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Karyotypic Description of Six Species of *Clarias* (Siluriformes: Clariidae) from South West Nigeria

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Abstract: Data on karyotype of fishes in Nigeria is limited. In this study, chromosome analysis of *Clarias anguillaris* (Linnaeus, 1758), *C. pachynema* (Boulenger, 1903), *C. gariepinus* (Burchell, 1862) *C. camerunensis* (Lonnberg, 1895), *C. jaensis* (Boulenger, 1909) and *C. macromystax* (Gunther, 1864) from South-West Nigeria was carried out using the kidney cells. *C. anguillaris* have variable 2n = 48 and 56. The spread with 2n = 48 comprised 27 metacentric, 10 submetacentric, 3 subtelocentric and 8 telocentric chromosomes; while the spread with 2n = 56, which is the modal diploid number, comprised 33 metacentric, 12 submetacentric, 2 subtelocentric and 9 telocentric chromosomes. *C. pachynema* with 2n = 66 comprised 30 metacentric, 10 submetacentric, 16 subtelocentric and 10 telocentric chromosomes. The 2n = 56 for *C. gariepinus* comprised 25 metacentric, 14 submetacentric, 14 subtelocentric and 3 telocentric chromosomes; while same diploid number for *C. camerunensis* included 22 metacentric, 20 submetacentric, 9 subtelocentric and 5 telocentric chromosomes. *C. jaensis* had a 2n = 54, comprising 22 metacentric, 12 submetacentric, 5 subtelocentric and 15 telocentric chromosomes, while the 2n = 49 for *C. macromystax* comprised 27 metacentric 10 submetacentric, 11 subtelocentric and 1 telocentric chromosomes. Idiograms were prepared for each species based on the chromosome measurements. Several reasons were adduced for the karyotypic variability in *C. anguillaris*. This study is of importance in the evolution, classification and taxonomy of *Clarias* species and also in monitoring aquatic toxicity.

Key words: Chimera, chromosome, *Clarias*, karyotype, taxonomy

INTRODUCTION

Karyological studies in fish have shown potentials in increasing knowledge in the fields of genetics, taxonomy, evolution, systematics, mutagenesis, environmental toxicology and aquaculture (Amemiya, 1986; Kligerman and Bloom, 1977; Cucchi and Baruffaldi, 1990). Several methods have been developed for karyological studies in fish (Webb, 1974; Cucchi and Baruffaldi, 1990) but the direct *in vivo* method has been extensively used till date. The air-drying technique, originally developed for mammalian organisms is the most common procedure used for chromosome preparations in fish. While the main steps are the same, modifications have been applied to this technique for each species (Foresti *et al*., 1993).

Fish fauna can be characterized either by the occurrence of stable karyotypic groups or by divergent ones with an extensive chromosome diversity (De Rosa *et al*., 2007). While several taxa show a chromosome evolution relatively divergent concerning the karyotype macrostructure, other fish groups share a common karyotype structure and equal number of chromosomes (Bertollo *et al*., 1986; Oliveira *et al*., 1988; Arefjev, 1990; Galetti *et al*., 1994; Sczepanski *et al*., 2007). Catfishes (Siluriformes) have been known to show a great diversity in the organization of the genome; this includes the karyotype as well as the amount of DNA included in each nucleus. They are characterized by a very dynamic history of cytogenetics. Polyploidization and chromosome rearrangements have occurred among and within families on several occasions (Volckaert and Agnèse, 1996).

Among the various species of catfish, members of the genus *Clarias* are the most cultured in Nigeria. Their importance is due mainly to their rapid growth rate, large sizes, sedentary lifestyle, low bone content, hardiness, high yield, omnivorous feeding habits, good flesh quality, tolerance to poor water quality (even in the larvae) and high protein content. They also contribute largely to the fisheries in many basins of Africa. They are therefore in high demand and feature prominently in the diet of Africans partly because they are comparatively a cheaper source of protein than those from animal husbandry. Their culture has been favoured by the relatively simple technique for their artificial reproduction (Viveen *et al*., 1986). *Clarias* sp. has high species diversity in Nigeria (Reed *et al*., 1967; Idodo-Umeh, 2003; Olaosebikan and

