KARYOMORPHOLOGY OF THE MORMYRID FISH *Brevimyrus niger:* THE SOLE REPRESENTATIVE OF THE GENUS *Brevimyrus* FROM OLUWA RIVER, NIGERIA.

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ABSTRACT
The present study analysed the chromosome composition of the Brevimyrus niger population in the Oluwa River in Nigeria. It provides karyotypic data, including C- and $AgNO_3$ banding, to describe its karyotype and highlight the differences between it and a previously studied Ethiopian population. Metaphase chromosomes were processed from the kidney following a peritoneal 0.05% colchicine injection at 1 ml per 100g of fish mass. The chromosome metaphase slide was stained with 6% Giemsa solution for 20 minutes; C-banding and location of the nucleolar organising region followed standard cytogenetic procedure. Karyotyping was done by classifying the metaphase chromosomes as metacentric, submetacentric, and acrocentric. Chromosome homologues were subsequently arranged in descending order of size. The diploid chromosome number, 2n = 48, included heteromorphic chromosomes comprised of a metacentric and an acrocentric. C-bands were positive at the centromere of all the chromosomes and the telomere of an acrocentric. The evolution of the karyotype probably involved the centric fission of a metacentric chromosome and the subsequent telomere-telomere fusion of one of the fission products with an acrocentric while maintaining the chromosome number. While the Nigerian and the Ethiopian fish share the same diploid number of 2n = 48, the presence of heteromorphic chromosomes in the Nigerian fish differentiates it from its Ethiopian counterpart as a distinct population. However, the possibility of speciation within the Brevimyrus genus cannot be ruled out. The study contributes to understanding chromosome evolution in the family Mormyridae and demonstrates the significance of cytogenetics in discovering biodiversity in the Brevimyrus genus.

Keywords: Osteoglossiformes, Mormyridae, Karyomorph, heteromorphism, biodiversity.

INTRODUCTION

The family Mormyridae belongs to an old Teleostean fish superorder, the Osteoglossomorpha, also known as bonytongues; the common name originated from a tongue-like structure on the floor of the buccal cavity, which aids the fish in prey swallowing (Helfman *et al*., 2009). Fossil records revealed that the bonytongues once had a near-global distribution; however, extant members of the group are now confined to North America and the continents of Gondwana extraction: Africa, South America, Asia and Australia (Hilton and Lavoué, 2018). The near basal position of the Osteoglossimorpha in the general Teleostean phylogeny, its intercontinental geographical distribution, and its rich fossil record make them good models for the evolutionary and biogeographical study of freshwater fish distribution (Hilton and Lavoué, 2018; Barby *et al*., 2018). In addition, Osteoglossiformes are of substantial economic interest as food and aquarium fish; the Asian arowana *Scleropages formosus,* the African *Pantodon buchholzi*, and *Gnathonemus petersii* are attractive aquarium fish and play significant roles in the International Aquarium trade (Kuřiková *et al*., 2015; Bian *et al*., 2016).

Although the Mormyridae belongs to a relatively small fish order, the Osteoglossiformes, it comprises 22 genera and 230 species, portraying it as the most diverse Osteoglossiformes family and among the most speciose African freshwater fish families (Fricke *et al*., 2025; Froese and Pauly, 2025). The mormyrids are distributed in the Northernmost Cape Provinces to North Africa, including the Zambezi, the Gambia, Volta, Congo, Niger, Senegal, Queme, Mono, Ogoouen and the Nile, but have not been reported in the Sahara and northernmost Maghreb (Roberts, 1975; Bigorne, 2003; Adjibade *et al*., 2009). Throughout its range, mormyrids support artisanal fisheries, thus providing a good source of cheap, high-quality animal proteins and a means of livelihood to many riverine dwellers, thus contributing to the region's food security.

