

KARYOTYPE OF THE AFRICAN WEAKLY ELECTRIC FISH, *GYMNARCHUS NILOTICUS* (OSTEOGLOSSIFORMES: GYMNARCHIDAE) FROM OLUWA RIVER, NIGERIA

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ABSTRACT

Bonytongue fishes of the order Osteoglossiformes provide an interesting clade for evolutionary study considering their wide distribution and their basal position in the general Teleostei phylogeny. Lack of adequate information on the cytogenetics of this group of fishes has been a limiting factor to the understanding of karyotype evolution of the clade. In this study, karyotype of *Gymnarchus niloticus*, the only species in the family Gymnarchidae collected from Oluwa River was investigated in order to increase the knowledge of karyotype pattern in the family. Metaphase chromosomes were obtained from the anterior portion of the kidney after injecting the fish intraperitoneally with 0.05% colchicine. Slide preparation followed the conventional Giemsa staining technique while digital images of the chromosomes were processed using Microsoft Excel and Photoshop. The result revealed a karyotype of $2n = 54$ (26m+14sm+14sta) which was significantly different in chromosome number and in chromosome macrostructure from the karyotype of $2n = 34$ (34m/sm) reported for *G. niloticus* from Lekki Lagoon. The wide difference in chromosome number and in chromosome structure observed in *G. niloticus* from these two locations is not a common occurrence among closely related fish species. These two karyotypes therefore raise a question of the possibility of another species in the family Gymnarchidae.

Keywords: Cytogenetics, Chromosomes, Bonytongues, Osteoglossomorpha

INTRODUCTION

The superorder Osteoglossomorpha (bonytongues) composed of the orders Hiodontiformes and Osteoglossiformes is an ancient group of bonyfishes (Inoue *et al.*, 2001; Austin *et al.*, 2015; Bian *et al.*, 2016) distributed in North America and in all the major continents of the southern hemisphere (Lavoue and Sullivan, 2004). The Hiodontiformes, restricted to the Northern America, consists of a single family, the family Hiodontidae which is a monogeneric clade composed of two species, *Hiodon tergius* and *Hiodon alosoides* (Li and Wilson, 1996) while the Osteoglossiformes on the other hand is made up of six families (Arapaimidae, Osteoglossidae, Pantodontidae, Notopteridae, Mormyridae and Gymnarchidae) distributed in the tropical region of all the major continents of the southern hemisphere (Bera, 2007). Their wide geographical distribution (Nelson *et al.*, 2016), basal position in the teleostean phylogeny (Inoue *et al.*, 2001; Austin *et al.*, 2015; Bian *et al.*, 2016), coupled with their restriction to freshwater make them an interesting clade for evolutionary and biogeographical studies

(Inoue *et al.*, 2009).

Although osteoglossiforms are a relatively primitive fish group, they display some advanced or derived characteristics including aerial respiration and parental care among some of the species (Helfman *et al.*, 2009). The super family mormyroidea, made up of the mormyridae and the Gymnarchidae families possess electroreceptor cells in their caudal muscles which enables them to generate and sense weak electric discharges (Carlson 2002; Kawasaki and Guo, 1996). The electric organ discharge system is used for social interaction and location of prey and is presumably coordinated by a large cerebellum (Helfman *et al.*, 2009).

Till date, information on the evolutionary relationship among the bonytongues have relied largely on morphological, osteological (Li and Wilson, 1996; Hilton, 2003) and molecular data (Kumasawa and Nishida, 2000; Lavoue and Sullivan, 2004; Mu *et al.*, 2014). A more robust approach should be one that incorporates

cytogenetic information in addition to these other sources of data. Studies on fish chromosomes are still scarce in comparison with other vertebrates. There is no method of obtaining metaphases that has been successful for all the different group of fishes. Consequently, different methods of obtaining metaphase chromosome have to be developed for different group of fishes (Ozouf-Costaz *et al.*, 2015a).

