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CHROMOSOME STUDIES IN A SMALL AFRICAN BARB SPECIES *ENTEROMIUS* CF. PUNCTITAENIATUS FROM OPA RESERVOIR, ILE-IFE, SOUTHWESTERN, NIGERIA

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ABSTRACT

Enteromius cf. *punctitaeniatus* is a Cyprinid fish obtained from the Opa Reservoir, Obafemi Awolowo University, Ile-Ife, Nigeria. Using the conventional Giemsa staining procedure, we provided the first cytogenetic information of *E.* cf. *punctitaeniatus*. Gill cells were used to make mitotic chromosome preparations. The diploid number (2n) of 50 chromosomes was identified in *E.* cf. *punctitaeniatus* and consistent with the diploid chromosome number recorded for small African barb species. Also, the number of chromosomal arms (NF) was 80 and within the range known for small African barb species. The karyotypic formula was 18m + 2sm + 2st + 28T. The longest and shortest chromosomes in the diploid set are 0.65 m and 0.24 m long, respectively. There was no karyological evidence of sex chromosomal dimorphism discovered in this study.

Keywords: Chromosome, Cyprinid, Diploid number, *Enteromius*.

INTRODUCTION

The polyphyletic assemblage of the genus *Barbus* has been of great concern over the years to taxonomists (Berrebi and Rab, 1998). However, according to Agnèse *et al.* (1990), some delineations have been established within the group. One is that members of the genus are categorized as either small or large, the small *Barbus* being rarely longer than 10 cm, while the large *Barbus* is longer than 50 cm, respectively. In addition, the large *Barbus* species are characterized by their scales with many parallel striae and a dorsal fin with nine to eleven branched rays. However, the small *Barbus* species have scales with a small number of divergent striae and a dorsal fin with seven or eight branched rays (Lévêquc and Guégan, 1990). Another delineation is that the members are diploid or tetraploid. Incidentally, the small African *Barbus* are diploid, while the large African *Barbus* are tetraploid (Golubtsov and Krysanov, 1993; Gue´gan *et al.*, 1995; Rab *et al.*, 1995). Nonetheless, this delineation has proved insufficient to establish further internal relationships within the genus. Hence, Yang *et al.* (2015) proposed a revalidation of the genus *Enteromius* Cope 1867 to accommodate all small African *Barbus* species.

Karyological data is essential in understanding the taxonomy of plants and animals. However, data is scarce for many species, including *E.* cf. *punctitaeniatus*. Also, there is a paucity of karyotyping data on African cyprinids (Oellermaan and Skelton, 1990). Karyological data are unique evolutionary signatures that can unravel historical patterns and events to infer even the dispersal pattern within the distribution range of any species. This present study presents the karyotype of a species of small African *Barbus*, presently temporarily identified as *Enteromius* cf. *punctitaeniatus* (Paugy *et al.*, 2003). The fish shares affinity with *E. punctitaeniatus* but differs in having four scales (4) between the insertion of the dorsal fin and the lateral line instead of the three and a half (3.5) scale known for *E. punctitaeniatus* (Paugy *et al.*, 2003).

MATERIALS AND METHODS

In January 2018, live *Enteromius* cf. *punctitaeniatus* samples (Figure 1) were collected from the Opa Reservoir spillway (7°50'17.3"N and 4°52'96.6"E), Obafemi Awolowo University Ile-Ife, Nigeria. Beach seine nets and fish traps were used to collect the samples. The specimens were kept alive in sets of aquaria at the Department of Zoology, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, before chromosome extraction. The fishes were identified using identification keys described previously (Paugy *et al.*, 2003). The study comprised female (n=15) and male fish samples (n=15).