Mormyrids are also notable for their ability to transmit and detect low electric voltage signals used for prey electrolocation, communication with conspecifics, sex mates, and social interactions. Possessing an electric organ discharge system is a significant adaptation that enhances navigation at night and in turbid waters with poor visibility. The electric organ discharge (EOD) system is believed to be a major driver of population diversity and speciation in the group; its species and sex specificity have been employed in the species and sex differentiation of many cryptic species within the group (Arnegard and Calson, 2005; Hopkins *et al*., 2007). In addition, mormyrids EOD has become a model in neuroscience studies (Skeels *et al*., 2023)

It has long been discovered that genomic variation does not instantly translate to external morphological differentiation (Bickford *et al*., 2007); thus, biodiversity may exist in a taxon without identifiable morphological

differences. Therefore, cytogenetics promises additional tools for studying fish species' biodiversity, phylogeny, taxonomy, and sustainable utilisation (Kretschmer *et al*., 2021). Indeed, cytogenetics plays crucial roles in fisheries science by providing data on unique reproductive modes, sex determination mechanism and their evolution; identification of distinct populations by elucidating intra and interpopulation chromosome diversity as demonstrated in the South American Erythrinidae family, especially *Hoplias malabaricus* and *Erythrinus erythrinus* species (de Souza *et al*.,2022; Souza *et al*., 2024). In a cytogenetic analysis of three unmorphologically identifiable allopatric populations of *Hypostomus tietensis* in the Upper Parana River Basin, Paula *et al*. (2012) uncovered a cryptic species. The 'type species' sampled from the Pirai River with a chromosome formula of 2n = 72 (8m+8sm+56st/a); the two other populations belong to the same evolutionary lineage, constituting a cryptic species (*Hypostomus ff, tietensis*), possessing similar karyotype of 2n = 76 (8m+6sm+62st/a)

In a cytogenetic study of the family Arapaimidae, *Heterotis niloticus*, the African Arapaimidae representative, displayed a karyotype of 2n = 40 of entirely bi-armed chromosomes; 2n = 40 (40m/sm), while its South American counterpart, *Arapaima gigas*, possesses 2n = 56 chromosomes of equal number of bi and uni-armed elements, 2n = 56 (28m/sm+28a) (de Oliveira *et al*., 2019). The monospecific African Gymnarchidae, represented by *Gymnarchus niloticus*, presents an interesting chromosome organisation of two strains: a strain sampled from Lekki Lagoon displayed a karyotype consisting of 2n = 34, bi-armed; 2n = 34 (26m+8sm) (Hatanaka *et al*., 2018) and another from the Oluwa River which had a diploid chromosome number of 2n = 54 composed of bi and uni-armed elements; 2n = 54 (26m+14sm+14a) (Jegede *et al*., 2018). Thus, *Gymnarchus niloticus* from the two water bodies represents different evolutionary lineages and are potentially different species. Similarly, the mormyrid fish *Hyperopisus bebe* in Asejire Reservoir presented a different chromosome composition from the same fish in the White Nile Basin. In contrast, the Nigerian fish consists of a karyotype of 2n = 40 (24m + 6sm + 10a) and the Ethiopian fish karyotype is composed of 2n = 40 (24sm+2sm+14a) (Simanovsky *et al*., 2021a; Jegede, 2022a).

Cytogenetics complements other taxonomic and evolutionary tools to differentiate cryptic and closely related species. (Milhomem *et al*., 2008; Cioffi *et al*., 2018; Borges *et al*., 2019; Paula *et al*., 2022). In aquaculture, cytogenetics helps polyploid production, monitors chromosome compatibility in hybridisation programmes, and improves farmed fish stock by enhancing the selection process (Goes *et al*., 2020; Rossi *et al*., 2021; Ajithkumar *et al*., 2025)

Application of cytogenetics to fisheries management, taxonomy, conservation and aquaculture is still limited due to the paucity of cytogenetic data. Given its immense diversity, mormyrids cytogenetic data is sparse in the literature since only 14 genera out of 22 and 19 of the 230 mormyrid species have been karyotyped (Simanovsky *et al*., 2021a; Jegede, 2022a); this implies that about 90% of its species lack karyotype information. However, the little available cytogenetic data on mormyrids' karyotypes exposed their chromosome diversity, as revealed by the fundamental chromosome number range (FN 42-84), which aligns with its enormous species diversity.

The genetic diversity of the Mormyridae species must be clearly understood and described to enhance its sustainable exploitation. In this regard, morphological, osteological, molecular and EOD patterns have been employed to provide a multiprong approach to its diversity study, which has uncovered about 10 new species within the past two decades (Kramer *et al*., 2013., Maake *et al*., 2014., Sullivan *et al*., 2016., Fricke *et al*., 2025). A better comprehension of mormyrids' chromosome diversity requires sampling more species and populations.

