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CYTOLOGICAL STUDIES ON *ARCHACHATINA MARGINATA* (SWAINSON, 1821) FROM ILORIN, NIGERIA

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ABSTRACT

Chromosomal information on molluscs is scanty in Nigeria. This study was done to determine the chromosome number of Archachatina marginata populations from Ilorin, Nigeria. Ten samples were obtained from popular markets in Ilorin and humid areas within the University of Ilorin campus, Nigeria. The snails were treated with 0.075M KCl and 0.02% colchicine. The ovotestis was used for chromosomal preparation. The chromosomes examined were dot-like and acrocentric. The haploid chromosomes were found to be within the range of x=22 to x=29 with the modal haploid chromosome number x=28 (2n = 56). Polyploids and chromosomal aberrations were not observed in the cells. These results contribute to cytological information on A. marginata.

KEY WORDS: *Archachatina marginata, snail, ovotestis, chromosome number*

INTRODUCTION

Archachatina marginata commonly called Giant African snail belongs to the Family Achatinidae. This gastropod snail is native to West Africa (Bequaert, 1950) occurring in the high forests and in the fringing forests of the derived Guinea Savanna regions (Ajayi *et al*., 1978). It is also well distributed in less humid areas (Raut and Barker 2002). Achatinidae are hermaphrodites, nocturnal and generally regarded as herbivorous, feeding primarily on living and decaying vascular plant materials (Raut and Barker, 2002). However, *A. marginata was reported* to be omnivorous, subsisting on fresh and decaying plant and animal materials (Ajayi *et al.,* 1978). In Nigeria, *A. marginata* is a choice gastropod in terms of dietary preference. The flesh is rich in protein, low in fat and of additional value as a source of iron (Ajayi *et al.,* 1978). Reduction of wild populations of this species is rampant and attributed to destruction of the snails' habitat by deforestation, overharvesting, bush burning and the increased use of agricultural pesticide (Ajayi *et al.,* 1978; Okorie & Ibeawuchi, 2004).

Morphologically, Archachatina snails are distinguished by the possession of a blunt apex and a V-shape on their tail that is raised, serrated bump (Bequaert, 1950, https://www.petsnails.co.uk). Cytogenetic parameters such as chromosome number and morphology are valuable in species characterization and deciphering phylogeny evolution and taxonomic relationships (Poonam *et al*., 2013). Research works on karyotype analysis of molluscs are scarce due to difficulties of obtaining mitotic fields

with enough quality for chromosome studies (Park *et al*., 1999). Chromosomal information on molluscs is scanty in Nigeria. Search from literature revealed only two cytological studies on populations of *A. marginata* obtained from South-western part of Nigeria (Fagbuaro *et al*., 2002; Awodiran *et al*., 2012). Those studies reported dissimilar chromosome numbers for the giant land snail *Archatina archatina*. Occurrence of different karyotypes reported in same gastropod species maybe due to polymorphism among different geographical populations or differences in methods of chromosome preparation (Thiriot-Quievreux, 2003). This necessitates the cytological studies of *A. marginata* from other populations in Nigeria. Hence, this study is aimed at providing more cytological information on *A. marginata* from North-central Nigeria in order to confirm the chromosome number. This will expand existing cytological knowledge and valuable in taxonomy of *A. marginata*.

MATERIALS AND METHODS

Sample collection. The land snails were purchased from the *Ipata* and *Ganmo* markets, Ilorin, Kwara State, Nigeria. Samples were collected also from the Dam area and Zoological Garden, University of Ilorin, Nigeria. Their weights ranged between 54 and 142 g. The centres of purchase and capture are located within the range of about 10 km from the city centre (8.50^{o} latitude and 4.54^{o} longitude, 320 meters above sea level). Snails were identified using keys and descriptions (Bequaert, 1950; https://www.petsnails.co.uk).

Figure 1. Samples *Archachatina marginata* from Ilorin, Nigeria.

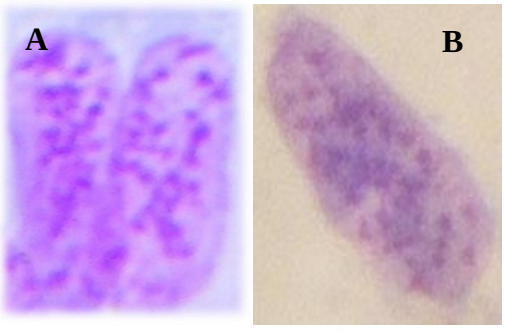

Figure 2. A-B: Metaphase spread of A*rchachatina marginata* from Ilorin, Nigeria.