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Raji, 2004), but there are limited studies on the chromosomal characterization. Ozouf-costaz *et al.* (1990) were the first to report the karyological analysis of three strains of *C. gariepinus* used in aquaculture, and they established a chromosome formula of 2n = 56. Teugels *et al*. (1992) carried out the karyological analysis of *C. gariepinus*, *Heterobranchus longifilis* and the artificial hybrid of the two species. They reported a chromosome formula of 2n = 56 for *C. gariepinus*, 2n = 52 for *H. longifilis*, and 2n = 54 for the hybrid. Ergene *et al*. (1999) reported a chromosome diploid number of 2n = 56 for *C. lazera* from the Goksu Delta. Eyo (2005) studying the cytogenetic variation in four species of *Clarias* species in Anambra River, Nigeria reported a diploid chromosome value of 2n = 48 for *C. ebriensis* and *C. albopunctatus* and 2n = 56 for *C. anguillaris* and *C. gariepinus*. The increasing importance of chromosomal studies on fish and lack of data on karyotype of several species of *Clarias* from Nigerian water bodies led to the present investigation. In this study, we characterized the chromosomes of six species of *Clarias* obtained from selected water bodies in South West, Nigeria.

MATERIALS AND METHODS

Sampling site and sample collection: Fish specimens used for this study were obtained from Oyo State Fish Farm, Agodi, Ibadan; Asejire Lake, Osun State and Lekki lagoon, Lagos State, Nigeria. Asejire Lake, a man-made lake with coordinates of 04º05′ E and 07º21′ N was constructed in 1970 on River Oshun. It has an impounded area of 2342 ha with gross storage capacity of 7403 million litres and located about 30 km East of Ibadan, Southwest Nigeria at an altitude of 137 metres (Egborge, 1979; Ekpo,1993; Ayoade *et al*., 2006). The impoundment was created primarily for the provision of public water supply with fisheries development as a major ancillary benefit (Ayodele and Adeniyi, 2006). Epe lagoon, Lekki is located about 48 km east of Lagos lagoon between longitude 4º00′ and 4º15′ E and latitude 6º22′ and 6º37′ N. The water is completely fresh with reported highest salinity of 0.30‰ because of limited and restricted tidal impacts (Ikusemiju, 1976; 1983; Fagade, 1978). The Oyo State Fish Farm is a Government owned commercial fish farm located in Agodi, Ibadan, Oyo State, Nigeria. The geographical area of sample collection is tropical, characterized by two annual seasons of wet (April-September) and dry (October to March) seasons.

Live samples of different *Clarias* species were bought from the fish farm, and also from landing centres of local fishermen. They were transported to the Hydrobiology and Fisheries Laboratory of the Department of Zoology, University of Ibadan, for identification using Reed *et al*. (1967); Idodo-Umeh (2003) and Olaosebikan and Raji (2004).

Chromosome preparation: Chromosomes were prepared from five specimens of each species following slight modification of a standard procedure (Nagpure *et al*., 2005). The specimens were kept in well aerated aquaria for 5 days in the laboratory to acclimatize. Each fish sample was injected with 0.05% colchicine intraperitoneally at 1 mL/100 g of body weight and kept for 2-3 h before sacrifice. They were decapitated, the kidney tissues removed, and homogenized separately in 7 mL 0.5% KCL for 30 min. Hypotonic action was stopped by overlaying each cell suspension with 1 mL carnoy fixative (3:1 ethanol:Glacial acetic acid, v/v), which was mixed gently and then centrifuged at 2000 rpm for 10 min.

The supernatant was discarded and permanent fixation was done by overlaying with 2.5 mL of carnoy fixative. The suspensions were kept at 4ºC for 30 min for thorough fixation. Slides were prepared by the addition of drops of each suspension at about 1.5 feet on pre cleaned slides. They were air-dried and stained with 5% Giemsa (v/v, stock Giemsa stain/distilled water) for 20 min. Metaphase spreads were observed and photographed at ×1000 magnification. Chromosomal morphology was determined according to Levan *et al*. (1964). The idiograms were prepared using Microsoft Excel 2007® software.

RESULTS

The six species of *Clarias* for the study are *Clarias pachynema*, *C. anguillaris*, *C. jaensis*, *C. gariepinus*, *C. camerunensis* and *C. macromystax*. The chromosome number for *C. pachynema*, 2n = 66, comprised of 30 metacentric 10 submetacentric, 16 subtelocentric and 10 telocentric chromosomes (Fig. 1). The size (total length) of chromosomes varied from 0. 37 to 1.6 cm based on the mean values of the measurement of best mitotic metaphases; the idiogram is also given in Fig. 1. The diploid value of 2n = 56 for *C. gariepinus* comprised of 25 metacentric, 14 submetacentric, 14 subtelocentric and 3 telocentric chromosomes (Fig. 2). The chromosome size ranged from 0.62 to 1.26 cm; the idiogram is also given in Fig. 2. For *C. camerunensis*, with chromosome number 2n = 56, included 22 metacentric, 20 submetacentric, 9 subtelocentric and 5 telocentric chromosomes (Fig. 3). The chromosome size ranged from 0.37 to 1.22 cm and the idiogram is given in Fig. 3. Sixty one percent of the metaphase spreads from *C. anguillaris* showed a chromosome diploid value of 2n = 56 while 39% showed a diploid value of 2n = 48. The spread with 2n = 56 comprised of 3 metacentric, 12 submetacentric, 2 subtelocentric and 9 telocentric chromosomes, while the spread with 2n = 48 comprised of 27 metacentric, 10 submetacentric, 3 subtelocentric and 8 telocentric chromosomes (Fig. 4 and 5). The chromosome size varied

