KARYOTYPIC ANALYSIS OF *LABEO COUBIE* (RUPPELL, 1832) (AFRICAN CARP) FROM UNIVERSITY OF ILORIN DAM, ILORIN, NIGERIA

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ABSTRACT

Karyotypic information is a useful endpoint in environmental monitoring and breeding programme. Data on karyotype of Labeo coubie is needful for environmental assessment and genetic improvement breeding projects. This study was aimed at determining the karyotype of L. coubie. 10 specimens were obtained from the University of Ilorin dam, Ilorin, Nigeria. Each specimen received intraperitoneally 0.02% colchicine (1ml/100g body weight) and left for 4 hours before sacrificing. Chromosome preparation was made from the kidney and liver. A total of 200 metaphase spreads were scored. The diploid chromosome numbers ranged from 2n = 44 to 2n = 50. The modal diploid number was found to be 2n = 50 and this represents 56 %. The kidney tissue gave better chromosome preparation. These results contribute to the karyotypic data on L. coubie.

Keywords: *Labeo coubie*, Karyotype, Chromosome

INTRODUCTION

Labeo coubie is one of the common species of fishes of the family Cyprinidae found in Nigeria, West Africa. It is a highly valued food in Nigeria and other West African countries (Ayotunde *et al.,* 2007). *L. coubie* lives on the bottom of seas or lakes. It inhabits rivers and lakes, particularly sheltered bays and it migrates within freshwater only. It feeds on mud, plant debris and diatoms (Azeroual *et al.*, 2010). The University of Ilorin dam is a structure of immense importance. It is located in the University of Ilorin Main Campus. It is a small dam created for the function of water supply. Apart from its function to supply water, it contains different species of fishes like *Clarias, Labeo, Oreochromis* and *Sarotherodon* (Omotosho, 1993; Achionye-Nzeh and Isimaikaiye, 2010).

Nigeria is one of the top 25 fish producers; however it had not been able to meet its local demand. Jamu and Ayinla (2003) reported that fish yields from Nigerian inland waters to be on decline. Appropriate conservation strategies are needed to ensure sustainable yields. Pertinent to this is karyotype analysis of Nigerian fishes. Cytogenetic data on fishes from Nigerian waters is very lacking. Identification of fishes has been based on the traditional morphological method.

Karyotype is a test to identify and evaluate the size, shape, and number of chromosomes. Karyotype analysis can be used for many purposes such as classification, evolution and fish breeding (King *et al.,* 2006). Few workers venture into studying fish chromosomes. This is due to large number of small chromosomes in fish (Golubtsov and Krysanov, 1993).

Scanty works are available on the karyotype of *L. coubie.* Study was carried out by Paugy *et al.* (1990) reported a modal diploid number of 2n = 50 for *L. coubie* which is the most common diploid number in the Cyprinidae (Vasiliev, 1985). In the majority of cyprinid karyotype, all the chromosomes are acrocentric but the occurrence of metacentric has also been reported in *L. gonius* and *L. fimbriatus* (Nayyar,

1966; Biswal, 2010). Cytology on *L. coubie* is insufficient, and further study is needed to evaluate karyological characteristics of the species (Paugy *et al.*, 1990). The aim of this study was to provide knowledge on the karyotype of *L. coubie.*

MATERIALS AND METHODS

Study Area: Ten specimens of *L. coubie* (Figure 1) were collected from the University of Ilorin dam, Ilorin, Kwara State, Nigeria, and identified based on the identification key of Paugy *et al.* (2003).

Figure 1: *Labeo coubie* collected from the University of Ilorin dam, Ilorin, Nigeria

The dam is located on latitude 8° 30″ N and longitude 4° 32″ E. Two seasons predominate in Ilorin: wet (March to October) and dry (November to February). The annual rainfall ranges from 1000 mm to 1500 mm. Temperature ranges between 25°C and 30°C in the wet season, while the dry season has a range of 33°C to 34°C (Sule *et al.*, 2011; Akpenpuun and Busari, 2013). The fishes were transported to the laboratory very early in the morning.

