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The micronucleus test in *Ameiva ameiva* (Squamata: Teiidae) in riparian forests in the Central Cerrado, Brazil

O teste do micronúcleo em *Ameiva ameiva* (Squamata: Teiidae) em matas ciliares do Cerrado Central, Brasil

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Free-living species can be used as indicators to estimate the impact of environmental stressors. In this study, the heliophilous lizard *Ameiva ameiva* was used for the first time for the micronucleus test and other nuclear abnormalities. This biomarker is rapid and reliable in evaluating populations environmentally exposed to genotoxic agents. Thus, two animal populations were collected in the Cerrado Central riparian forests with pasture influence. The results indicated the presence of micronuclei and different nuclear erythrocyte abnormalities: cells with nuclear bud, segmented nucleus, notched nucleus, lobed nucleus, and reniform nucleus. There was no statistical difference in the frequency of DNA damage between the populations in the pasture areas. The inclusion of these species in biomonitoring programs seems relevant to shed light on the susceptibility to expression of genotoxic damage. This species has a wide geographic distribution in the Neotropical region, which makes it promising for research on environmental mutagenesis.
Keywords: lizard, micronucleus, biomarker.

Espécies de vida livre podem ser usadas como indicadoras para estimar o impacto dos estressores ambientais. Neste estudo, o lagarto heliófilo *Ameiva ameiva* foi utilizado pela primeira vez para teste de micronúcleo e outras anomalias nucleares. Este biomarcador é rápido e confiável na avaliação de populações ambientalmente expostas a agentes genotóxicos. Assim, foram coletadas duas populações de animais nas matas ciliares do Cerrado Central com influência de pastagem. Os resultados indicaram a presença de micronúcleos e diferentes anormalidades eritrocitárias nucleares: células com botão nuclear, núcleo segmentado, núcleo entalhado, núcleo lobado e núcleo reniforme. Não houve diferença estatística na frequência de danos ao DNA entre as populações nas áreas de pastagem. A inclusão destas espécies em programas de biomonitoramento parece relevante para esclarecer a suscetibilidade à expressão de danos genotóxicos. Esta espécie possui ampla distribuição geográfica na região Neotropical, o que a torna promissora para pesquisas em mutagênese ambiental.
Palavras-chave: lagarto, micronúcleo, biomarcador.

1. INTRODUCTION

Native species are widely used as indicators to estimate the impact of environmental pollution. Reptiles, although little explored in ecotoxicological studies, have shown to be excellent bioindicators. These animals are ectothermic and have a slower metabolism, which can directly influence their recovery power and, therefore, becomes more sensitive to xenobiotic effects [1].

In this sense, the application of less invasive biomarkers that do not require the death of animals are strongly recommended for a perspective of environmental sustainability. The micronucleus (MN) test is a simple and sensitive method to detect chromosomal fragments and delayed whole chromosomes [2, 3] during the cell division process. The test has become an important pollution biomarker and has been a tool for predicting the potential for long-term and short-term effects of xenobiotics in wild species [4]. In reptiles, Poletta and Siroski [5] point out that the MN test proved to be a sensitive tool for the detection of genotoxic effects induced by chemical and physical agents, as already observed in other vertebrates.

Recognizing the importance of this test, in the present study, the species *Ameiva ameiva* was selected for research not only in order to expand knowledge for the group, but also to demonstrate the importance of reptiles in ecotoxicological studies. *A. ameiva* is a species of heliophile lizard that has a wide geographic distribution in the Neotropical region. It is commonly known as "green calango" or "calango", with an average back-cloacal length of 96.5 ± 23.1 mm [6], is quite active and feeds on a variety of prey including arthropods [7] and small vertebrates [8]. They occur in different types of habitats, from open and forested areas [9]. In general, lizards are potential predators of high trophic levels of invertebrates, forming a significant connection [10] that can generate disturbance reflexes in DNA by contact with xenobiotics, either by ingestion, dermal contact, or inhalation. Thus, with fragmentation and loss of habitats increasingly reducing their living areas, these animals become more vulnerable to resource scarcity and soil pollutants [11], which require ecotoxicological studies.