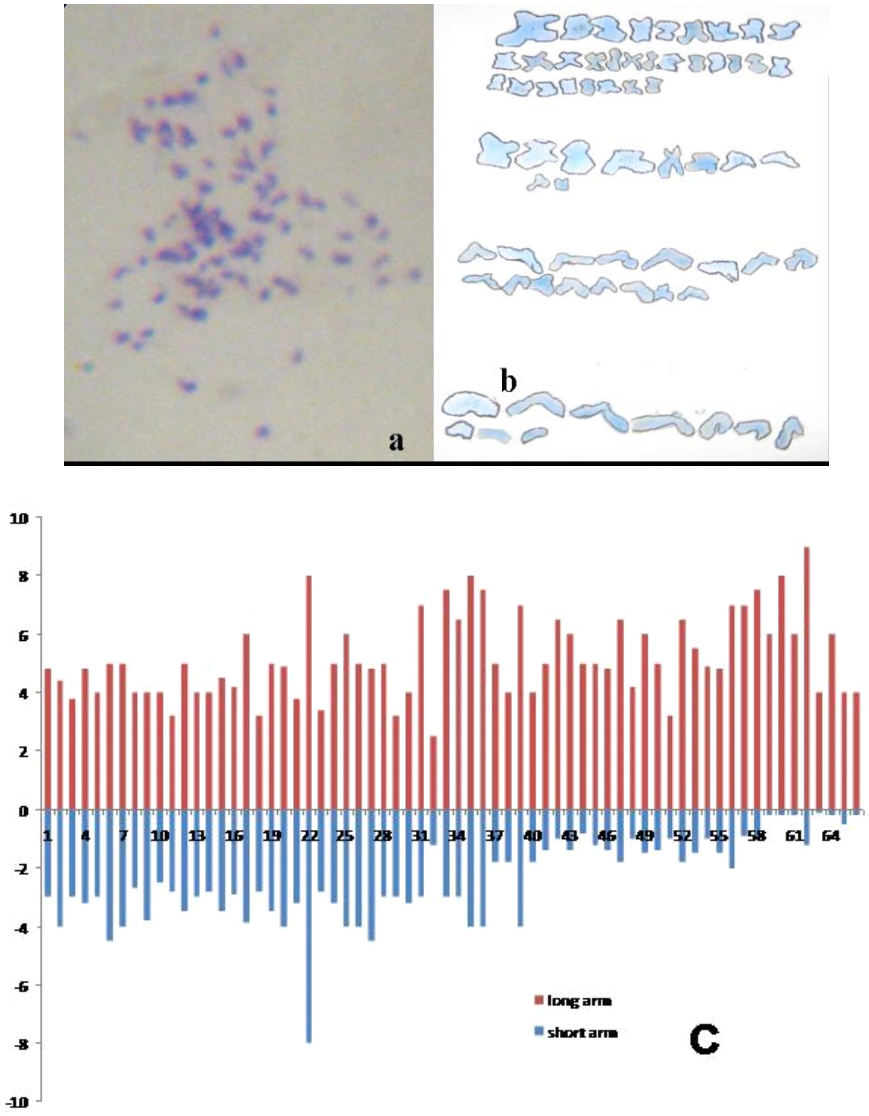
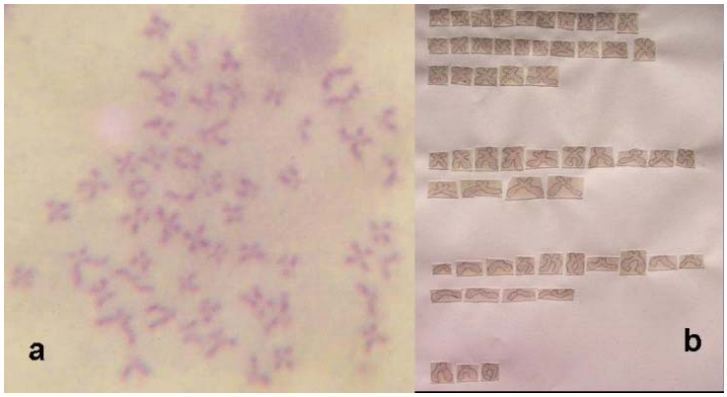


