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Cuad. herpetol. 34 (2): 271-274 (*2020*)

Cytogenetic analysis of *Rhinella jimi* (Stevaux, 2002) (Anura, Bufonidae) from northeastern Brazil

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Recibida: 11 Diciembre 2019
Revisada: 29 Mayo 2020
Aceptada: 10 Agosto 2020
Editor Asociado: D. Baldo

doi: 10.31017/CdH.2020.(2019-065)

ABSTRACT

In this work we analyzed the karyotype of *Rhinella jimi* (Stevaux, 2002) (Anura, Bufonidae) from Picos (Piauí) in Northeastern Brazil. The chromosomes were examined using classical cytogenetic approaches (Giemsa, C-banding, and Ag-NOR staining). This species has 2n = 22 chromosomes, all metacentric or submetacentric. Heterochromatic segments were visualized at the centromeric region and the nucleolus organizer regions (NOR) were restricted to terminal regions of the short arms in pair 7. There was no evidence of heteromorphic sex chromosomes. The chromosomal analysis of *R. jimi* allowed us to identify a karyotype that is similar to many other species of *Rhinella*, in which the diploid number remains unchanged and without evidences of structural rearrangements.

Key Words: Amphibians; Cytogenetic markers; C-banding; Karyotype evolution; Nucleolar organizing region.

Rhinella Fitzinger, 1826 is a genus that comprises 92 valid species of frogs and that is distributed from Lower Rio Grande Valley region of southern Texas (USA) and southern Sonora (Mexico) south, through tropical Mexico and to southern South America; one species in particular (*Rhinella marina*) is widely introduced (Antilles, Hawaii, Fiji, Philippines, Taiwan, Ryukyu Is. (Japan), New Guinea, Australia, and many Pacific islands) (Frost, 2020).

All *Rhinella* species were previously grouped in the polyphyletic genus *Bufo* Laurenti, 1768, and currently are arranged in several species groups (i.e., *R. crucifer*, *R. festae*, *R. granulosa*, *R. margaritifera*, *R. marina*, *R. spinulosa*, and *R. veraguensis*) Pramuk, 2006; Chaparro *et al.*, 2007; Moravec *et al.*, 2014). However, there are species that have not been assigned to none of the existing groups, thus showing a taxonomic confusion in the group (Chaparro *et al.*, 2007).

Chromosomal data of *Rhinella* were reported as a relatively conserved karyotype, composed by a diploid number of 2n= 22 and NF= 44, such as in *R. achalensis* (Cei, 1972), *R. achavali* (Maneyro, Arrieta, and de Sá, 2004), *R. arenarum* (Hensel, 1867), *R. crucifer* (Wied-Neuwied, 1821), *R. diptycha* (Cope, 1862), *R. fernandezae* (Gallardo, 1957), *R. granulosa* (Spix, 1824), *R. henseli* (Lutz, 1934), *R. hoogmoedi* Caramaschi and Pombal, 2006, *R. icterica* (Spix, 1824), *R. jimi* (Stevaux, 2002), *R. margaritifera* (Laurenti, 1768), *R. marina* (Linnaeus, 1758), *R. ornata* (Spix, 1824), *R. proboscidea* (Spix, 1824), *R. pygmaea* (Myers and Carvalho, 1952), and *R. rubescens* (Lutz, 1925) (Kasahara *et al.*, 1996; Baldissera *et al.*, 1999; Azevedo *et al.*, 2003; Amaro-Ghilardi *et al.*, 2008; Baraquet *et al.*, 2011; Kolenc *et al.*, 2013; Bruschi *et al.*, 2019).

Rhinella jimi (*R. marina* group) is mainly distributed in northeastern Brazil, however, it occurs from the state of Pará (Municipality of Bujaru) and Maranhão to Piauí, to the state of Espírito Santo, at altitudes of 15 to 500 m (Frost, 2020). Thus, in this work, we cytogenetically analyzed a population of *R. jimi* from northeastern Brazil by conventional staining, to better understand chromosomal characteristics and contribute to understanding the evolution of chromosomes in this widely distributed anuran species.

