Karyotype analysis of a *Sarotherodon cf. galilaeus* fish, Wesafu and two conspecifics, *Sarotherodon melanotheron melanotheron* Ruppel and *Sarotherondon galilaeus galilaeus* (Linnaeus)

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Abstract: The chromosome sets of a *Sarotherodon cf. galialeus* commonly called Wesafu was investigated while the chromosome of *S. melanotheron melanotheron* and *S. galilaeus galilaeus* were re-evaluated. The mitotic chromosome spread of Wesafu, *S. g. galilaeus* and *S. m. melanotheron* were 2n=44, with a characteristics tilapia marker of a long pair of chromosome. However, the autosomal fundamental numbers of the fishes were 76, 80 and 72 respectively. The karyotype of Wesafu showed 1nm + 2nsm + 1sm + 9nsm + 3nst + 6t while *S. g. galilaeus* has 2m + 6nsm + 10nst + 4t and *S. m. melanotheron* has 3nsm + 11nsm + 6 nst + 2t. The length of diploid set of the chromosome of Wesafu ranges between 1.81µm and 0.59µm for the longest and the shortest chromosomes respectively. However, the karyotype obtained for *S. m. melanotheron* and *S. g. galilaeus* indicated cytoforms of their respective species.

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1. Introduction

The family Cichlidae is one of the most species-rich teleost families (Kocher, 2004) and is divided into four subfamilies: Etroplinae in India and Madagascar; Ptychochrominae in Malagasy, Cichlinae in Neotropics; and Pseudocrenilabrinae in Africa (Spark *et al*., 2004). The Cichlid attracts a lot of attention in various fields of biology due to their enormous diversity in morphology, behaviour and ecology (Fryer and Iles, 1972); therefore, becoming a prime model system in evolutionary biology, especially in speciation research (Kocher, 2004; Salzburger and Meyer, 2004; Seehausen, 2006). Moreover cichlids play an important role in tropical and subtropical aquaculture (Pullin, 1991).

In Nigeria, the cichlid fishes have received the most scientific attention compared to any other fish groups (Adesulu and Syndenham 2007). Ten genera of cichlid fishes occur in the freshwaters of Nigeria. These are *Tilapia* Smith, 1840, *Sarotherodon* Ruppel 1852, *Oreochromis* Gunther 1889, *Hemichromis* Peters 1858, *Pelmatochromis* Steindachner 1895, *Chromidotilapia* Boulenger 1898, *Pelvicachromis* Thys van den Audenaerde 1968, *Thysochromis* Daget 1988, *Gobiochilia* Kanazawa 1951 and *Tylochromis* Regan 1920; with about nineteen species in total (Paugy *et al*., 2003). However, a *Sarotherodon cf. galilaeus* locally called 'Wesafu' is reportedly found only in Epe lagoon (Fashina *et al*., 2012, Fashina-Bombata and Megbowon, 2012). Generally, in terms of colouration and even reproductive behaviour (mouthbrooding), Wesafu can easily be taken as *Sarotherodon galilaeus galilaeus* (Fashina-Bombatta *et al*., 2015, Fashina-Bombatta and Megbowon, 2012). However, 'Wesafu' grows bigger than other Oreochromini and Coptodinni fish species (*Sarotherodon melanotheron melanotheron*, *Coptodon mariae, Coptodon guinenesis*) found in the lagoon (up to 1500g) making it of higher demand for commercial purposes (Fashina-Bombata *et al.,* 2005, 2008, Fashina-Bombata and Megbowon, 2012). This great economic value of the fish has elicited scientific interest towards its development for aquaculture. However, its taxonomic identity is still unclear (Megbowon, *et al.,* 2010 and Megbowon *et al*., 2009). Wesafu has been characterised using protein and random amplified DNA techniques (Hammed *et al*., 2011; Megbowon and Fashina-Bombatta, 2013). Its proximate, chemical and amino acid composition has also been determined (Fashina-Bombatta and Megbowon, 2012; Fashina-Bombatta and Oduntan, 2012, Hammed *et al*., 2010).

Grouping of cichlid fishes into taxonomic units has been difficult over the years and so their taxonomy has been reviewed several times. The major difficulty is that the level of morphological innovation in cichlids is muted when compared to other percomorph clades. Much of the morphological differentiation associated with cichlid diversification facilitates trophic diversification without major structural modification (Burress 2014). Such situation has made it difficult to delineate the inter and intra familial relationships among cichlids using morphological characters alone as there are only few

morphological characters that can be of use in that regard (Stiassny 1991).

Although, DNA sequence data are exerting an increasingly strong influence in modern fish systematics, the place of cytogenetic studies cannot be overemphasized. In fact, in some instances, analysis of karyotypes can have an edge over DNA sequences (Arai 2011). This study therefore, assessed the chromosome of Wesafu in order to provide further information for a robust basis for its taxonomic identification. In addition, the chromosome of two related species, *Sarotheron melanotheron melanotheron* and *Sarotheron galilaeus galilaeus* were re-evaluated. This study is important for the efficient utilization of the fish in aquaculture, its conservation and also for bio-geographical inferences.