Fig. 1: Metaphase spread from kidney cells (a), giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias* pachynema



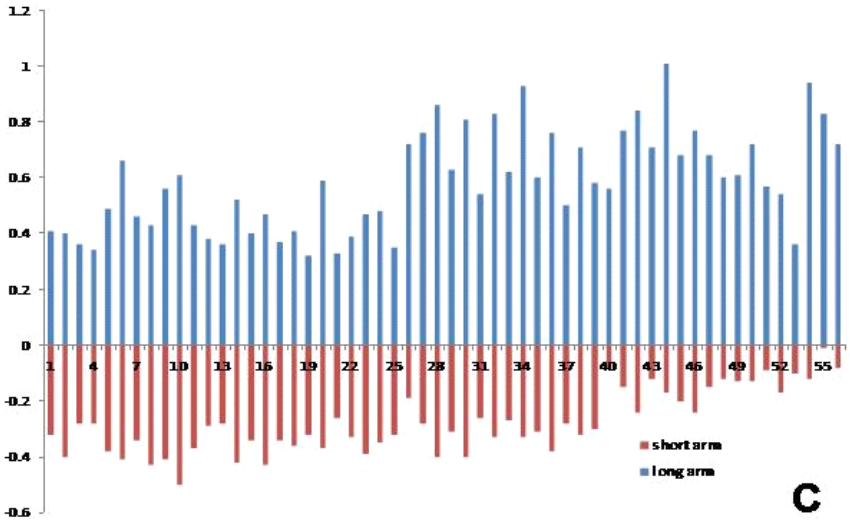


Fig. 2: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias* gariepinus

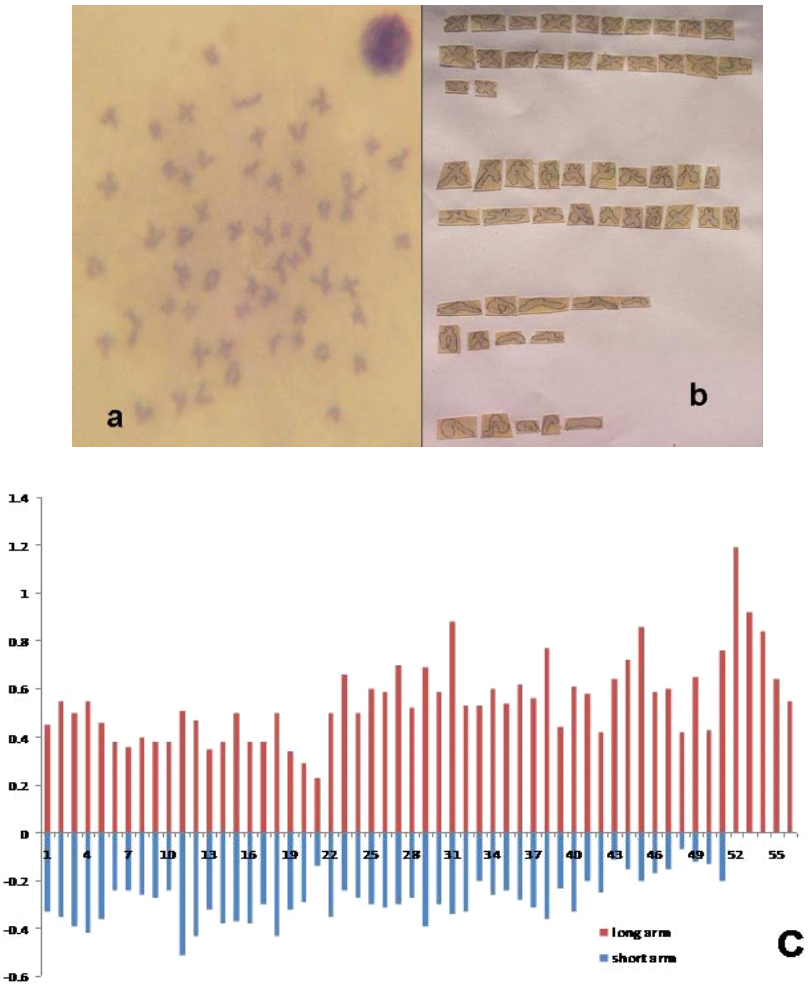