Brevimyrus niger is a small mormyrid distributed mainly across West and Central Africa and the Nile; it represents the only species in the genus *Brevimyrus*; and has been recorded in several river systems, including the Niger, Ogun, Oluwa, Benue, and Cross Rivers in Nigeria, as well as in river basins across Benin, Togo, Ghana, Cameroon, and Gabon (Roberts, 1975; Adjibade *et al*., 2009; Olaosebikan and Raji, 2013), where it is usually hunted for food and as baits of large fish predators such as *Gymnarchus niloticus* and *Lates niloticus*. Simanovsky *et al*. (2020) presented the first karyotype of *B. niger* in Tida River, Ethiopia. The present study provides karyotypic data, including C- and $AgNO_3$ banding on a Nigerian population of *B. niger* from the Oluwa River in Western Nigeria, to elucidate its chromosome structure, highlight population similarities and differences between the karyotype of the Ethiopian and Nigerian populations.

MATERIALS AND METHODS

Sample collection

Ten live specimens of *Brevimyrus niger* (Fig. 1) were purchased from fishers hunting in the Oluwa River, Okitipupa, located in the forest belt of Ondo State, Nigeria, at approximately (4.79'01"E, 6.61' 45"N). The river is in the tropical forest region of southwestern Nigeria, running from Ayepe-Olode in Osun State and eventually discharging into the Atlantic Ocean at Ayetoro village through the Gulf of Guinea. Despite pollution emanating from anthropogenic activities such as sand mining, domestic wastes, agricultural runoffs, palm oil mill effluent and seasonal variation in water quality, the river supports thriving artisanal fisheries, thus contributing to the economy and nutritional status of the dwellers of the region (Ayedun, 2021; Olaniyan and Okeke, 2024).

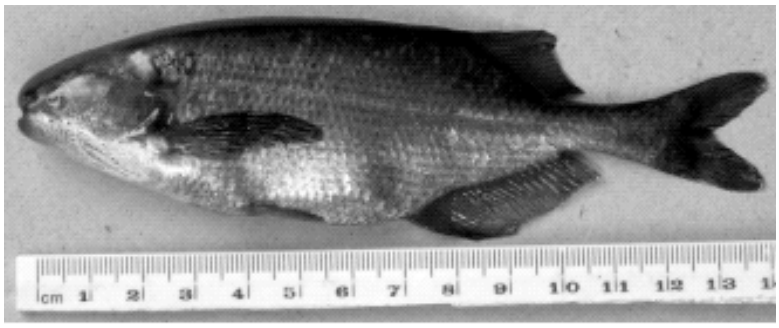

Fig. 1: *Brevimyrus niger* from the Oluwa River, Ondo State, Nigeria

Metaphase chromosomes preparation
Upon collection, the fish was transferred to an aerated plastic aquarium and kept at the river bank for 5 hrs. The water was replaced with fresh water from the river to reduce pollution during transportation. The fish was later conveyed in the aerated plastic aquarium to a private facility and acclimatised for a day before the laboratory procedure. Chromosome preparation was carried out at the Department of Zoology, Obafemi Awolowo University, Ile-Ife, while slide preparation to locate the constitutive heterochromatin and Silver nitrate bands was done at the Fish Cytogenetic Laboratory, Department of Genetics and Evolution, Federal University of Sao Carlos, Brazil.

Chromosome preparation was as described by Bertollo *et al*. (1978; 2015); the fish was injected intraperitoneally with a yeast suspension; the yeast treatments, which lasted for 24 hrs, served as a mitogen meant to stimulate mitosis. The mitotic cell was arrested at the metaphase stage by another intraperitoneal injection with 0.05% colchicine solution at 1 ml per 100g of the fish mass for 1hr. After the colchicine injection, the fish was euthanised and dissected to locate and remove the kidney; the fish cephalic kidney contains haematopoetic cells analogous to Mammalian bone marrow. The kidney was transferred to a beaker containing 5 ml of 0.56% KCl solution and incubated for 30 minutes at an ambient temperature of about 35 °C. The kidney was teased out, and significant kidney tissue remnants were removed before the suspension was homogenised using a hypodermal syringe without a needle. Then, the tissue was centrifuged to obtain cell precipitates. After centrifugation, the supernatant was decanted, and the precipitated cell was suspended in 7 ml of 3:1 methanol: acetic acid fixative and centrifuged at 1000 rpm for ten minutes; this procedure was repeated twice. After the last centrifugation, the precipitated cells were suspended in 1 ml of the fixative and kept in a freezer until slide preparation.

