CHROMOSOMAL ANALYSIS OF THE FRESHWATER FISH *Mormyrus rume* (MORMYRIDAE) FROM ASEJIRE RESERVOIR, OYO STATE, NIGERIA

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ABSTRACT

There is sparse cytogenetic information on 7 genera out of 22 genera and about 206 out of 225 species of the family: Mormyridae. To enhance the understanding of chromosome patterns in the family, cytogenetic data on these genera and species are essential. In this study, the first of such data on Mormyrus rume, including the standard karyotype, and C- and Ag-NOR banding~~,~~ were determined and presented. This was to investigate their chromosomal patterns and provide additional information for comparative analyses. The results showed that, M. rume exhibited 2n = 50 (24m+10sm+16a), NF = 84. C-bands were observed in the centromeric portion of all the chromosomes. Telomeric C-bands were noticed in a pair of sub-metacentric chromosomes while nucleolar organiser regions were located in the interstitial position of a pair of metacentric chromosomes. Pericentric inversion and Robertsonian events appeared to be the major evolutionary mechanisms responsible for chromosome evolution in the species.

Keywords: Elephant snout, karyotype, metaphases, osteoglossiformes,

INTRODUCTION

The Osteoglossiformes are an interesting fish order for evolutionary study owing to their early-divergent position in the Teleostean phylogeny, and their wide geographical distribution (Austin~~,~~ *et al*., 2015). Among Osteoglossiformes families, the Mormyridae also called elephant snout fish is the most species-rich, found exclusively in the inland freshwaters of tropical Africa. Cytogenetic data have enhanced the understanding of karyotype organisation, evolution, and biogeography of most osteoglossiformes families. However, cytogenetic data are sparse for most representatives of the family: Mormyridae. The family comprises of over 225 scientifically described species, classified into 22 genera (Fricke *et al*., 2020). This makes the family: Mormyridae the most speciose osteoglossiform group amongst Africa's freshwater fish families. Mormyrids have their highest diversity in the freshwaters of Central and West Africa but are absent in the Sahara, northernmost Maghreb, and southernmost Cape provinces (Roberts, 1975). The genus: *Mormyrus* composed of 22 species is the third most speciose Mormyrid~~ae~~ genus (Froese and Pauly, 2020). In Nigeria, the genus is represented by four species; *Mormyrus rume, Mormyrus hasselquistii, Mormyrus tapirus, and Mormyrus macrophthalmus* (Olaosebikan and Raji, 2004).

Despite their great diversity, Mormyrids have not been well studied cytogenetically (Rab *et al*., 2016). Cytogenetic data are important biodiversity tools and complement other genetic and morphological data for the study of comparative genomic organisation, species evolution, taxonomy, and biogeography (Mazzuchelli *et al*., 2011; Cioffi *et al*., 2012; Barby *et al*., 2018). A basic requirement of all cytogenetic analysis is karyotyping, which reveals the pairing and arrangement of an organism's homologous chromosomes. Modern chromosome banding and molecular cytogenetic techniques make the pairing of homologous chromosomes easier and more reliable and allow karyotypic comparison at a level of detail that is not feasible with uniformly stained chromosomes. Constitutive heterochromatin characterisation as revealed by C- banding is thus, very useful in fish karyotyping and has played important roles in the karyotype differentiation and inference of evolutionary relationships among closely related species, especially those with similar karyotype macrostructure, but with differences in the distribution of constitutive heterochromatin (Kavalco *et al*., 2004; Arzu and Ergene, 2009).

Cytogenetic data are available for only nineteen of the over 225 scientifically described mormyrid species (Simanovsky *et al*., 2020; 2021b), but data on constitutive heterochromatin (C-banding), and nucleolar organiser region (Ag-NOR) are documented for only six species (Ozouf-Costaz *et al*., 2015). Simanovsky *et al*. (2021a), provided information on the karyotype of three out of the 22 species in the genus: *Mormyrus*; *M. caschive, M. hasselquistiii, and M. kannume*. Information on the *Mormyrus rume* or any of the Nigerian representatives of the family: Mormyridae were yet to be found. In this study, cytogenetic data for the *M. rume* were determined and presented. The data included standard karyotype, C- and Ag-NOR banding~~,~~ which can be used to study chromosomal patterns in the Mormyrids and provide additional information for comparative analyses.