G. niloticus, the only species in the monotypic Osteoglossiformes family, Gymnarchidae is widely distributed in tropical African freshwaters and in River Nile (Nelson *et al.*, 2016). The fish is unique by its lack of anal and pelvic fins and by its possession of a modified caudal fin that resembles the tail of rat (Reed *et al.*, 1967; Paugy *et al.*, 2003). Its large size, reaching up to 19 kg in weight (Ayoola and Abotti, 2010), cultural significance, solid flesh and high palatability (Reed *et al.*, 1967, Ayoola and Abotti, 2010) makes it one of the most highly valued freshwater fishes in Nigeria. (Hatanaka *et al.*, in press) recorded a karyotype of

$2n = 34$ composed solely of bi-armed chromosomes from *G. niloticus* in Lekki Lagoon, Nigeria. Other than this, information on the cytogenetics of *G. niloticus* is scarce in literature. This study presents a report on the chromosome composition of *G. niloticus* from Oluwa River, Ondo State, Nigeria, with a view to enhancing the understanding of chromosome composition of the fish.

MATERIALS AND METHODS

Fish Collection and Husbandry

A total of twenty specimens of *G. niloticus* (Figure 1) were procured from local fishermen operating in Oluwa River, Okitipupa, Ondo State, Nigeria. The fish was usually caught between August and January, a period that coincided with their breeding season. The specimens were transported to the Zoology laboratory, Obafemi Awolowo University, Ile-Ife. Less than five specimens were usually collected per trip. Owing to the territorial habit of the fish, each specimen was maintained in separate aquarium.



Figure 1: *Gymnarchus niloticus* from Oluwa River, Nigeria

Technique for Obtaining Metaphase Chromosome Spread

Metaphase chromosomes were obtained from the cephalic portion of the fish kidney (Bertollo *et al.*, 2015) and from gill filaments, by injecting the fish intraperitoneally with 0.05% colchicine at the rate of 1 ml per 100 g of fish. The injected fish was then sacrificed 1 hour later, dissected, and fragments of the anterior portion of the kidney and gill filaments were removed and placed in centrifuge tubes containing 0.075 M potassium chloride solution for 20 minutes. The fragments were thereafter thoroughly squashed in a beaker using a 10 ml syringe without needle to obtain a

homogenous cell suspension, after which, the cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded and the pelleted cells re-suspended in 7 ml of freshly prepared fixative (made up of 3:1 methanol: glacial acetic acid) and centrifuged again at 1000 rpm for 10 minutes. Washing in the fixative by centrifugation was repeated twice. After the last centrifugation, the pelleted cells were re-suspended in 1 ml of the fixative and refrigerated at -20°C pending slide preparation.

Slide Preparation and Photo Microscopy

One or two drops of the fixed cells were placed on

different parts of the slides from a height of approximately 12 to 20 cm. The slides were stained for 30 minutes in a staining jar containing 6% Giemsa stain in phosphate buffer pH 6.8. The prepared slides were viewed under a binocular light microscope to locate metaphase spreads. Digital images of those adjudged good were captured using the microscope camera AmScope MT version 3.0.0.1. The modal diploid number obtained from the best ten spreads was recorded as the diploid chromosome number.

Karyotyping and Preparation of Idiogram

Centromeric position and nomenclature followed the criteria of Levan *et al.* (1964). Both karyotyping and idiogram construction were done electronically using Adobe Photoshop CS6. Chromosome lengths were measured using the measuring tools in Photoshop. For curved chromosome arms, the arms were divided into segments, each segment was measured separately and the sum of the lengths of the segments of a

chromosome arm was recorded as the chromosome arm length. To construct the idiograms, chromosome lengths were plotted in Microsoft Excel 2007 and later processed in Photoshop and arranged using the cut and selection tools in Photoshop.

RESULTS

Diploid Chromosome Number (2n)

Most of the fish died from the injuries they sustained at the time of capture and from the wounds they inflicted on each other when more than one fish were kept in an aquarium. Metaphase chromosomes were obtained from five specimens but the best metaphases were obtained from the gill filaments of one of the specimens. Most of the specimens used were juveniles, hence it was difficult to differentiate the sexes. The diploid chromosome frequency counts revealed a diploid number of $2n = 54$ composed mainly of bi-armed chromosomes (Figure 2) as the modal chromosome number.

