

KARYOTYPE OF A NIGERIAN POPULATION OF *Hyperopisus bebe* (OSTEOGLOSSIFORMES: MORMYRIDAE) REVEALED ANOTHER EVOLUTIONARY LINEAGE IN THE MONOSPECIFIC GENUS: *HYPEROPISUS*

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ABSTRACT

This study provides data on the gross chromosome morphology, including centromeric and silver nitrate banding of a Nigerian population of *Hyperopisus bebe*, sampled from Asejire Reservoir, Osun River basin. The chromosomes of the fish samples were extracted using the Giemsa staining technique, and the centromeric and silver nitrate bandings were analyzed based on standard cytogenetic methods. The *H. bebe* population had a chromosome number of $2n = 40$ and a karyotype formula of $2n = 40 (24m+6sm+10st/a)$, FN = 70. C-bands were restricted to the centromeric regions, suggesting that Robertsonian events are the major mechanism in the karyotype evolution in the population. Currently, *H. bebe* represents the only species in the genus *Hyperopisus*. The karyomorph data from this study suggest that the fish sample belongs to another species. However, other systematic approaches are needed to establish its taxonomic status.

Keywords: Cytogenetics, Chromosome rearrangements, Mormyrids, Robertsonian events, karyomorphs.

INTRODUCTION

Osteoglossiformes, also known as bonytongues, is an ancient Teleostei order characterized by the presence of a toothed tongue and only one ovary (Near *et al.*, 2012; Bian *et al.*, 2016; Nelson, 2016). The members of this relatively species-poor order consist of six families (Osteoglossidae, Arapaimidae, Notopteridae, Pantodontidae, Gymnarchidae, and Mormyridae) with 36 genera and 262 species (Fricke *et al.*, 2022; Froese and Pauly, 2022) distributed in the tropical freshwaters of the major continents in the southern hemisphere. The representatives of the family Osteoglossidae have been identified on three continents. They include South America, South East Asia, and Australia, Arapaimidae in Africa and South America, and Notopteridae in Africa and South East Asia. The remaining three families: Pantodontidae, Gymnarchidae, and Mormyridae are restricted to Africa. The intercontinental distribution of these freshwater fishes, long evolutionary history, and unique adaptations make them good models for biogeographical and evolutionary studies (Barby *et al.*, 2018; Barby *et al.*, 2019; Cioffi *et al.*, 2019; de Oliveira *et al.*, 2019).

Within this relictual order is a highly successful family, the Mormyridae (Hopkin *et al.*, 2007), consisting of 22 genera and 227 species (Fricke *et al.*, 2022; Froese and Pauly, 2022), making it one of

the most speciose freshwater fish families in Africa. The Mormyridae and its sister family, Gymnarchidae, possess a remarkable ability to sense and generate weak electric signals to identify the presence and location of prey, mate, navigation, and social interaction (Lavoué *et al.*, 2012). The Mormyridae electric organ discharge is both species and sex-specific and has been used as a key for species identification and taxonomy of the group (Lavoué *et al.*, 2000; Lavoué and Sullivan, 2004; Hopkins *et al.*, 2007; Kramer *et al.*, 2013; Sullivan *et al.*, 2016).

One of the consequences of the rich species diversity of mormyrids is the taxonomical challenge, which had traditionally relied on morphological and meristic characteristics (Bigorne, 2003). However, the osteological studies, molecular phylogeny, and analysis of the electric organ discharge system have enhanced the understanding of their species diversity and phylogeny (Sullivan *et al.*, 2000; Hilton, 2003; Hilton and Lavoué, 2018).

Cytogenetics is essential in resolving taxonomic issues for distinct fish groups (Cioffi *et al.*, 2018). However, Mormyridae is the least studied, with only 20 (~8%) of the total 257 species karyotyped (Simanovsky *et al.*, 2021a). Previous data on constitutive heterochromatin (C-band) and

Nucleolar Organizer Regions (NORs) using 18S rDNA mapping provided information on six mormyrids species (*Gnathonenus petersii*, *Ivindomyrus opdenboschi*, *Marcusenius moorii*, *Brienomyrus sp.*, *Stomatorbinus walker*, and *Petrocephalus microphthalmus*) (Ozouf-Costaz *et al.*, 2015). Apart from this study, cytogenetic data on mormyrids has been investigated by determining diploid chromosome numbers and describing gross chromosome morphologies. Although there is a paucity of cytogenetic data compared to the species diversity, recent investigations revealed extensive variations in the Mormyridae karyotype, as evident in their fundamental numbers (FN = 42–84) (Simanovsky *et al.*, 2021a; 2021b).

