Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.12, No.20, 2022

Karyotype of the Mormyrid Baby Whale Fish (Osteoglossiformes: Mormyridae): Brienomyrus Brachyistius in Oluwa River, Nigeria

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Abstract
The mormyrid fishes of the order Osteoglossiformes are abundant in the freshwaters of tropical Africa; the enormous species diversity of the group requires many systematics tools to comprehend this diversity better. Cytogenetics data can complement other systematics procedures to accomplish the above-stated objective, but information on many mormyrids' karyotypes is unavailable. In this study, *Brienomyrus brachyistius* from the Oluwa River was studied to enhance the understanding of the karyotype organization in the Mormyridae. Metaphase chromosomes were processed from the fish kidney following a conventional cytogenetic procedure; the fish was subjected to an intra-peritoneal colchicine injection at the rate of 1ml per 100g of fish mass. Hypotonic treatment was in 0.56% KCl solution, and cells fixation and washes were in 3: 1 methanol: acetic acid. The slides were stained with Giemsa solution, while the constitutive heterochromatin was revealed from the previous Giemsa stained slide using the C-banding sequential method. Capturing of metaphase images was done with an Olympus BX50 microscope. The karyotype of 2n = 50 (50sta) obtained in the species was the first mormyrid karyotype composed of 2n = 50 acrocentrics, supporting the proposed ancestral karyotype for the Mormyridae.
Keywords: Cytogenetics, chromosomes, Osteoglossiformes, karyotype, Mormyrids, elephantfish
DOI: 10.7176/JBAH/12-20-04
Publication date: October 31st 2022

Introduction

The African Osteoglossiformes of the family Mormyridae, commonly called freshwater elephantfish, are widely distributed in the inland and coastal freshwaters of tropical Africa (Roberts, 1975). The family constitutes a monophyletic group characterized by the possession of an electric organ discharge system (EOD)s generated from the muscle of the caudal peduncle (Boden *et al*., 1997; Hopkins *et al*., 2007; Kramer & Wink, 2013). Mormyrids (EOD)s are employed for orientation, communication among conspecifics, prey detection, and social interaction in their usually dark and turbid habitats (Lissmann, 1958; Arnegard & Carlson, 2005; Hopkins *et al*., 2007; Sullivan *et al*., 2016) The Mormyridae (EOD)s is both species and sex-specific thus providing a valuable tool for biodiversity discovery and systematics study (Hopkins *et al*., 2007; Lamanna *et al*., 2016). Furthermore, mormyrids possess a relatively large brain that coordinates their elaborate electrical system (Helfman *et al*., 2009).

Mormyridae is the most prominent Osteoglossiformes family, harbouring 22 genera and 255 species (**Fricke *et al*., 2022)**. The continual discovery of new species and the presence of many undescribed mormyrids suggests an underestimation of the present mormyrid fish diversity (Lavoué *et al*., 2003; Maake *et al*., 2014; Sullivan *et al*., 2016). Although Mormyridae is monophyletic, some genera within the family, such as *Marcusenius*, *Pollimyrus*, *Hippotamyrus* and *Brienomyrus*, have been discovered to be polyphyletic (Alves-Gomes & Hopkins, 1997; Sullivan *et al*., 2000; Lavoue & Sullivan, 2004). Therefore, a better comprehension of the diversity, taxonomy and phylogeny of the Mormyridae requires additional tools to complement the use of morphometric, osteological and analysis of mormyrids' electric organ discharge systems (Boden *et al*., 1997; Hopkins *et al*., 2007; Hilton, 2003; Kramer *et al*., 2013; Hilton. & Lavoué, 2018)