For the chromosome extraction, the fishes were administered with 0.02 mL/gram of colchicine from a stock of 0.05% weight/volume colchicine to stop cell division at the metaphase stage. The injection was given intraperitoneally at the base of the dorsal fin muscle mass. After 3 h of the colchicine treatment, the fish samples were sacrificed, and the gills were removed. Thereafter, they were placed in a hypotonic solution of 0.56% KCl for 30 min and squashed to homogeneity with a mortar and pestle. The suspension was centrifuged at 1000 rpm for 10 min. The pellets were fixed in Carnoy's fixative (ethanol/acetic acid: 3:1 dilution). To promote cell spreading, the cell suspension was transferred to a clean, cold, and wet glass microscope slide, and dried in a warmer (Photax Dish warmer 2a Model, USA) at 60 ºC for 24 h. Subsequently, the slides were stained with 6% Giemsa stain for 25 min. The slides were examined using a trinocular light microscope (Omax G013055005 Model, USA) and photographed using a camera (Omax A3514OU Model, USA) mounted to the microscope. The chromosome number with the highest frequency was selected as the species chromosome complement. The karyotype 2.0 software was used to determine the following: the chromosome number and size, arm ratio, centromeric index, relative length (chromosome measurement and nomenclature), and idiogram as previously described (Altinordu *et al.*, 2016). The metacentric (m) or submetacentric (sm) chromosomes, telocentric (t), or subtelocentric (st) chromosomes were classified according to Levan *et al.* (1964).

Figure 1: Picture of *E.* cf. *punctitaeniatus.*

RESULTS

The chromosome spread for *E.* cf. *punctitaeniatus*, and the karyogram is described in Figures 2 and 3, respectively. The karyotype formula was 18m + 2sm + 2st + 28T, with a diploid chromosomal number of 50 and a fundamental number of autosomal arms (NFa) of 80. *E.* cf. *punctitaeniatus* possesses nine pairs of metacentric chromosomes, and these are chromosomes 1, 2, 5, 6, 8, 9, 10, 11, and 12 (Table 1). Chromosome 23 was found to be Submedian, while 3, 7, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, and 25 were telocentric. The only chromosome that was subterminal was chromosome 6. The length of the longest and shortest diploid set of chromosomes was 0.65 m and 0.24 m, respectively. The karyotype of *E.* cf. *punctitaeniatus* revealed that the chromosomes could be classified into four size groups.

The big chromosomes, which include chromosomes 1 and 2, belong to the first class (Group A). The medium-sized chromosomes, comprising chromosomes 3 to 11, belong to the second class (Group B). The first group of small chromosomes, i.e., chromosomes 12 to 21, were classified in the third class (Group C). The fourth class (Group D), which consists of chromosomes 22 to 25, is the second batch of small chromosomes. The graph of the length of the chromosomes and the different size groups is described in Figure 4. Also, the idiogram of the chromosomes, which includes nine metacentric chromosomes, one submetacentric chromosome, fourteen telocentric chromosomes, and one subtelocentric chromosome is illustrated in Figure 5.

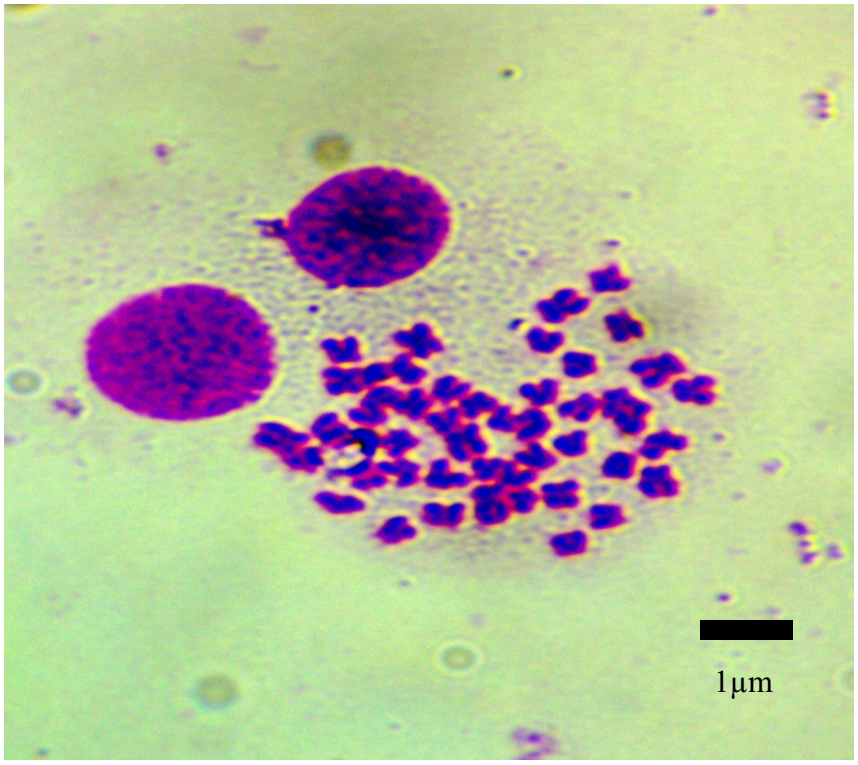

Figure 2: Mitotic metaphase chromosome spread of *Enteromius* cf. *punctitaeniatus.*