Fig. 3: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias camerunensis*

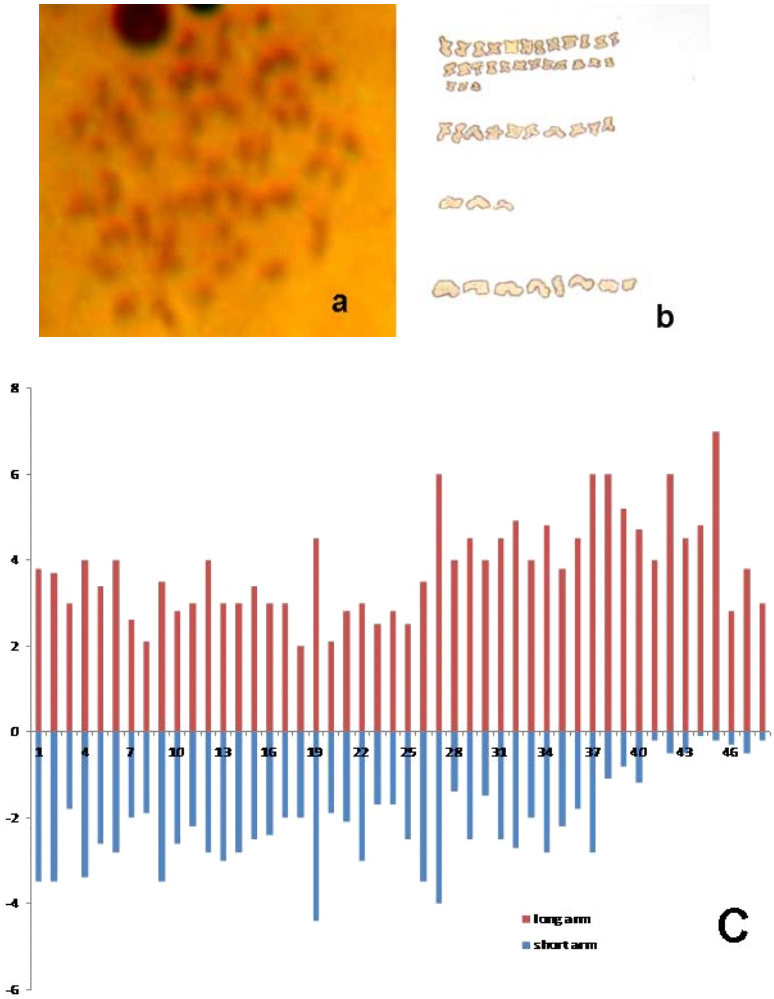
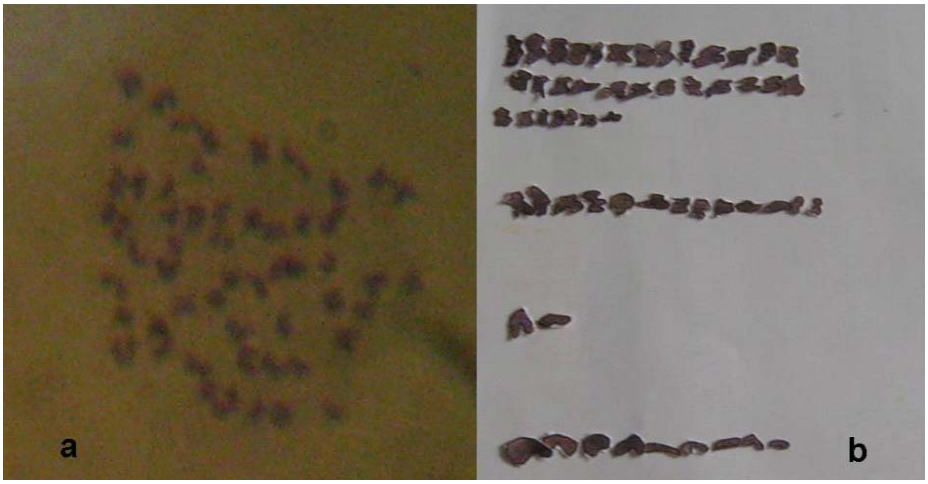


Fig. 4: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias anguillaris* (2n = 48)



