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## PRELIMINARY CYTOLOGICAL STUDY OF *AMIETOPHRYNUS REGULARIS* (REUSS, 1833) FROM AFON, KWARA STATE, NIGERIA

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### ABSTRACT

**Information on the karyotype of *Amietophrynus regularis* is scarce. 8 specimens of *A. regularis* were collected from Afon, Kwara State, Nigeria. The specimens were injected intraperitoneally with 0.5 % colchicine and left for 5 hours before they were sacrificed. Chromosomes were prepared from the bone marrow of the femur. 80 metaphase spreads were scored and the diploid chromosome number ranged from  $2n = 38$  to  $2n = 42$ . The modal number of chromosome was found to be  $2n = 40$  with percentage occurrence of 47.5 %.**

**Keywords:** *Amietophrynus regularis*, Afon, Chromosome, Metaphase, Karyotype

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### INTRODUCTION

*Amietophrynus regularis* commonly known as African common toad belongs to the Bufonidae family. Originally, *A. regularis* was included in the genus *Bufo*. This species is native to Africa where it is widely distributed. Aridity is known to restrict its range. It is found in savannahs and other habitats often in association with rivers. It breeds in rivers around the shallow edges away from the main current (IUCN, 2014; Frost, 2016).

In Nigeria, *A. regularis* is one of many amphibian species that is consumed as a food. Toads are an integral part of the economy in areas with large populations. Aside from their value as an essential food source, they also serve for cultural and traditional medicine values in rural areas (Mohneke *et al.*, 2010). Studies on amphibians in Nigeria are limited to their morphology, ecology, parasites (Adediran *et al.*, 2014) and nutritional composition (Oduntan *et al.* 2012 a, b).

Studies on amphibian cytology in Nigeria are very scarce. Knowledge of amphibian cytogenetics is valuable in defining species groups and evolution. Members of the genus *Amietophrynus* have a diploid

chromosome number of 20 except *A. pardalis* (Cunningham and Cherry, 2004; Frost *et al.*, 2006). Polyploidy with chromosome numbers of 40 and 44 have been reported in this genus (Bogart and Tandy, 1976; King, 1990; Kuramoto, 1990). This study was carried out to determine the chromosome number of *A. regularis* from Afon, Kwara State, Nigeria.

### MATERIAL AND METHODS

**Study Area:** The specimens were collected from Afon, Kwara State, Nigeria. It is located at an elevation of 307 meters above sea level and its population amounts to 174,152. Its coordinates are 8°19'0" N and 4°31'60" E in DMS (Degrees Minutes Seconds) or 8.31667 and 4.53333 (in decimal degrees). Afon has an annual rainfall range of 1,000 to 1,500 mm. Its vegetation comprises Guinea savannah, derived savannah and rainforest. The major occupations of the people are farming and trading (Adewumi *et al.*, 2012; Tunde *et al.*, 2013). Eight samples of *A. regularis* comprising of five males and three females collected at random were used for the study. Sampled *A. regularis* were maintained in insect cages and fed with life insects until they were sacrificed.

**Chromosomal Preparation:** Chromosomal preparation was carried out following the conventional colchicine-hypotonic-acetic-alcohol air-drying Giemsa staining technique (Tijo and Whang, 1965) with slight modification. Each specimen received intramuscular injection of 0.5 % colchicine solution. Five hours post injection with colchicine; sampled *A. regularis* were sacrificed and the femur bone dissected to locate the marrow. Bone marrow was flushed out with 0.56 % of KCl and subjected to 0.56 % KCl treatment for 40 minutes. Tissue was fixed in freshly prepared Carnoy's fixative for 30 minutes and after every 10 minutes fixative was changed. Air drying dabbing method was used for preparing slides. Tissue was dabbed on clean and dry slides and eventually air-dried. Slides were stained in 3 % Giemsa stain in phosphate buffer pH 6.8 for 30 minutes. 4 slides were prepared from each specimen. Metaphase spreads from each specimen were examined and photographed under a light microscope at a magnification of x100.

**RESULTS AND DISCUSSION**

This report is the first on the cytogenetics of *A. regularis* from Afon, Kwara State, Nigeria. The results of the diploid chromosome number and their percentage occurrence are presented in Table 1.

