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Karyological and Chromosomal Study of Catfish (Clariidae, *Clarias gariepinus*, Burchell, 1822) from Anambra River, Anambra State, Nigeria

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Abstract: Karyological and Chromosome analysis of *Clarias gariepinus* (Burchell, 1822) inhabiting Anambra River, Anambra State of Nigeria was carried out using Modified Air-drying technique. *Clarias gariepinus* was found to have standard karyotype and diploid chromosome number of $2n = 56$. The study further revealed that the males and females catfish consist of 8 metacentric, 24 and 25 submetacentric and 24 and 23 acrocentric chromosomes, respectively. The work documented the karyotypic polymorphism of *Clarias gariepinus* resident in the River.

Key words: Karyotype, chromosome, *Clarias gariepinus*

INTRODUCTION

Clarias gariepinus is widely considered as the most tropical catfish species ideal for aquaculture in West Africa (Clay, 1979). It has a pan African distribution, being preponderance from Nile to West Africa and from Algeria to South Africa as the Orange system and Umtamvuna (east coast), going by Teugels (1986) report. The species is also preponderance in Asia Minor and Potamodromous (Teugels, 1986). In Nigeria, *Clarias gariepinus* is known by different names among various ethnic groups; Tarwada (Hausa), Imunu (Ijaw), Ejengi (Nupe), Aro (Yoruba) and Arira (Igbo). According to Ozouf-Costaz *et al.* (1990), in *Clarias gariepinus*, the karyotype and chromatin materials are very stable. They observed no detectable karyotypic differences among the *Clarias gariepinus* populations from three different geographical locations. Working on the chromosomal structure, Levan *et al.* (1964) maintained that fish chromosomes with centromere index less than 35 were classified as acrocentric, while those between 35 and 45 were regarded as submetacentric and those within 45-50 were the metacentric chromosomes.

Data on chromosomal constitution, architecture and by extension, the photomicrograph of the chromosome morphology of catfish is rather limited compared to other vertebrates because of the small size and limitations of techniques employed (Klinkhardt, 1991; Ergene *et al.*, 1999). Air-drying technique which was originally developed for the study of mammals' chromosomes (Serap and Tolga, 2004) can now be applied to chromosome studies in other species. According to the authors, while the basic steps are the same, few modifications have been applied to this technique for different species (Foresti *et al.*, 1993; Cucchi and Barufaldi, 1990). Doussau and Ozouf-Costaz (1985) has

described a technique for studying fish chromosomes using anterior kidney and in some cases, stimulation of fish sample with yeast suspension is advocated for induction of mitosis (Lee and Elder, 1980). However, the use of Air-drying technique appears to be simpler, cheaper and more reliable when compared to the existing techniques. The study was therefore, designed to determine the karyotype, chromosomal architecture, the proportion of acrocentric, submetacentric and metacentric chromosomes and number of chromosomes in *Clarias gariepinus*, Burchell, 1822 (Pisces, Clariidae) in Anambra River, Anambra State, Nigeria using Air Drying Technique.

MATERIALS AND METHODS

Live *Clarias gariepinus* specimens were collected from Anambra River, Anambra State, Nigeria; brought to the laboratory in air-tight containers and put into aquariums where they were kept for several days. They were fed twice a day and 0.5% Colchicine was injected intra-peritoneally into the fish sample to disrupt spindle formation at mitosis and prevent the replicated chromosomes from migrating to their respective poles (Hartwell *et al.*, 2000). Four hours later, the fish sample was sacrificed by decapitation and dissected. Gill arches and kidney tissues were extracted and crushed to obtain an epithelial cell suspension. The suspension was aspirated into centrifuge tubes and after hypotonic treatment with 0.75% KCL solution for 30 min (at room temperature); they were centrifuged at 2000 rpm for 10 min.

The supernatants were recovered and the cell buttons were broken up with the tip of pipette. Cells were fixed in Glacial Acetic Acid (GAA) and Methanol at the ratio of 1:3. The solution was allowed to stand for 30 sec and the fixative changed by three successive centrifugations for

10 min. Cells from the fixative were transferred to clean and cold slides, air-dried and stained with 10% giemsa solution in 6.8 phosphate buffer. Finally, the slides were rinsed with distilled water and air-dried. Slides were covered, microscopic studies were performed and well-separated metaphase chromosomes photographed. A total of sixty slides were prepared at the rate of five slides from a fish sample. A karyogram was prepared by high-contrast chromosome photographs (Fig. 1) and the individual chromosomes were cut out of the photographs. Classification and karyogram of the chromosomes were performed according to the techniques described by Levan *et al.* (1964) and Ergene *et al.* (1998a,b). The final karyogram was scanned and printed.

RESULTS AND DISCUSSION

The diploid chromosome number of *C. gariepinus* in Anambra River was found to be twenty-eight pairs ($2n = 56$) and autosomes basic arm number (NF) is 88 for males and 89 for females. It was also found that the male *Clarias gariepinus* has 8 metacentric, 24 submetacentric and 24 acrocentric chromosomes, while the female recorded 8 metacentric, 25 submetacentric and 23 acrocentric chromosomes. Figure 1 presents diploid metaphase chromosomes from gill epithelial cells of *C. gariepinus*, while Fig. 2 and 3 display the male and female Karyotypes, respectively of gill epithelial cells of *C. gariepinus*. The percentage occurrences of metacentric, submetacentric and acrocentric chromosomes in sixty-four samples examined is shown Table 1.