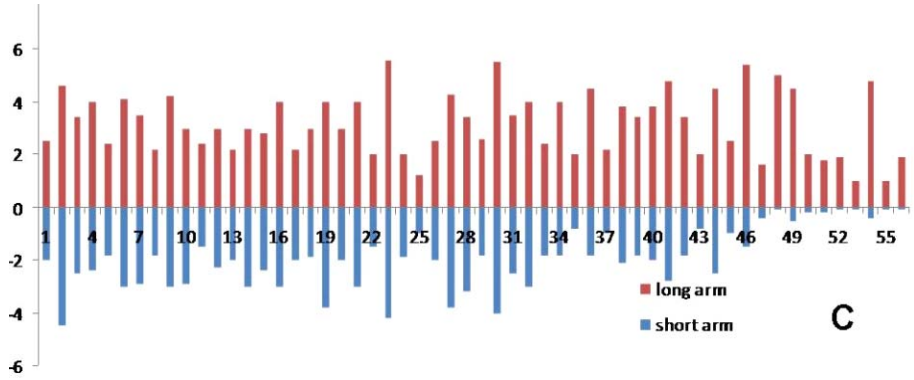
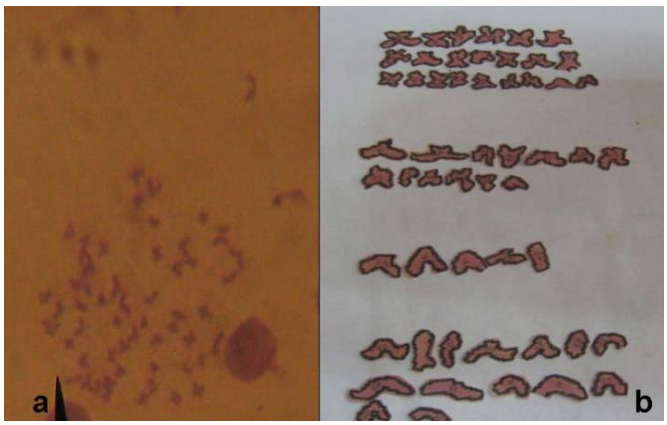


Fig. 5: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias anguillaris* (2n = 56)



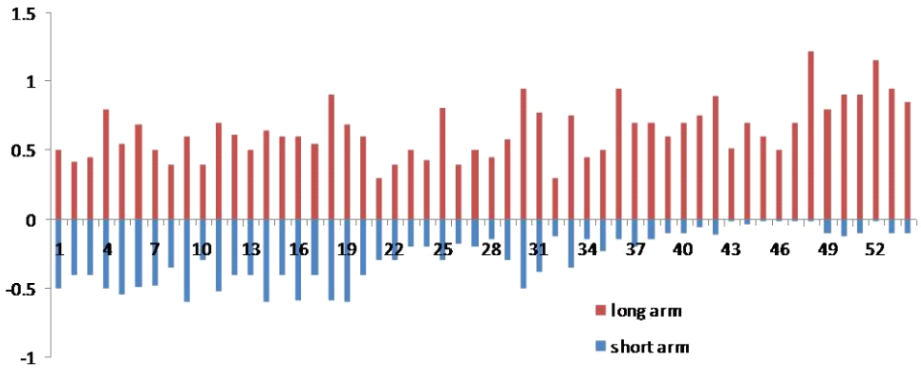


Fig. 6: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias jaensis*

from 0.11 to 0.95 cm (for 2n = 56) and 0.31 to 1.0 cm (for 2n = 48) the idiograms are shown in Fig. 4 and 5. The chromosome number for *C. jaensis* of 2n = 54 comprised of 22 metacentric, 13 submetacentric, 5 subtelocentric and 14 telocentric chromosomes (Fig. 6). The chromosome size ranged from 0.42 to 1.29 cm; and the idiogram is given in Fig. 6. The diploid value of 2n = 49 for *C. macromystax* had 27 metacentric 10 submetacentric, 11 subtelocentric and 1 telocentric chromosomes (Fig. 7). The chromosome size varied from 0.51 to 1.39 cm; the idiogram is given in Fig. 7.

DISCUSSION

This study investigated the chromosome structure and number of six species of *Clarias* from selected water bodies in South West Nigeria. The kidney had been reported severally to give the best quantity and quality of

