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Conventional Karyotypic Lengths in African Giant Rat (*Cricetomysgambianus*, Waterhouse-1840)

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Abstract
Background: *Karyotypic studies were carried out on the African giant rat, Cricetomysgambianus, Waterhouse-1840 with the aim of determining its chromosomal lengths and chromosomal arm lengths.*
Methods*: The chromosomes were prepared from the conventional bone marrow of four (4) African giant rats – males and females treated intra-peritoneally with 2 ml of 0.04% colchicines for 3 hours. Chromosomes in well spread mitotic metaphase cells were counted and measured using KaryoType computer software. Chromosomal numbers were identified and arm lengths were determined from these measurements done in micrometer (µm). Ideograms were also constructed from the measurements. Data were collected and analysed using SPSS version 20.*
Results*: There was gradual decrease in length from one chromosome pair to another. The mean chromosomal arm lengths were statistically significant, with the males having higher values compared to the female. The X chromosomes were medium-sized metacentric and small acrocentric while the Y chromosome was small acrocentric.*
Conclusion*: Cricetomysgambianus was found to have an identifiable autosomal diploid number, and chromosomal lengths that are higher among the male counterparts. The chromosomal lengths of Cricetomysgambianus showed a clear way of differentiating this genus from other genus within its family and from variable rodents within the genera that resembled those in Benin, Senegal, Niger, Cameroun and other countries.*
Keywords*: Cricetomysgambianus, Conventional, Length, Chromosomal arm length.*

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I. Introduction

The African Giant rat (AGR) also known as Gambian pouched rat belongs to the order *Rodentia*, Suborder *Myomorpha*, family *Cricetidae*, subfamily *Cricetomyiane* and genus *Cricetomys*[1]. It is a wild rodent consumed by the rural population in Nigeria. Two species have been recorded in Nigeria, *Cricetomysemni* and *Cricetomysgambianus*, Waterhouse-1840[2].Other Gambian species exist in South Africa and they include *Cricetomysgambianusadventor*, *C. gambianusselindensis*and *C. gambianuscunator*. *Cricetomysemni* is distributed naturally in the rain forest zone and is not associated with human habitation. It is less common than the Gambian Giant rat[2].

The African giant pouched rat, because of its exceptional size and other interesting attributes, is an economically important rodent within Africa.

It is one of the most common mammals exploited as bush meat[3-5] and has been trained to aid in the detection of landmines[6]and also in the medical diagnosis of pulmonary tuberculosis[7].

Several publications, employing alternative techniques such as karyotyping of the African Giant rat[8-11]; plasma biochemical properties of the African Giant rat[12-13]; stereological estimation of the cerebral layers of African Giant rats[14]; multivariate craniometry[15]anatomical and histological studies of the digestive system of the African Giant rat[16] ;morphologic, morphometric and histologic studies of cerebellum and forebrain of the African Giant rat[17] ; morphometric studies of the cerebellum and forebrain of the African Giant rat;[18]morphometric characterization of the African Giant rat (*Cricetomysgambianus*,Waterhouse 1840) in the forest zone of south western Nigeria;[19] weight assessment of some accessory digestive organs in the adult African pouched rat; [20] gross anatomical, histological and histochemical studies of the oesophagus of the African Giant rat; [21] histological and histochemical studies of the colon of the African rat [22] and gross anatomical aspect of gastro-intestinal tract of the wild African giant rat –*Cricetomysgambianus*,[23] have

attempted to provide additional information useful for characterization of the various giant pouched rat species. However, the taxonomic impact of these studies has been of restricted importance because they were conducted on limited specimen collections, underscoring the need for more investigations covering the entire range of these rodents.