The advent of molecular cytogenetic methods has paved the way for the discovery of many cryptic species, species complexes, and hybrid characterization and provided data for taxonomic, evolutionary, biogeographical, and phylogenetic studies of many fish taxa (Nakayama *et al*., 2001; Milhomem *et al*., 2008; Poletto *et al*., 2010; Cioffi *et al*., 2018; Barby *et al*., 2018; Toma *et al*., 2019; da Costa *et al*., 2019; Borges *et al*., 2019; Goes *et al*., 2020; Kretschmer *et al*., 2021; Paula *et al*., 2022 Machado *et al*., 2022). However, the conventional cytogenetic approach is still valuable and can provide insight into the species diversity of some fish groups (Reviewed in Cioffi *et al*., 2019; Jegede *et al*., 2018). The conventional cytogenetic procedure has been the major approach to investigating the chromosome composition of the Mormyridae (Krysanov & Golubtsov, 2014; Simanovsky *et al*., 2020; Simanovsky *et al*., 2021a; Simanovsky *et al*., 2021b; Jegede, 2022). However, karyotype knowledge of the mormyrids is still sparse: till now, karyotype information is known for only 14 genera and 19 species of the 22 genera and over 255 mormyrids' species (Simanovsky *et al*., 2021a); the paucity of cytogenetic information on this fish group has been a limiting factor to its application to their evolution and phylogeny.

Species of the genus *Brienomyrus* also known as baby whales in the aquarium pet trade owing to their

resemblance to the sperm whale, consist of three species: *B. adustus*, *B. longianalis* and *B. brachyistius*: *B. longianalis* found in the coastal waters of Mali, Nigeria, Cameroon, Guinea, Niger and Sierra Leone, while *B. brachyistius* distribution stretches from the Gambia to the Democratic Republic of Congo, but *B. adustus* is endemic to the coastal waters of Cameroon (Bigorne, 1990; Froese & Pauly, 2022). Uyeno (1973) described the karyotype of *Marcusenieus brachistius* (now *Brienomyrus brachyistius*) as 2n = 48 (1m+4sm+43a), while Ozouf-Costaz (2015) recorded 2n = 50 (2m+6sm+42a) for an unidentified *Brienomyrus* species from Ebeigne, Woleu River, Gabon. *Brienomyrus* is represented in Nigeria by *B. longianalis* and *B. brachyistius*; this study provides the basic karyotype, including C-banding information on *Brienomyrus brachyistius* from a Nigerian coastal river; supporting the proposed ancestral karyotype for the Mormyridae and provided insight into the ancestral karyotype condition and karyotype diversity of the *Brienomyrus* genus.

Materials and methods

Sample Collection

Five samples of *Marcusenius brucii* (Figure 1) were procured at one of the landing sites of the local fishers hunting in the Oluwa River Okitipupa, Ondo State, Nigeria (4.79'01"E, 6.61' 45"N). The Oluwa River is a relatively short river located within the forest belt of southwestern Nigeria and discharges directly into the Nigerian coastal lagoon system towards the western border of the Niger Delta. After collection, the fish was transported in an aerated plastic container and acclimatized in a private facility for a day before the laboratory procedure. The experiment was done at the Department of Zoology, Obafemi Awolowo, University, Ile-Ife, but C-banding was at the Fish Cytogenetic Laboratory, Department of Genetics and Evolution, Federal University of Sao Carlos, Brazil.

Metaphase Chromosomes Preparation

After acclimatization, the fish was injected intraperitoneally with a yeast suspension to stimulate mitosis (Bertollo *et al*., 2015); another intraperitoneal injection followed this with 0.05% colchicine solution at the rate of 1% of the fish mass. After 24 hrs., the fish was euthanized and dissected to remove the kidney. The kidney was teased out in 5ml of 0.56% KCl solution and incubated for about 30 minutes at about 35^0 C. After removing the kidney tissue remnants, the suspension was homogenized and centrifuged to obtain cell precipitates. The precipitates were washed three times in 3:1 methanol: acetic acid fixative. After the last centrifugation, the precipitated cells were stored in 1 ml of the fixative pending slide preparation.

Slide Preparation C-banding and karyotyping.

Slide preparation followed Bertollo *et al*. (2015). One or two drops of the chromosomes preparation were placed on different parts of a pre-cleaned slide and dried on a slide warmer. Giemsa staining was in 6% Giemsa solution for 20 minutes; excess stains were washed under a running tap and air-dried. The slides were checked under a microscope to detect metaphases. Good ones were further examined at a magnification of 100 X under immersion oil, and the images of metaphase chromosomes were captured with an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan) with CoolSNAP. Constitutive heterochromatin was detected by the C-banding method of Sumner (1972) but using chromosome slides previously Giemsa stained following the sequential C-banding technique of Rábová *et al*. (2015). Karyotyping was according to the criteria of Levan *et al*. (1964); the chromosomes were arranged and paired in descending order of size.

