

Chromosomal Study of two Cyprinid fishes of the genus *Barbus*; *Barbus callipterus* Boulenger 1907 and *Barbus parablables* Daget 1957

Popoola Michael Olaoluwa, Irewole Ezekiel Ariyo

Obafemi Awolowo University, Ile-Ife, Nigeria

hestillguides@yahoo.com

Abstract: The chromosomes of two cyprinid fishes of the genus *Barbus*; *B. callipterus* and *B. parablables* were assessed in this study with a view to providing baseline information about the diploid chromosome number, karyotype and morphology. This study reveals that *B. callipterus* has a diploid chromosome number of $2n = 50$, fundamental number of autosomal arms (NFa) of 66, and karyotype formula: $2n = 4m + 6sm + 6st + 34T$. On the other hand, *B. parablables* has a diploid chromosome number of $2n = 50$, fundamental number of autosomal arms (NFa) of 50, and karyotype formula: $2n = 2t + 48T$. The chromosome number obtained for both species confirms that they belong to the diploid group of African barbs. However, contrary to the general chromosome morphology described for small African barbs, the two species studied are mostly telocentric.

[Popoola MO, Irewole EA. **Chromosomal Study of two Cyprinid fishes of the genus *Barbus*; *Barbus callipterus* Boulenger 1907 and *Barbus parablables* Daget 1957.** *Nat Sci* 2018;16(12):101-107]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). <http://www.sciencepub.net/nature>. 17. doi:[10.7537/marsnsj161218.17](https://doi.org/10.7537/marsnsj161218.17).

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

1. Introduction

The taxonomy of the African *Barbus* species has received scientific attention over the years. However, the controversies over the internal groupings are yet to be resolved. Leveque and Daget (1984) suggested that the name *Barbus* should be reserved for European species and several North African species. Based on morphological data, Leveque and Guegan (1990) and Daget and Iltis (1985) grouped the African barbs into small and large barbs. However, cytogenetic data provided serious evidence in support of the above hypotheses and thereby demonstrated the great importance of karyological data in *Barbus* taxonomy. 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 Europeans *Barbus* while the small African *Barbus* are related to Asian *Barbus* genus *Puntius* and allied genera. The possibility that the *Barbus* species can be characterized along ploidy levels shows that karyological data hold strong potentials in resolving relationships among the taxonomic groupings (Berrebi and Ran, 1998).

Unfortunately, out of the 300 African barb species recognized (Leveque and Daget, 1984), only the karyotypes of 76 have been reported (Arai, 2011). Twenty five of the karyotype had $2n = 48-50$; eighteen had $2n = 100$; and thirteen had $2n = 148-150$. There are about 24 small barbs species in Nigeria (Paugy et al., 2003) but only the karyotypes of 3 have been assessed (Arai, 2011). This gap in knowledge of the karyotype of the small African barbs species therefore,

needs to be bridged. This will provide a robust cytogenetic data that can be used to make valuable inferences concerning their taxonomy (Agnese, 1990; Berrebi and Rab, 1998). This study assesses for the first time, the karyotype of *B. parablables* and *B. callipterus* in order to add to existing cytogenetic data on the African barbs.

2. Materials and methods

Live samples of *B. callipterus* and *B. parablables* were collected from the spillway section of Opa dam (Figure 1), Obafemi Awolowo University, using beach seine net. The chromosome extraction procedure was initiated by injecting the fishes with 0.02 ml/gram of colchicine from a stock of 0.05% wt/vol colchicine. The injection was made intraperitoneal at the muscle mass of the base of the dorsal fin. The specimens were sacrificed three hours after the colchicine treatment and the gills excised. The tissues excised were placed in a hypotonic solution of 0.56% KCl for 30 minutes, fixed in Carnoy's fixative; the cell suspension dropped onto microscope slides, air-dried, and stained with 6% Giemsa stain for twenty-five minutes. The length of each chromosome and idiogram were determined using KaryoType software (version 2.0). The measurements and nomenclature were according to Levan et al. (1964).

