

CYTOGENETIC CHARACTERIZATION OF *Ameivula ocellifera* (SPIX, 1825) (SQUAMATA, TEIIDAE) FROM THE BRAZILIAN NORTHEAST

CARACTERIZAÇÃO CITOGENÉTICA DE *Ameivula ocellifera* (SPIX, 1825) (SQUAMATA, TEIIDAE) DO NORDESTE BRASILEIRO

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ABSTRACT: *Ameivula* is as a new genus of Teiidae family that emerged after extensive revision of species that comprised the former complex of species called *Cnemidophorus* group. Its species has a wide distribution from the northeast of Brazil to northern Argentina. Cytogenetic studies in the Teiidae family have shown that karyotypical data are important tools in phylogenetic and systematic studies within this group allowing to determine the position of species in the family. Thus, this study aimed to describe the karyotype of *Ameivula ocellifera* (Spix, 1825) from Picos, Piauí state in the Brazilian Northeast. Specimens were collected from August 2014 to October 2015 using interception traps and pitfalls, mounted randomly along the Caatinga area. The animals were collected and transported to Federal Institute of Piauí, campus Picos, where was carried out all laboratory procedures. Individuals analyzed showed a diploid number of $2n = 50$ for both sexes, with karyotype composed by 30 macrochromosomes and 20 microchromosomes of telocentric and subtelocentric types. There were no heteromorphic sex chromosomes in the studied specimens. C-band technique evidenced the heterochromatic blocks in pericentromeric and telomeric regions of chromosomes. The nucleolar organizing regions appeared as a simple unit located at the terminal portion of the long arm of chromosomal pair number 5. The chromosomal characteristics of *A. ocellifera* analyzed do not show divergences regarding individuals from other regions. However, the nucleolar organizing regions seems to be a good chromosomal marker that permits to distinguish the species already studied.

KEYWORDS. *Ameivula ocellifera*. C Banding. Cytogenetic markers. Karyotype. NOR.

INTRDUCTION

The Teiidae family is a group of lizards comprising 160 species distributed in three subfamilies: Teiinae integrates the genera *Ameiva* Meyer 1795, *Ameivula* (Spix, 1825), *Aurivela* (Bell, 1843), *Aspidoscelis* Fitzinger 1843, *Contomastix* (Dumésil & Bibron, 1839), *Cnemidophorus* Wagler 1830, *Dicrodon* Duméril & Bibron 1839, *Holcosus* (Cope, 1862) *Kentropyx* Spix 1825, *Medopheos* (Bocourt, 1874) and *Teius* Merrem 1820; Tupinambinae includes the genera *Crocodylurus* Spix 1825, *Dracaena* Daudin 1802, *Salvator* (Dumésil & Bibron, 1839) and *Tupinambis* (Daudin, 1802); and finally Callopiistinae with only *Callopiistes* Gravenhorst 1838 (HARVEY; UGUETO; GUTBERLET, 2012; UETZ, 2019).

The *Ameivula* genus emerged from extensive systematic revisions in Teiidae family totaling 11 valid species of small animals (UETZ, 2019). Its species are widely distributed throughout Brazil and can be found from the Brazilian

Northeast to the north of Argentina (VANZOLINI; RAMOS-COSTA; VITT, 1980). In Brazil, this lizard occurs in several environments, such as the Caatinga (VITT, 1983; MENEZES et al. 2011), Cerrado (MESQUITA; COLLI, 2003), and restinga of Atlantic Forest (SANTANA et al. 2010). *Ameivula ocellifera* (Spix, 1825) is a species of active foraging habit, with ecological and morphological characteristics identical to other species of the genus (SILVA; ARAÚJO, 2005).

In the '70s Teiidae was divided into three groups based on the chromosomal characteristics of its members: (1) a group consisting of the genera *Dracaena*, *Callopiistes*, *Crocodylurus* and *Tupinambis*, with a diploid number of $2n = 34-38$, divided into five to six pairs of metacentric macrochromosomes and one satellite in pair 2; (2) a group formed by the genera *Ameiva*, *Dicrodon*, *Kentropyx*, *Teius*, and *Cnemidophorus* of which of the latter, some species became *Ameivula*, with diploid number $2n = 46-56$ with many acrocentric chromosomes; and (3) a group with the microteiids

with a diploid number of $2n = 46$ (GORMAN, 1970).

