulare.

Fig. 3. UERN PV-50, right tarsal of Rhinella jimi. A. dorsal view; B. plantar view. Scale bar = 5 mm.

In proximal view, the articular surface of the head of the tibiale has an area twice the size of the fibulare one. The lateral portion of its head presents a prominence that extends along the entire lateral border. In distal view, only the distal end of the tibiale is present and the plantar tuberosity is semilunar.

Comparisons. The morphological comparisons between UERN PV-50 and other large-sized anurans are summarized in the Table 1. Among the bufonids, we exclude Melanophryniscus Gallardo, 1961 from comparisons because this genus presents distal tarsal elements four and five fused with the fibulare (McDiarmid, 1971; Gandee, 2005) and the articulation for distal tarsal elements is not observed in the fibulare of UERN PV-50. Clearly, UERN PV-50 shares a set of characters with the Bufonidae species used in our comparisons, mostly with R. jimi, to which we assign it.
Tab. 1. Morphological comparison between fossil specimen and large-sized anurans.

<table>
<thead>
<tr>
<th>Variables</th>
<th>UERN PV-50</th>
<th>Rhinella jimii (UFRJ DG 86 AC)</th>
<th>Rhinella schneideri (UERJ AC-01)</th>
<th>Rhinella icterica (UFRJ DG 4 AC)</th>
<th>Leptodactylus sp. (UFRJ DG 5 AC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum length</td>
<td>32 mm</td>
<td>35 mm</td>
<td>24 mm</td>
<td>25 mm</td>
<td>21 mm</td>
</tr>
<tr>
<td>Maximum width (in the proximal end)</td>
<td>14 mm</td>
<td>14 mm</td>
<td>10 mm</td>
<td>10 mm</td>
<td>5 mm</td>
</tr>
<tr>
<td>Proximal head of fibulare in relation to fibulare one</td>
<td>3 mm higher</td>
<td>Proximal head of the fibulare is 4 mm higher than the tibiale one</td>
<td>Proximal head of the fibulare is 1 mm higher than the tibiale one</td>
<td>Proximal head of the fibulare is 1 mm higher than the tibiale one</td>
<td>Proximal head of the fibulare is 0.5 mm higher than the tibiale one</td>
</tr>
<tr>
<td>Plantar tuberosity (in anterior view)</td>
<td>Partially visible</td>
<td>Partially visible</td>
<td>Not visible</td>
<td>Not visible</td>
<td>Absent</td>
</tr>
<tr>
<td>Shape of plantar tuberosity (in distal view)</td>
<td>Semilunar-shaped</td>
<td>Semilunar-shaped</td>
<td>Straight</td>
<td>Straight</td>
<td>Absent</td>
</tr>
<tr>
<td>Shape of tibiale (in anterior view)</td>
<td>Slightly curved</td>
<td>Tibiale is slightly curved</td>
<td>Tibiale is slightly curved</td>
<td>Tibiale is more curved than UERN PV-50</td>
<td>Tibiale is slightly curved</td>
</tr>
<tr>
<td>Shape of tibiale (in anterior view)</td>
<td>Slightly curved</td>
<td>Fibulare is slightly curved</td>
<td>Fibulare is slightly curved</td>
<td>Fibulare is more curved than UERN PV-50</td>
<td>Fibulare is straight</td>
</tr>
<tr>
<td>Presence of a crest on tibiale (in medial view)</td>
<td>Small crest on the diaphysis of the tibiale</td>
<td>Crest on the diaphysis of the tibiale; larger than R. icterica and smaller than UERN PV-50</td>
<td>Crest on the diaphysis of the tibiale; larger than R. icterica and smaller than UERN PV-50</td>
<td>Small crest on the diaphysis of the tibiale; smaller than UERN PV-50 and R. jimii</td>
<td>No crest on the tibiale</td>
</tr>
<tr>
<td>Presence of crest on fibulare (in medial view)</td>
<td>Small crest on the diaphysis of the fibulare</td>
<td>Crest on the diaphysis of the fibulare; smaller than UERN PV-50</td>
<td>No crest on the fibulare</td>
<td>No crest on the fibulare</td>
<td>No crest on the fibulare</td>
</tr>
<tr>
<td>Area of the articular surface of tibiale (in proximal view)</td>
<td>Articular surface of the head of the tibiale has area 2x larger than the fibulare one</td>
<td>Articular surface of the head of the tibiale has area 2x larger than the fibulare one</td>
<td>Articular surface of the head of the tibiale has area 3x higher than the fibulare one</td>
<td>Articular surface of the head of the tibiale has area 1.5x larger than the fibulare one</td>
<td>Articular surface of the head of the tibiale has area 3x larger than the fibulare one</td>
</tr>
<tr>
<td>Robustness of the tibiale and fibulare</td>
<td>Tibiale and fibulare are robust, like in UERN PV-50</td>
<td>Tibiale and fibulare are robust, like in UERN PV-50</td>
<td>Tibiale and fibulare are robust, like in UERN PV-50</td>
<td>Tibiale and fibulare are slighter than UERN PV-50</td>
<td>Tibiale and fibulare are slighter than R. icterica</td>
</tr>
</tbody>
</table>

