

A Cytogenetic Analysis of *Enteromius parablabe* (Daget, 1957) from Aho Stream, Ile-Ife, Nigeria

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ABSTRACT

In this study, *Enteromius parablabe* (Daget, 1957) was analyzed with the aim of providing base line information regarding the diploid chromosome number and karyotype differences of both sexes. The diploid chromosome number ($2n$) was 50 for both sexes; and this corresponds to the diploid chromosome number reported for most small African *Barbus* species. The fundamental number (NFa) of the male and female was 81 and 98 respectively. The first pair of metaphase chromosome which has been suggested to be a marker for the small African *Barbus* group was conspicuously larger in the female karyotype. The karyotype of the female consisted more of metacentric ($39m + 7sm + 2st + 2t$) which is common in the group while the karyotype of the male which consisted more of telocentric chromosomes ($10m + 21st + 19T$) is scarce. The chromosomal number obtained for *E. parablabe* demonstrates its diploid status in the context of the ploidy lines characteristic of the African *Barbus* assemblage.

Keywords: Karyotype; *Barbus*; Taxonomy; Africa, Chromosome

INTRODUCTION

The taxonomic identity of the small African *Barbus* (Presently known as *Enteromius* Cope 1867) species remains unresolved (Hayes and Ambruster, 2017). However, the uncertainty around the taxonomy of the genus used to be deeper, as the genus formerly consisted of more unrelated members from Europe, Asia and the Mediterranean compared to the present composition. The earlier composition was due to the morphological criteria used which turned out to be of less systematic value. The criterion used to group the species were possession of two pairs of barbels and the presence/absence of ossified and serrated rays in the dorsal fin (Berrebi, 1990).

On the basis of body size, African *Barbus* are generally recognized as either large or small. Large *Barbus* are characterized by an adult body size greater than 20 cm standard length (SL) and the presence of parallel or converging striae on their scales. In contrast, small African *Barbus* usually reach an adult size of less than 20 cm SL and have divergent scale striae (Agnese *et al.*, 1990). Studies by Agnese *et al.*, (1990), Golubtsov and Krysanov, (1993); and Oellerman and Skelton, (1990) showed that the small and large African *Barbus* are distantly

related to each other; and that the large African *Barbus* are closely related to the European *Barbus* while the small African *Barbus* are related to Asian *Barbus* genus *Puntius* and allied genera.

Karyological data became a valuable tool in understanding the internal relationships within the small African *Barbus* when Berrebi, (1990) successfully divided the members into either diploid or tetraploid lineages, although, a third lineage of African hexaploid *Barbus* was later reported by Oellerman and Skelton, (1990). The small African *Barbus* was found to be diploid while the large African *Barbus* were either tetraploid or hexaploid. Yang *et al.* (2015) confirmed the groupings made along ploidy lines, thereby further demonstrating the importance of karyological data in *Barbus* taxonomy (Berrebi and Ran 1998). Yang *et al.*, 2015 proposed the revalidation of the genus *Enteromius* Cope, 1867 to accommodate all African diploid 'Barbus' species.

Despite the importance of karyological data in the taxonomy of the genus, such data are scarce, as it is only available for very few of the 300 African *Barbus* species recognized (Leveque and Daget 1984). There are about 24 small African *Enteromius* species in Nigeria (Paugy *et*

al., 2003) but only the karyotypes of 3 have been assessed (Arai 2011). This study assessed the karyotype of *Enteromius parablabe* (Daget, 1957).