All the individuals used were collected in the

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field under a governmental license issued by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) number 47710-1/2015. Cytogenetic analyzes were performed on 10 specimens of *Rhinella jimi* (6 males and 4 females) collected in Picos (6° 54' 22.9"; S 41° 33' 49.8" W) in the Brazilian state of Piauí. Cellular suspensions were obtained from bone marrow using an in vitro colchicine 1% treatment for four hours according to Bertollo *et al.* (1978). Metaphase spreads were stained with 10% Giemsa in order to determine the standard karyotype of the sampled animals. The active NOR sites were detected by Ag-NOR staining according to Howell and Black (1980) and C-banding was carried out as described by Sumner (1972). Metaphases were photographed with a Nikon Eclipse microscope coupled to Thiachron camera and processed using the AMscope 3.7® software. The chromosomes were ordered in decreasing according to Levan *et al.* (1964), with modifications of Guerra *et al.* (1986).

While few *Rhinella* species have been analyzed cytogenetically, the karyotypic macrostructure and the diploid number of 2n= 22 was observed in all populations studied so far. Here, we found the same characteristic for a new population of *R. jimi* from Northeastern Brazil (Fig. 1 A and B), suggesting that this chromosome number is common to the genus and has been maintained over time in all analyzed groups, such as the *R. crucifer*, *R. granulosa* and *R. marina* groups (see Kasahara *et al.*, 1996; Baldissera *et al.*, 1999; Baraquet *et al.*, 2011; Amaro-Ghilardi *et al.*, 2008; Kolenc *et al.*, 2013; Bruschi *et al.*, 2019).

The conserved aspect of *Rhinella* species karyotype is not restrict to the chromosome number aspects. In all *Rhinella* species analyzed so far, a highly conserved chromosomal morphology composed by biarmed chromosomes of metacentric and subcentric types were yet observed (Bruschi *et al.*, 2019). However, some subtle differences such as the proportion between metacentric and submetacentric pairs have been reported (Amaro-Ghilardi *et al.*, 2008; Baraquet *et al.*, 2011). Nevertheless, the application of different criteria for chromosome classification may have interfered in such differences, rather than any actual variation between species in their chromosomal assemblies, and a thorough revision is needed.

Heterochromatic blocks were detected in the centromeric regions of chromosomes (Fig. 2, A and B) and no secondary constrictions were evident. Regarding nucleolar organizing regions, only one pair corresponding to the short arm of pair 7 was observed (Fig. 1, C). Regarding the pattern of constitutive heterochromatin distribution in the *R. jimi*, the karyotype shows positive marks at the centromeric region of all chromosomes, corroborating previous results observed for some *Rhinella* species (e.g., Kasahara *et al.*, 1996; Amaro-Guilardi *et al.*, 2008; Bruschi *et al.*, 2019). In our analysis, the NOR located in the short arm of chromosomal pair 7 in *R. jimi* are coincident with the NORs found for the species in others of the *Rhinella marina* group: *R. icterica*, *R. rubescens* and *R. diptycha* by Kasahara *et al.* (1996) and Amaro-Ghilardi *et al.* (2008), differing from these only in the location occupied on the chromosome.