In this context, in the present study, we aimed to determine the frequency of Micronucleus (MN) and other nuclear abnormalities (ENAs) in *A. Ameiva* lizard in riparian forests under the influence of pastures in the Cerrado of Central Brazil. In addition, we tested the hypothesis of whether there is a difference in the frequency of MNs and ENAs among lizard populations living in pasture areas. These reference values serve as a basis for other studies involving environmental mutagenesis.

2. MATERIAL AND METHODS

2.1 Study area

Ameiva ameiva individuals were obtained in two riparian forest environments from the Brazilian Cerrado, one associated with the Preto River, municipality of Unaí, State of Minas Gerais, and the other with the Verdinho River, municipality of Serranópolis, State of Goiás (Figure 1). The individuals were collected in interception and fall traps (Pitfall traps) in the months of May 2018, December, and January 2019. A total of seventeen animals were captured for the micronucleus test. For the two collection areas, the main anthropic activity in the surroundings is livestock. However, there is no history of poisoning farmed animals or humans by possible xenobiotics (pesticides), although they have been used for many decades for small-scale livestock. Considering that the animals were obtained through environmental licensing, the work was approved by the Chico Mendes Institute for Biodiversity Conservation (nº 22694-1 and 77735-1).

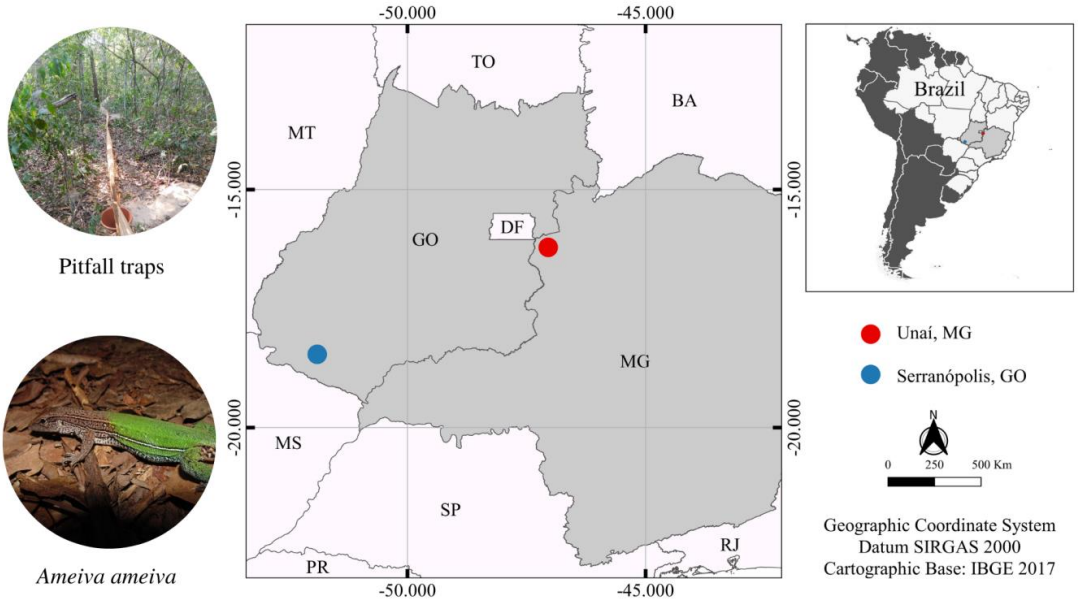

Figure 1: Sampling points in Unaí, Minas Gerais, and Serranópolis, Goiás, Brazil.

2.2 Mutagenic analysis

The micronucleus test was used for mutagenic evaluation in erythrocytes. The blood was obtained with the aid of heparinized syringes applied to the caudal vein of the animals collected in the Unaí-Baixo region, and then they were released at the same capture site. In Serranópolis, the animals were euthanized and the blood was obtained by means of cardiac pulse and the animals were deposited at the Laboratory of Ecotoxicology and Animal Systematics (IFGoiano, Rio Verde, Goiás). Anesthesia was performed with Xylazine and Ketamine administered intramuscularly in the thigh. Anesthetic deepening was performed with Lidocaine. Two blood smears were performed on clean glass slides for each animal, fixed with methanol for 10 minutes, and then stained with Giemsa 10% for 10 min. A total of 1000 mature erythrocytes were analyzed with an optical microscope by a single observer (1000x). MNs were considered to be those that presented structures similar to the main nucleus; absence of connection with the main nucleus; same texture and color intensity of the main nucleus. In addition to micronucleus, other nuclear erythrocyte abnormalities were scored [4].