The Bebe mormyrid (*Hyperopisus bebe*) is a medium-sized freshwater fish found in all African Sahelo-Sudanese river basins and the Nile River basin, and its karyotype structure was described recently (Simanovsky *et al.*, 2021a). In Nigeria, *H. bebe* is distributed, in most inland and coastal freshwaters, including the Niger, Benue, Ogun, Osun, and Chad basins (Bigorne, 2003; Olaosebikan and Raji, 2004), providing a source of cheap protein for the riverine dwellers. However, unlike most mormyrids' species-rich

genera, *Hyperopisus* and six other genera: *Boulengeromyrus*, *Brevimyrus*, *Cyphomyrus*, *Genyomyrus*, *Heteromormyrus*, and *Isichthys*, are monotypic (Fricke *et al.*, 2022). Simanovsky *et al.* (2021a) provided the first cytogenetic data on *H. bebe* by analyzing the chromosome complement of the Alvero River population in Ethiopia. The species presented with $2n = 40$, with 24 metacentrics (m), 2 submetacentrics (sm), and 14 acrocentrics (a). This study described the chromosome characteristics of a Nigerian bebe mormyrid population in Asejire Reservoir, including the distribution pattern of its constitutive heterochromatin (C-) and Nucleolar Organizer Region (NOR) bands.

MATERIALS AND METHODS

Sample Collection

A total of five samples of *Hyperopisus bebe* (Figure 1) were purchased from a fisherman in Asejire Reservoir (4°52'56"E, 7°51'91"N), Osun River basin. However, the chromosome preparation was obtained from three samples. The 267 km river flows southwards through the rainforest belt of southwestern Nigeria into the Lagos Lagoon system (Anifowose and Oyeboode, 2019). Upon collection, the fish was transported in a well-aerated aquarium and acclimatized for 24 h.



Figure 1: *Hyperopisus bebe* from Asejire Reservoir, Osun River basin, Nigeria.

Metaphase Chromosomes Preparation

The laboratory procedure was at the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria. The chromosomes were prepared from the anterior portion of the fish kidney as described in Bertolo *et al.* (1978; 2015). The fish was injected intraperitoneally with 0.5% colchicine solution for about 2 h and euthanized. The anterior kidney was removed and transferred to a hypotonic solution containing 0.56% KCl at

room temperature for 20 min. The kidney fragments were homogenized followed by subsequent washing steps in methanol: acetic acid (3:1) fixative and standard cytogenetic procedures. After the final centrifugation, the cells were re-suspended in 1 mL of the fixative solution.

Slide Preparation and Chromosome Banding

Two drops of the chromosome preparation were dispensed on a pre-cleaned glass slide and dried on

a slide warmer. Then, the slide was stained with 6% Giemsa solution, the excess stain washed off, and dried on a slide warmer. The chromosome banding was performed at the Fish Cytogenetic Laboratory, Department of Genetics and Evolution, Federal University of Sao Carlos, Brazil. The constitutive heterochromatin (C-banding) was performed as described previously (Sumner, 1972). The slide was immersed in 1N HCl for 3 min at room temperature, briefly rinsed in distilled water, and subsequently incubated in a freshly prepared (5%) barium hydroxide solution for 3 min. Then, the slide was incubated in 2X SSC (0.3 M sodium chloride, 0.03 M tri-sodium citrate) at 50 °C for 1 h, rinsed in distilled water, air-dried, and stained with 6% Giemsa solution for 30 min. Silver nitrate staining of the Nucleolar Organizer Region (Ag-NOR) was performed as described previously (Howell and Black, 1980; Rábová *et al.*, 2015). The slide was placed on a hotplate, covered with filter paper, and maintained at 45 °C for 5 min. With a Pasteur pipette, three drops of 2% gelatin solution were dispensed, followed by six drops of freshly prepared 50% Ag-NO₃ solution, and then covered with a coverslip till the mixture turned golden brown. The chromosomes were viewed with an Olympus BX50 microscope (Olympus Corporation, Ishikawa, Japan) with

CoolSNAP.