2. Methods

Life samples of the *Sarotherodon* sp. "Wesafu" and *S. m. melanotheron* were collected from the Lekki lagoon while life samples of *S. g. galilaeus* were collected from the riverine portion of the Erinle reservoir. The life samples were transported to sets of glass aquaria at the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria, in a bucket of water aerated with a battery powered bubble box air pumps. The fishes were injected with 0.1- 1ml of fermented yeast solution per 100g at the base of the dorsal fin to increase the mitotic index. The injected fishes were left in a well aerated aquarium for 24 hours. However, 0.1- 1ml solution of colchicines per 100g was injected into the base of the dorsal fin to arrest the cells at the 21st hour of the yeast treatment. Then, the fishes were dissected to remove the gills of each fishes which were squashed to homogeneity separately in 0.56% KCl solution of KCl. The resultant mixture was decanted into a centrifuge tube and centrifuged at 1000 rev/min for 10 minutes; the supernatant discarded, leaving a residue of the cells. The cell residue was fixed by addition of about 6ml of Carnoy fixative (3:1 ethanol - glacial acetic acid). A portion of the fixed cell suspension was drawn with a Pasteur pipette and two or three drops were droped unto clean, cold and wet glass slides. The cells were stained in a 6% solution of stock Giemsa stain for 25 minutes. The stained slides were dried on a Photax dishwamer 2a slide warmer set at 60°C. Slides were viewed and photomicrography taken using an Omax microscope model G013055005 fitted with a camera. The morphology of chromosomes, the diploid number (2n) and the autosomal fundamental number of chromosome arms (FNa) were then determined. The karyotyping of the chromosome was carried out from the printed photomicrographic copies of the mitotic metaphase spread. The karyotype of the chromosome spread was scanned and the length of each pairs of chromosomes was determined using CorelDraw version 17. The chromosome measurement and classification measurement was according to Abraham and Prasad (1983).

3. Result

The mitotic metaphase chromosome spreads of the *Sarotherodon sp.* Wesafu is shown in plate 1 while the mitotic chromosome number and the autosomal fundamental number observed are shown in Table 1. The karyotype is shown in plate 2. The karyotype of "Wesafu" comprise of 1 nearly median (nm) - chromosome 8; 2 nearly submedian (nsm) (-) - chromosomes 14 and 16; 1 submedian (sm) - chromosome 3; 9 nearly submedian (nsm) (+) - chromosomes 2, 4-7, 11, 12, 17 & 18; 3 nearly subterminal (nst) (-) - chromosomes 1, 13, 15; and 6 terminal T - chromosomes 9, 10, 19-22 as shown in table 2. The length of its diploid set of chromosomes ranges between 1.81µm and 0.59µm for the longest and the shortest chromosomes respectively (Table 2). The karyotype of "Wesafu" shows that the chromosomes can be grouped into four classes on the basis of size. The first class, group A is the large sized chromosomes consisting of two pairs (Chromosomes 1 and 2). The second class, group B are the medium sized chromosomes consisting of fourteen pairs (Chromosomes 3 to 16). The remaining chromosomes are small but chromosomes 17 to 20 are bigger compared to chromosomes 21 and 22. Therefore, chromosomes 17 to 20 are categorized as group C while chromosome 21 and 22 are in group D. No sex chromosome was observed. The chromosome morphology in form of an ideogram is shown in Figure 3 having relatively the small arms (p) shorter than the long arms (q) across board. Variation in the length of the chromosome is shown in Figure 2.

The mitotic metaphase chromosome spreads of *S. g. galilaeus* and *S. m. melanotheron* are shown in plates 3 and 5 while plates 4 and 6 shows their karyotype respectively. The karyotype of *S. g. galilaeus* consists of 2 median (m) - chromosomes 8 & 12; 6 nearly submedian (nsm) (+) - chromosomes 2, 9, 11, 13, 14 & 21; 10 nearly subterminal (nst) (-) - chromosomes 1, 3-7, 10, 16-18 and 4 terminal (T) - chromosomes 15, 19, 20 & 22 – Table 2; while the length of its diploid set of chromosome ranges between 1.66µm and 0.53µm for the longest and the shortest chromosomes respectively. However, the karyotype of *S. m. melanotheron* is made up of 3 nearly submedian (nsm) (-) - chromosomes 6, 8, 21; 11 nearly submedian (nsm) (+) - chromosomes 2, 3, 7, 9, 10, 12-14, 17, 18 and 20; 6 nearly subterminal (nst) (-) - chromosomes 1, 4, 5, 11, 15 and 16; and 2 terminal (T) - chromosomes 19 and 22. The length of its diploid set of chromosome ranges between 2.28µm

and 0.76µm for the longest and the shortest chromosomes respectively. The ideogram of *S. g. galilaeus and S. m. melanotheron* is depicted in figures 4 and 6 while the variation in the chromosome length is shown in figure 5 and 7 respectively.

4. Discussion

With the chromosome number of 44 obtained for Wesafu in this study, the fish shares similar chromosome number with members of the tribe tilapinnii and agrees with the report that the African cichlid lineage show limited variation both in diploid number and chromosomal structure (Felberg 2003). Wesafu exhibits biparental mouthbrooding behaviour; it may therefore, be placed with the *Sarotherodon* genus and its current grouping will be under the tribe Oreochromini (Dunz and Schliewen 2013). The karyotype of Wesafu also show the characteristic long chromosome described as the tilapia marker (although an exception is found in the *Stomatepia pindu*) (Kornfield *et al*., 1980). However, the karyotype formula determined for Wesafu (1nm + 2nsm + 1sm + 9nsm + 3nst + 6t) in this study was different from the formula reported for conspecifics like *S. g. galilaeus* by Sofy et al. (2008) – 1 m, 6 sm, 7 st and 8 t or *S. melanotheron* by Li Si-Fa et al. (2011) – 1 m, 2 sm, 12 st, and 7t.