Figure 1. *Brienomyrus brachyistius* from Oluwa River, Nigeria.

Results

Thirteen metaphases were obtained from three specimens of *B. brachyistius*; almost all had a diploid number of 2 n = 50 acrocentrics (st/a) (Figures 2 and 3) and were considered the chromosome number of the species. No heteromorphic differentiated chromosome was observed in any of the metaphase spreads. C-bands were located in the centromeric region of all the chromosomes; chromosome 6 showed a sizeable C-band signal; in addition, C-band signals were noticed in the telomeric region of chromosome 14. Figures 4 and five displays the karyotype of the un-banded and the C-banded chromosomes, respectively.

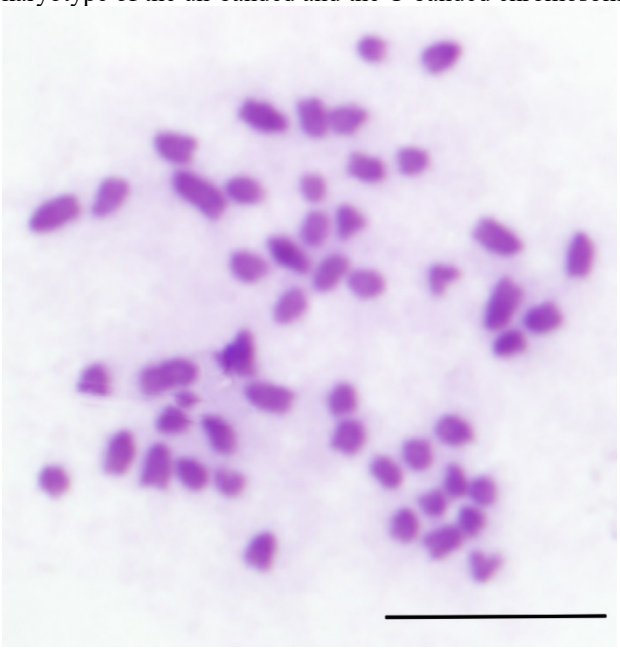

Figure 2. Giemsa stain Chromosomes of *Brienomyrus brachyistius* in Oluwa River, Nigeria. Bar = 10µ

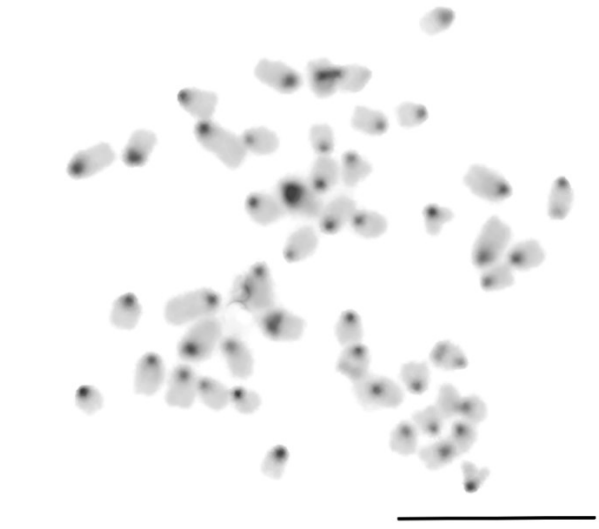

Figure 3. C-banded Chromosomes of *Brienomyrus brachyistius* in Oluwa River, Nigeria. Bar = 10µ