Slide preparation
Slide preparation was performed after Bertollo *et al*.'s method (2015). The slide was washed, rinsed with distilled water, and air-dried. One or two drops of the extracted chromosome preparation were dispensed on different portions of the slide and dried on a slide warmer. Slide staining was done using 0.05% Giemsa solution for 20 minutes; excess stains were washed under a slow-running tap and dried on a hot plate (Jegede, 2022a). The slide was stained in a staining jar containing 0.05% Giemsa solution for 30 minutes. The slide was then rinsed to remove excess stain and dried on a hot plate.

Chromosome C-banding
The constitutive heterochromatin (C-band) was detected using the method of Sumner (1972). A previously Giemsa-stained slide was destained and subjected to the sequential staining technique of Rábová *et al*. (2015). The destained slide was immersed in 1 N HCl for 3 min at ambient temperature, rinsed in distilled water and then inserted in a freshly prepared 5% barium hydroxide solution for 3 min. The slide was subsequently incubated in 2x SSC (0.3 M sodium chloride, 0.03 M tri-sodium citrate) at 50 °c for an hour, then rinsed in distilled water, air-dried and stained with 6% Giemsa solution for 30 min.

Nucleolar Organiser Region (Ag-NOR) detection
The Nucleolar Organiser Region (Ag-NOR) was evidenced by the Silver nitrate staining technique described by Howell and Black (1980) and 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 on the slide, followed by six drops of freshly prepared 50% Ag-NO_3 solution, and then covered with a coverslip till the mixture turned golden brown. The slide was then viewed under a microscope to locate the nucleolar organising chromosomes.

Microscopy and karyotyping
The slides were viewed under a microscope at 10X and 60X magnifications seeking metaphase spreads; good metaphases were further examined at 100X under immersion oil, and the metaphase chromosome images were snapped with a BX50 Olympus microscope with CoolSNAP. Chromosome nomenclature and characterisation were based on the centromeric positions as described by Levan *et al*. (1964); the chromosomes were categorised as metacentric (m), submetacentric (sm), and acrocentric (sta). Chromosome homologues were paired considering centromeric positions and chromosome size; the homologues were arranged in descending order of size.

RESULTS

Chromosome spreads were successful in five specimens, of which 31 metaphases were counted; the modal diploid number was 2n = 48 (Figure 2), and no sexually distinguishable chromosomes were found. A heteromorphic metacentric chromosome was recovered from the metaphases. Positive C-bands were observed at the centromeric region of all the chromosomes and the telomeric portion of an acrocentric (chromosome 4) chromosome, which was suspected to be the rearranged heteromorphic metacentric pair of chromosomes 3 (Figs. 2 and 4). Therefore, the karyotype formula of the fish was recorded as 2n=48 (5m+43a), FN = 53. The nucleolar organiser region was located in the centromeric and pericentromeric part of an acrocentric chromosome suspected to be chromosome 8 (Figs 3 and 4).

Fig. 2: Spread of unbanded Giemsa stained chromosomes of *Brevimyrus niger* from the Oluwa River, Nigeria.

Fig. 3: Chromosome spread of C-banded chromosomes of *Brevimyrus niger* from the Oluwa River, Nigeria.

Fig. 4: Chromosome spread of *Brevimyrus niger* from the Oluwa River, Nigeria. Arrows show nucleolar organising chromosomes evidenced by Silver Nitrate stain ($AgNO_3NOR$)

Fig. 5: Karyogram of C-banded *Brevimyrus niger* chromosomes from Oluwa River, Ondo State, Nigeria. Chromosomes 3 and 4 lack homologous pairs. Inboxed are the nucleolar organising chromosomes.

DISCUSSION

The karyotype of 2n = 48 (5m+43sta) herein recorded for *B. niger* is substantially close to the 2n = 48-50 and 2n= 50-52 dominated by acrocentrics, a condition that has been hypothesised as the ancestral karyotype form of Osteoglossiformes and Mormyridae respectively (Uyeno, 1973; Canitz *et al*., 2017; Simanovsky *et al*., 2020). The studied *B. niger* population also shares similar chromosome numbers with its Ethiopian White Nile population by possessing 2n = 48 chromosomes. They, however, differ in the distribution of Uni and bi-armed elements. The Ethiopian population consists of 6 bi-armed chromosomes composed of 4 metacentrics, two submetacentrics and 42 acrocentrics; 2n = 46 (4m+2sm+42a); at the same time, the Nigerian population has five bi-armed chromosomes made up of five metacentrics and 43 acrocentrics (2n = 48 (5m+43sta). The presence of heteromorphic chromosomes recorded in this study represents the second scenario among cytogenetically analysed mormyrids. Uyeno (1973)