The use of molecular methods to provide more insight into the taxonomy and phylogeny of *Cricetomys*was recommended. Preliminary molecular studies involving this genus have helped to clarify its position and relationships with regard to other groups within the rodent superfamily *Muroidea.* [24]

In Benin, a list of rodent species has been published, but uncertainties remain about the taxonomic status of specimens from several genera, such as *Cricetomys*, *Tatera*, *Mastomys* or *Mus*, due to the absence of cytotaxonomic data. [25] Some chromosomal data also exist for the rodents of Benin, concerning the genera *Cricetomys*,[9]*Tatera*, [9,26]*Arvicanthis.* [27, 28]

Until recently karyological investigations on rodents of Senegal have mainly focused on *Gerbillidae*, and particularly the genera *Tatera*and*Taterillus.* These karyological studies made it possible to distinguish two sibling species of *Taterillus*, namely *T. gracilis*, with a diploid number of 2n = 36/37, and *T. pygargus*with (2n = 22/23), [29-31] and to characterize the two species of *Tatera*, *T. gambiana*(2n = 52)and *T. guineue*(2n = 50).[29, 32, 33] In the family *Muridae*, data on the two previously known species of *Mastomys*, *M. erythroleucus*(2n = 38) and *M. huberti*(2n = 32) have been published, [34] but recent extensive studies of *Mastomys* genus in Senegal revealed the presence of a third species, *M. cfnatalensis*, morphologically indistinguishable from *M. huberti*, and having the same diploid number, but with distinct ecological preferences and a specific autosomal fundamental number (NFa = 54 versus 44) for *M. huberti.*[35-37]

II. Materials And Methods

This study was conducted on 4 adult African Giant rats (*Cricetomysgambianus*, Waterhouse-1840) of both sexes that were captured alive in the wild around Zaria City, Kaduna State, Nigeria using a local metal cage traps without inflicting injuries on them. They were housed in customized laboratory rat cages in the animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria and fed with fruits, groundnut pellets and water was given *ad libitum* for a week prior to commencement of study. [38-40]

The rats were euthanized using anaesthetic chloroform in a confined container and weighed using a balance (EMPEROR model p.1210), made in Chandler, Arizona, United State of America (USA), with a sensitivity of 0.1 g. The live specimens were taken to the laboratory and injected intraperitoneally with 2 ml of 0.04% colchicine solution) prepared with sterile physiological saline. After a period of 3 hours of injection, the specimens were sacrificed using cervical dislocation.

Dividing cells were obtained from the marrow of the femur and tibia, dissected out in accordance with the methodology described by. [41- 43] Both ends of the femur and tibia were cut open, and a hypodermic needle attached to a syringe containing 1 – 1.5 ml of freshly prepared and pre-warmed (37^oC) hypotonic buffer (0.55% KCl) was inserted. The marrow was flushed out into a 15 ml centrifuge tube.

Freshly prepared fixative (3:1 methanol – glacial acetic acid) was added drop wise, with quick agitation after each drop to re-suspend the cells. A total of 2.0 – 2.5 ml of the fixative were added.

From a height of about 1 metre, two or more drops of the cell suspension were allowed to fall on the slides uniformly. The slides were blown quickly across its length and placed on a slide warmer set at 60^0C, and were allowed to dry for about 24 hours before staining. The slides were flame dried for normal metaphase cells and air dried completely before staining. [44]

Giemsa powder (0.5 g) was dissolved in 33 ml glycerol and put in Erlmyer bottle in a dark compartment overnight. It was heated in a water bath set at 60^oC for 2 hours and was allowed to cool, after which 33 ml of methanol was added. This solution was stored in an amber coloured bottle as the stock Giemsa stain. G-bands were performed according to Seabright. [45] The slides were stained for 20 minutes, and rinsed in distilled water to remove the stain.

The chromosomes of an African giant rat were arranged in order of decreasing size, and the karyotype of*Cricetomysgambianus*were arranged in accordance with the report of Granjon*et al*.[8]

Ethical approval and permission were obtained from Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Ethics Committee for conducting the study.