MATERIALS AND METHODS

Samples of *Enteromius parablabe* were collected from Aho stream, (7°31'23.7"N and 4°31'44.5"E) using frame nets and fish traps and kept alive in sets of aquaria at the Department of Zoology, O.A.U., Ile-Ife, Osun State. The identity of *E.parablabe* was determined based on diagnostic characters provided by Paugyet *al.*, (2003). The sexes of the samples were determined majorly by cutting them opened and examining the gonads. Cell division was arrested by injecting the fishes with 0.02 ml of colchicine per gram wet weight. The specimens were sacrificed three hours after the colchicine treatment and the gills removed. The tissues from the specimen of each sexes were treated separately. The tissues excised were placed in a hypotonic solution of 0.56% KCl for 30 minutes. The pellets were suspended in freshly prepared Carnoy's fixative. Cell suspension was dropped on a clean, cold and wet glass microscope slide and dried on Photax Dish warmer 2a Model slide warmer set at a temperature of 60° C for about 24 hours. The cells were stained with 6% stock Giemsa stain. The slides were viewed under the Omax G013055005 Model trinocular light microscope while photomicrography of the spreads were done using Omax A3514OUModel camera

attached to the microscope. The morphology, length of each chromosome and the ideogram were determined using Karyotype software (version 2.0). Chromosomes were classified according to centromere reposition (Levan *et al.*, 1964) as metacentric (m), submetacentric (sm) and telocentric (t) and subtelocentric (st). Metacentric and submetacentric chromosomes are grouped together as metacentric while telocentric and subtelocentric are grouped together as telocentric.

RESULTS

The chromosome spread obtained for the male and female *E.parablabe* is shown in Plate 1 and 2 while Plate 3 and 4 shows their karyotype respectively. The diploid chromosome number of both is 50 while the autosomal fundamental number (NFA) for the male and female is 81 and 98 respectively. The chromosome nomenclature shows that the male's chromosomes 1-10 are metacentric; 11, 12, 21, 22, 25-28, 31 - 34, 39, and 43 - 50 are sub-telocentric while 13 - 20, 23, 24, 29, 30, 35 - 38, and 40 - 42 are telocentric. On the other hand, chromosomes 1, 3 - 6, 9 - 17, 19 - 24, 27 - 35, and 37 - 46 of the female are metacentric; 2, 7, 8, 18, 25, 26, 36 are sub-metacentric; 49 and 50 are sub-telocentric while chromosomes 47 and 48 are telocentric. The morphology of the chromosomes of the male and female sexes in form of an ideogram is presented in Fig. I. and II respectively

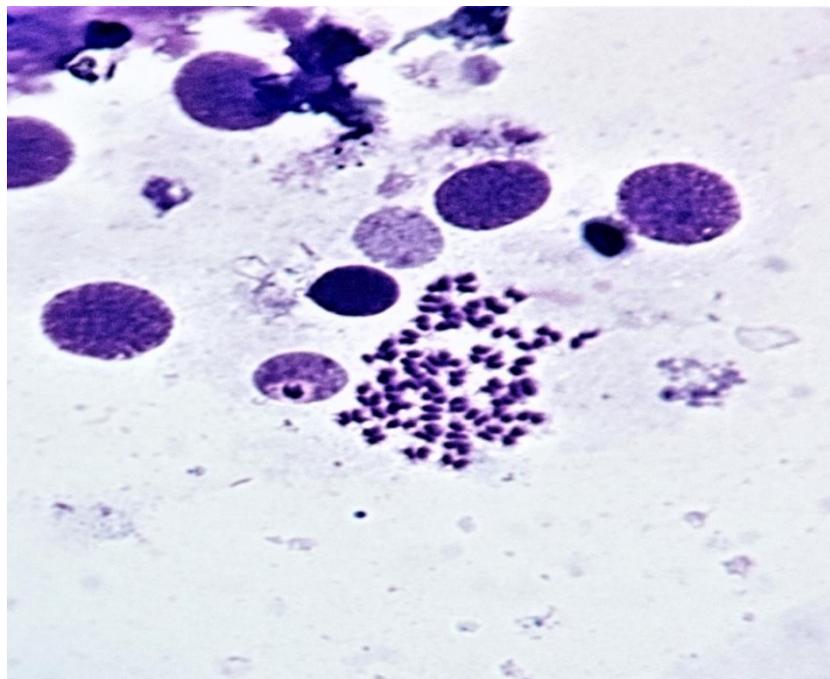


Plate1: Mitotic chromosomes spread of male *E. parablabe* ($2n = 50$).