On the other hand, the karyotype of both *S. g galilaeus* and *S. m. melanotheron* obtained in this study are different from what was earlier reported by Sofy et al. (2008) and Li Si-Fa et al. (2011). The karyotype reported for *S. g galilaeus* was 14m, sm/30st, a; while the karyotype reported for *S. m. melantheron* was 6m, sm/38st, a. However, the karyotype determined in this study for *S. g. galilaeus* was 16m, sm/28st, a; while the karyotype determined for *S. m. melanotheron* was 28m, sm/16st, a. Differences in chromosome morphology or karyotype are common occurrences among populations of fishes (Feldberg *et al*., 2003). In fact, different populations of the same species may show different karyotype formula. In some cases however, differences in karyotype might be that a karyotype consists of chromosomes that cannot easily be distinguished as either SM or ST, such that different authors may report different information (Arai 2011). For example, the karyotype of *Geophagus brasiliensis* was 4m, 44sm/st, a; by Thompson, (1979), 2m, 46sm/st, a; by Felberg and Bertollo, (1985a,b), 8m, 40sm/st, a; by Oliveira *et al*., (1994) and Brum *et al*., (1998); 8m, 40sm/st, a; by Martins *et al*., (1995), 8m, 40sm/st, a; by Couto *et al*., (1998) and 4m, 44sm/st, a; by Mizoguchi and Martins-Santos, (2000). Also, an account of the karyotype of *Oreochromis niloticus* was 6sm/38st, a; by Li Si-Fa *et al*., (2011) which was different from 2m/42st, a; by Sofy *et al*., (2008). In some other instances, these differences may be from artifacts of preparation technique or taxonomic problems, rather than representing real polymorphisms. Other factors include differences in degrees of chromosome condensation, lack of a uniform terminology among authors, or even to some miscalculations (Aria 2011).

Nonetheless, the chromosomes of fishes possess an inherent difficulty that limits cytogenetics analysis especially when the common giemsa technique is used. Their chromosomes are generally very small and this limits the resolution power of the giemsa staining technique in unraveling the underlying chromosomal arrangements - pericentric inversions (leaving the chromosome diploid number unchanged but increasing the FN); centric fusions (producing large meta- or submetacentric elements decreasing the chromosome diploid number but leaving the FN unchanged; and tandem (centromere-telomere), (decreasing the chromosome diploid number and the FN).

The resolution of the cytogenetic approach used in this study therefore, can only confirm that Wesafu belongs to the tilapiine group.

Sampling of specimens was conducted on different field-trips between 2016 and 2017 under Obafemi Awolowo University ethical approval and following all applicable international and national guidelines of animal use and ethical standards. Fish individuals were either bought freshly from local fishermen or caught using different methods.

Material examined

Sarotherodon cf. galilaeus: PMOC 6 (20, Lekki lagoon - 2016), Nigeria (Lat. 6°25'37.99"N, Long. 4° 6'5.11"E).

Sarotherodon galilaeus galilaeus (Linnaeus): PMOC 7 (15, Erinle dam - 2017), Nigeria, no exact locality data available, purchased from fishermen landings (7°45'24.90"N, Long. 4°27'13.74"E).

Figure 1. Geographic origin of the samples studied.

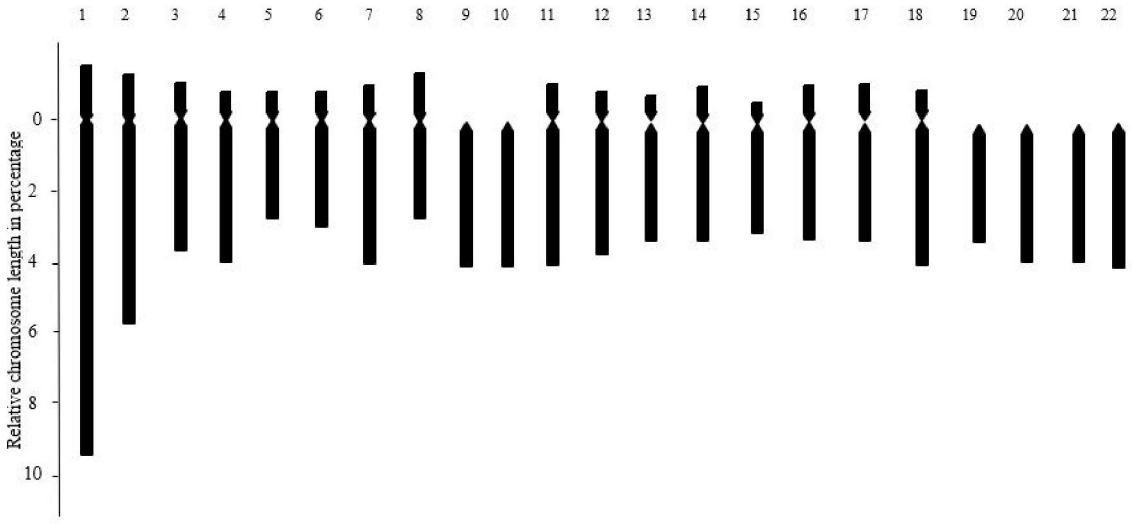

Figure 2. An ideogram of the karyotype of the *Sarotherodn sp*. Wesafu showing the morphology of the chromosome. The 0 represents the centromere

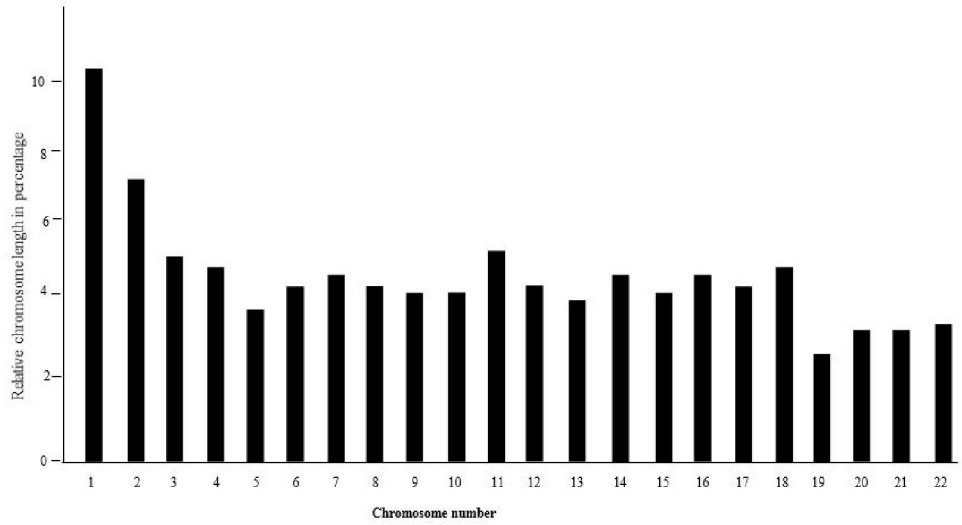

Figure 3. Relative length of the chromosome of the *Sarotherodon sp*. Wesafu showing the size variation