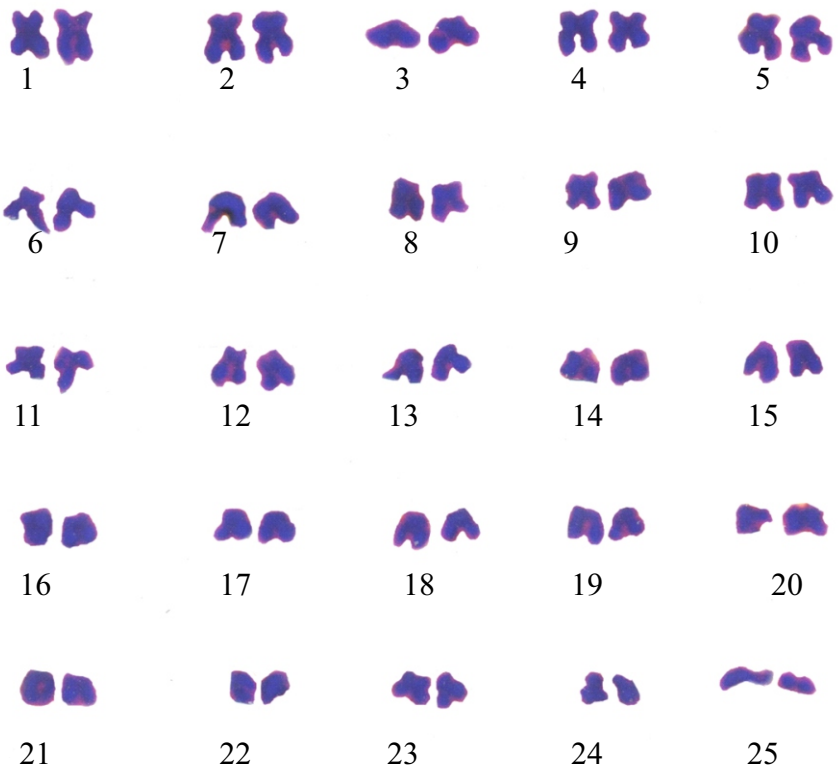

Figure 3: Karyotype of *Enteromious* cf. *punctitaeniatus.*

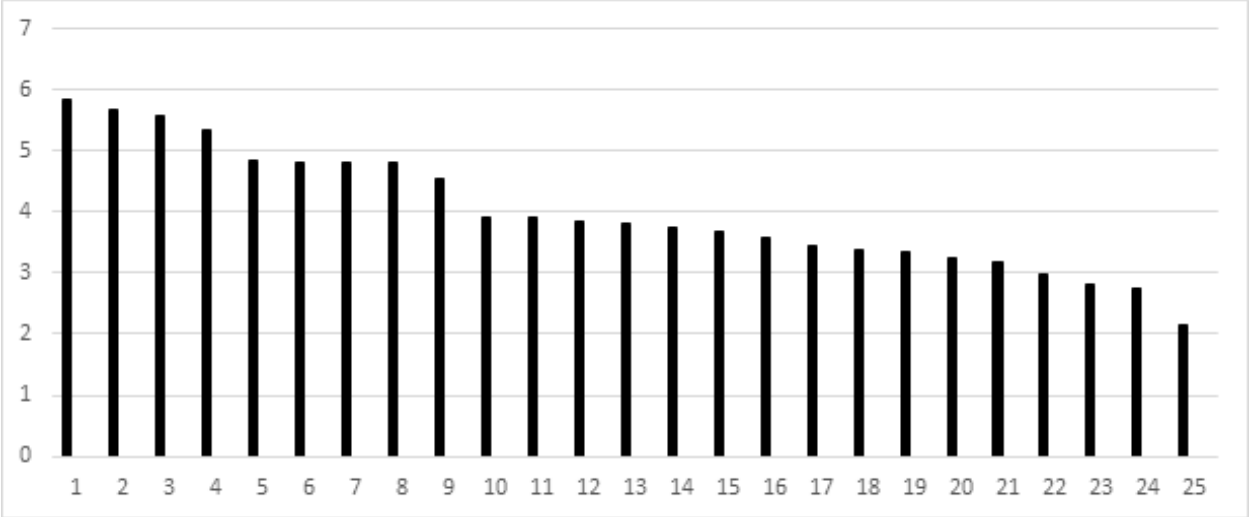

Figure 4: Relative length of the chromosomes of *Enteromious* cf. *punctitaeniatus* showing the size variation.

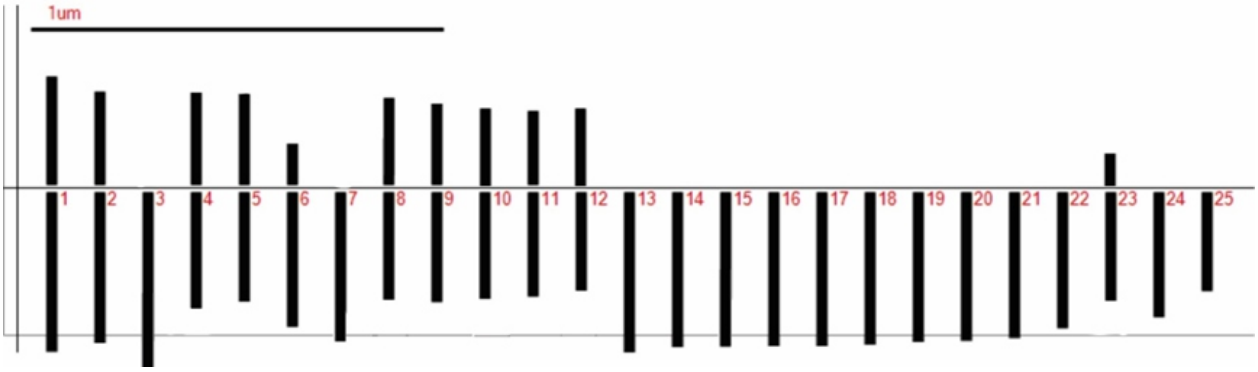

Figure 5: An idiogram of the karyotype of *Enteromious* cf. *punctitaeniatus* showing the variation in the morphology of the chromosomes.

Table 1: The Chromosome measurement and nomenclature of *E.* cf. *punctitaeniatus.*

	Measurement (µm)			Relative length (%)						
Chromosome No.	**Short arms (µm)**	**Long arm l (µm)**	**Total Length c (µm)**	**Short arm s` (%)**	**Long arm l ` (%)**	**Total length c` (%)**	**Centromeric Index (CI 1)**	**Arm Ratio r**	**Nomenclature**	
1	0.39	0.26	0.65	3.48	2.36	5.84	40.44	1.47	**median**	**M**
2	0.4	0.23	0.63	3.64	2.03	5.67	35.81	1.79	**median**	**M**
3	0.36	0.26	0.62	3.24	2.34	5.58	42.01	1.38	**terminal**	**T**
4	0.33	0.26	0.59	2.96	2.37	5.33	44.44	1.25	**median**	**M**
5	0.29	0.25	0.54	2.6	2.25	4.85	46.33	1.16	**median**	**M**