Comments. Bufonidae is one of the most known anuran families. It has a worldwide geographic distribution in comparison to other extant vertebrates (Pramuk et al., 2001). This family occurs naturally in all continents, except Antarctica and Oceania. In America, natural populations of Bufonidae occur from southern Texas and northwestern
Mexico to Southern Brazil (Cei, 1968; Zug and Zug, 1979; Garcia et al., 2007). According to Frost (2013), about 53 genera and 576 species are included in Bufonidae and, according to Segalla et al. (2012), around 70 of them occur in Brazil. In Brazil, fossils of Bufonidae were mentioned by Estes (1970) and Báez and Nicoli (2004) to the Itaboraí Basin (late Paleocene), though those specimens were not formally described or figured. Recently, Araújo-Júnior and Moura (2014) described remains of *R. jimii* and *R. schneideri* recovered from a tank deposit at Itapipoca, Ceará State, northeastern Brazil.

*Rhinella jimii* is one of the largest species of neotropical anurans (Stevaux, 2002), a condition that may facilitates its preservation in comparison to other anuran species. It is restricted to the northeastern Brazil (Stevaux, 2002), suggesting that this species is endemic of the Brazilian Intertropical Region (BIR).

According to Maciel et al. (2010), the origin of *R. jimii* is related to the arising of geographic barriers and dispersion routes during the uplift of the Brazilian Central Shield in the late Miocene (~8.08 My, based on molecular data).

**Fig. 4.** 3D reconstruction of UERN PV-50 *Rhinella jimii*. A. dorsal view; B. medial view of the tarsal in diagonal position; C. plantar view; D. lateral view of the tarsal in diagonal position; E. cranial view; F. caudal view. Scale bar: 5 mm.
5. Paleocological and paleoenvironmental implications

Anurans are environmentally related and their autoecology is relatively well defined, making them useful in paleoenvironmental analyses. This group is carnivore, more specifically insectivore (Toft, 1980, 1981; Duellman and Trueb, 1994), usually preying insects, arachnids and even small vertebrates (Pough et al., 2004; Batista et al., 2011). In general, they gather in swampy areas or lentic environments for breeding (Haddad and Prado, 2005). In the case of Riacho Verde, the stagnation of waters inside the tank during raining periods may have favored their gathering around the tank areas in the past, thus facilitating their preservation in the tank deposit.

According to Sousa et al. (2008), bufonids are bioindicators of the diversity of other groups in the communities. In the case of Riacho Verde, the presence of *R. jimi* suggests the paleobiodiversity in the region of Riacho Verde may have been higher than the observed in its Quaternary fossil record, which is composed of five genera of mammals and one anuran species until now.

The skin of individuals of *R. jimi* is heavy hough and keratinized, a likely adaptation to arid climate (Maciel et al., 2010) and the geographic distribution of extant individuals of this species (Stevaux, 2002) suggests that this species is adapted to areas of seasonality where dry and rainy periods are well delimited. Thus, its presence in Riacho Verde paleontological site suggests that similar climatic conditions occurred in that area during the Late Pleistocene-early Holocene. Finally, based on actualistic observations, it is likely that tanks – including the tank of Riacho Verde – may have functioned as a breeding, foraging and death site for the paleonurofauna of the region.

The occurrence of a fragmented and moderately abraded anuran remain in a conglomeratic level suggests that the individual experienced some degree of transport. Therefore, it is likely that the animal has died in nearby areas and was later transported to inside the tank by tractive events. Sudden events – such as gravitational flows or flash floods – likely deposited the vertebrate remains inside the tank (Araújo-Júnior et al., 2013). In this sense, the tank deposit of Riacho Verde preserved a peripheral assemblage (sensu Araújo-Júnior, 2016).

6. Conclusions

Anurans are reported for the first time for Quaternary deposits of Rio Grande do Norte State. Before this work, formal reports of Quaternary anurans were restricted to the Ceará State. The geographic distribution of fossil specimens of *R. jimi* extends 470 km toward southeast the area of the previous report (Itapipoca, Ceará State).

The presence of *R. jimi* in Riacho Verde paleontological site indicates that: (i) the paleobiodiversity in that area was higher than the observed in the fossil record; (ii) the tank was an area of aggregating anurans during raining periods; (iii) an arid climate with marked seasonality occurred in the Riacho Verde area during the Late Pleistocene-early Holocene; (iv) the Riacho Verde paleontological site preserved a peripheral assemblage.

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