3. Results

Chromosomes of *B. callipterus*

The chromosome spread obtained for *B. callipterus* is shown in Plate 1. On the other hand, Plate 2 shows the karyotype of *B. callipterus* with diploid number of $2n = 50$, fundamental number of

autosomal arms (NFa) = 66, and karyotype formula of $2n = 4m + 6sm + 6st + 34T$. The chromosome nomenclature in Table 1 shows that *B. callipterus* has two pairs of metacentric chromosomes with centromeres in the median region (m), that is, chromosomes 1 and 11; three pairs of submetacentric chromosomes with centromeres in the submedian region (sm), that is, chromosomes 2, 9 and 12; three pairs of subtelocentric chromosomes with centromeres in the subterminal region (st), that is, chromosomes 3, 8 and 13; Chromosomes 4 to 7, as well as chromosomes 14 to 25 are telocentric chromosomes with centromeres at the terminal point (T). The length of its diploid set of chromosomes ranges between 0.41 μm and 0.10 μm for the longest and the shortest chromosomes respectively.

The karyotype of *B. callipterus* shows that the chromosomes can be grouped into four classes on the basis of size. The first class, group A, are the large sized chromosomes consisting of two pairs, chromosome 1 and 2. The second class, group B are the medium sized chromosomes consisting of sixteen pairs, chromosomes 3 to 17. The third class, group C is made of the first set of the small sized chromosome consisting of seven pairs, chromosomes 18 to 24. The fourth class, group D is the second set of the small sized chromosomes, comprising only a pair, chromosome 25. The morphology of the chromosomes in form of an idiogram is presented in Figure 2, showing two metacentric chromosomes and twenty-three telocentric chromosomes. The graph of the relative length of the chromosomes is presented in Figure 3.

Chromosomes of *B. parablables*

The chromosome spread obtained for *B. parablables* is shown in Plate 3. Plate 4 shows the karyotype of *B. parablables* with diploid number of $2n = 50$, fundamental number of autosomal arms (NFa) = 50, and karyotype formula of $2n = 2t + 48T$. Table 2 shows that *B. parablables* has twenty-five telocentric chromosomes. One of the chromosome pairs has centromere in the terminal region (t), that is, chromosome 1. Chromosomes 2 to 25 are telocentric chromosomes with centromeres in the terminal point (T). The length of its diploid set of chromosomes ranges between 0.21 μm and 0.11 μm for the longest and the shortest chromosomes respectively.

The karyotype of *B. parablables* shows that the chromosomes can be grouped into three classes on the basis of size. The first class, group A are the medium sized chromosomes consisting of fifteen pairs, chromosome 1 to 15. The second class, group B is made of the first set of the small sized chromosome consisting of eight pairs, chromosomes 16 to 23. The third class, group C is the second set of the small sized

chromosomes, comprising two pairs, chromosomes 24 and 25.

The morphology of the chromosomes in form of an idiogram is presented in Figure 4, showing twenty-five telocentric chromosomes. The graph of the relative length of the chromosomes is presented in Figure 5.

3. Discussions

The karyotype of *B. callipterus* and *B. parablables* obtained in this study exhibit some basic characteristics that have been reported for African small barbs. The first pair of chromosome is larger and the chromosomes are generally small in size. The diploid chromosome number was $2n = 50$ and thus fall within the expected range of $2n = 48$ to $2n = 50$ (Arai, 2011; Rab *et al.*, 1995). Generally, the chromosome numbers of Cyprinidae have been reported to appear unsusceptible to chromosome structural changes such as fissions or fusions that precipitate (non-polyploid) changes in chromosome number. With the diploid number of $2n = 50$, our findings further support the viewpoint that the African small barbs cannot be assigned phylogenetically to the true polyploid genus *Barbus* sensu stricto.

However, contrary to the general morphology known of the karyotypes of diploid cyprinids (Lee *et al.*, 1986; Ueda *et al.*, 2001; Luca *et al.*, 2010), the karyotypes obtained for *B. parablables* and *B. callipterus* were mostly telocentric; *B. callipterus* has two pairs of metacentric chromosomes with median centromere positions, three pairs of submetacentric chromosomes with submedian centromere positions, three pairs of subtelocentric chromosomes with subterminal centromere positions, and seventeen pairs of telocentric chromosomes with terminal centromere positions while the twenty-five chromosome pairs of *B. parablables* are telocentric chromosomes with terminal centromere positions. Using the documentation of Arai (2011) as basis; this study is reporting for the first time, African small barbs species that are rather rich in telocentric chromosomes.