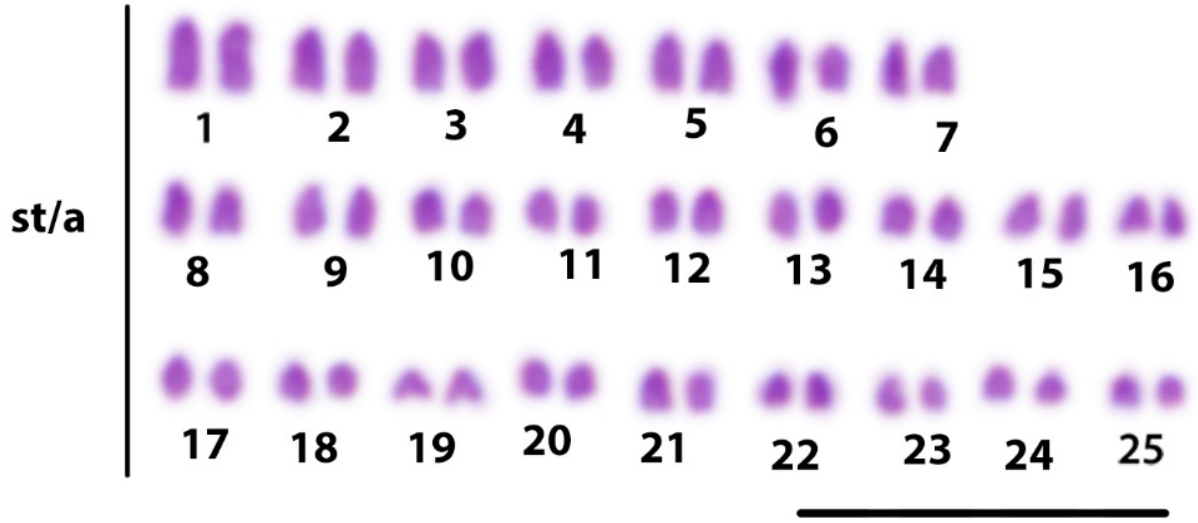

Figure 4. Karyogram of Giemsa Stained Chromosomes of *Brienomyrus brachyistius* in Oluwa River, Nigeria.Bar = 10µ

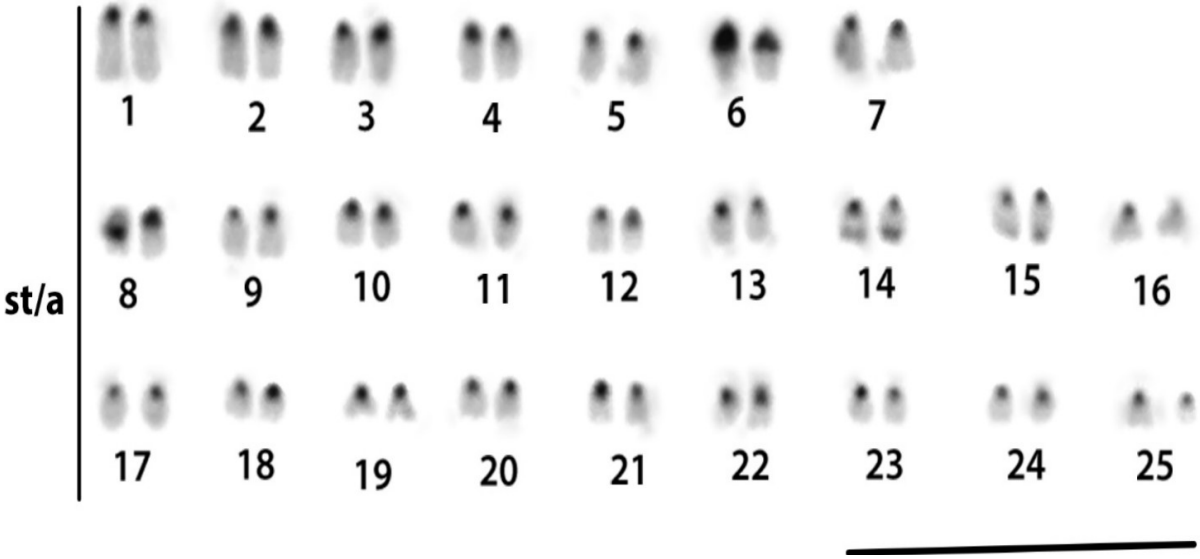

Figure 5. Karyogram of C-banded Chromosomes of *Brienomyrus brachyistius* in Oluwa River, Nigeria.Bar = 10µ