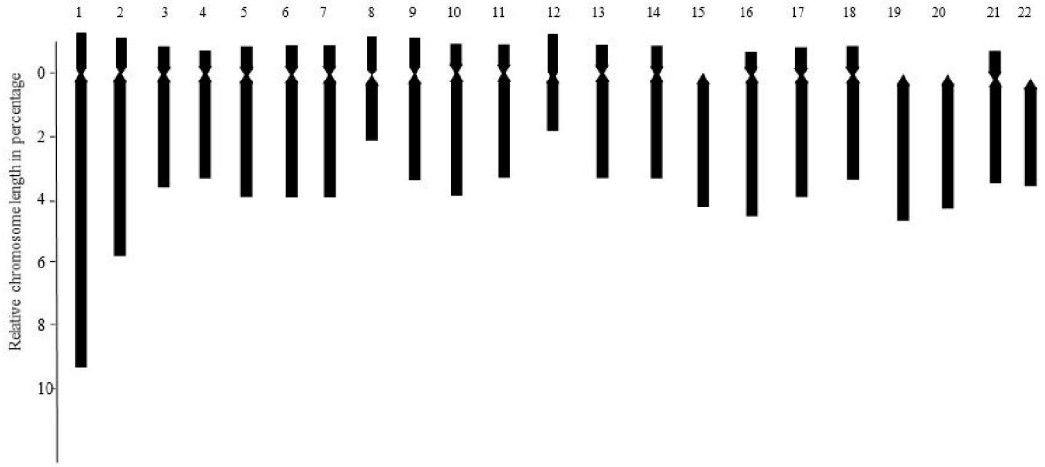

Figure 4. An ideogram of the karyotype of *S. g. galilaeus*. The 0 represents the position of the centromere.

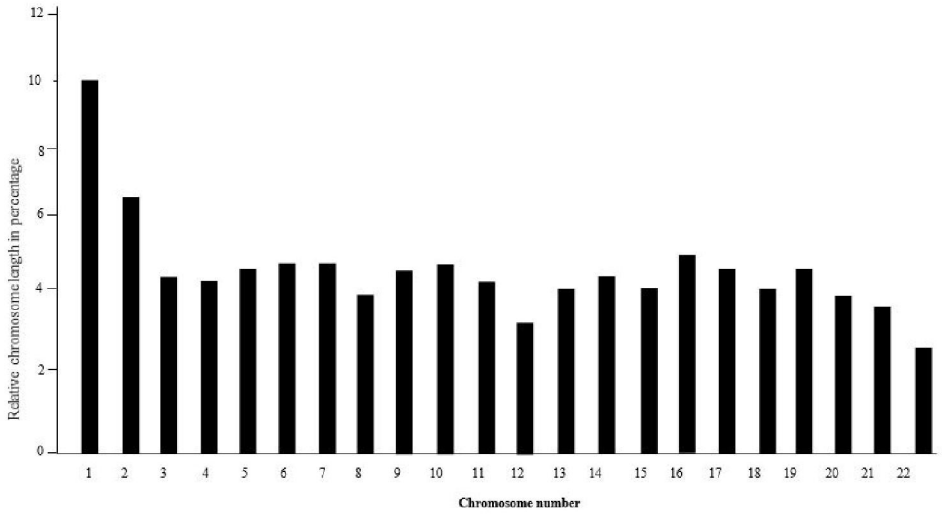

Figure 5. Relative length of the chromosome of *S. g. galilaeus* showing size variation

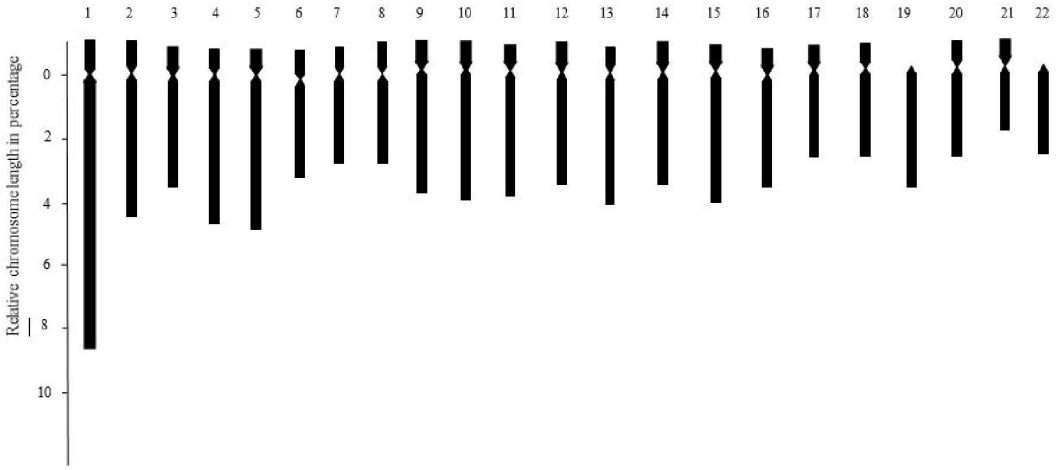

Figure 6. An ideogram of the karyotype of S.m. melanotheron. The 0 represents the position of the centromere