Karyotyping

The criteria described by Levan *et al.* (1968) were used to characterize and group the chromosomes into three categories: metacentric (m), submetacentric (sm), and acrocentrics (a). Homologous chromosomes were paired based on the size and position of the centromere of 15 metaphase spreads of three unsexed fish.

RESULTS

The most frequent diploid number was $2n = 40$ (Figures 2 and 3), with no sexually heteromorphic chromosomes observed. The chromosome complement consisted of 24 metacentrics, 6 submetacentrics, and 10 acrocentrics, with a chromosome formula of $2n = 40: (24m+6sm+10st/a)$, FN = 70 (Figure 4). The shorter arm of the largest metacentric chromosome (chromosome 1) harbours the nucleolar organizer regions in the interstitial portions of the chromosome pair (Figure 4). C-bands occurred at or around the centromere of all the chromosomes, and no interstitial or telomeric C-bands were observed (Figure 3). The details of chromosome measurements are described in Table 1.



Figure 2: Metaphase plate of silver nitrate banded chromosomes of *Hyperopisus bebe* from Asejire Reservoir, Nigeria. Bar = 5 μ ; arrows indicate nucleolar organiser region.

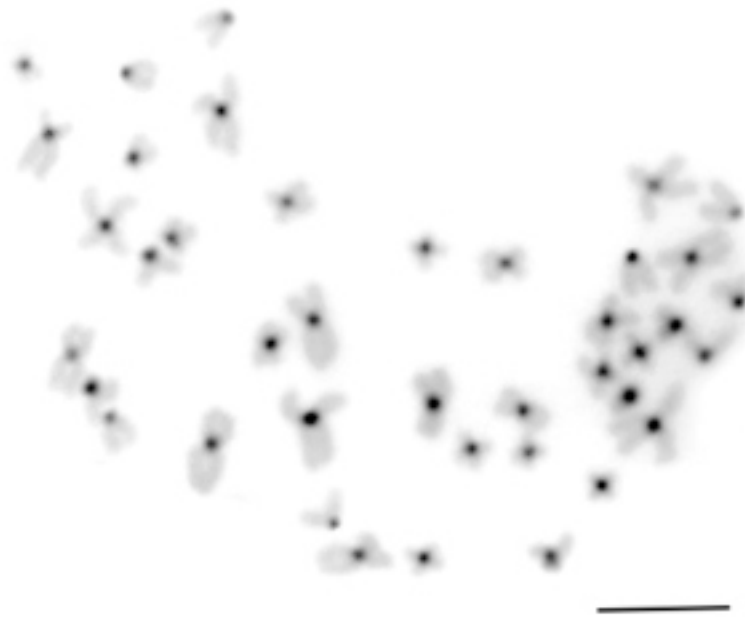


Figure 3: Metaphase plate of C-banded chromosomes of *Hyperopisus bebe* from Asejire Reservoir, Nigeria. Bar = 5 μ .

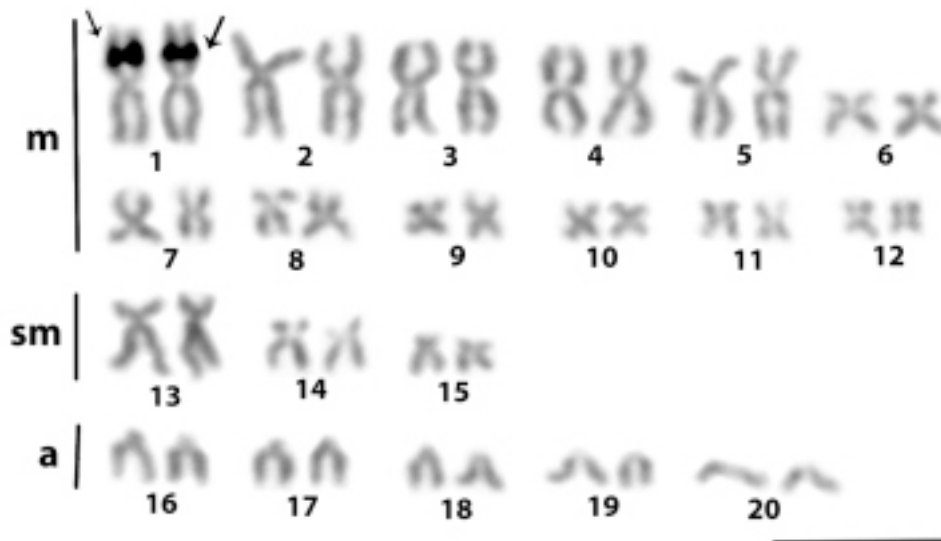


Figure 4: Karyogram of *Hyperopisus bebe* from Asejire Reservoir, Osun River basin, Nigeria. Arrowed are the Nucleolar Organizing chromosomes, Bar = 5 μ .