In addition, although *B. callipterus* and *B. parablables* both have diploid chromosome number of $2n = 50$, they both present different fundamental number of autosomal arms (NFa) and karyotype formulae (k) different from previous works already documented by Arai (2011). The NFa number reported so far are generally higher; above 80. For instance, *B. ablables* has NFa = 98, *B. anema* has NFa = 92 and *B. macrops* has NFa = 92 while *B. callipterus* has NFa = 66 and *B. parablables* has NFa = 50. *B. ablables* has $k = 18m + 30sm + 2st$, *B. anema* has $k = 42m, sm + 8t$ and *B. macrops* has $k = 14m + 28sm + 8st$ while *B. callipterus* has $k = 4m + 6sm + 6st + 34T$ and *B. parablables* has $k = 2t + 48T$. From the chromosome

measurements presented in Table 1 and Table 2, and the graph of relative length of chromosomes in Figure 2 and Figure 4, the chromosomes of *B. parablabe*s (0.21 μm - 0.11 μm) are relatively small than those of *B. callipterus* (0.41 μm - 0.10 μm).

Acknowledgements:

Authors are grateful to Dr Denloye for help in microscopy and Mr Akintoye for useful discussions during the project.

Corresponding Author:

Dr. Michael Olaoluwa Popoola
Department of Zoology
Obafemi Awolowo University
Ile-Ife, Nigeria
Telephone: +2348129481927
E-mail: hestillguides@yahoo.com

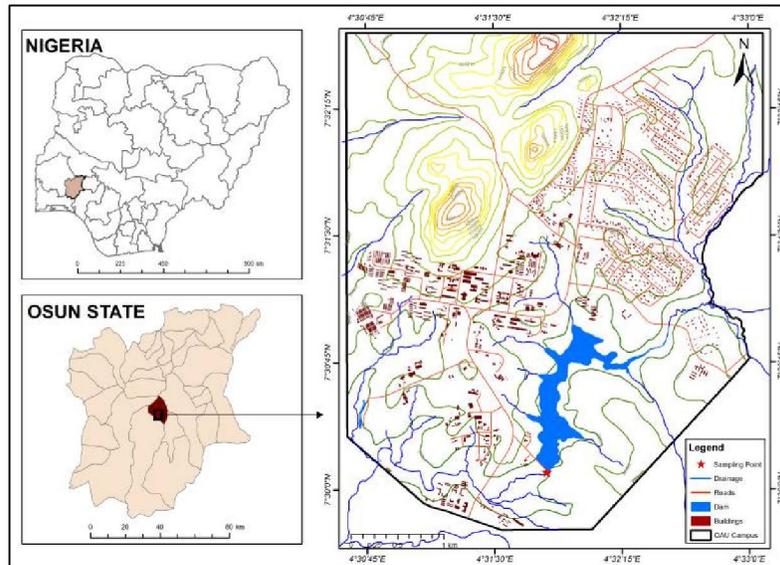


Figure 1: Map of Obafemi Awolowo University showing sampling point on the Opa Dam