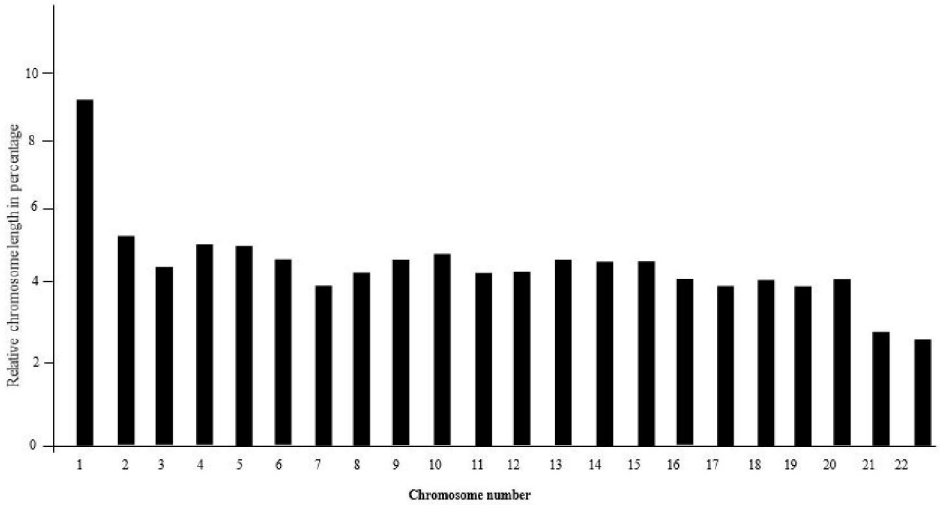

Figure 7. Relative length of the chromosome of *S.m. melanotheron* showing the size variation

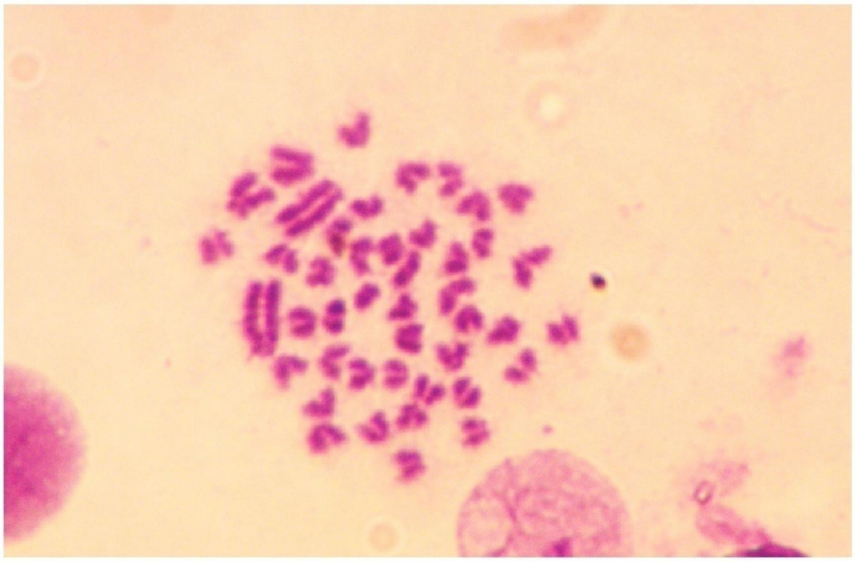

Plate 1. Mitotic metaphase chromosome spread of *Sarotherodon cf. galilaeus* (Wesafu)

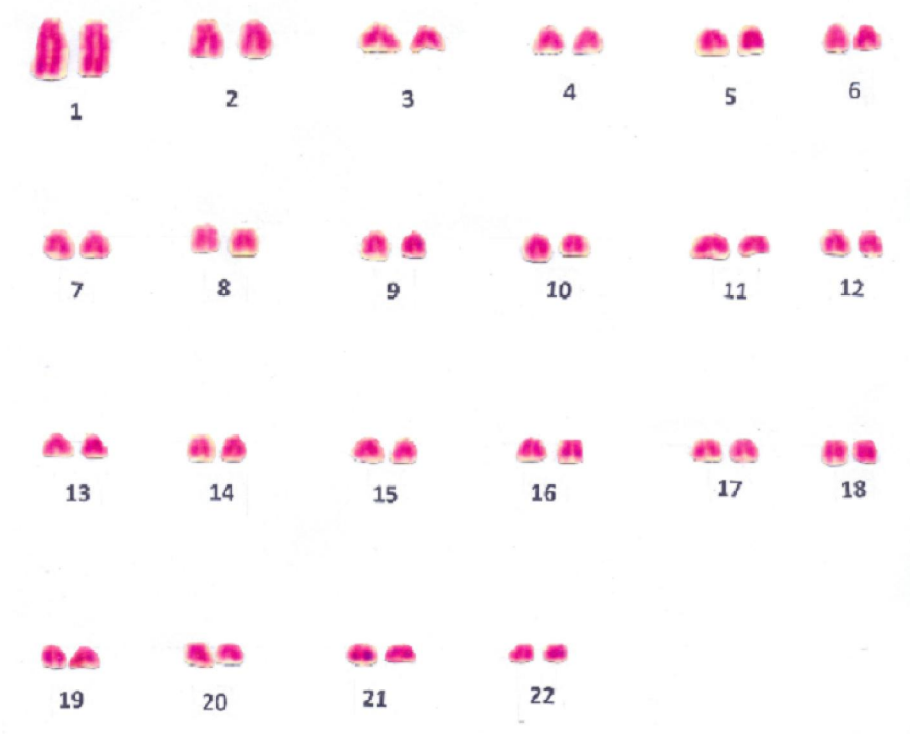

Plate 2. Karyotype of *Sarotherodon cf. galilaeus* (Wesafu)