6	0.33	0.21	0.54	2.93	1.89	4.82	39.21	1.55	**subterminal**	st
7	0.29	0.24	0.53	2.62	2.2	4.82	45.69	1.19	**terminal**	**T**
8	0.42	0.11	0.53	3.78	1.02	4.8	21.33	3.68	**median**	**M**
9	0.27	0.24	0.5	2.42	2.12	4.54	46.77	1.14	**median**	**M**
10	0.43	0	0.43	3.91	0	3.91	0	4344.56	**median**	**M**
11	0.25	0.18	0.43	2.29	1.63	3.92	41.54	1.41	**median**	**M**
12	0.43	0	0.43	3.84	0	3.84	0	4267.39	**median**	**M**
13	0.42	0	0.42	3.8	0	3.8	0	4220.64	**terminal**	**T**
14	0.41	0	0.41	3.74	0	3.74	0	4148.15	**terminal**	**T**
15	0.26	0.15	0.41	2.32	1.36	3.68	36.89	1.71	**terminal**	**T**
16	0.4	0	0.4	3.57	0	3.57	0	3968.32	**terminal**	**T**
17	0.38	0	0.38	3.46	0	3.46	0	3836.45	**terminal**	**T**
18	0.38	0	0.38	3.38	0	3.38	0	3754.07	**terminal**	**T**
19	0.37	0	0.37	3.33	0	3.33	0	3691.51	**terminal**	**T**
20	0.36	0	0.36	3.26	0	3.26	0	3615.36	**terminal**	**T**
21	0.35	0	0.35	3.18	0	3.18	0	3531.15	**terminal**	**T**
22	0.33	0	0.33	2.98	0	2.98	0	3308.42	**terminal**	**T**
23	0.31	0	0.31	2.8	0	2.8	0	3110.05	**submedia**	Sm
24	0.3	0	0.3	2.74	0	2.74	0	3038.79	**terminal**	**T**
25	0.24	0	0.24	2.16	0	2.16	0	2401.8	**terminal**	**T**