analysed the karyotype of a mormyrid identified as *Marcusenius brachyistius* (a synonym of *Brienomyrus brachyistius*), which had similar chromosome configuration in terms of 2n, FN, and the presence of heteromorphic chromosomes (2n = 48 (1m+4sm+43sta), FN = 53); the nature of the heteromorphic chromosomes could not be decipher in the previous study.

When viewed in the context of available Mormyridae karyotype data (Table1), the species analysed by Uyeno shares a similarity in chromosome number with *Brevimyrus* (2n = 48) rather than *Brienomyrus* (2n = 50) (Ozouf-Costaz *et al*., 2015). In addition, except for the species analysed by Uyeno (1973), the seven Mormyridae genera on which more than one cytogenetic study has been reported suggest that each mormyrid genus has a characteristic chromosome number (Table 1). Karyotypic differences between some of the species studied by Uyeno (1973) and more recent studies have also been observed; these were attributed to population differences or misidentification (Barby *et al*., 2019; Jegede, 2022b). From the foregoing, the species studied by Uyeno (1973) and the one analysed in the present study seem the same and were probably sampled from a closely related population.

Table 1: Available karyotype data on Mormyridae species, banding status, locality of collection and reference. C- = banding, NOR* = nucleolar organising region = detected with $AgNO_3$; NOR, ** = detected with rDNA probe.

Species	2n	Karyotype	FN	banding	Locality of collection	Reference
Brienomyrus brachyistius	48	1m+4sm+43sta	53	none	Aquarium store, locality not reported	Uyeno, 1973
Unidentified *Brienomyrus* sp	50	2m+6sm+42a	58	C-, NOR	Woleu River, Gabon	Ozouf-Costaz *et al*., 2015
Brevimyrus niger	48	4m+2sm+42a	54	None	White Nile Basin, Ethiopia	Simanovsky *et al*., 2020
Brevimyrus niger	48	5m+43sta	53	C-, NOR	Oluwa River, Nigeria	Present study
Campylomormyrus rhynchophorus	48	26m+4sm+18a	78	None	Aquarium store, locality not reported	Canitz *et al*., 2016
Cyphomyrus petherici	50	18m+4sm+28a	72	None	White Nile Basin, Ethiopia	Simanovsky *et al*., 2020
Gnathonemus petersii	48	10m+6sm+32a	64	None	Aquarium store, locality not reported	Uyeno, 1973
Gnathonemus petersii	48	18m+2sm+28a	68	C-, NOR	Aquarium store, locality not reported	Ozouf-Costaz *et al*., 2015
G. petersii	48	18m+2sm+28a	68	None	Oluwa River, Nigeria	Jegede, 2022b
Hippopotamyrus pictus	50	24m + 4sm + 22a	78	None	White Nile Basin, Ethiopia	Simanovsky *et al*., 2020
Hyperopisus bebe	40	24m+2sm+14sta	66	None	Alvero River, Ethiopia	Simanovsky *et al*., 2021a
Hyperopisus bebe	40	24m+6sm+10sta	70	C-, NOR	Asejire Reservoir, Nigeria	Jegede, 2022a
Ivindomyrus opdenboschi	50	10m + 2sm + 38a	62	C-, NOR	Ntem River, Gabon	Ozouf-Costaz *et al*. 2015
Marcusenius moorii	50	4sm + 46a	54	C-, NOR	Ntem River, Gabon	Ozouf-Costaz *et al*. 2015
Marcusenius cyprinoides	50	22m + 4sm + 24a	76	C-, NOR	White Nile Basin, Ethiopia	Simanovsky *et al*., 2020
Mormyrops anguilloides	52	52a	52	None	White Nile Basin, Ethiopia	Simanovsky *et al*., 2020
Mormyrus caschive	50	20m+14sm+16a	84	None	White Nile Basin, Ethiopia	Simanovsky*et al*., 2021b
Mormyrus hasselquistii	50	20m+14sm+16a	84	None	Alvero River, Nile Basin, Ethiopia	Simanovsky *et al*., 2021b
Mormyrus kannume	50	20m+14sm+16a	84	None	Gibe River, Omo-Turkana basin, Ethiopia	Simanovsky *et al*., 2021b
Mormyrus rume	50	24m+10sm+16a	84	C-, NOR	Asejire Reservoir, Nigeria	Jegede, 2022c
Paramormyrops Unidentified sp	50	2m + 6sm + 42a	58	C-, NOR	Ebeigne, Woleu River, Gabon	Ozouf-Costaz *et al*. 2015
Pollimyrus nigricans	40	2m + 38a	42	None	White Nile and Omo-Turkana basins, Ethiopia	Krysanov and Golubtsov 2014
Pollimyrus isidori	40	26m + 6sm + 8a	74	None	White Nile Basin, Ethiopia	Simanovsky *et al*., 2021a
Stomatorhinus walkeri	50	2sm + 48a	52	C-, NOR	Ogooué Basin, Gabon	Ozouf-Costaz *et al*. 2015