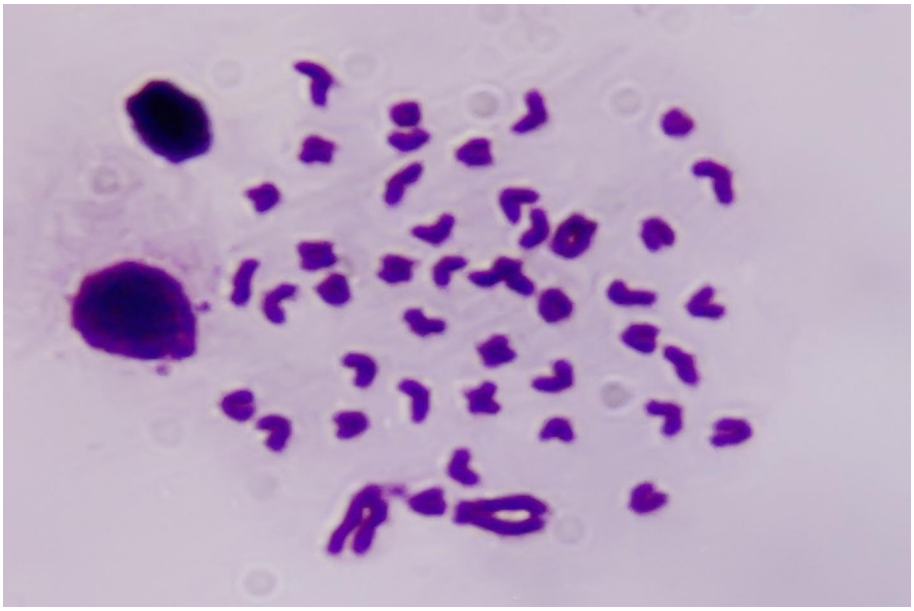

Plate 3. Mitotic metaphase chromosome spread of *Sarotherodon galilaeus galilaeus*

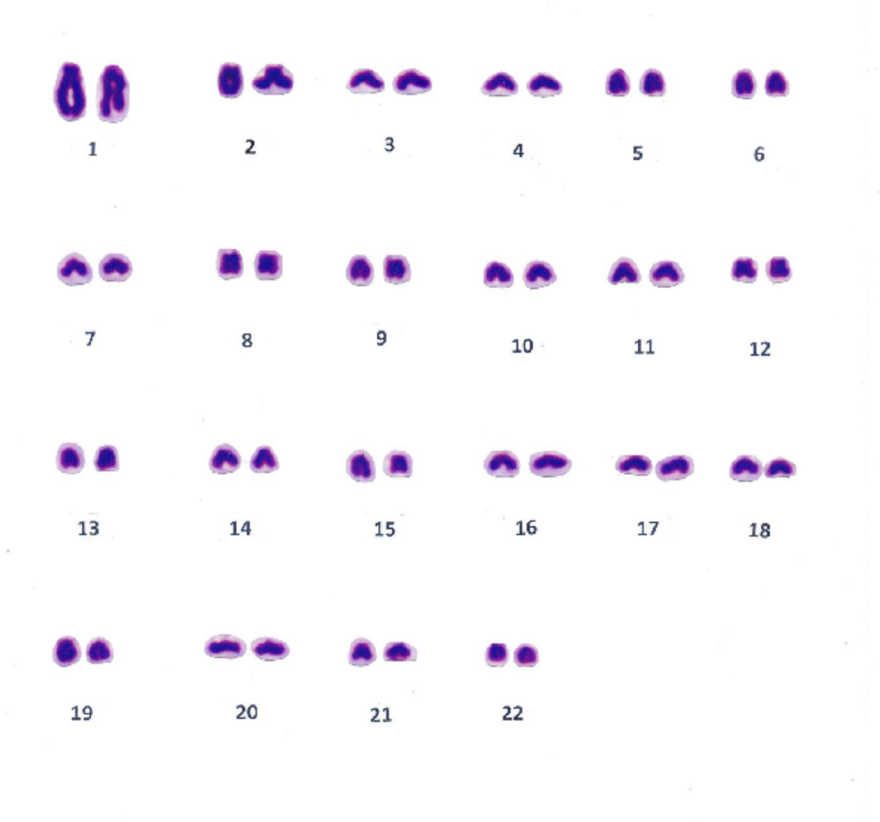

Plate 4. Karyotype of *Sarotherodon galilaeus galilaeus*

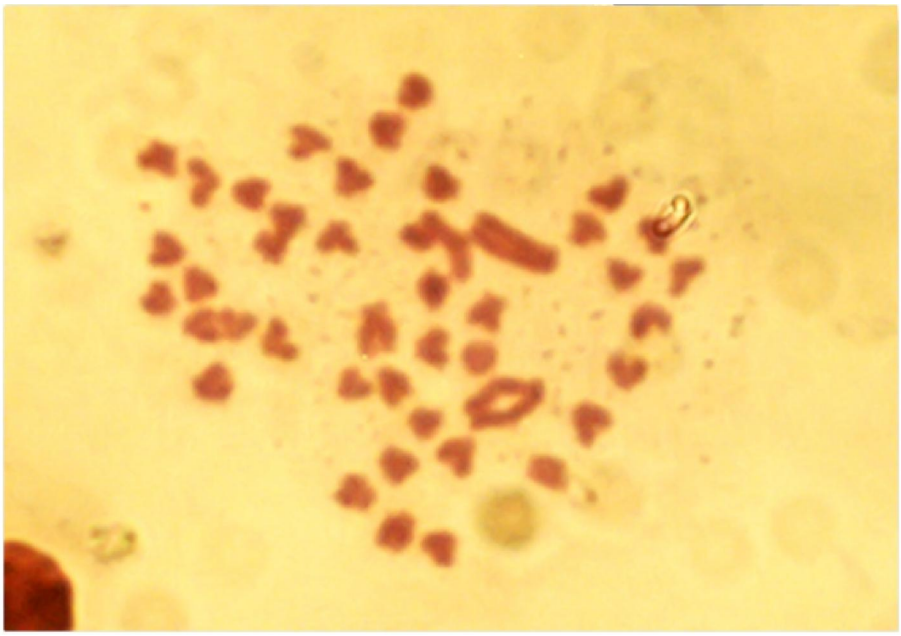

Plate 5. Mitotic metaphase chromosome spread of *Sarotherodon melanotheron melanotheron*