The ancient karyotype of Teiidae would have originated from centric fission of macrochromosomes of an ancestral karyotype of Tupinambinae with $2n = 50$ (GORMAN, 1973). Additionally, pericentric inversions, fusions and fissions may have been responsible for the karyotypic diversification of some of the species, e.g., those belonging *Cnemidophorus* genus, in which chromosome numbers in diploid species vary between $2n = 46$ and 52. In triploid species, they vary from $3n = 69$ to 71, and it is of $4n = 92$ chromosomes in tetraploid species (GORMAN, 1970; PECCININI-SEALE, 1981, 1989).

Thus, considering the karyotype variation already defined for different genera and species of the Teiidae family, this study aimed to describe the karyotype of a population of *Ameivula ocellifera*, looking for genetic patterns that can be used as a tool to establish differences between species.

MATERIAL AND METHODS

The specimens were collected from August 2014 to October 2015, in areas of Caatinga in the city of Picos, state of Piauí, Brazil. The collection used pitfalls mounted in places where the presence of lizards was observed. The traps were checked three times a day and the captured animals were collected to prevent them from dying due to excessive heat.

Thus, the collected animals were taken to the Biology Laboratory of the Federal Institute of

Piauí, campus Picos, where all procedures were performed for the obtention of cytogenetic material, cataloguing, recording and subsequent fixation and preservation. All under license of collection and processing of the animals, issued by the Chico Mendes Institute for Biodiversity Conservation - ICMBIO through the SISBIO platform, number: 47710-1.

Chromosomal preparations were obtained through the air-drying method as described by Bertollo, Takahashi and Moreira-Filho (1978). C-banding (Sumner, 1972) was used to visualize Heterochromatin regions and silver nitrate staining to detect NORs, according to the procedure described by Howel, Black, (1980).

Chromosomes were observed using a microscope coupled to a camera, and the best images captured. The chromosomal nomenclature was based on the system of Levan, Fredga and Sandberg (1964) taking into account only macrochromosomes (M) and microchromosomes (m).

RESULTS

Cytogenetic analyses were performed on three males and four females of *Ameivula ocellifera*. The individuals analyzed showed a diploid number of $2n = 50$ for both sexes, with a karyotype composed of 30 macrochromosomes and 20 microchromosomes of telocentric and subtelocentric types (Figure 1). There were no heteromorphic sex chromosomes observed in the specimens studied.

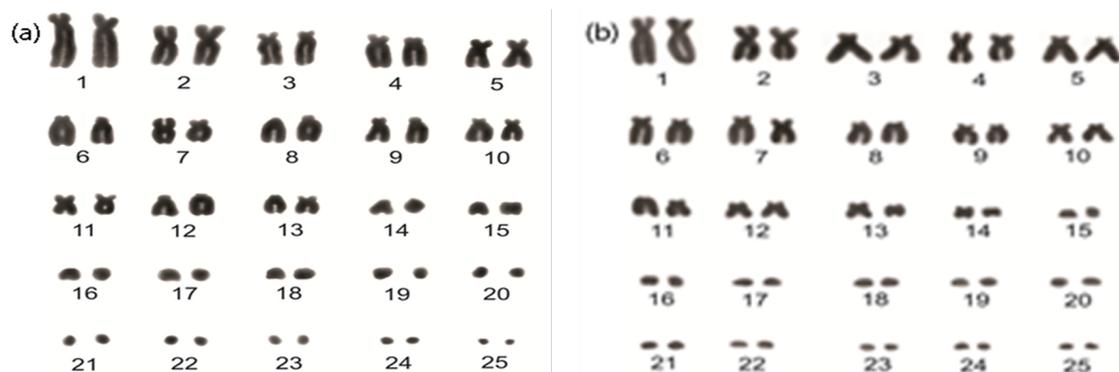


Figure 1. Karyotype of *A. ocellifera* in conventional staining with Giemsa showing $2n = 50$ chromosomes. a) Male individual. b) Female individual.