2. 3 Data analysis

Micronucleus counts and other abnormalities were counted as the mean frequency ± standard error. Quantitative variables were subjected to normality tests (Shapiro-Wilk) and Levene. The Mann-Whitney U and Student's t tests were applied according to the normality, non-parametric and parametric requirements. A value of $p < 0.05$ was considered significant.

3. RESULTS

Ameiva ameiva was first examined for the micronucleus test. This biomarker, also recognized as a mutagenicity test, favored the detection of micronuclei and other erythrocyte nuclear abnormalities (Figure 2). The main nuclear abnormalities observed were nuclear sprout, cell with segmented nucleus, cell with notched nucleus, cell with lobed nucleus and cell with reniform nucleus. When comparing two populations of riparian forests, no significant differences were found for the frequency of micronucleus (Table 1). There was also no difference in the comparison between other erythrocyte nuclear abnormalities, as well as their sum.

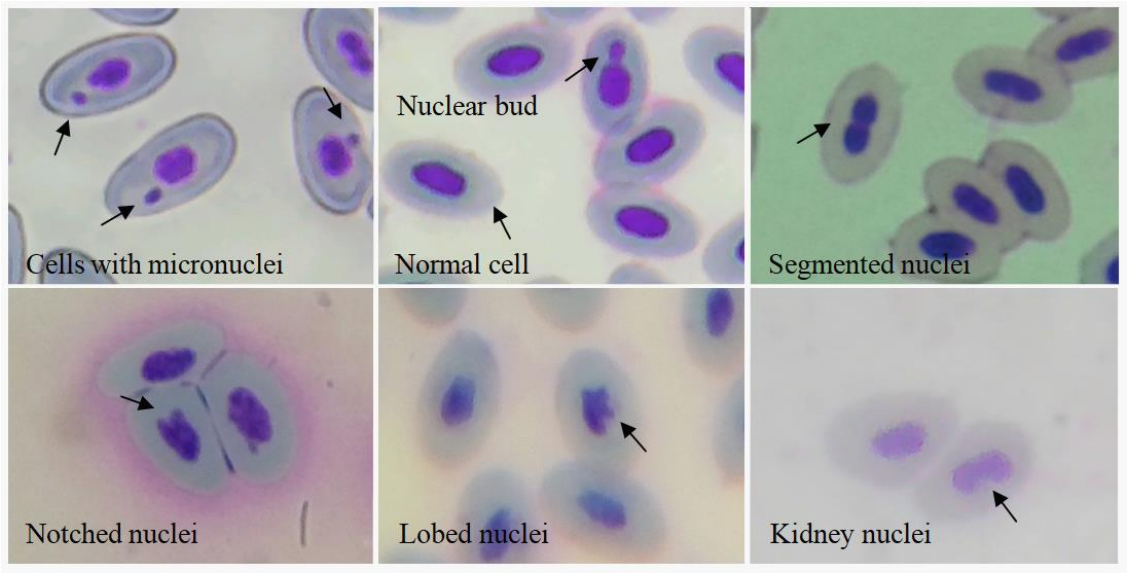

Figure 2: Micronucleus photomicrograph and erythrocyte nuclear abnormalities in Ameiva ameiva.

Table 1: Mean frequency ± standard error of micronucleus frequency and other nuclear abnormalities in Ameiva ameiva from riparian forests.

Erythrocyte alterations	**Frequency in 1000 cells ± standard error**		***p***
	Serranópolis (n = 10)	**Unaí (n = 7)**	
Micronucleus	0.03±0.02	0.16±0.07	0.14
Nuclear bud	0.46±0.12	0.37±0.12	0.62
Reniform nucleus	0.03±0.02	0.06±0.03	0.46
Lobed nucleus	2.51±0.64	0.94±0.23	0.08
Notched nucleus	0.48±0.13	0.63±0.26	0.92
Segmented nucleus	0.04±0.02	0.10±0.06	0.58
Total AENs	3.55±0.95	2.26±0.77	0.19

Mann-Whitney U and Student's t tests. A value of p <0.05 was considered significant.