All statistical analyses were performed on an SPSS version 20.0 software package (SPSS Inc., Chicago, Illinois, USA). Data were presented as Mean ± SEM. One way analysis of variance (ANOVA) was used to compare the mean differences. P-values of less than 0.05 were considered to be statistically significant.

III. Results

Figure 1 shows a combination of 12 groups of chromosomes forming a heterogenous pattern with first group having large chromosomes numbered 1-6, which were apparently clearly visible and identifiable on the

metaphase spread measuring 15.02 µm, 10.12 µm, 5.01 µm, 4.14 µm, 3.36 µm and 2.96 µm, respectively. It is followed by other chromosomal groups appearing in decreasing order of size, 7 to 8, 9 to 11, 12 to 13, 14 to 15, 16 to 19, 20 to 22, 23 to 24, 25 to 27, 28 to 29, 30 to 33 and 34 to 38. The lengths were as follows; 2.77 µm, 2.57 µm, 1.79 µm, 1.58 µm, 0.80 µm, 0.79 µm, 0.62 µm, 0.59 µm, 0.39 µm, 0.28 µm, 0.26 µm and 0.19 µm, respectively. X chromosome measured 5.73 µm while Y chromosome measured 1.58 µm.

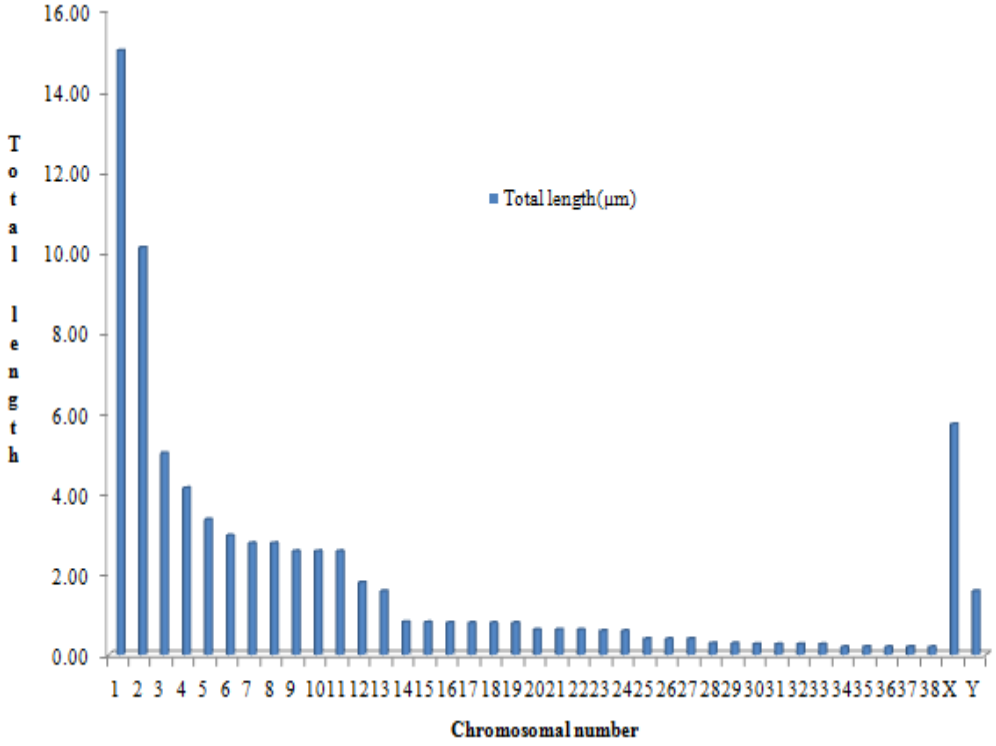

Figure 1: Total chromosomal length and number of a male *C. gambianus*

Figure 2 shows the morphology of the chromosome for *C. gambianus.* The chromosome 1 was the largest chromosome in the series and the chromosomes 34 to 38 were the smallest within the complement.