MATERIALS AND METHODS

Technique for obtaining Metaphase Chromosomes

Two specimens of *M. rume* were sampled from Asejire Reservoir (Osun River drainage) Nigeria. Metaphase chromosomes were obtained from mitotic cells of the cephalic kidney following *in-vivo* injection of 0.05% colchicine (Bertollo *et al*., 2015). The cephalic kidney contains the haematopoetic cells analogous to bone marrow in mammals. Twenty-four hours before colchicine administration, the fish was injected with yeast suspension following the method of Bertollo *et al*., (1978). Yeast suspension is mitogenic and served to induce mitosis in the fish. The fish was sacrificed one hour after colchicine injection and fragments of the cephalic kidney were homogenised in 0.56% Potassium chloride solution using a syringe without a needle. Potassium chloride solution is hypotonic which makes the cells turgid and the chromosomes to be well dispersed. Hypotonic treatment lasted for about 20minutes. The suspension was transferred into a centrifuge tube and centrifuged for ten minutes at 1000rpm. The supernatant was discarded and the pelleted cells were re-suspended in a 5ml (3:1, methanol: acetic acid) fixative and centrifuged. This was repeated twice. After the last centrifugation, the pelleted cells were re-suspended in 1ml of the fixative and stored in Eppendorf tubes at -20^{0}C pending slide preparation.

Slide Preparation and Microscopy.

Slide preparation followed the standard Giemsa staining technique (Bertollo *et al*., 2015). Two drops of the chromosome preparations were placed on different parts of clean slides and dried on a slide warmer. The slides were stained in 6% Giemsa solution for 20minutes. Excess stains were rinsed under running tap water and air-dried. The slides were examined under a binocular microscope to locate metaphase spreads. Good metaphases were examined at a magnification of 100 X under immersion oil. Images of metaphase chromosomes were captured with an epifluorescence microscope (Olympus BX50) and processed in Adobe Photoshop (CC 2019 Version 20). Chromosome nomenclature followed Levan *et al*. (1964).

Karyotyping

Chromosome arm measurements and pairing of homologous chromosomes were done using the tools provided in Adobe Photoshop (CC 2019 Version 20). Centromeric position and chromosome classification was according to Levan *et al*.~~,~~ (1968).

Chromosome Banding

Constitutive heterochromatin and nucleolar organiser region were detected by C- banding (Sumner, 1972) and by silver nitrate staining (Ag-NOR) (Howell and Black, 1980) respectively. Previously G- stained chromosome slides were subjected to the sequential C-banding technique by first de-staining the slides following the method of Rábová *et al*.~~,~~ (2015).

RESULTS

Thirty-one metaphases obtained from the two specimens of *M. rume* were analyzed. The most frequent chromosome number recorded for the species was 2n = 50 (Plate 1) with a preponderance of bi-armed chromosomes. Its karyotype was formed by 24m+10sm+16st/a, and a fundamental number (FN) = 84 (Figure~~.~~1). The largest chromosome was a sub-metacentric (chromosome 13), followed by a metacentric (chromosome 1). The majority of the bi-armed chromosomes were similar in size. For instance, each of chromosomes 3-12 was 0.04 percent of the total chromosome length. Details of chromosome arm measurements and classification are presented in Table 1.

C-bands were detected in the centromeric regions of all the chromosomes except in one of the pairs of a metacentric chromosome (chromosome 4), and also in one of the pairs of an acrocentric chromosome (chromosome 21) which did not show visible heterochromatic block. In some of the chromosomes, notably; chromosomes 3, 9, and a pair of chromosome 2, the C-band spread to a large portion of the chromosome arms. A faint C-band signal was observed in the telomeric portion of chromosome 16. The nucleolar organiser region was detected in a pair of metacentric chromosomes. No sex-differentiated chromosomes were noticed (Figure~~.~~ 1).