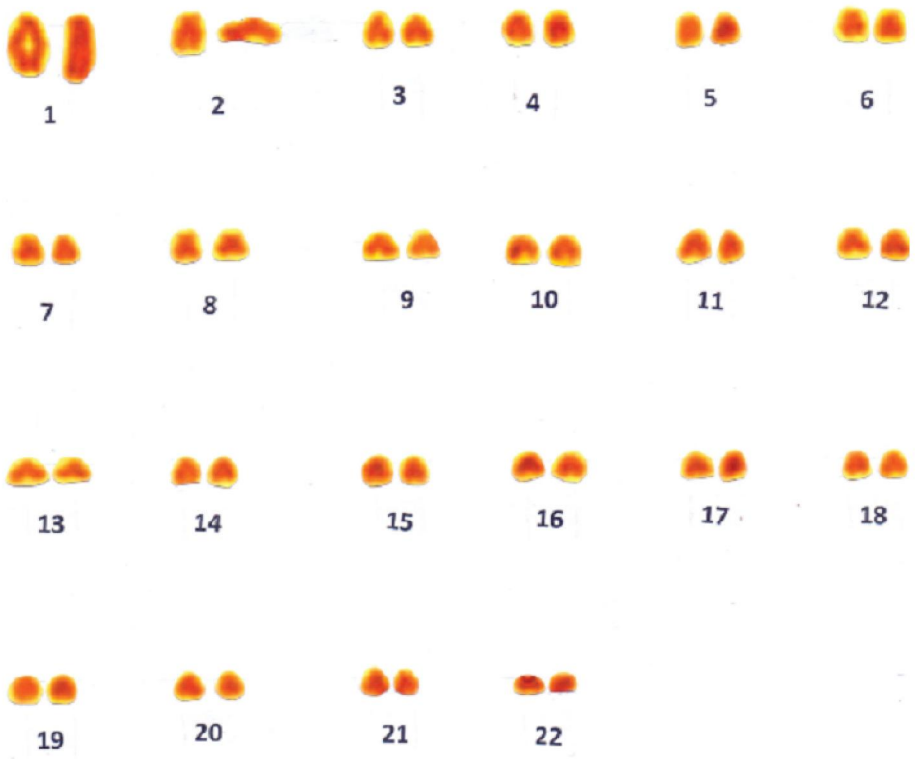

Plate 6. Karyotype of *Sarotherodon melanotheron melanotheron*

Table 1. Diploid Chromosome Number and Autosomal Fundamental Number (NFa)

Species	Diploid Chromosome Number (2n)	NFa
***Sarotherodon cf. galilaeus* (Wesafu)**	44	76
Sarotherodon galilaeus galilaeus	44	80
Sarotherodon melanotheron melanotheron	44	84

Table 2. The chromosome measurement and nomenclature of *Sarotherodon cf. galilaeus* (Wesafu) using the centromeric index (CI 1) = 100s/c and (CI 2) = 100l/c.

	Measurement (µm)			Relative length (%)					
Chromosome No.	Short arm s (µm)	Long arm l (µm)	Total Length c (µm)	Short arm s' (%)	Long arm l' (%)	Total length c' (%)	Centromeric Index (CI 1)	Centromeric Index (CI 2)	Nomenclature
1	0.32	1.50	1.81	1.78	8.47	10.25	17.36	82.64	**nearly subterminal nst (-)**
2	0.30	0.95	1.25	1.69	5.36	7.05	23.96	76.04	**nearly submedian nsm (+)**
3	0.22	0.67	0.88	1.22	3.76	4.98	24.55	75.45	**submedian SM**
4	0.17	0.68	0.84	0.94	3.83	4.77	19.75	80.25	**nearly submedian nsm (+)**
5	0.15	0.46	0.61	0.85	2.62	3.47	24.46	75.54	**nearly submedian nsm (+)**
6	0.16	0.54	0.71	0.93	3.07	4.00	23.13	76.87	**nearly submedian nsm (+)**
7	0.20	0.60	0.80	1.13	3.40	4.52	24.89	75.11	**nearly submedian nsm (+)**
8	0.29	0.43	0.73	1.66	2.44	4.09	40.45	59.55	**nearly median nm**
9	0	0.70	0.70	0	3.94	3.94	0	100	**terminal T**
10	0	0.69	0.69	0	3.91	3.91	0	100	**terminal T**
11	0.21	0.68	0.89	1.17	3.84	5.02	23.40	76.60	**nearly submedian nsm (+)**
12	0.15	0.62	0.77	0.84	3.50	4.34	19.34	80.66	**nearly submedian nsm (+)**
13	0.12	0.57	0.69	0.65	3.24	3.89	16.82	83.18	**nearly subterminal nst (-)**
14	0.21	0.59	0.79	1.18	3.30	4.49	26.34	73.66	**nearly submedian nsm (-)**
15	0.09	0.52	0.60	0.49	2.92	3.41	14.34	85.66	**nearly subterminal nst (-)**
16	0.21	0.58	0.79	1.19	3.26	4.45	26.66	73.34	**nearly submedian nsm (-)**
17	0.18	0.58	0.76	1.03	3.25	4.28	24.11	75.89	**nearly submedian nsm (+)**
18	0.17	0.67	0.85	0.99	3.80	4.78	20.64	79.36	**nearly submedian nsm (+)**
19	0	0.59	0.59	0	3.34	3.34	0	100	**terminal T**
20	0	0.64	0.64	0	3.63	3.63	0	100	**terminal T**
21	0	0.65	0.65	0	3.67	3.67	0	100	**terminal T**
22	0	0.66	0.66	0	3.72	3.72	0	100	**terminal T**

Table 3. The chromosome measurement and nomenclature of *Sarotherodon galilaeus galilaeus* using the centromeric index (CI 1) = 100s/c and (CI 2) = 100l/c.