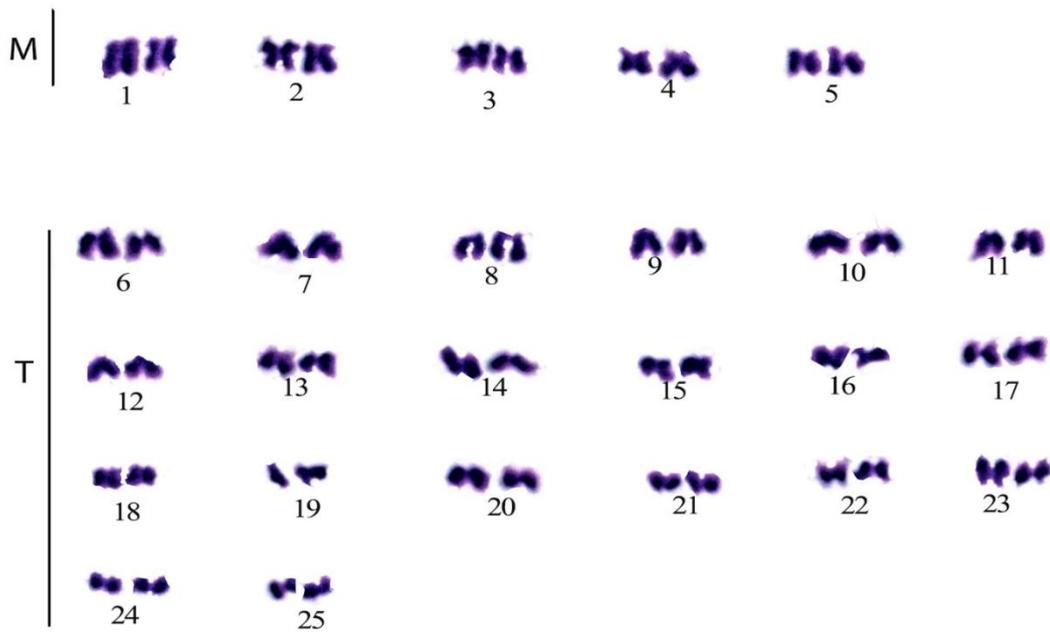


Plate 2: Karyotype of *E. parablabe* male.

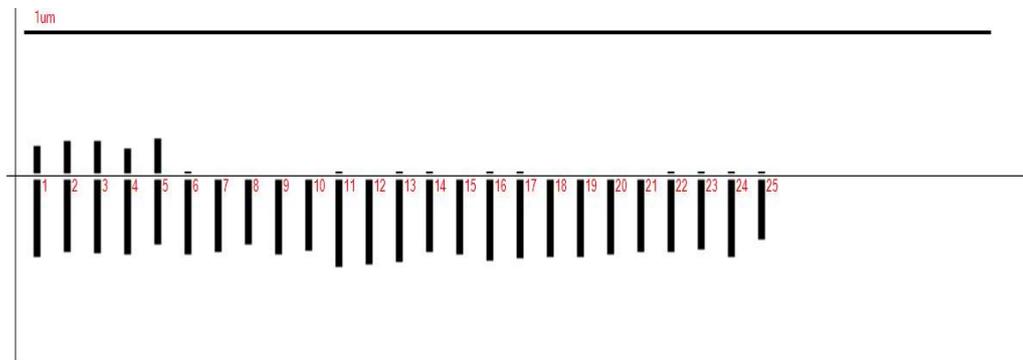


Fig I: An ideogram of the karyotype of male *Enteromius parablabe*.