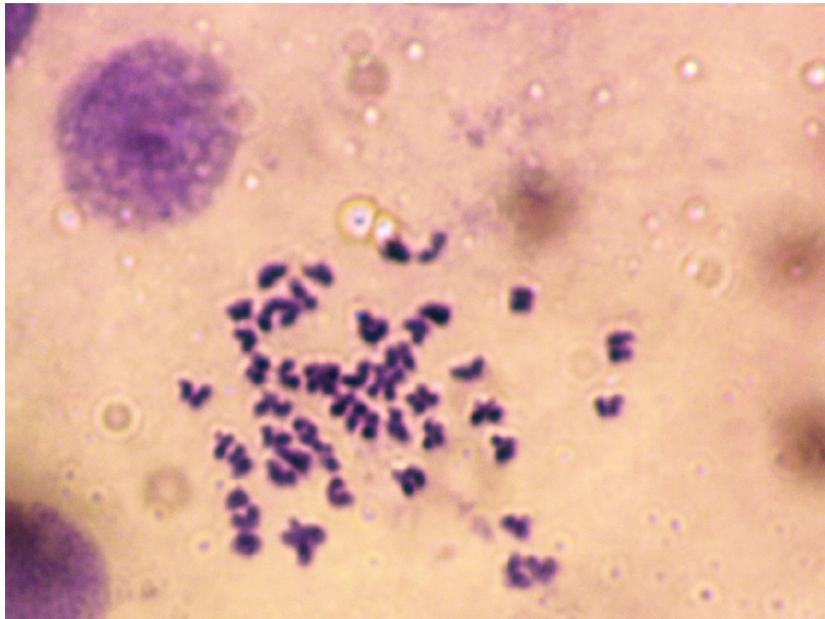


Plate 1: Mitotic metaphase chromosome spreads of *B. callipterus* ($2n=50$)

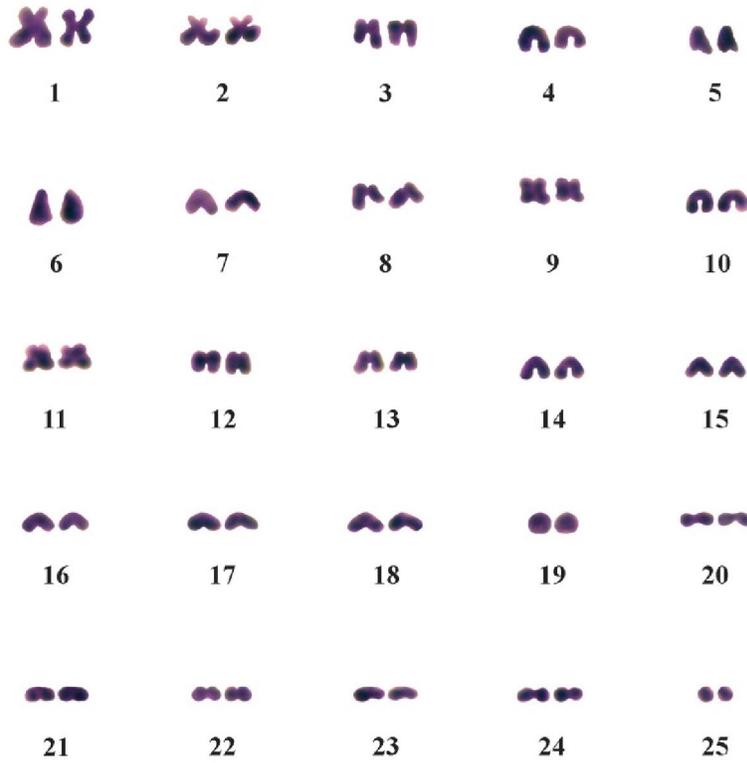


Plate 2: Karyotype of *B. callipterus*

Table 1: The chromosome measurement and nomenclature of *B. callipterus*

Chromosome (n)	Measurement (µm)				Relative length (%)				Centromeric index (100 s/c)	Nomenclature
	Long arm (l)	Short arm (s)	Total length (c)	Relative length (l')	Relative length (s')	Relative length (c')	Relative length (s/c)			
1	0.22	0.19	0.41	4.27	3.85	8.12	46.34	Median (m)		
2	0.21	0.12	0.32	4.11	2.30	6.41	37.50	Submedian (sm)		
3	0.21	0.05	0.26	4.22	0.98	5.20	19.23	Subterminal (st)		
4	0.24	0	0.24	4.80	0	4.80	0	Terminal (T)		
5	0.27	0	0.27	5.27	0	5.27	0	Terminal (T)		
6	0.25	0	0.25	4.95	0	4.95	0	Terminal (T)		
7	0.19	0	0.19	3.86	0	3.86	0	Terminal (T)		
8	0.19	0.05	0.24	3.70	0.97	4.67	20.83	Subterminal (st)		
9	0.13	0.07	0.20	2.55	1.36	3.91	35.0	Submedian (sm)		
10	0.22	0	0.22	4.32	0	4.32	0	Terminal (T)		
11	0.12	0.10	0.22	2.80	1.63	4.43	45.45	Median (m)		
12	0.15	0.06	0.21	3.02	1.14	4.16	28.57	Submedian (sm)		
13	0.16	0.04	0.20	3.12	0.89	4.01	20.0	Subterminal (st)		
14	0.18	0	0.18	3.48	0	3.48	0	Terminal (T)		
15	0.17	0	0.17	3.35	0	3.35	0	Terminal (T)		
16	0.16	0	0.16	3.19	0	3.19	0	Terminal (T)		
17	0.15	0	0.15	3.02	0	3.02	0	Terminal (T)		
18	0.15	0	0.15	2.98	0	2.98	0	Terminal (T)		
19	0.15	0	0.15	2.99	0	2.99	0	Terminal (T)		
20	0.14	0	0.14	2.88	0	2.88	0	Terminal (T)		
21	0.14	0	0.14	2.87	0	2.87	0	Terminal (T)		
22	0.14	0	0.14	2.86	0	2.86	0	Terminal (T)		
23	0.13	0	0.13	2.59	0	2.59	0	Terminal (T)		
24	0.12	0	0.12	2.39	0	2.39	0	Terminal (T)		
25	0.10	0	0.10	2.06	0	2.06	0	Terminal (T)		