CI = s/(s+l), CI 1 = 100s/c; (CI 2) = 100l/c; c=l+s; c`= s`+l`, r=l/s% (Altinordu *et al.*, 2016).

DISCUSSION

There has been an increase in the study of fish chromosomes due to their importance in evolution, heredity, fish breeding, rapid production of inbred lines, and cytotaxonomy (Ganai *et al.*, 2011). The diploid chromosome number is a conservative characteristic used to indicate the closeness of species inter-relationships within families (Buth *et al.*, 1991). In this study, the diploid chromosome number of 2n = 50 observed for *E.* cf. *punctitaeniatus* is consistent with previous reports of chromosome complement of members of the genus *Enteromius* (Arai, 2011). The modal number of diploid chromosome number for the family Cyprinidae is 2n=50. Accordingly, chromosome number in this genus is conserved as in other genera of the family Cyprinidae. The conservation of the chromosome number within the family Cyprinidae suggests resistance to chromosomal structural alterations such as fissions or fusions, which result in fast chromosome number changes. However, chromosomal structural changes like pericentric inversions, translocations, and heterochromatic addition and or deletion have been inferred to be responsible for the conservation of chromosome numbers (Buth *et al.*, 1991). Another characteristic of karyotypes within the Cyprinidae is that they consist mainly of small chromosomes, making morphological orientation identification problematic (Saenjundaeng *et al.*, 2018). The chromosome length of *E.* cf. *punctitaeniatus* range from 0.24 to 0.65 m, showing that the chromosomes are small.

Most of the chromosomes in this study were telocentric. Similar observations have been reported of karyotypes of *Barbus callipterus* (Popoola and Irewole, 2018), and *E. perince* (Adeniran *et al.*, 2021). The number of metacentric, submetacentric and acrocentric chromosomes within the family Cyprinidae has been reported to differ across species (Buth *et al.*, 1991). Some previous investigations on small African *Enteromius* species show a largely metacentric karyotype (Rab, 1981; Golubtsov and Krysanov, 1993, Rab *et al.*, 1995). Cyprinid chromosomes, both evolutionary diploid and polyploid, are often small in size, and most authors have difficulties establishing their karyotype (Rab *et al.*, 1995). The telocentric karyotype reported for the fish in this study showed a reduced number

of chromosomal arms (NF). Hence, the NF (80) differs from the NF of *B. ablabes* and *B. macrops* which have NF values of 98 and 92, respectively (Rab *et al*., 1995).

Sex chromosomes have been described in a few cyprinid species. While the genetic evidence of male heterogamety has been reported, sex chromosomes in cyprinids, as in most fishes, appear to be undifferentiated (Buth *et al*., 1991). This study did not find any evidence of sexual dimorphism in the chromosomes. In conclusion, the chromosomal number and karyotype characteristics of *Enteromious* cf. *punctitaeniatus* show that they are diploid small African barb species.

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