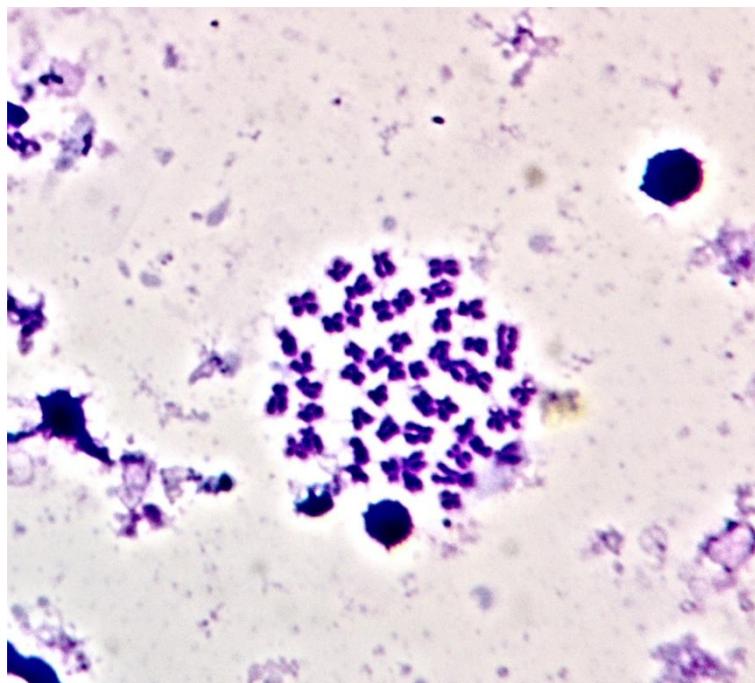


Plate 3: Mitotic chromosomes spread of female *Enteromius parablabe* ($2n = 50$).

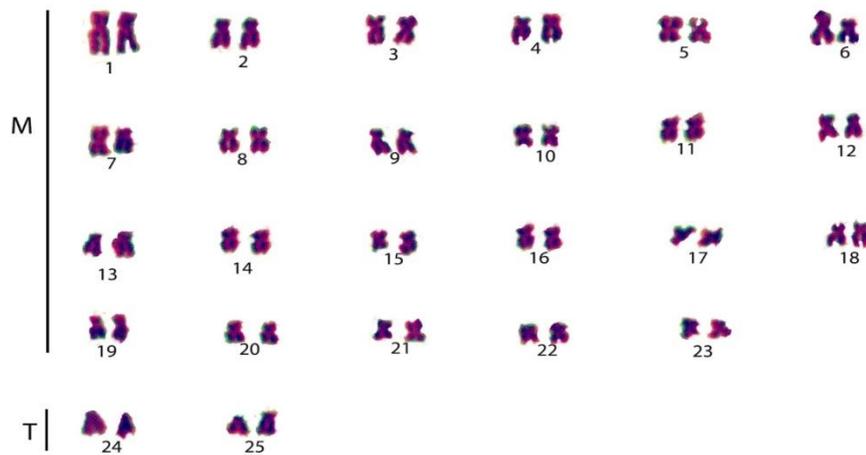


Plate4: Karyotype of *Enteromius parablabe* female.

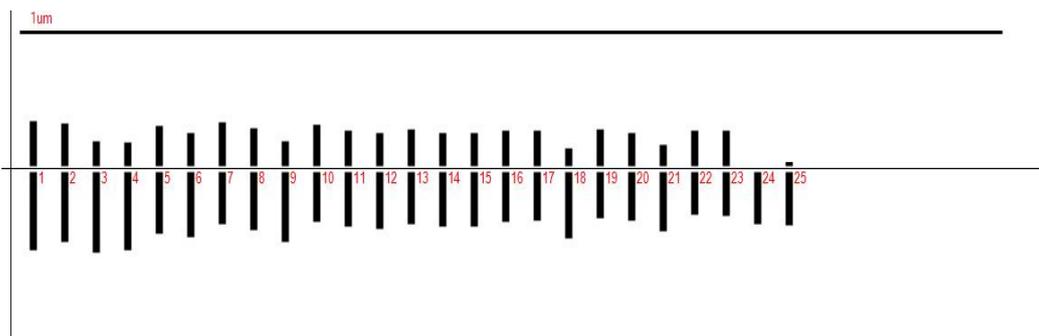


Fig II: An ideogram of the karyotype of female *Enteromius parablabe*.