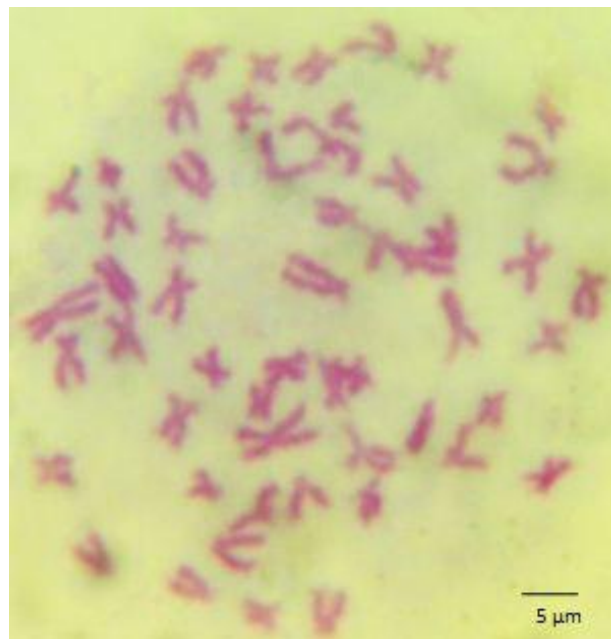


Figure 2: Metaphase Chromosomes of *Gymnarchus niloticus* from Oluwa River, Nigeria

Chromosome Morphology and Chromosome Nomenclature

Karyotype of $2n = 54$ (26m+14sm+14sta), FN = 94 (Figure 3) was recorded in the fish. Chromosome 1 was a very large metacentric. Chromosomes 2 to 4 were medium metacentrics, while chromosomes 5 to 13 were small

metacentrics. The submetacentrics were composed of five large (chromosomes 14 to 18), one medium (chromosome 19) and one small (chromosome 20) chromosomes. The uni-armed chromosomes were represented by seven subtolocentrics, of which chromosome 21 was a large, chromosomes 22 to 24 were medium and

chromosomes 25 to 27 were small. No morphologically differentiated sex chromosome was observed in the specimens. Idiogram of the karyogram is presented in Figure 4.