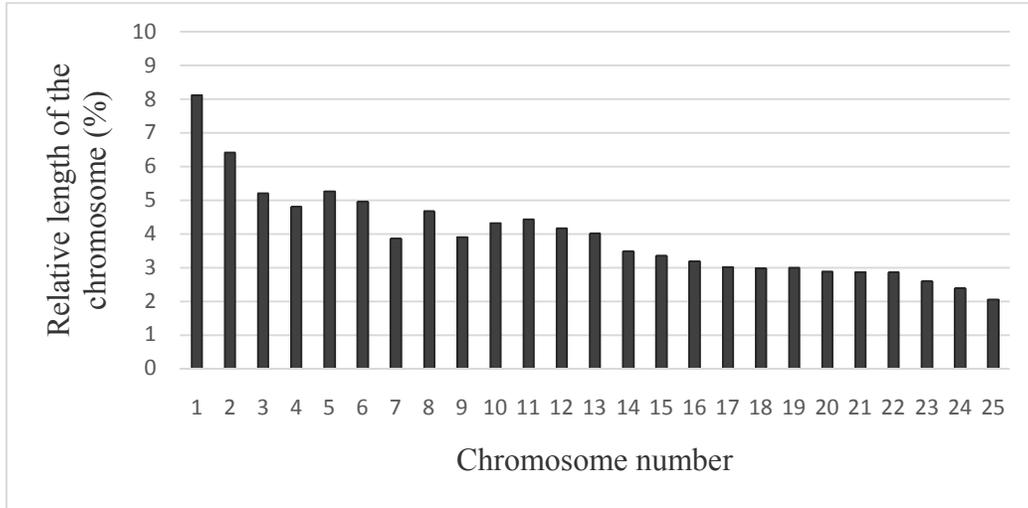


Figure 2: Relative length of the chromosomes of *B. callipterus* showing the size variation

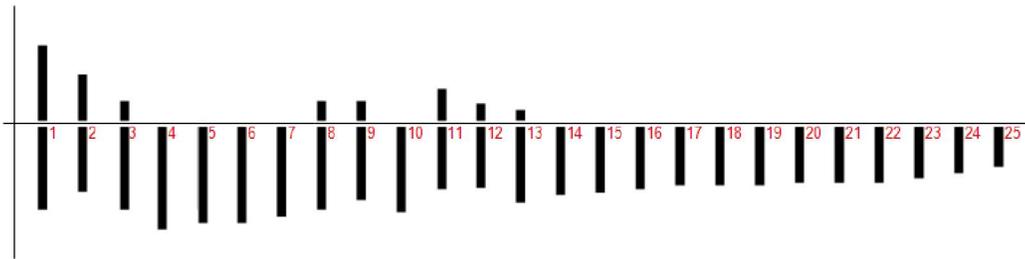


Figure 3: An idiogram of the karyotype of *B. callipterus* showing the morphology of the chromosomes. The arrow represents the position of the centromere.