Chromosomal preparation was done following the method of Awodiran *et al*. (2012) with little modifications. Snail samples received 0.075M KCl hypotonic solution to enable swelling of the cells (2ml per 100g weight). After two hours, they were injected with 0.02% colchicine (metaphase arresting agent) and allowed to stay for 3-4 hours. The shells were then broken at the apex to access the ovotestis. The ovotestis which was located at the posterior part of the apex was found within three (3) to four (4) whorls of the shell. The ovotestis was distinguished by its white, gray or pale yellow color in contrast to the surrounding brown color of the digestive glands (Patterson & Burch, 1978). The

ovotestis was removed and immersed in freshly prepared Carnoy's fixative (3:1 methanol: acetic acid v/v) and left for 24 hours. Ovotestis was cut into pieces and placed in a beaker with addition of 3 - 4 drops of 40% acetic acid and squashing to enhance fixing of the cells. Drops of the cell suspension were placed on the slide and allowed to dry for an hour. After air drying, the slides were then stained with 10% Giemsa stain solution for 45 minutes. It was then rinsed several times in water to remove excess stain. The slides were then left over night to dry. Chromosome counting was done using X100 objective of the microscope. Cells adjudged to contain well spread metaphase chromosomes were photographed under oil immersion. Ten spreads were scored for each of the four slides prepared from each of the samples.

RESULTS AND DISCUSSIONS

Ovotestis cells revealed haploid chromosome number of *A. marginata* ranged between x=22 and x=29 with the modal haploid chromosome of 28 (Table 1). Most of the chromosomes were dot-like and acrocentric (Figure 2). Polyploids and chromosomal aberrations were not observed in the cells scored. A haploid number of 28 chromosomes was reported for *A. marginata* in this study. The modal diploid chromosome complement of *A. marginata* was 56. Similar result was obtained by Awodiran *et al*. (2012) for populations obtained from South-west Nigeria. Obviously, the chromosome number of *A. marginata* is conserved.

Table 1. Percentage occurrence of haploid chromosome numbers of *Archachatina marginata f*rom Ilorin, Nigeria.

	Haploid Chromosome Number					
Samples	22	25	26	27	28	29
1	1	2	8	11	15	3
2	-	-	2	20	17	1
3	-	1	5	14	20	-
4	-	-	7	12	19	2
5	-	1	6	13	19	1
6	-	3	10	8	19	-
7	-	1	10	5	21	3
8	-	2	11	4	18	5
9	-	2	9	8	21	-
10	-	3	6	8	23	-
% Occurrence	0.25	3.75	18.50	25.75	48.00	3.75

Most cytological studies on land molluscs have been limited to establishing their chromosome number as their chromosomes are small, numerous, and difficult to see (Page, 1978). The chromosomes of *A. marginata* were found to be majorly dot-like and acrocentric. The acrocentric nature of this chromosome often makes them difficult to describe morphologically. Therefore, the chromosomal length and arm ratios were not resolved. Also, fundamental numbers could not be obtained due to inability in figuring out the chromosomal arms. Awodiran *et al*. (2012) reported chromosomes of *A. marginata* as

metacentric and acrocentric but their studies failed to describe the morphology and karyotype of *A. marginata*.

Even though the number of chromosomes frequently observed were x=28, few cells were observed with chromosome numbers of less than 28 and some were found to be 29. The resulting deviation could be from loss or addition of chromosomes during preparation or inaccuracy in chromosome count (Ademola *et al*., 2017). Polyploids and chromosomal aberrations were not observed in the cells scored. Polyploidy has not been reported in *A. marginata* (Fagbuaro *et al.*, 2002; Awodiran *et al*., 2012). Previously, pollution has been implicated to cause chromosomal aberrations in molluscs (Barsiene, and Bucinskiene, 2001). Therefore, the absence of chromosome aberrations in populations of *A. marginata* from this study indicate that the snails may have not been sourced or obtained from contaminated environment.

CONCLUSIONS

The result of this study revealed the haploid (n) chromosome number of the Giant African snail to be x=28 without description of the chromosomes. It therefore recommended that further studies employing techniques such as banding and fluorescent *in situ* hybridization (FISH) will allow description of the chromosomes of *A. marginata*.

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