The C-banding technique showed blocks of heterochromatin in the pericentromeric and telomeric regions in both arms in several pairs of chromosomes of both sexes (Figure 2).

The nucleolar organizing region is present in the terminal portion of the long arm of number 5 pair in individuals of both sexes (Figure 3). It was not possible to observe secondary constriction in the analyzed metaphases.

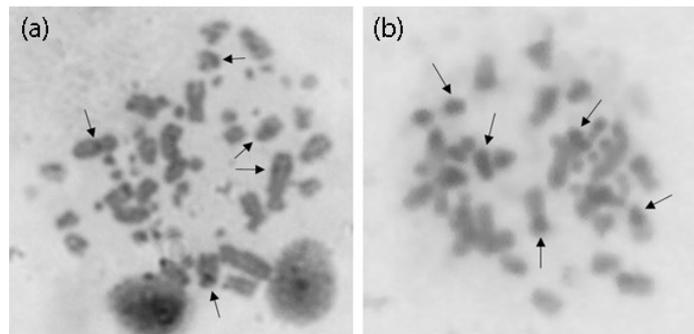


Figure 2. Metaphases of *A. ocellifera* showing the constitutive heterochromatin. a) Male individual. b) Female individual. The arrows indicate the most visible heterochromatic blocks.

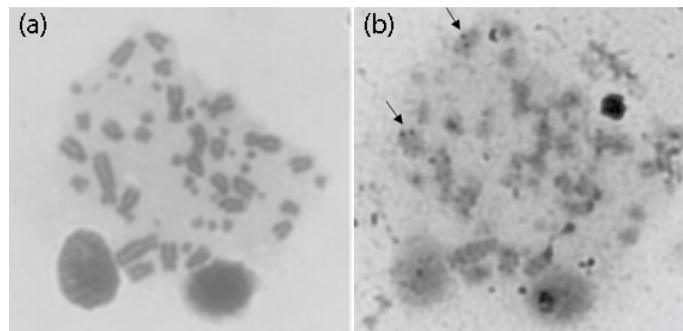


Figure 3. Metaphase of *A. ocellifera*. a) conventional staining with Giemsa. b) Impregnation with Silver Nitrate showing in the arrows the chromosomal pairs carrying the nucleolar organizing regions.

DISCUSSION

In recent years, the Teiidae family and its genera have undergone major changes that have led to the repositioning of species into complexes and the creation of new genera. *Ameivula* is a new genus that accommodates the species that belonged to the former group *Cnemidophorus ocellifera*. The complexity in the precise determination involving the species *Cnemidophorus ocellifera* arouse works based on phylogenetic evidence and molecular data. These works positioned these species in the new genus *Ameivula*, even though the latter having many features shared with the *Ameiva* species (GIUGLIANO; CONTEL; COLLI, 2006).

The diploid number of $2n = 50$ that includes biarmed chromosomes observed in *Ameivula ocellifera* in this study remains unaltered despite its wide geographic distribution. This fact also corroborates the studies in individuals also attributed to the Northeast and Southeast of Brazil (SANTOS et al. 2007). However, there is a reduction in the karyotype of *Ameivula nativo* (ROCHA; BERGALLO; PECCININI-SEALE, 1997) that have $2n = 48$ (ROCHA; BERGALLO; PECCININI-SEALE, 1997) and *Ameivula littoralis*

(ROCHA; ARAÚJO; VRCIBRADIC; COSTA, 2000) with $2n = 46$, and an XY sex chromosome system (PECCININI-SEALE et al. 2004) (Tab. 1). Signs of chromosome rearrangements in the species of *Ameivula* are evident when comparing the *A. littoralis* karyotype from the Brazilian Southeast, with other South American species (PECCININI-SEALE et al. 2004). According to the latter authors, the decrease in diploid number observed can be attributed to centric fusions of microchromosomes. In addition, pericentric inversions in the first and second pair of chromosomes occurred in *A. littoralis*, making these pairs of the submetacentric type.