	Measurement (µm)			Relative length (%)					
Chromosome No.	**Short arm s (µm)**	**Long arm l (µm)**	**Total Length c (µm)**	**Short arm s' (%)**	**Long arm l' (%)**	**Total length c' (%)**	**Centromeric Index (CI 1)**	**Centromeric Index (CI 2)**	**Nomenclature**
1	0.26	1.40	1.66	1.59	8.49	10.04	15.81	84.19	**nearly subterminal nst (-)**
2	0.19	0.87	1.07	1.18	5.28	6.46	18.19	81.81	**nearly submedian nsm (+)**
3	0.11	0.59	0.70	0.68	3.58	4.26	15.88	84.12	**nearly subterminal nst (-)**
4	0.10	0.58	0.68	0.61	3.50	4.11	14.86	85.14	**nearly subterminal nst (-)**
5	0.11	0.63	0.74	0.68	3.82	4.49	15.03	84.97	**nearly subterminal nst (-)**
6	0.12	0.63	0.75	0.75	3.82	4.56	16.40	83.60	**nearly subterminal nst (-)**
7	0.14	0.62	0.76	0.84	3.78	4.62	18.15	81.85	**nearly subterminal nst (-)**
8	0.31	0.31	0.62	1.88	1.86	3.74	50.27	49.73	**median M**
9	0.17	0.56	0.73	1.06	3.39	4.45	23.83	76.17	**nearly submedian nsm (+)**
10	0.12	0.62	0.74	0.71	3.76	4.48	15.93	84.07	**nearly subterminal nst (-)**
11	0.16	0.54	0.70	0.96	3.26	4.21	22.69	77.31	**nearly submedian nsm (+)**
12	0.27	0.26	0.53	1.63	1.55	3.18	51.31	48.69	**median M**
13	0.14	0.54	0.67	0.82	3.25	4.07	20.26	79.74	**nearly submediannsm (+)**
14	0.14	0.59	0.72	0.82	3.56	4.38	18.72	81.28	**nearly submediannsm (+)**
15	0	0.67	0.67	0	4.05	4.05	0	100	**terminal t**
16	0.10	0.69	0.79	0.61	4.18	4.79	12.70	87.30	**nearly subterminalnst (-)**
17	0.12	0.62	0.74	0.73	3.76	4.49	16.17	83.83	**nearly subterminalnst (-)**
18	0.12	0.55	0.66	0.71	3.31	4.02	17.57	82.43	**nearly subterminalnst (-)**
19	0	0.76	0.76	0	4.57	4.57	0	100	**terminal t**
20	0	0.65	0.65	0	3.93	3.93	0	100	**terminal t**
21	0.13	0.51	0.64	0.76	3.09	3.85	19.83	80.17	**nearly submediannsm (+)**
22	0	0.53	0.53	0	3.18	3.18	0	100	**terminal t**

Table 4. The chromosome measurement and nomenclature of *Sarotherodon melanotheron melanotheron* using the centromeric index (CI 1) = 100s/c and (CI 2) = 100l/c.

	Measurement (µm)			Relative length (%)					
Chromosome No.	Short arm s (µm)	Long arm l (µm)	Total Length c (µm)	Short arm s' (%)	Long arm l' (%)	Total length c' (%)	Centromeric Index (CI 1)	Centromeric Index (CI 2)	Nomenclature
1	0.31	1.97	2.28	1.26	7.96	9.22	13.69	86.31	**nearly subterminal nst (-)**
2	0.31	1.03	1.34	1.26	4.16	5.42	23.20	76.80	**nearly submedian nsm (+)**
3	0.25	0.83	1.08	1.01	3.37	4.38	23.07	76.93	**nearly submedian nsm (+)**
4	0.24	1.04	1.28	0.97	4.21	5.18	18.68	81.32	**nearly subterminal nst (-)**
5	0.20	1.08	1.28	0.80	4.37	5.16	15.46	84.54	**nearly subterminal nst (-)**
6	0.37	0.77	1.15	1.51	3.12	4.64	32.65	67.35	**nearly submedian nsm (-)**
7	0.23	0.75	0.98	0.94	3.02	3.96	23.78	76.22	**nearly submedian nsm (+)**
8	0.31	0.74	1.04	1.24	2.98	4.22	29.37	70.63	**nearly submedian nsm (-)**
9	0.28	0.89	1.17	1.17	3.59	4.73	24.16	75.84	**nearly submedian nsm (+)**
10	0.29	0.91	1.20	1.17	3.67	4.84	24.17	75.83	**nearly submedian nsm (+)**
11	0.20	0.88	1.08	0.79	3.56	4.35	18.13	81.87	**nearly subterminal nst (-)**
12	0.25	0.81	1.06	1.03	3.25	4.28	23.96	76.04	**nearly submediannsm (+)**
13	0.21	0.92	1.14	0.86	3.73	4.59	18.72	81.28	**nearly submediannsm (+)**
14	0.26	0.84	1.09	1.03	3.37	4.40	23.43	76.57	**nearly submediannsm (+)**
15	0.19	0.92	1.11	0.78	3.72	4.50	17.28	82.72	**nearly subterminalnst (-)**
16	0.18	0.83	1.01	0.72	3.35	4.06	17.59	82.41	**nearly subterminalnst (-)**
17	0.20	0.77	0.97	0.81	3.12	3.92	20.53	79.47	**nearly submediannsm (+)**
18	0.24	0.76	0.99	0.95	3.06	4.01	23.78	76.22	**nearly submediannsm (+)**
19	0	0.96	0.96	0	3.86	3.86	0	100	**terminal T**
20	0.24	0.75	0.99	0.97	3.03	4.00	24.22	75.78	**nearly submediannsm (+)**
21	0.21	0.57	0.79	0.86	2.32	3.18	27.01	72.99	**nearly submediannsm (-)**
22	0	0.76	0.76	0	3.08	3.08	0	100	**terminal T**

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