Ozouf-Costaz *et al*. (2015) analysed C-bands in six mormyrids: *Ivindomyrus opdenboschi*, *Marcusenius moorii*, *Gnathonemus petersii*, *Brienomyrus* sp, *Stomatorhinus walkeri*, *Petrocephalus microphthalmus*, all the species displayed C-bands in the centromeric or pericentromeric and interstitial portions. In addition, *Petrocephalus microphthalmus* exhibited positive telomeric bands in a few acrocentric chromosomes while maintaining a 2n = 50, suggesting Robersonian events as one of the most significant mechanisms driving chromosome diversity in the species. Similarly, Jegede (2022c) observed positive C-bands in the centromere of all chromosomes and the telomeric part of an acrocentric pair of *Mormyrus rume* while the chromosome number remained 2n=50. In the *M. rume* case, the two acrocentrics that displayed telomeric C-bands were probably products of telomeric inversions (Jegede, 2022c). Ag-NOR band data are unavailable for more than one species per genus, making specific inferences from the data unfeasible (Table 1).

In the present study, one metacentric lacking a visible homologue and an acrocentric chromosome exhibiting a conspicuous telomeric C-band, which also lacked a homologue, was recorded. However, the chromosome number remained unchanged, ruling out Robertsonian fusion. These observations suggest that the heteromorphism involved two events: centric fission of a metacentric chromosome, followed by telomere-to-telomere fusion of one of the fission products with an acrocentric chromosome. The telomeric C-band, possibly indicating heterochromatin accumulation at the fusion point, supports this hypothesis. Such telomeric fusion events, although less common than centric fusions, have been reported in other vertebrate taxa and represent an alternative route of karyotype evolution (Garagna *et al*., 1995; Lukhtanov and Kuznetsova, 2010).

Karyomorpholocal differences between a Nigerian and an Ethiopian population of *Hyperomyrus bebe* have been previously reported (Simanovsky *et al*., 2021a; Jegede, 2022a) (Table 1). The presence of heteromorphic chromosomes in the Nigerian *B. niger* population differentiates it from its Ethiopian counterpart, pointing to distinct populations or speciation within the *Brevimyrus* genus. Karyotype differentiation between the Nigerian and the Ethiopian populations of *H. bebe* and *B. niger* may have resulted from the impact of vast geographical distance and differences in the ecology of the water bodies, causing reproductive isolation. However, further studies incorporating morphometric and molecular genetics data are essential to defining the taxonomic status of the two *Brevimyrus* populations.

CONCLUSION

The karyotype of *B. niger* analysed in the present study included heteromorphic chromosomes, which probably involved centric fission of a metacentric chromosome and the subsequent telomere-telomere fusion of one of the fission products, while the chromosome number remained unaltered. The karyomorph of the Nigerian *B. niger* population differentiates it from its Ethiopian counterpart as a distinct population. However, the possibility of speciation within the *Brevimyrus* genus cannot be ruled out. The study has contributed to understanding chromosome organisation in the family Mormyridae and demonstrates the significance of cytogenetics in discovering biodiversity. However, further studies are essential to clarify the taxonomic status of *B. niger* from the two populations.

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AUTHORS CONTRIBUTION

J.O.I. Conceptualised the project, collected samples, extracted chromosomes, performed microscopy and karyotyping, and wrote the manuscript.
M.A.A. prepared the slides, carried out microscopy, funding, and karyotyping

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