The constitutive heterochromatin patterns observed in *Ameivula ocellifera* shows pericentromeric and telomeric regions with numerous blocks of heterochromatin in the two arms of several pairs of chromosomes (Table 1). This is very similar among the species of the Teiidae family such as *Ameiva ameiva* (LINNAEUS, 1758) and *Kentropyx paulensis* (BOETTGER, 1893) (SANTOS et al. 2007). The constitutive heterochromatin is restricted to the telomeric and centromeric region in *Ameiva* and *Cnemidophorus* species from other regions of the

country. In specimens of these regions, the microchromosomes were almost entirely

heterochromatic, and this fact appears being common among those related groups.

Table 1. Chromosome data available for Brazilian *Ameivula* species.

Specie	Chromosomal number and karyotypic formulae	NOR	Constitutive heterochromatin	Brazilian region	Reference
<i>Ameivula ocellifera</i> (Spix, 1825)	50. Gradual series of acrocentric chromosomes	Terminal, long arm pair 5	Centromeric and Telomeric regions	Northeast	Santos et al. (2007)
	50. 24 metacentric/telocentric; 26m subtelo-centric			Southeast	
<i>Ameivula littoralis</i> (ROCHA, ARAÚJO, VRCIBRADIC; COSTA, 2000)	46. 5 submetacentric; 41 telocentric/subtelocentric (XX; XY)	Terminal, long arm pair 8		Northeast	Present paper
				Southeast	
<i>Ameivula nativo</i> (ROCHA, BERGALLO; PECCININI-SEALE, 1997)	50. 5 submetacentric; 19 telocentric/subtelocentric; 24 microchromosomes	Multiple undefined pairs		Southeast	Rocha, Bergallo and Peccinini-Seale (1997)

Regarding the NORs, the number and position seem remaining conserved in *Ameivula ocellifera* in accordance with other populations previously analyzed (Table 1). The exception is the absence of size polymorphism as those observed by Santos et al. (2007). It was also detected in the congeneric species *Ameivula littoralis* a single pair carrying the NOR (PECCININI-SEALE et al. 2004). However, in *Ameivula nativo*, multiple NOR were noticed in a different chromosome pair (ROCHA BERGALLO; PECCININI-SEALE, 1997). This differentiation maybe due rearrangements involving the nucleolar organizer regions among the *Ameivula* species from Brazil.

In this way, despite the stability in the diploid number proven with this study, the NORs pattern is a useful tool to establish differences among the *Ameivula* species, contributing to correct identification of regional forms of these lizards.

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RESUMO: *Ameivula* é um novo gênero da família Teiidae que surgiu após extensa revisão de espécies que compuseram o antigo complexo de espécies chamado grupo *Cnemidophorus*. Suas espécies têm uma ampla distribuição do nordeste do Brasil ao norte da Argentina. Estudos citogenéticos na família Teiidae mostraram que os dados cariotípicos são ferramentas importantes em estudos filogenéticos e sistemáticos dentro deste grupo, permitindo determinar a posição das espécies na família. Assim, este estudo tem como objetivo descrever o cariótipo de *Ameivula ocellifera* (Spix, 1825) de Picos, no nordeste brasileiro. Os espécimes foram coletados de agosto de 2014 a outubro de 2015 utilizando armadilhas de interceptação e armadilhas, montadas aleatoriamente ao longo da área da Caatinga. Os animais foram coletados e transportados para o Instituto Federal do Piauí, campus Picos, onde foram realizados todos os procedimentos laboratoriais. Os indivíduos analisados apresentaram um número diploide de $2n = 50$ para ambos os sexos, com cariótipo composto por 30 macrocromossomos e 20 microcromossomos dos tipos telocêntrico e subtelo-cêntrico. Não houve cromossomos

sexuais heteromórficos nos espécimes estudados. A técnica da banda C evidenciou os blocos heterocromáticos nas regiões pericentroméricas e teloméricas dos cromossomos. As regiões organizadoras de nucléolos apareceram como uma unidade simples localizada na porção terminal do braço longo do par cromossômico número 5. As características cromossômicas de *A. ocellifera* analisadas não mostram divergências em relação a indivíduos de outras regiões. No entanto, as regiões organizadoras de nucléolos parecem ser um bom marcador cromossômico que permite distinguir as espécies já estudadas.

PALAVRAS-CHAVE. *Ameivula ocellifera*. Bandamento C. Marcadores citogenéticos. Cariótipo. RON.

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