Chromosomal Preparation: The experiment was carried out following the air-dry method of Bertollo *et al.* (1978). Each specimen was injected intraperitoneally with 0.02 % colchicine (1ml/100g body weight) to arrest cell division at metaphase stage. The fishes were then sacrificed after 4 hours by pitching. The kidney and liver were carefully removed and washed in an isotonic solution of 0.9 % NaCl. The purpose of this is to prevent osmotic effect and consequent damage to the cell. Small pieces of tissues were transferred to hypotonic solution of 0.56 % KCl. This helps to increase the volume of the cells. Using a Pasteur pipette, the tissue and solution were transferred into a centrifuge tube and homogenized after which centrifugation was carried out for 7 minutes at 1000 rpm. After that, the supernatant was removed.

Fixation was carried out by adding a cold mixture of freshly prepared Carnoy's fixative (methanol: acetic acid in the ratio of 3:1). This helps to preserve the internal structure of the cells. Thereafter, it was centrifuged again and the supernatant was removed. Re-fixation was then carried out twice as above (Bertollo *et al.*, 1978). Cell suspension was spread with the aid of a Pasteur pipette on clean slides. Eight (8) slides were prepared for each specimen. After drying, the slides were stained in freshly prepared Giemsa stain. Excess stains were rinsed off in distilled water. Ten metaphase spreads from each specimen were examined and photographed under a light microscope at magnification of x1000. For kidney and liver, ten good metaphase spreads were studied.

Data analysis: Chromosomes were classified according to the nomenclature of Levan *et al.* (1964). KaryoType was used to determine the length of chromosomal arms and idiogram (Altınordu *et al.*, 2016).

RESULTS

Metaphase spread, karyotype and idiogram for *L. coubie* are presented in Figures 2, 3 and 4 respectively.

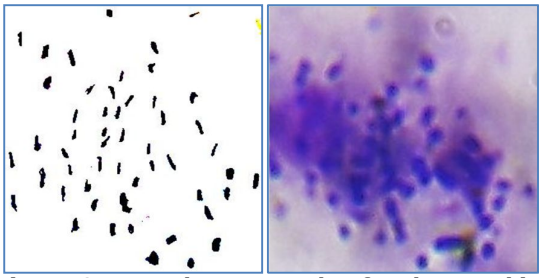

Figure 2: Metaphase spreads of *Labeo coubie* from the University of Ilorin dam, Ilorin, Nigeria. (a) Traced, (b) Normal metaphase view

The kidney cells gave better chromosome spreads than the liver cells. The diploid chromosome numbers obtained ranged from 2n = 44 to 2n = 50.

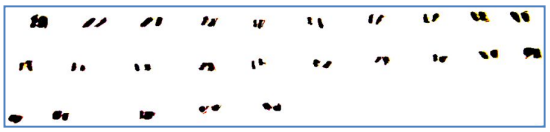

Figure 3: Karyotype of *Labeo coubie* from the University of Ilorin dam, Ilorin, Nigeria

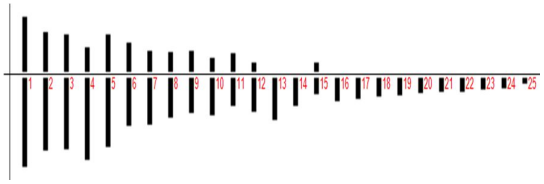

Figure 4: An idiogram of *Labeo coubie* from the University of Ilorin dam, Ilorin, Nigeria constructed on basis of chromosome numbers and centromere position

The percentage occurrence of the diploid chromosome numbers from the kidney cells showed that the modal chromosome number was 2n = 50 (Table 1). The percentage occurrence of the diploid chromosome numbers from the liver cells showed that the modal chromosome number was 2n = 50 (Table 2). Sex chromosomes were not detected. The karyotype formula for *L. coubie* was found to be 2n = 10m + 11sm + 5st + 24t. Most of the chromosomes were telocentric (Table 3).

DISCUSSION

Chromosomes of fishes are smaller in size and more compacted in structures when compared to those of mammals, thus studying and measuring fish chromosomes is somewhat more difficult than those of mammals (Gül *et al.,* 2004). In karyotype studies of Cyprinids, the diploid chromosome numbers ranges from 2n = 44 to 2n = 50, with a modal diploid number of 2n = 50 (Manna and Khuda-Bukhsh, 1977; Manna, 1984; Rishi, 1989). Although, in the Cyprinids, there has been recorded cases of polyploidy in chromosome numbers ranges from 2n = 94 to 2n = 200 in *Carassius carassius* and *C. auratus* (Raicu *et al.,* 1981), in the present study the modal diploid chromosome number of *L. coubie* was found to be 2n = 50. The diploid chromosome number of the species is in agreement with the work Paugy *et al.* (1990) for *L. coubie* and *L. senegalensis* from defined and described from the Upper Niger River and Upper Senegal (Baoulé) River basins.