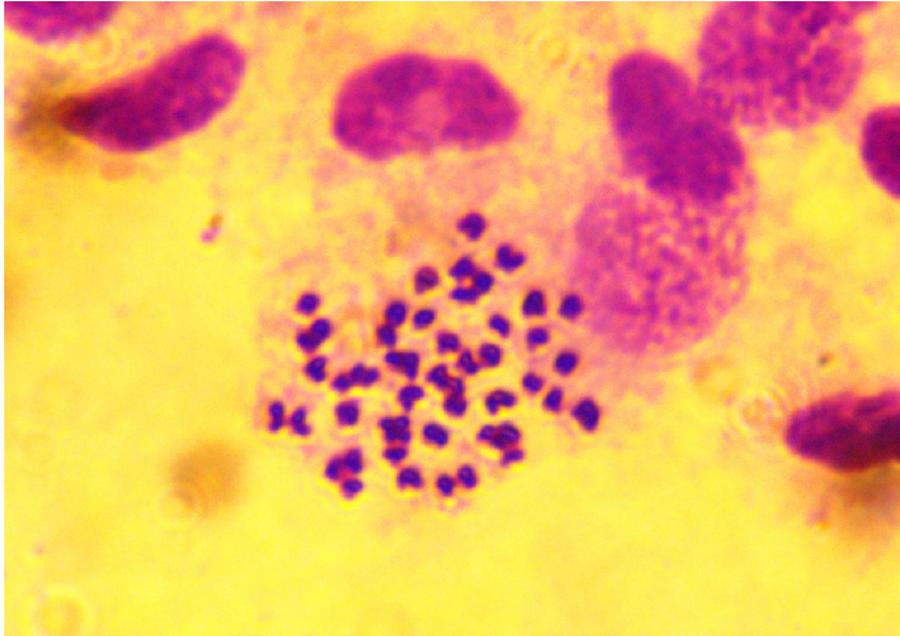
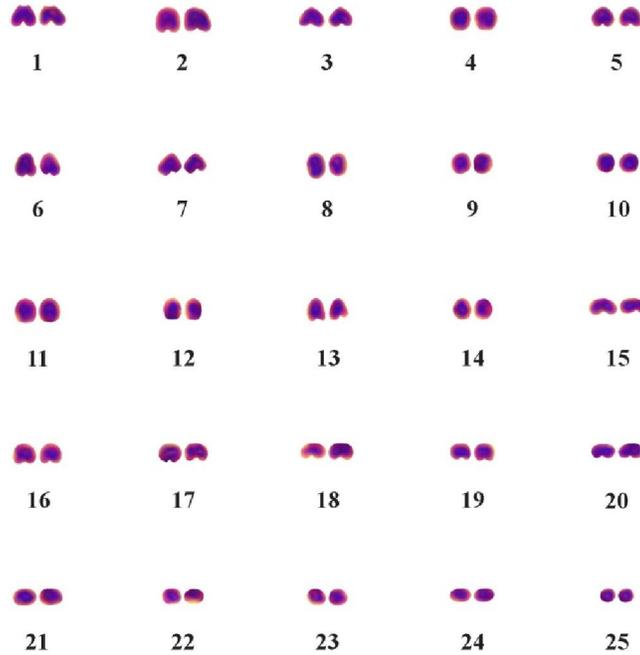


Plate 3: Mitotic metaphase chromosome spreads of *B. parablabe* ($2n=50$)

Plate 4: Karyotype of *B. parablabe*Table 2: The chromosome measurement and nomenclature of *B. parablabe*

Chromosome (n)	Measurement (μm)			Relative length (%)			Centromeric index (100 s/c)	Nomenclature
	Long arm (l)	Short arm (s)	Total length (c)	Long arm (l')	Short arm (s')	Total length (c')		
1	0.2	0.01	0.21	4.96	0.14	5.1	5	Terminal (t)
2	0.19	0	0.19	4.69	0	4.69	0	Terminal (T)
3	0.19	0	0.19	4.69	0	4.69	0	Terminal (T)
4	0.19	0	0.19	4.68	0	4.68	0	Terminal (T)
5	0.19	0	0.19	4.68	0	4.68	0	Terminal (T)
6	0.19	0	0.19	4.66	0	4.66	0	Terminal (T)
7	0.18	0	0.18	4.57	0	4.57	0	Terminal (T)
8	0.18	0	0.18	4.51	0	4.51	0	Terminal (T)
9	0.18	0	0.18	4.41	0	4.41	0	Terminal (T)
10	0.17	0	0.17	4.22	0	4.22	0	Terminal (T)
11	0.17	0	0.17	4.25	0	4.25	0	Terminal (T)
12	0.17	0	0.17	4.22	0	4.22	0	Terminal (T)
13	0.16	0	0.16	4.09	0	4.09	0	Terminal (T)
14	0.16	0	0.16	3.94	0	3.94	0	Terminal (T)
15	0.16	0	0.16	3.92	0	3.92	0	Terminal (T)
16	0.15	0	0.15	3.75	0	3.75	0	Terminal (T)
17	0.15	0	0.15	3.65	0	3.65	0	Terminal (T)
18	0.14	0	0.14	3.53	0	3.53	0	Terminal (T)
19	0.14	0	0.14	3.51	0	3.51	0	Terminal (T)
20	0.13	0	0.13	3.39	0	3.39	0	Terminal (T)
21	0.13	0	0.13	3.3	0	3.3	0	Terminal (T)
22	0.13	0	0.13	3.29	0	3.29	0	Terminal (T)
23	0.13	0	0.13	3.19	0	3.19	0	Terminal (T)
24	0.12	0	0.12	2.96	0	2.96	0	Terminal (T)
25	0.11	0	0.11	2.67	0	2.67	0	Terminal (T)