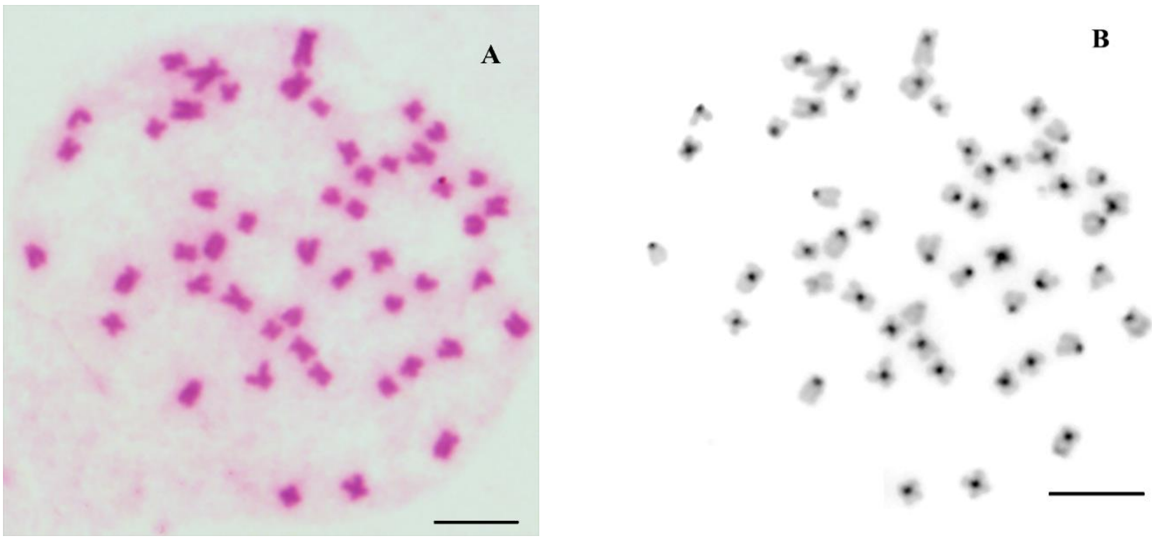

Plate 1: Metaphase chromosomes of *M. rume* from Asejire Reservoir, Nigeria.
A = G-stained chromosomes, B = C-banded chromosomes, bar = 5µ

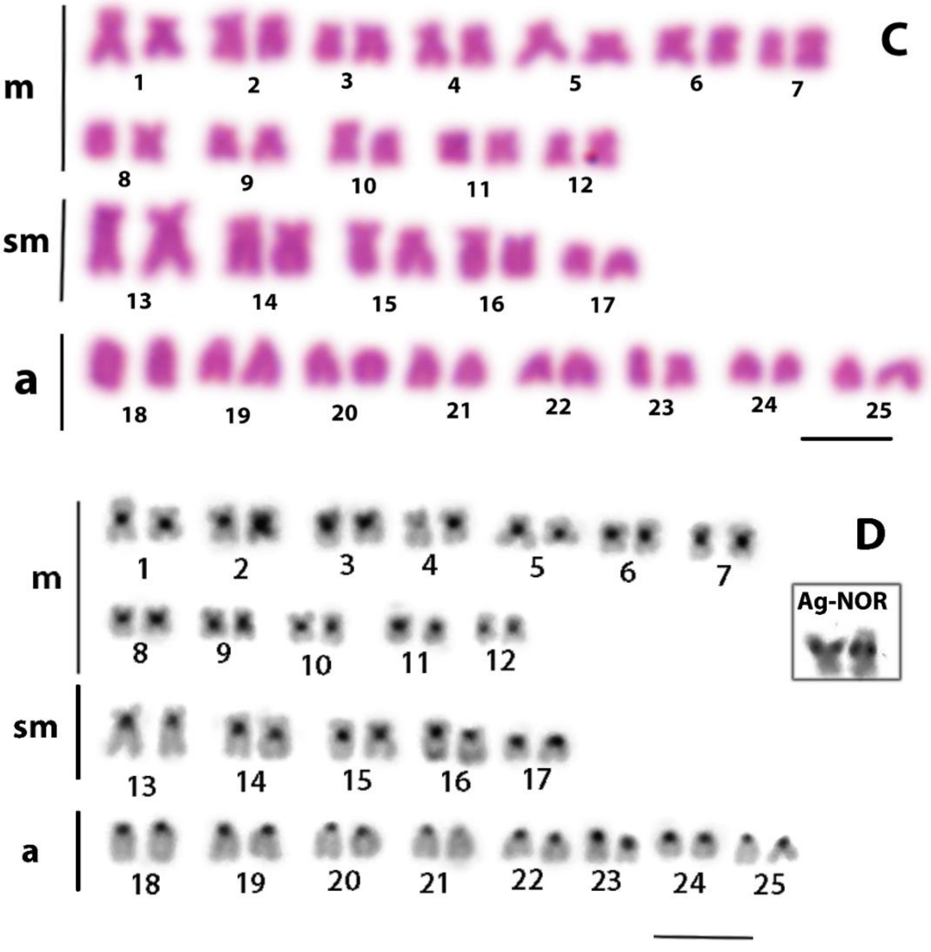

Figure 1: Karyogram of *M. rume* chromosomes from Asejire Reservoir, Nigeria.
C = G-stained, D = C-banded, inboxed are the nucleolar organising chromosomes, bar = 5µ