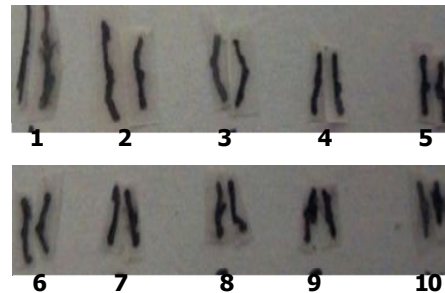
**Table 1: Diploid chromosome number and their percentage occurrence in *Amietophrynus regularis* from Afon, Kwara State, Nigeria**

| Specimen            | 2n=18 | 2n=19 | 2n=20 | 2n=21 |
|---------------------|-------|-------|-------|-------|
| 1                   | 4     | 4     | 10    | 2     |
| 2                   | 4     | 5     | 9     | 2     |
| 3                   | 3     | 7     | 9     | 1     |
| 4                   | 3     | 5     | 10    | 2     |
| <b>Total</b>        | 14    | 21    | 38    | 7     |
| <b>% occurrence</b> | 17.5  | 26.25 | 47.5  | 8.75  |

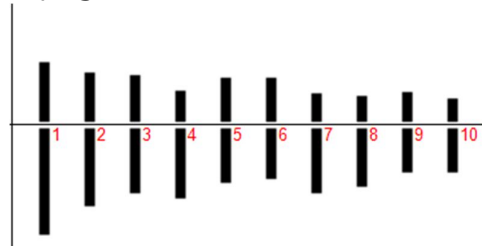
Diploid number ranged from 2n =19 – 2n = 21. The modal diploid number was found to be 2n = 20, represented by 47.5 %. The mitotic metaphase spread, karyotype and idiogram of *A. regularis* are presented on Figures 1, 2 and 3.



**Figure 1: Metaphase spread of *Amietophrynus regularis* from Afon, Kwara State, Nigeria**



**Figure 2: Chromosomal karyotype of *Amietophrynus regularis* from Afon, Kwara State, Nigeria**



**Figure 2: An idiogram of *Amietophrynus regularis* constructed on the basis of chromosome numbers and the position of centromere**

The chromosomes are metacentric and submetacentric (Table 2). A karyotype formula of 2n = 10m + 10sm was found for *A. regularis* following the nomenclature by Levan *et al.* (1964). The fundamental number for *A. regularis* was 40. Sex chromosomes were not observed as it is expected for anurans as they do not have heteromorphic sex chromosomes (Gileva and Shchupak, 2015). Polyploidy was not observed. African toads have a diploid number of 2n = 20. The diploid chromosome number of *A. regularis* was found to be 2n = 20. This agreed with the works of Bogart (1968) for members of the genus *Bufo* and Al-Shehri and Al-Saleh (2008) for *B. regularis* in Saudi Arabia.

**Table 2: Arm ratios and types of centromeres of *Amietophrynus regularis***

| Chromosome number | Long arm (q) | Short Arm (p) | p + q | Arm ratio p/q | Type of centromere |
|-------------------|--------------|---------------|-------|---------------|--------------------|
| 1                 | 2.95         | 1.66          | 4.61  | 1.77          | M                  |
| 2                 | 2.17         | 1.38          | 3.56  | 1.57          | SM                 |
| 3                 | 1.83         | 1.3           | 3.12  | 1.41          | M                  |
| 4                 | 1.94         | 0.88          | 2.82  | 2.22          | SM                 |
| 5                 | 1.52         | 1.22          | 2.74  | 1.24          | SM                 |
| 6                 | 1.4          | 1.21          | 2.62  | 1.16          | SM                 |
| 7                 | 1.8          | 0.81          | 2.61  | 2.22          | M                  |
| 8                 | 1.63         | 0.73          | 2.36  | 2.24          | SM                 |
| 9                 | 1.25         | 0.83          | 2.08  | 1.5           | SM                 |
| 10                | 1.23         | 0.65          | 1.87  | 1.9           | M                  |

The chromosome number of *A. regularis* is conserved. Chromosomal aberrations such breaks, ring and dicentric chromosomes were not observed during scoring. The absence of aberrations in the chromosomes of *A. regularis* suggested that it is not significantly exposed to clastogens in Afon. *A. regularis* can accumulate heavy metals when exposed to pollutants (Said *et al.*, 2015). Pollutants such as heavy metals have been reported cause to chromosomal aberrations in toad (Gileva and Shchupak, 2015). Obviously, anthropogenic activities such as mining, heavy pesticide and fertilizer usage are not common in this locality.

**Conclusion:** This study found the modal diploid number of *A. regularis* to be  $2n = 20$ . This confirms the diploid number as reported in previous works. This shows that the chromosome number of this species is conserved.

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