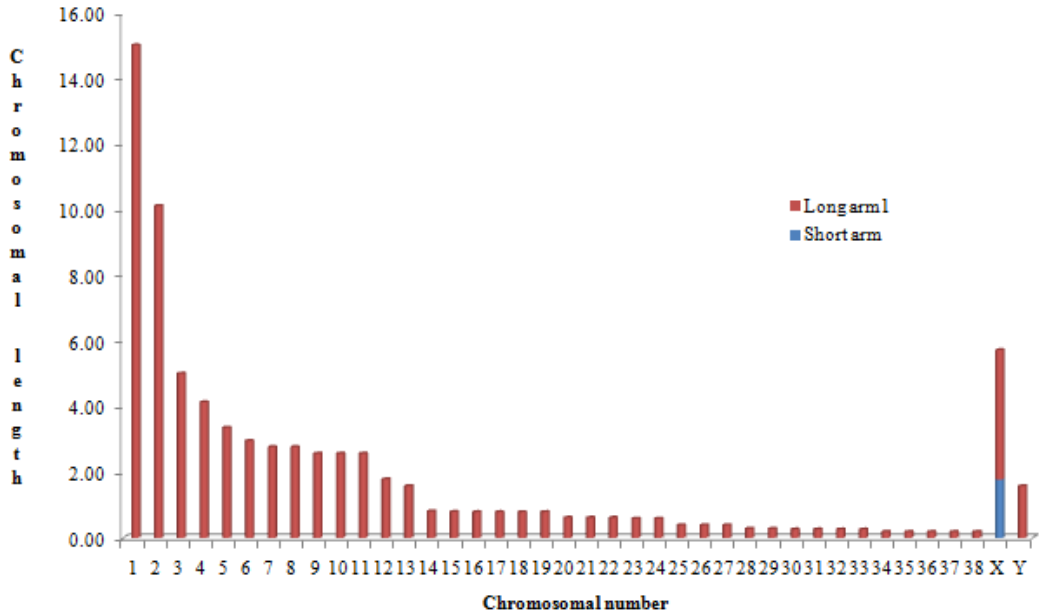

Figure 2: Ideograms of a male *C. gambianus* showing the chromosomal number and length. The colour demarcated area presented the centromeric position

Figure 3 shows a combination of 23 groups of chromosomes forming a heterogenous pattern with first group having large chromosomes numbered 1-5, which were always clearly visible and identifiable on the metaphase spread measuring 9.71 µm, 6.52 µm, 6.31 µm, 5.33 µm and 5.33 µm, respectively. It is followed by other chromosomal groups appearing in decreasing order of size, 6, 7, 8, 9, 10, 11, 12, 13, 14 to 15, 16 to 17, 18 to 19, 20, 21, 22, 23 to 26, 27, 28, 29 to 30, 31, 32, 33 to 35, 36, 37 having the following lengths; 4.61 µm, 4.54 µm, 3.79 µm, 3.55 µm, 3.40 µm, 3.36 µm, 3.23 µm, 3.16 µm, 2.76 µm, 2.59 µm, 2.57 µm, 2.56 µm, 2.37 µm, 2.36 µm, 2.17 µm, 2.03 µm, 1.98 µm, 1.97 µm, 1.82 µm, 1.79 µm, 1.18 µm, and 0.79 µm, respectively. The X chromosome measured 9.48 µm while Y chromosome measured 5.97 µm.