DISCUSSION

The diploid chromosome number of 50 exhibited by both sexes of the *E. parablabe* is consistent with the reported chromosome number of the species in the genus *Enteromius*. Studies undertaken so far regarding the karyotype of cyprinids including the genus *Enteromius*, have shown a very low variability of their chromosome number (Luca *et al.*, 2010) as majority of the species examined presented a diploid number of 50. The high level of conservation of the karyological pattern of the majority of the cytogenetically analyzed fishes is however, a great departure from their speciation and high morphological diversity (Collares-Pereira 1990).

In addition, karyotyped diploid cyprinid have been found to be mostly made up of small sized chromosomes and this makes identifying their morphological orientations difficult (Saenjundaen *et al.*, 2018). Another challenge in karyotyping cyprinid species is chromosome arm contraction due to temporal and dose colchicine treatment (Rab, (1991). Like other cyprinid species, the chromosomes of the *E. parablabe*, both male and female were very small and karyotyping was difficult. Our final karyotype for the male *E. parablabe* was

arrived at with great difficulty as some of the chromosomes were too small for us to assign them to a precise category. However, we suspect that more of the chromosome of the male *E. parablabe* will ultimately have their centromere at the terminal region. On the other hand, the chromosomes of the female *E. parablabe* are clearer and legible. The female chromosomes are mostly (92%) with their centromere at the median region, while only few (8%) have their centromere at the terminal region. This karyotypic composition of both male and female *E. parablabe* falls within the range described for cyprinid fishes. The composition of the karyotype of cyprinid consists of the centromere positions being placed gradually from a median point to a terminal point (Rabet *et al.*, 1995; Naran, 1997; Luca *et al.*, 2010). A typical karyotype for the cyprinids consists of 6-8 pairs of metacentric chromosomes (m), 12-17 pairs of submeta- and subtelocentric chromosomes (sm, st) and 3-4 pairs of acrocentric chromosomes

Majority of cyprinids, including small African barbs present karyotype rich in metacentric and sub-metacentric chromosomes (Lee, *et al.*, 1986; Luca *et al.*, 2010). However, a telocentric rich karyotype was reported for *B. callipterus* by Popoola and Irewole (2018). The differences in

karyotype composition of the male and female *E. parablades* could have been precipitated by chromosomal arrangements, such as centric fusions and pericentric inversions, which have played an important role in karyotype evolution. Such chromosomal evolution have been shown to lead to numerous chromosomal rearrangements in the position of centromere on the chromosome and in chromosome numbers. The incongruence between the chromosome morphology of the male with previous report might also be attributed to population differences. Similar differences in karyotype of male and female fish species have been reported by Karahan and Ergene (2010). However, a distinctly large metacentric chromosome suspected to be a marker element for the small African barb (Rabet *et al.*, 1995), was found in both the male and female chromosome spread.

Sexual dimorphism at the chromosome level has been characterized among organisms (Quanqiet *et al.*, 2009). There are the XX and XO, XY and XX and ZZ and ZW types. In the XY and XO type, XX is female while XY is male. In this present study, both sexes have the same diploid chromosome number of $2n = 50$ but although, earlier report did not find distinguishable sexual dimorphic chromosome in *Enteromius* species, the result of this study suggests otherwise. Two chromosomes, which are telocentric and acrocentric in the female are thought to be sex chromosomes. However, caution is generally suggested in the determination of sexual system in cyprinids due to their characteristic small sized chromosomes (Rabet *et al.*, 1995). A more advanced cytogenetic approach like chromosome painting and banding techniques are therefore suggested to confirm the sexual dimorphism of *E. parablades* at the chromosome level.

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Citation: Popoola, Michael Olaoluwa and Awe, Oluwatomisin, "A Cytogenetic Analysis of *Enteromius parablabe* (Daget, 1957) from Aho Stream, Ile-Ife, Nigeria *Journal of Genetics and Genetic Engineering*, 5(1), 2021, pp 1-6. DOI: <https://doi.org/10.22259/2639-1953.0501001>

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