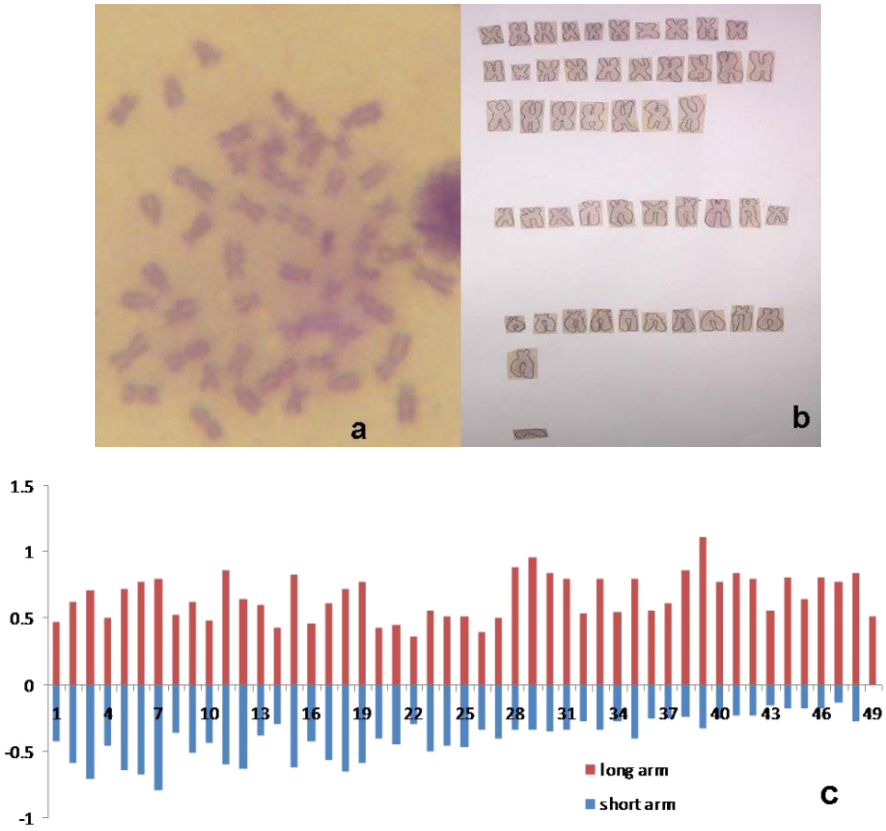


Fig. 7: Metaphase spread from kidney cells (a) giemsa stained karyotypes (b) and haploid idiogram (c) of *Clarias* macromystax

metaphase chromosome spread in fish. The kidney does not have the problem of dirt like the intestine and the stomach, and usually it is easier to tease than the gills. These conforms with the choice of workers in the use of the kidney when karyotyping fish (Ozouf-Costaz *et al*., 1990; Teugels *et al*., 1992; Margarido *et al*., 2007; Esmaeili *et al*., 2007; Sczepanski *et al*., 2007; Vasconcelos and Molina, 2009).

The *Clarias* species used for this study have large numbers and small sizes of chromosomes. The 2n = 56 for *C. gariepinus* agrees with the report of Ozouf-costaz *et al*. (1990), Teugels *et al*. (1992), Ergene *et al*. (1999), Eyo (2005) and Okonkwo and Obiakor (2010). However, the chromosome formula differs from those of these authors. This is not exceptional as the chromosome formula reported by these authors differs from one another. This indicates that different karyotypic forms exist from one population of *C. gariepinus* to another. The 2n = 56 obtained for *C. camerunensis* differed from 2n = 54 available on Fishbase (2004) from unspecified countries and localities. There was however, no information on the chromosome morphology of this species. *C. pachynema* is very abundant while *C. jaensis* is a very rare species in the freshwater bodies of South-Western Nigeria. *C. macromystax* is rarely used in aquaculture in Nigeria. Prior to this report, there was no information on the karyotype of *C. pachynema*, *C. jaensis* and *C. macromystax*.

All the *C. anguillaris* samples used in this study showed the mosaic diploid number of 2n = 48 and 56 and were obtained from Asejire Lake. Previous studies have also shown that more than one diploid number can exist in a fish sample, but the modal numbers have been chosen as the standard diploid number (Ozouf-costaz *et al*., 1990; Vitturi *et al*., 1991; Ergene *et al*., 1999; Eyo, 2005; Naran *et al*., 2006; Esmaeili *et al*., 2007). No case of mosaicism has earlier been reported in the genus *Clarias* although karyotype polymorphism was reported in *C. lazera* (Ergene *et al*., 1999). In this study, the modal diploid number of 2n = 56 is considered as the diploid number of *C. anguillaris*. This is the same as reported so far for *C. gariepinus*, *C. lazera*, *C. mossambicus* and *C. anguillaris* (Ozouf-costaz *et al*., 1990; Ergene *et al*., 1999; Eyo, 2005; Okonkwo and Obiakor, 2010), the first three been classified as synonymous by Teugels (1986).