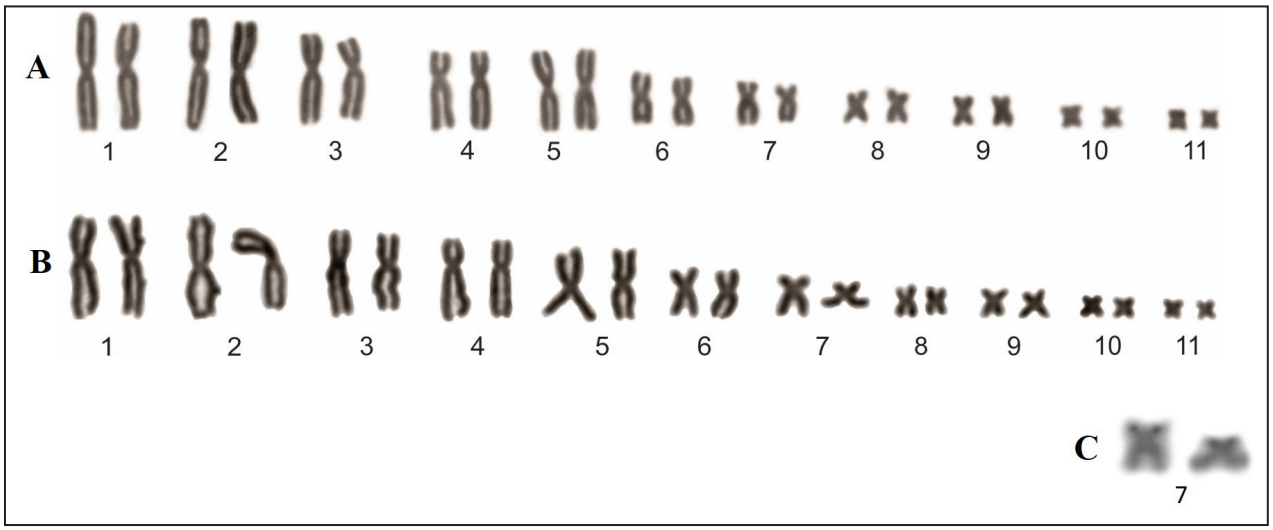

Figure 1. Karyotypes of *Rhinella jimi* based on Giemsa staining: A, male metaphase and B, female metaphase; C, stained by the Ag-NOR method, showing the pair 7.

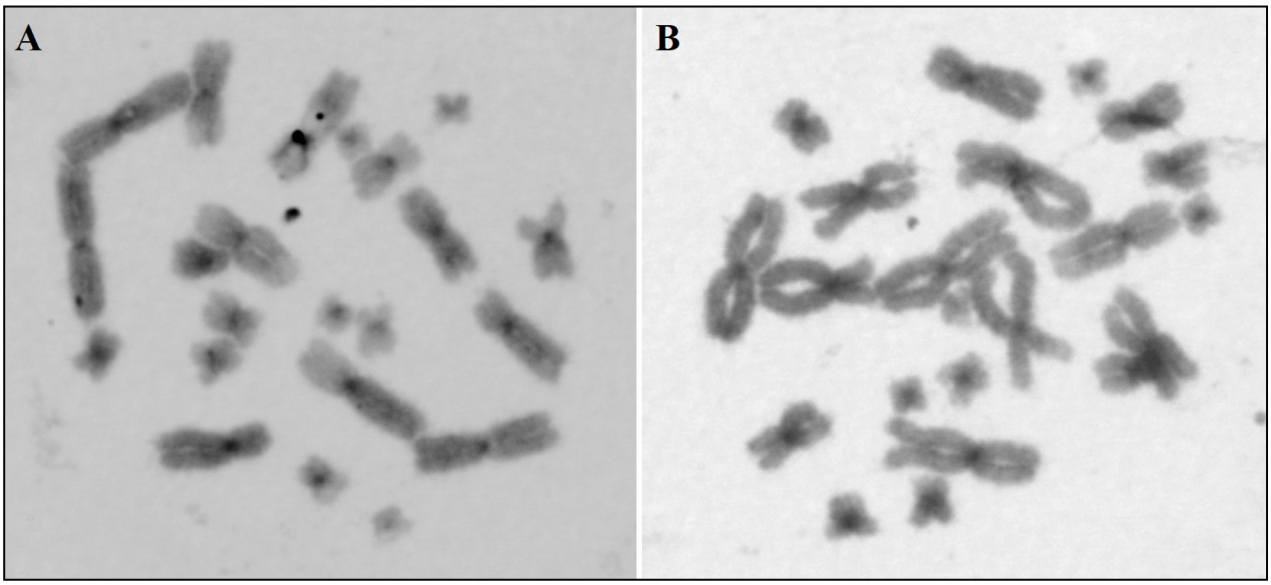

Figure 2. Karyotypes of *Rhinella jimi* based on C-banding: A, male metaphase and B, female metaphase.

In conclusion, the chromosomal analysis of *Rhinella jimi* in this work allowed us to identify a karyotype very close to that observed in other *Rhinella* species, and in particular in the *R. marina* group, in which the diploid number remains unchanged and without evidence of structural rearrangements. Further studies considering more resolutive cytogenetic techniques are needed to test for the occurrence of hidden variation in the conserved karyotypes of *Rhinella*.

Acknowledgements

The authors are grateful to Instituto Federal de Educação, Ciência e Tecnologia do Piauí (IFPI), campus Picos (PROAGRUPAR-INFRA 033/2014) for logistic and financial support and to the anonymous reviewers for suggestions to our manuscript.

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