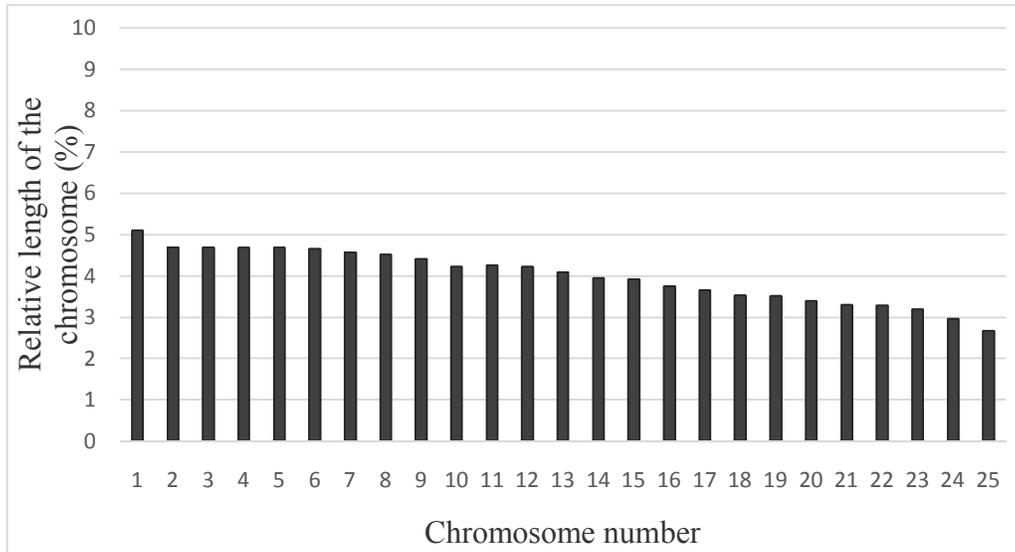


Figure 4: Relative length of the chromosomes of *B. parablades* showing the size variation

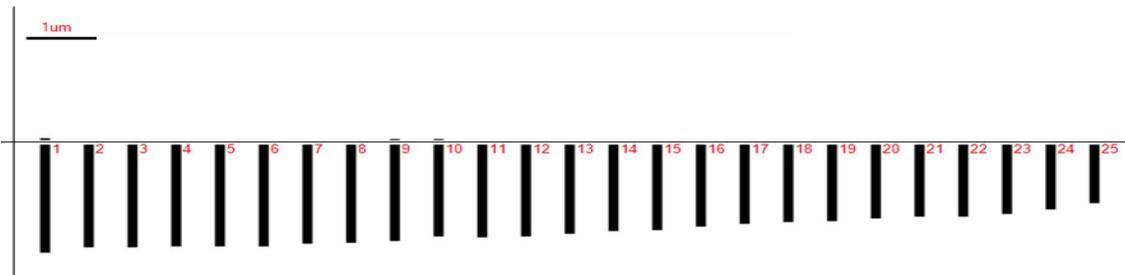


Figure 5: An ideogram of the karyotype of *B. parablades* showing the morphology of the chromosomes. The arrow represents the position of the centromere.

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