Table 1: Chromosome measurements of *Hyperopisus bebe* from Asejire Reservoir, Osun River basin, Nigeria, based on centromeric positions.

sn	p	q	cl	r	ci	d	tcp	cn
1	159.04	170.26	329.30	1.07	48.30	0.34	9.76	m
2	131.24	168.46	299.70	1.28	43.79	1.24	8.88	m
3	126.11	139.5	265.61	1.11	47.48	0.50	7.87	m
4	125.61	138.77	264.38	1.10	47.51	0.49	7.84	m
5	108.00	140.44	248.44	1.30	43.47	1.31	7.36	m
6	72.26	85.19	157.45	1.18	45.89	0.82	4.67	m
7	70.96	82.32	153.28	1.16	46.29	0.74	4.54	m
8	68.39	81.75	150.14	1.20	45.55	0.89	4.45	m
9	57.75	62.83	120.58	1.09	47.89	0.42	3.57	m
10	54.68	55.88	110.56	1.02	49.45	0.11	3.28	m
11	45.44	51.17	96.61	1.13	47.03	0.59	2.86	m
12	42.00	52.47	94.47	1.25	44.46	1.11	2.80	m
13	77.31	154.01	231.32	1.99	33.42	3.32	6.86	sm
14	57.64	104.89	162.53	1.82	35.46	2.91	4.82	sm
15	41.48	77.94	119.42	1.88	34.73	3.05	3.54	sm
16	22.72	120.8	143.52	5.31	15.83	6.83	4.25	st
17	19.07	111.36	130.43	5.84	14.62	7.08	3.87	st
18	11.21	92.61	103.82	8.26	10.80	7.84	3.08	t
19	0	96.52	96.52	∞	0	10	2.86	T
20	0	95.48	95.48	∞	0	10	2.83	T

*p = short arm length, q = long arm length,

cl = p + q, r = $\frac{p}{q}$, ci = $(\frac{p}{cl})100$, d =

$(\frac{q}{cl} - \frac{p}{cl}) 10$,

tcp = total chromosome percent $(\frac{cl}{778.92})100$,

cn = centromeric nomenclature, m = metacentric, sm = submetacentric, T = terminal point, st = subterminal region, t = terminal region, px = pixels.

DISCUSSION

The cytogenetic data of the five Osteoglossidae species as described previously (Cioffi *et al.*, 2019) are as follows: *Osteoglossum bicirrhosum* (2n = 56: 56sta), *Osteoglossum ferreirai* (2n = 54: 2m+52sta), *Scleropages formosus* (2n = 50: 8m/sm+42); *Scleropages jardinii* (2n 48: 20m/sm+28sta) and *Scleropages leichardti* (2n = 44: 24m/sm+20sta). However, the karyotype of *H. bebe* in the Asejire Reservoir herein investigated is 2n = 40 (24m+6sm+10a), which is different from the general karyotype trends in Osteoglossiformes. Furthermore, following the previous chromosomal studies in Osteoglossiformes (Ozouf-Costaz *et al.*, 2015; Ráb *et al.*, 2016; Canitz

et al., 2017; Cioffi *et al.*, 2019), no sexually differentiated chromosome was found in the karyotype.

Uyeno (1973) and Canitz *et al.* (2017) proposed 2n = 48, dominated by acrocentrics as the ancestral karyotype of Osteoglossiformes since many species exhibit karyotypes around this number. While many species within this order show varying degrees of similarity to the basal form, the family Notopteridae touts a high degree of conservation and conformity to the basal karyotype in their structure. The nine notopterids species are composed of 2n = 48 acrocentrics, except for *Papyrocranus afer* (2n = 50 (2m+48a)), and *Chitala lopsi* (2n = 38: (38a)). Subtle rearrangements, like paracentric inversions and the spreading of repetitive DNAs (Barby *et al.*, 2018; Barby *et al.*, 2019), could have promoted karyotype stasis among this group. Unlike the karyotype situation in the Notopteridae, the remaining Osteoglossiformes families, except the monospecific family Pantodontidae represented by *Pantodon buchholzi*, show substantial karyotype differentiation. The African representative of the family Arapaimidae; *Heterotis niloticus* has a