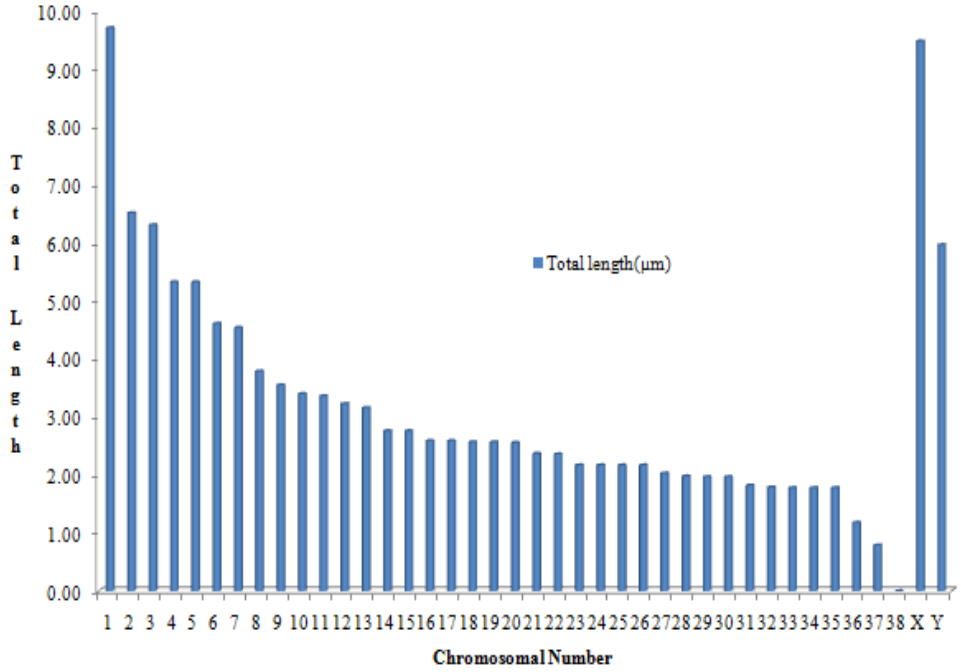

Figure 3: Total chromosomal length and number of a male *C. gambianus*

Figure 4 shows the morphology of the chromosome for *C. gambianus* having 30 terminal, 2 subterminal, 6 median and 1 submedian nomenclature. The chromosome number 1 was the largest chromosome in the series, while the chromosomes number 37 was the smallest within the complement.

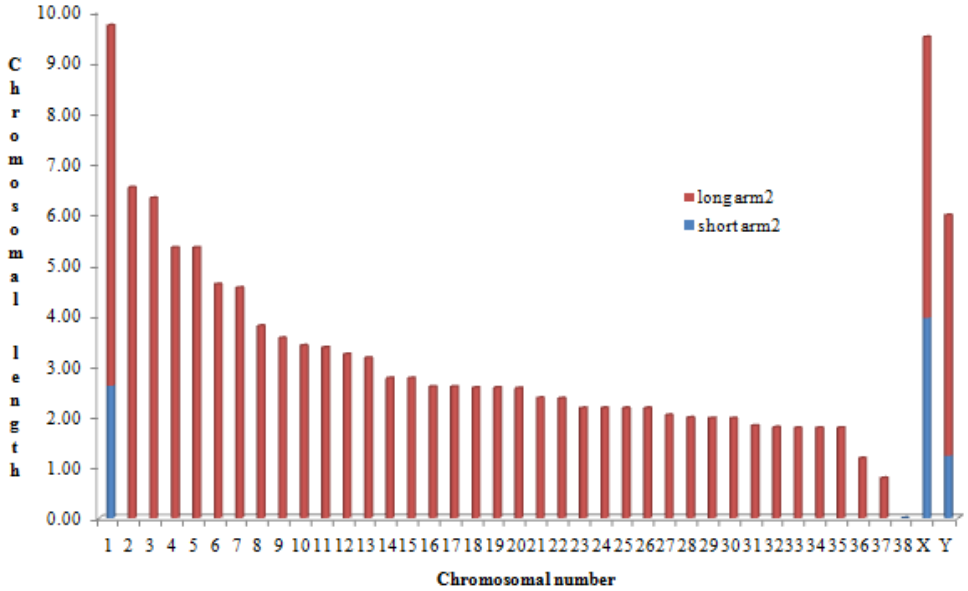

Figure 4: Ideograms of a male *C. gambianus* showing the chromosomal arm lengths and number.Thecolour demarcated area presented the centromeric position