4. DISCUSSION

This study was the first to apply the test for micronuclei and other erythrocyte nuclear abnormalities in the lizard species *A. ameiva*. The tests carried out in the two populations in riparian forests did not indicate any difference in the frequency of micronucleus and other AEN´s. However, the answer can be explained by the fact that the environments present similar phytophysiognomies. Anthropization close to the sampling sites is basically pasture with livestock. However, this environmental variable did not provide an explanation for the observed micronucleus frequency. On the other hand, it is known that manure produced by cattle can increase the number of metabolites of steroid hormones in the soil. According to Silva et al. (2020) [1], a study with lizards from organic farms exhibited liver biosynthetic changes typical of estrogenic contamination from manure-based fertilizer. The authors emphasize that exogenous estrogens, resulting from manure, can affect the welfare of wild animals, leading to bioaccumulation in the food chain.

Considering this fact, many chemicals are released into the environment regularly and can reach non-target organisms and cause chromosomal changes. Genotoxic damage in the medium term can impact the survival of animals. These damages can be verified by the formation of MN, which are cytoplasmic portions of chromatin of round or oval shape that is located near the main nucleus [12]. The manifestation of MNs depends on the intensity of the exposure to the disturbance, reaching responses in the short term. Basically, chemical contaminants reach the

bloodstream and induce the formation of MNs, which are the result of chromosomal fragments or entire chromosomes injured in anaphase. There may also be the formation of other nuclear erythrocyte abnormalities such as those detected in the present study, such as Nuclear bud, Reniform nucleus, Lobed nucleus, Notched nucleus, Notched nucleus and Segmented nucleus. The physiological aspect of the animals must also be considered in the expression of these nuclear abnormalities.

In view of these observations, we can see that *A. ameiva* can be considered important in the biomonitoring of genotoxicity due to the sensitivity in the expression of different erythrocyte alterations. In addition, it is worth considering that the organisms have an average baseline number of 2MN/1000 for the expression of abnormalities, as found in the present study. Considering that lizards are ectothermic organisms, with a low metabolic rate, they are possibly more sensitive to the effects of xenobiotics than other non-reptile species [1]. Thus, they can be sentinel organisms and, above all, generate indicators of environmental pollution.

Studies with the MN test in lizards are scarce. Zúñiga-González et al. (2001) [13] performed the tests on the lizard species *Iguana iguana* and *Ctenosaura pectinata*, showing numbers of spontaneous MN at different ages, while Schaumburg et al. (2012) [14] reported the use of the MN test in a natural environment, in a pioneering way in *T. merianae.* Schaumburg et al. (2014) [15] performed the MN test on the giant teiú (*Tupinambis merianae*) using hydrogen peroxide as a genotoxic agent and, in this case, the authors observed a high number of cells with MN. Schaumburg et al. (2016) [16] demonstrated that one of the most common formulations based on glyphosate, has genotoxic capacity in the teiú lizard and that the DNA damage induced in the erythrocytes of this native species after exposure to sublethal concentrations during the embryonic period, can interfere with the development and survival of embryos and young. In this way, the use of biomarkers can provide a useful early warning system for the presence of pollutants before any irreversible damage to the environment occurs [17, 18].

5. CONCLUSION

In summary, no difference was observed in the frequency of micronucleus and other nuclear abnormalities in populations of *A. Ameiva* lizards in riparian forests under the influence of pasture. The biomarker showed promise in the evaluation of this species due to the sensitivity of detection of DNA damage and other nuclear abnormalities. Future work may consider standardizing the sampling effort in different environments, in addition, whenever possible, consider analyzing the sex of animals for genotoxicity. Another important point will be the use of other biomarkers linked to the micronucleus test, in order to generate more information about the sensitivity of this important species in the neotropical region.

6. ACKNOWLEDGEMENTS

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