That only *C. anguillaris* showed chimeric condition out of the species analyzed in this study could be due to species sensitivity. And there are many reasons to account

for this observation. The condition may arise as a result of numerical chromosomal aberration which may be due to exposure of the fish population to xenobiotics in their dwelling place (Ergene *et al*., 1998; Cavas and Ergene-Gozukara, 2005). Lameed and Obadara (2006) reported high level of Cadmium, Nickel, Iron and Lead in Asejire Lake. The interaction of these individually, additively, antagonistically and or synergistically could cause numerical chromosomal aberration in the fish population of Asejire Lake. Ale *et al*. (2005) reported numerical chromosomal alteration in *Oreochromis niloticus* exposed to lead nitrate. Hence, the chimeric condition found in *C. anguillaris* in this study may be as a result of numerical chromosome aberration which arose from the exposure of the population to heavy metals and or some other unidentified toxicants in Asejire Lake.

Brummett (2008) reported that interactions between farmed lines of *Clarias* and wild population may represent a significant threat to the genetic integrity of the wild population. Domestication or captive holding on fish farms has resulted in a certain amount of genetic change. Da Costa (1998) found a 20% difference between cultured and wild stocks of *C. anguillaris*, with the cultured stock performing significantly worse. The *H. longifilis* x *C gariepinus* hybrid, once thought to be sterile, has been recently shown to have the capacity to interbreed with wild *C. gariepinus*, creating what is effectively a transgenic clariid, with unpredictable consequences for the wild populations. The interaction of genetically modified fish with wild conspecifics has always led to genetic contamination of wild populations by domesticated escapees. The interaction of this genetically modified cultured *Clarias* with the wild population might have occurred in clariid species in Nigeria due to indiscriminate handling of fish by fish farmers. This could be responsible for the loss of genetic integrity leading to the chimeric condition observed in *C. anguillaris* herein. The mosaic condition could also be as a result of contaminated gene pool since such phenomenon was not observed in species that were not used in aquaculture. Williams *et al*. (2008) reported that due to the morphological and genetic similarity between *C. gariepinus* and *C. anguillaris*, many farmers in Nigeria are inadvertently culturing *C. anguillaris* and contaminating the gene pool of *C. anguillaris* and *C. gariepinus* through inter-specific hybridization. This suspicion was confirmed by a recent study on the genetic characteristics of the Dutch strain of *C. gariepinus* from five hatcheries in Nigeria that showed contamination of the exotic gene pool with local strains of *C. anguillaris* associated with unwholesome hatchery practices (Nwafili and Gao, 2007).

There is obviously a wide difference between the chromosome numbers of the different species of *Clarias* based on our observations and previous reports. Our results ranging from 2n = 48 to 2n = 66 contrast the generic chromosomal formula of 2n = 52±4 suggested by Eyo (2005) for the genus *Clarias*. From a phenetic approach *C. gariepinus*, *C. anguillaris*, *C. lazera*, *C. camerunensis* and *C. jaensis* are closely related, while *C. ebriensis*, *C. albopunctatus* and *C. maromystax* are also closely related but distantly related to the first five and *C. pachynema* appeared to be distantly related to all. However, the phenetic relationships inferred from karyotype do not necessarily reflect the true phylogeny. In a cladistic context, it is a mistake to assume that phenetic differences in karyotypes necessarily imply wide phyletic relationship or that cytogenetical data are more profound than morphological data. One could simply postulate that, all *Clarias* species share a common primitive karyotype and some species show specialized departure from this basic inheritance which can be as a result of centric fusions and pericentric inversions, which are considered to be the main mechanism of karyotypic evolution (Galetti *et al*., 2000). It is not unusual for members of the same species or genus to have different number of chromosomes. Catfishes (Siluriformes) have been known to show a great diversity in their karyotype (Volckaert and Agnèse, 1996). Variation in chromosome number of members of the same species (Lopez and Fenocchio, 1994; Vitturi *et al*., 1996; Völker *et al*. 2007; Margarido *et al*., 2007), genus (Scheel *et al*., 1972; Oliveira *et al*., 1990; 1992; Vitturi *et al*., 1996; Margarido *et al*., 2007) and family (Vasconcelos and Molina, 2009) has been commonly reported. Hence, differences in chromosome number may not imply distant phylogenetic relationship but could simply imply that while some fish families, genera and species have conserved chromosome numbers, others have divergent chromosome numbers due to evolutionary processes taking place over time.

This study has shown the chromosome structure and number of six species of *Clarias* from South West Nigeria; those for *C. maromystax*, *C. pachynema* and *C. jaensis* are reported for the first time. There is need for further genetic studies (both classical and molecular) on other species of *Clarias* and other fish species in the Nigerian waters. Considering the current trend of pollution of the aquatic environment, studies are required to evaluate the level of compromise of the genetic system of fishes in the Nigerian waters.

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