Table 1: Percentage occurrence of diploid chromosome numbers for kidney cells of *L. coubie* from University of Ilorin dam, Ilorin, Nigeria

Specimen	2n=44	2n=46	2n=48	2n=50
A	1	3	1	5
B	0	2	1	7
C	2	2	4	2
D	1	4	3	2
E	0	0	3	7
F	1	0	3	6
G	2	0	2	6
H	2	0	1	7
I	0	1	1	8
J	0	2	2	6
% Occurrence	9	14	21	56

Number of spreads = 10 for each specimen

Table 2: Percentage occurrence of diploid chromosome numbers for liver cells of *L. coubie* from the University of Ilorin dam, Ilorin, Nigeria

Specimen	2n=44	2n=46	2n=48	2n=50
A	1	3	2	4
B	1	4	2	3
C	2	2	2	4
D	0	1	2	7
E	0	2	4	4
F	4	2	1	3
G	2	2	3	3
H	1	2	4	3
I	1	4	3	2
J	1	5	3	1
% occurrence	13	27	26	34

Number of spreads = 10 for each specimen

Other than this, no previous work has been carried out on the karyotype of *L. coubie.*

The chromosome number of *L. coubie* is conserved as in other cyprinids (Arai, 2011; Sukham *et al*., 2015).

Cyprinid fishes are characterized by the presence of relatively small chromosomes with their centromere positions ranging gradually from median to nearly terminal, making it difficult or almost impossible to identify individual chromosomes (Rab and Collares-Pereira, 1995). This report is true when considering the karyotype of *L. coubie* in this study. It is characterized by the presence of nearly terminal chromosomes of approximately the same size, which lie closely packed at metaphase.

Table 3: Arm ratios and types of centromere in *Labeo coubie* from the University of Ilorin dam, Ilorin, Nigeria

Chromosome number	Long arm (q)	Short Arm (p)	p + q	Arm ratio (q/p)	Type of centromere
1	3.45	2.12	5.57	1.63	SM
2	2.79	1.53	4.32	1.82	T
3	2.77	1.43	4.20	1.93	T
4	3.16	0.96	4.12	3.28	T
5	2.66	1.45	4.10	1.84	SM
6	1.86	1.11	2.97	1.67	SM
7	1.81	0.81	2.63	2.23	T
8	1.53	0.78	2.30	1.97	SM
9	1.34	0.82	2.15	1.63	T
10	1.41	0.54	1.95	2.60	M
11	1.10	0.74	1.83	1.49	M
12	1.29	0.38	1.67	3.39	M
13	1.61	0	1.61	-	T
14	1.05	0	1.05	1.91	M
15	0.63	0.37	1.00	1.70	M
16	0.90	0	0.90	-	T
17	0.78	0	0.78	-	T
18	0.72	0	0.72	-	T
19	0.68	0	0.68	-	T
20	0.60	0	0.60	-	T
21	0.55	0	0.55	-	T
22	0.51	0	0.51	-	T
23	0.48	0	0.48	-	T
24	0.42	0	0.42	-	T
25	0.21	0	0.21	-	T

The chromosomes were small and of uniform thickness, condensed in nature and darkly stained. Cells not showing the modal counts were probably caused by loss during preparation or by chromosomes being obscured by the surrounding cell nuclei. It was observed that the chromosomal spread of *L. coubie* using the kidney was better than using the liver. The liver cells appeared to be pale and difficult to analyse, while the kidney cells were more distinct. The diploid number of chromosomes for this species occurred more in the kidney plates than the liver plates, although the diploid numbers were also observed in the liver plates. This was in conformity with the works of Margarido *et al.* (2007) and Vasconcelos and Molina (2009). They reported that the kidney is better used because it gives the best quantity and quality of metaphase chromosome spread in fish.

Conclusion: This present study is the first to describe the complete chromosomal characteristics of *L. coubie* from Nigerian waters. The result of this study revealed that *L. coubie* has a diploid number of 50. This chromosome number is highly conserved. Optimum results were obtained from kidney cells.

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