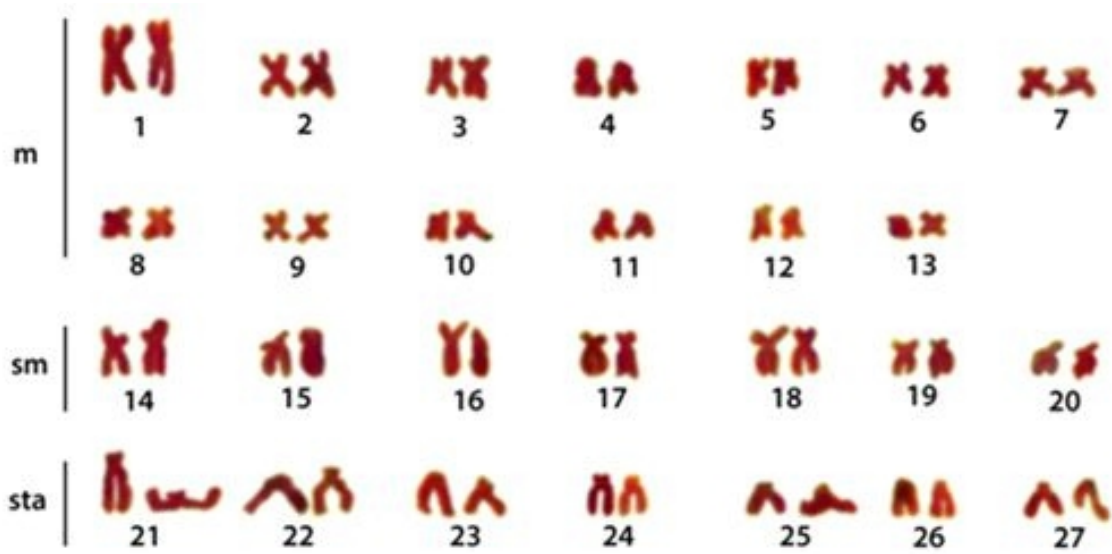


Figure 3: Karyogram of *Gymnarchus niloticus* from Oluwa River, Nigeria

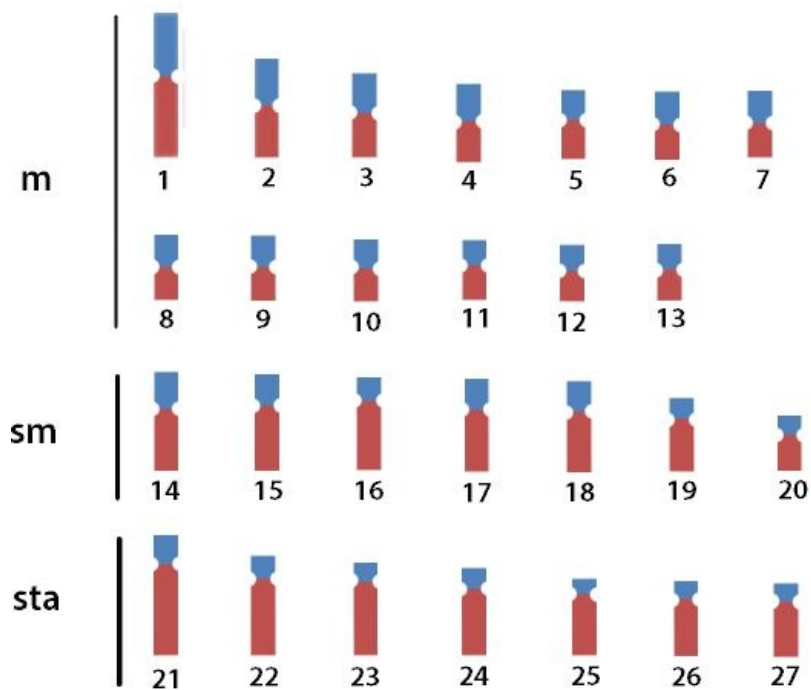


Figure 4: Idiogram of the karyotype of *Gymnarchus niloticus* from Oluwa River, Nigeria

DISCUSSION

Available cytogenetic information revealed that karyotype in the order Osteoglossiformes in general and in the suborder Notopteroidei in particular is highly conserved (Rab *et al.*, 2016, Canitz *et al.*, 2017; Barby *et al.*, (2018). Three families, Notopteridae, Mormyridae and Gymnarchidae constitute the suborder Notopteroidei. Among these families, Notopteridae consists of ten species distributed in four genera (*Chitala*, *Notopterus*, *Xenomystus* and *Papyrocranus*). Seven of the notopterids species drawn from the four genera have been cytogenetically studied. All the species except *Chitala lopsi* which bears a $2n = 38$ acrocentrics and *Papyrocranus afer*, which exhibits a $2n = 50$ ($2m+2sm+46sta$), are the only notopterids that differ from the characteristic Notopteridae karyotype of $2n = 42$ (42a) (Barby *et al.*, 2018).

The family Mormyridae, the most species rich Osteoglossiformes family, and the sister family of the Gymnarchidae, appears to be characterized by a karyotype that range between $2n = 48$ and $2n = 50$ composed of both uni-armed and bi-armed chromosomes (Ozouf-Costaz *et al.*, 2015b; Rab *et al.*, 2016; Canitz *et al.*, 2017). Of the nine mormyrids so far cytogenetically studied, only *pollimyrus nigricans* ($2n = 40$) possess chromosome number that differed from these two numbers (Krysanov and Golubtsov, 2014).

The karyotype of *G. niloticus* composed of uni and bi-armed chromosomes, $2n = 54$ ($26m+14sm+14sta$) recorded in this study was similar to the general karyotype pattern in the order Osteoglossiformes. Cytogenetic studies conducted so far on the osteoglossiforms other than *G. niloticus* species revealed a preponderance of acrocentrics and chromosome number range between $2n = 38$ in *Chitala lopsi* (Barby *et al.*, 2018) and $2n = 56$ in *Osteoglossum bicirrhosum* and in *Arapaima gigas* (Uyeno, 1973; Rosa *et al.*, 2009). The chromosome number of $2n = 34$ and the absence of acrocentrics recorded for *G. niloticus* collected in Lekki Lagoon (Hatanaka *et al.*, in press) was a significant departure from the karyotype pattern among the osteoglossiforms. The wide difference between the chromosome number and the chromosome characteristics of *G. niloticus* from Oluwa River and those reported for the same species from Lekki Lagoon is a rare occurrence

among fishes. In general, fishes are known to be cytogenetically conserved, with closely related species and even distantly related ones often sharing similar chromosome number and macrostructure. Karyotypic data of 615 Actinopterygian fishes revealed a diploid chromosome number range between $2n = 22$ and $2n = 250$ with about 54.7% of the species possessing a conservative diploid chromosome number that ranged from $2n = 48$ to $2n = 50$ (Mank and Avise, 2006). The karyotype of *G. niloticus* from Oluwa River obtained in this study was significantly different from those in Lekki Lagoon. Such wide intra-specific variation in fish karyotypes is an unusual occurrence. This result therefore suggests that *G. niloticus* in Oluwa River may be a different species from those in Lekki Lagoon.

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