Table 1: Chromosome arm lengths, centromeric positions~~,~~ and chromosome nomenclature in *Mormyrus rume* from Asejire Reservoir

SN.	P(px)	q(px)	cl(px)	r	d	ci	tcp	Ct	Cn
1	19.25	22.28	41.53	1.16	0.72	46.35	0.05	M	M
2	18.13	18.22	36.35	1.00	0.02	49.88	0.05	M	M
3	15.91	17.01	32.92	1.07	0.33	48.33	0.04	M	M
4	16.03	16.39	32.42	1.02	0.11	49.44	0.04	M	M
5	13.86	18.28	32.14	1.32	1.38	43.12	0.04	M	M
6	14.78	16.47	31.25	1.11	0.54	47.30	0.04	M	M
7	14.62	15.95	30.57	1.09	0.44	47.82	0.04	M	M
8	14.15	15.42	29.57	1.09	0.43	47.85	0.04	M	M
9	13.60	15.41	29.01	1.13	0.62	46.88	0.04	M	M
10	12.71	15.30	28.01	1.20	0.92	45.38	0.04	M	M
11	13.86	13.99	27.85	1.01	0.05	49.77	0.04	M	M
12	13.46	14.03	27.49	1.04.	0.21	48.96	0.04	M	M
13	13.31	31.39	44.70	2.36.	4.04	29.78	0.06	sm	sm
14	12.72	25.46	38.18	2.00	3.34	33.32	0.05	sm	sm
15	11.05	23.54	34.59	2.13	3.61	31.95	0.04	sm	sm
16	12.16	22.82	34.98	1.88	3.05	34.76	0.04	sm	sm
17	6.39	20.75	27.14	3.25	5.29	23.54	0.03	sm	sm
18	2.93	34.54	37.47	11.79	8.44	7.82	0.05	t	A
19	2.35	31.12	33.47	13.24	8.00	7.02	0.04	t	A
20	0.00	33.00	33.00	∞	10.00	0.00	0.04	T	A
21	0.00	27.95	27.95	∞	10.00	0.00	0.04	T	A
22	0.00	23.60	23.60	∞	10.00	0.00	0.03	T	A
23	2.70	24.90	27.60	9.22	8.04	9.78	0.03	t	A
24	2.16	24.31	26.47	11.25	8.37	8.16	0.03	t	A
25	0.00	26.40	26.40	∞	10.00	0.00	0.03	T	A

Key: p = short arm length, q = long arm length, cl = p + q, $d = \left(\frac{q}{cl} - \frac{p}{cl}\right)10$, r = $^{q}/_{p}$, $ci = (p/cl)100$, tcp = total chromosome percent (cl/778.92)100, ct = centromeric type, cn = centromeric nomenclature, M = median point, m = median region/metacentric, sm = sub-median region / sub-metacentric, T = terminal point, t = sub-terminal region, a = acrocentric, px = pixels.

DISCUSSION

Karyotype pattern in Mormyridae

The dearth of cytogenetic data on the family: Mormyridae poses a challenge to a comprehensive understanding of the karyotype evolution. Karyotype information is presently available on nineteen mormyrid~~s~~ species~~,~~ distributed in fourteen genera. This leaves seven genera and about 206 species without cytogenetic information. In this study, the karyotype of *M. rume* gave the most frequent chromosome number of 2n = 50, 24 metacentrics, 10 sub-metacentrics and 16 acrocentrics; 2n = 50 (24m + 10sm + 16a), and a fundamental number (FN) of 84.