Discussion

Canitz *et al*. (2017) and Simanovsky *et al*. (2020) proposed 2n = 48-50 and 2n = 50 all acrocentrics as the ancestral karyotype for the Mormyridae; most mormyrids karyotyped so far displayed chromosome numbers within this range; but till now, none has exhibited a 2n = 50 (50a) (Simanovsky *et al*., 2021a). The diploid number of 2n = 50 composed of uni-armed elements; 2n = 50 (50sta), FN = 50, obtained in this study for *Brienomyrus brachyistius* in Oluwa River, Nigeria, makes it the first mormyrid to display the Simanovsky *et al.'s*., (2020) proposed Mormyridae ancestral karyotype. The lack of heteromorphic sex chromosomes appears to be a general pattern of karyotype organization in the order Osteoglossiformes (Barby *et al*., 2018; Cioffi *et al*., 2019)

The Mormyridae phylogeny shows that the family consists of two subfamilies: Petrocephalinae, represented by the genus *Petrocephalus*, and Mormyrinae; represented by all other mormyrids' genera (Alves-Gomes & Hopkins, 1997; Lavoué et al., 2000). Since the Mormyridae forms a monophyletic clade, its ancestral karyotype would reside in one of the oldest mormyrid species. Therefore, if 2n = 50 (50a) is the hypothesized basal chromosome composition of the Mormyridae, then it is expected to be found in some *Petrocephalus* species in addition to its presence in some other old mormyrids genera. Furthermore, the only *Petrocephalus* species (*Petrocephalus microphthalmus*; 2n = 50 [2sm+48a]) that has been karyotyped (Ozouf-Costaz *et al*., 2015) exhibits a chromosome complement close to the hypothesized basal karyotype, raising the hope of its presence in

some of the remaining 45 *Petrocephalus* species that are yet to be karyotyped.

Compared to Uyeno (1973), who reported 2n = 48 (1m+4sm+43sta), FN = 53, as the chromosome complement of *B. brachyistius,* the result of 2n = 50 (50am), FN = 50 obtained for the same species in the present study differ in chromosome number, distribution of uni and bi-armed chromosomes and homologous pairs. The reduction in diploid chromosome number and the presence of a metacentric and an acrocentric without homologous pairs in the Uyeno's result may be attributed to incomplete chromosome spread, even though it was thought to be due to chromosome heteromorphism (Uyeno, 1973). Since the origin of Uyeno's (1973) specimen was uncertain, the possibility of sampling a cryptic or a different species cannot be ruled out. Indeed at least two previous studies on the Osteoglossiformes karyotype have exhibited differences from the ones reported by Uyeno (1973). The reason for the differences is not clearly understood but has been attributed to the presence of cryptic species, population differences or species misidentification (Rab *et al*., 2016; Barby *et al*., 2018)

The unidentified *Brienomyrus* species sampled by Ozouf-Costaz *et al*. (2015) had a karyotype of 2n = 50 (2m+6sm+42a), with positive C-band patterns in the centromeric position of all the chromosomes and the intercalary and telomeric regions of many chromosomes. Its chromosomal composition suggests a more complex intra-chromosomal re-organization than in the studied species, which displayed C-bands in the chromosomes' centromeres and the telomere of a single pair. Therefore, from a cytogenetic viewpoint, the studied *Brienomyrus* species occupies a basal position within the genus *Brienomyrus*.

Arnegard & Hopkins (2003) analyzed the (EOD)s of seven groups of morphologically similar *Brienomyrus* from a recently discovered mormyrids flock in Gabon. In the study, distinct species-typical EODs were discovered, suggesting the occurrence of many undescribed *Brienomyrus* species. Similarly, three *Brienomyrus* karyomorphs have been described, excluding the yet-to-be-described karyotypes of *B. longianalis* and *B. adustus*; this scenario is suggestive of more than three species in the *Brienomyrus* genus.

Conclusion

The karyotype of *Brienomyrus brachyistius* in Oluwa River, Nigeria, recorded in this study (2n = 50 [50st/a]) probably represents the basal *Brienomyrus* karyotype and supports the hypothesized ancestral karyotype condition for the Mormyridae. Furthermore, the analysis of EODs of the genus in a previous study and the available karyotype data suggest the occurrence of more than three species in the *Brienomyrus.* Further cytogenetics studies incorporating molecular cytogenetics data may uncover cryptic species in the genus.

Acknowledgement

The author acknowledges Marcelo de Bello Cioffi, Department of Genetics and Evolution, Federal University of São Carlos (UFSCar), Rodovia Washington Luiz, São Carlos, SP, Brazil, for his assistance in C-banding.

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