Karyotypes of *Clarias gariepinus* living in Anambra River, Anambra were $2n = 56$. The most recent and complete data summarizing silurid karyotypes have been produced by Rab (1981) and Vasiliev (1985). In silurid families, chromosome and/or chromosome arm numbers exhibit a great variability and we can assume that the karyotype is specific and that this criterion can be used for the species characterization.

It is a well known phenomenon that acrocentric chromosomes have a tendency to stick to each other by their centromeres and in this way they form metacentric chromosomes (Dogramaci *et al.*, 1994). If we consider the same condition for *C. lazera*, the metacentric number will increase but the acrocentric number will decrease; but since the acrocentric number would decrease and this would result in a decrease in chromosome number, this is considered to be a weak probability (Ergene *et al.*, 1999).

The variation seen in the karyotype of *C. gariepinus* in the Anambra River can be viewed as a small part of the main population and variations in chromosome number and chromosome morphology were equally determined for *C. gariepinus* inhabiting the River (Table 1). Variations such as these show that various karyotypic

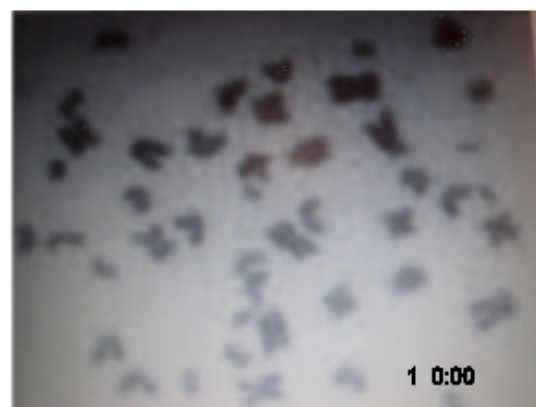


Fig. 1: Diploid metaphase chromosomes from gill epithelial cells of *C. gariepinus*, giemsa staining, x 1500



Fig. 2: Karyotype from gill epithelial cells of a male of *C. gariepinus*, giemsa staining, x 1500



Fig. 3: Karyotype from gill epithelial cells of a female of *C. gariepinus*, giemsa staining, x 1500

forms exist in this species, since the highest value in the count is accepted as the number of chromosomes and portray karyotypical polymorphism of the resident *C. gariepinus* species in Anambra River.

Table 1: Frequency distribution of diploid chromosome number of *Clarias gariepinus* in Anambra River, Anambra

Examined Specimen	Evaluated Metaphase Number	Diploid Chromosome Number (2n)	Males			Females			Occurrence (%)
			M	SM	A	M	SM	A	
-	2	49	8	12	14	10	23	20	3.10
-	3	51	12	25	12	10	23	20	4.70
-	6	52	9	23	12	12	22	19	9.40
-	2	53	13	23	12	6	18	17	3.10
-	5	54	12	18	25	12	17	18	7.80
-	3	55	12	22	24	8	18	24	4.70
-	17	55	8	23	24	8	12	22	26.60
-	24	56	8	24	24	8	25	23	37.50
-	2	58	5	24	24	8	25	20	3.10
10	64								100

M: Metacentric, SM: Submetacentric, A: Acrocentric

Ozouf-Costaz *et al.* (1990) supporting Teugels (1982) study, stated that *C. lazera* and *C. mosambicus* are synonymous with *C. gariepinus*. In order for *C. lazera* to be assumed synonymous to *C. gariepinus*, it must be determined whether they can breed and produce fertile individuals. However, taking only karyological and morphological characteristics into account in defining species has its shortcomings. Karyotypical studies on *C. gariepinus* living in other parts of Nigeria are necessary for more detailed knowledge about karyotypical forms.

The first successful hybridization between the two species of Clariidae, *Clarias gariepinus* and *Heterobranchius longifilis*, was done in 1985 by Hecht and Lublinkhof. But, karyotypes of the parental species and of their hybrids remained unknown. It is of interest to know the karyotype of the species used for this hybridization in order to estimate their genetic purity (Ozouf-Costaz *et al.*, 1990). A study of the phylogenetic relationships between these species by karyological and biochemical methods would allow a prediction of the possible genomes obtainable in the hybrids. Finally, the analysis of their karyotypes would clarify the mechanism involved in karyogamy, as well as why these hybrids are viable and relatively fertile (Ozouf-Costaz *et al.*, 1990). This study is the first in a series to characterize the chromosomes of clariid catfishes used for fish culture from Anambra River and will be followed by definition of the genetic structure of hybrids. Although studying chromosomes at meiosis are particularly difficult to undertake in the field, the marker chromosomes detected in the standard karyotypes of *Clarias gariepinus* appear to be useful in comparing different species. Furthermore, these markers may also be useful in identifying parental genetic input in hybrid karyotypes.

In addition to chromosomal architecture of *clarias gariepinus*, gene characterization and mapping and DNA analysis should be embarked upon to determine various

species, additional information on the fish cytogenetic database and provide clues for improvement of the economic species.

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