karyotype formula of $2n = 40$ (40 m/sm), FN = 80; but its South American counterpart displays $2n = 56$ (28m/sm+28), FN = 84. Mormyridae species may have witnessed extensive diversifications in karyotype organization, as reflected in their fundamental number (FN = 42–84) in consonance with the enormous species diversity in the group. However, while some species have maintained a few chromosome arrangements close to the hypothesized ancestral karyotype, *Pollimyrus nigricans*, *P. isidori*, and the herein investigated *H. bebe* have suffered reductions in their diploid numbers to $2n = 40$, attributed to fusion events (Simanovsky *et al.*, 2021a).

The hypothesized ancestral karyotype of either $2n = 48-50$ (Canitz *et al.*, 2017) or $2n = 50-52$ by acrocentrics (Simanovsky *et al.*, 2020) suggests the *H. bebe* karyotype could have resulted from centric fusions of several acrocentric chromosomes. This observation may also involve pericentric inversions within acrocentrics and some fission events. The occurrence of large metacentric chromosomes, and the restriction of C-bands to the pericentromeric region in the Bebe mormyrid, point to simple chromosomal rearrangements that involved Robertsonian events. Sember *et al.* (2020) observed a similar scenario in other freshwater fish groups, such as pencilfish and killifish (Krysanov and Demidova, 2018). Ozouf-Costaz *et al.* (2015) also observed interstitial and telomeric bands in a few chromosomes of all the five mormyrid species (*Gnathonemus petersii*, *Marcusenius moorii*, *Ivindomyrus opdenboschi*, *Stomatorhinus walker*, *Petrocephalus microphthalmus*, and *Brienomyrus sp*) analyzed, suggesting some more complex chromosomal rearrangements. When placed in a phylogenetic framework, the karyotypes of *Pollimyrus* spp. and *H. bebe* can be considered derived compared to other mormyrids investigated (Peterson *et al.*, 2022).

Before this study, Ag-NOR data were available for only seven mormyrids species (Ozouf-Costaz *et al.* 2015) corresponding to one species per genus, making it difficult to infer if the bands were genera or species-specific. In addition, the interstitial portion of the short arm of the largest

metacentric chromosome contains the Ag-NOR of the Bebe mormyrid karyotype, a position not similar to its location in any of the six species previously reported (Ozouf-Costaz *et al.*, 2015). However, a single pair with an interstitial Ag-NOR site observed in this study agrees with the pattern in other Osteoglossiformes (Barby *et al.*, 2018; Hatanaka *et al.*, 2018; de Oliveira *et al.*, 2019). Compared to its counterpart in the Alvero River, Ethiopia, reported as $2n = 40$: (24m+2sm+14st/a), FN = 66 (Simanovsky *et al.* (2021a), the Osun River population exhibits a $2n = 40$: (24m+6sm+10st/a), (FN = 70), which can be considered distinct karyomorphs for the genus. do Nascimento *et al.* (2018), de Souza *et al.* (2022), and Paula *et al.* (2022) proposed cryptic status species for species in several fish genera such as *Apareiodon*, *Erythrinus*, and *Hypostomus*. However, the poor number of studied populations (only two) across distinct river basins and even within the same river basin was insufficient to propose that those two $2n = 40$ populations that differ in karyotype organization were distinct species. At present, the direction of karyotype evolution in the genus cannot be ascertained; either from a $2n = 40$ (24+6sm+10st/a), FN = 70 (Nigerian population), to $2n = 40$ (24+2sm+14st/a), FN = 66 (Ethiopian population) or the reverse. To derive the karyotype of the Nigerian from the Ethiopian population would involve pericentric inversions within four acrocentrics, while the fusion of two bi-armed elements can explain the composition of the former. However, the high diversity of mormyrids in Central and West African waters (Roberts, 1975; Hopkins *et al.*, 2007; Kramer *et al.*, 2013) favours the Nigerian population karyotype $2n = 40$ (24+6sm+10st/a), FN = 70, as the older form.

CONCLUSION

The Osun and Alvero Rivers populations of *Hyperopisus bebe* are different in their gross chromosome morphologies, suggesting that they belong to different evolutionary lineages. However, whether the karyomorphs represent different species cannot be inferred based on karyotype differentiation alone. Therefore, other systematic and taxonomic tools are needed to establish its taxonomic status.

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