The osteoglossiformes are composed of karyotypes that involve both uni-armed and bi-armed chromosomes; with a preponderance of uni-armed elements. Most of the species in the Notopteridae exhibit conserved karyotypes of 2n = 42; all acrocentrics (Barby *et al*., 2018)~~,~~ except in *Papyrocranus afte*r and *Chitala lopsi*. *Papyrocranus afe*r differs from a typical notopterid karyotype by possessing a chromosome number of 2n = 50, and by carrying a pair of metacentrics; 2n = 50 (2m+48st/a). This karyotype structure is similar to the proposed ancestral karyotype of the Osteoglossiformes. On the other hand, *Chitala lopsi*; 2n = 38 (38st/a) suffered a reduction in chromosome number, which Barby *et al*. (2018), attributed to tandem fusion.

The families Arapaimidae (*Heterotis niloticus*) and Gymnarchidae (*Gymnarchus niloticus*) exhibit highly derived karyotypes by possessing chromosome numbers of 2n = 40 and 2n = 34, constituted entirely by bi-armed types (Hatanaka *et al*., 2018; Olivera *et al*., 2019). However, a strain of *Gymnarchus niloticus* from Oluwa River, Nigeria, did not exhibit a reduced karyotype as it had a karyotype composed of 2n = 54 (26m+14sm+14st/a), FN = 94. (Jegede *et al*., 2018)

Although, the mormyrids appear to have a conserved chromosome number of 2n = 48-50 (Canitz~~,~~ *et al*., 2017), they show a great divergence in their chromosome compositions. Their FN range from 42 in *P. nigricans* (Krysanov and Golubtsov, 2014) to FN = 84 in the genus: *Mormyrus*. However, *Pollimyrus isidori* and *Hyperopisus bebe* have highly reduced chromosome number of 2n = 40 representing the least number recorded so far in Mormyridae (Simanovsky *et al*., 2021b)

In congruence with the characteristic chromosome number (2n = 48 – 50) of this group (Ozouf-Costaz *et al*., 2015; Simanovsky *et al*., 2021a), the four *Mormyrus* species so far studied, portrayed chromosome complements of 2n = 50, constituted by both uni and bi-armed elements, but with a prevalence of bi-armed chromosomes (Table 2).

Table 2: Available karyotype of species in the genus Mormyrus

Species	2n	Karyotype	FN	Locality	Reference
M. caschive	50	20m + 14sm + 16a	84	White Nile Basin, Ethiopia	Simanovsky *et al*., 2021a
M. hasselquistii	50	20m + 14sm + 16a	84	White Nile Basin, Ethiopia	Simanovsky *et al*., 2021a
M. kannume	50	20m + 14sm + 16a	84	Omo-Turkana Basin, Ethiopia	Simanovsky *et al*., 2021a
Mormyrus rume	50	24m + 10sm + 16a	84	Nigeria, Asejire Reservoir	This study

The genus: *Mormyrus* has earlier been observed to be unique within the family: Mormyridae by possessing the same number of chromosomes and a high number of bi armed elements (Table 2). In addition, to exhibiting these features, the genus appears to maintain a kind of karyostasis in their karyotype macrostructure by possessing the same fundamental chromosome number that differs only in the distribution of bi-armed chromosomes. Furthermore, they exhibit the highest Fundamental Number (FN = 84).

C- and Ag-NOR bands

In addition to its localization in the centromere of almost all the chromosomes, and the presence of a less conspicuous telomeric band in chromosome 16, large C-bands which extended to chromosomes arms were found in 3 and 9, and also in one of the pairs of chromosome 2 (Figure~~.~~1). Similarly, Ozouf-Costaz *et al*. (2015) recorded C-bands in the cetromeric region of almost all the chromosomes of the six species studied and also in the intercalary, and or in the telomere of all the species. This implied a complex chromosomal re-organization in the course of the evolution of mormyrids karyotypes.

The Ag-NOR is situated in the interstitial segment of one of the large metacentric chromosomes. *M. rume* Ag-NOR bearing chromosomes and the site of the bands are not similar to any of the species recorded in Ozouf-Costaz *et al*. (2015). Indeed all the seven Mormyridae species (including the present study) for which Ag-NOR data are available displayed characteristic bands for each species. Since one species has so far been studied per genus, it ~~is~~ cannot be deduced if the bands were genera or species-specific.

CONCLUSION

The findings of this study have contributed to the understanding of chromosome organization in the family: Mormyridae. Chromosome number when used in combination with C-band and Ag-NOR band patterns may be of value in differentiating mormyrid~~s~~ species. These findings are important to the general understanding of chromosome characteristics of the genus: *Mormyrus* and may be important to the understanding of chromosome evolution in the family: Mormyridae.

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