Figure 5 shows a combination of 30 groups of chromosomes forming a heterogenous pattern with first group having large chromosomes numbered 1-6, which were apparently clearly visible and identifiable on the metaphase spread and measuring 15.22 µm, 9.29 µm, 8.06 µm, 7.10 µm, 6.01 µm and 5.91 µm, respectively. It was followed by other chromosomal groups appearing in decreasing order of size, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 to 17, 18, 19 to 20, 21, 22 to 23, 24, 25 to 27, 28, 29, 30, 31, 32, 33, 34 to 35, and 36 to 37. The lengths were as follows: 4.93 µm, 4.54 µm, 4.48 µm, 4.40 µm, 4.39 µm, 4.35 µm, 3.95 µm, 3.56 µm, 3.45 µm, 3.40 µm, 3.16 µm 2.96 µm, 2.81 µm, 2.77 µm, 2.76 µm, 2.57 µm, 2.56 µm, 2.37 µm, 2.18 µm, 1.98 µm, 1.97 µm, 0.81 µm, 0.39 µm and 0.28 µm respectively. Both X chromosomes measured 15.99 µm and 16.06 µm, respectively.

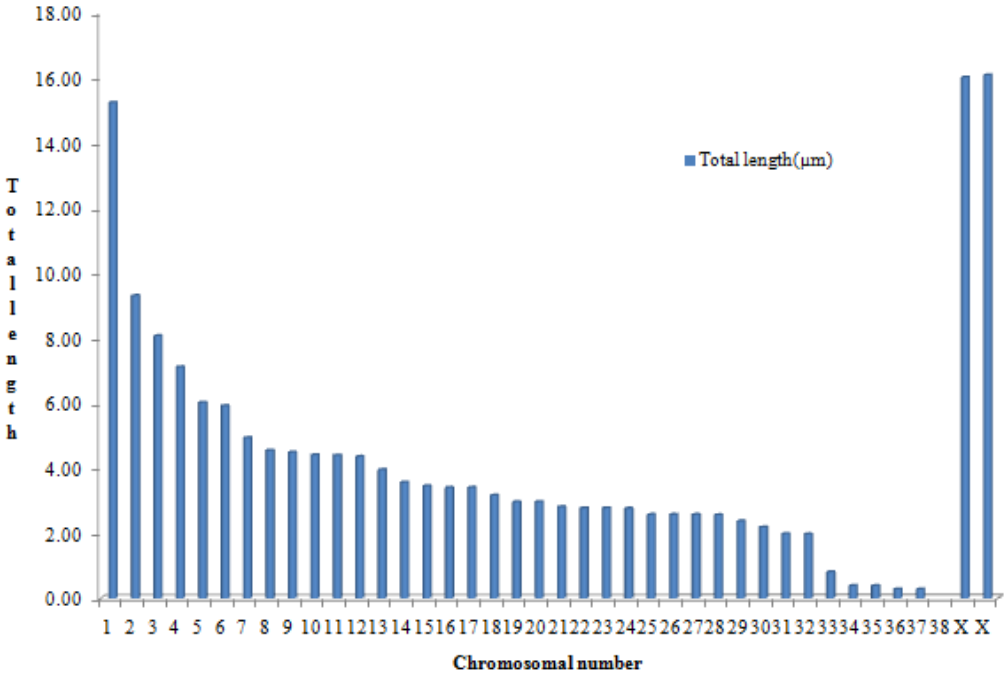

Figure 5: Total chromosomal length and number of a female *C. gambianus*

Figure 6 shows the morphology of the chromosome for *C. gambianus.* The sex chromosomes were the largest in the series and the chromosomes 36 to 37 were the smallest within the complement.

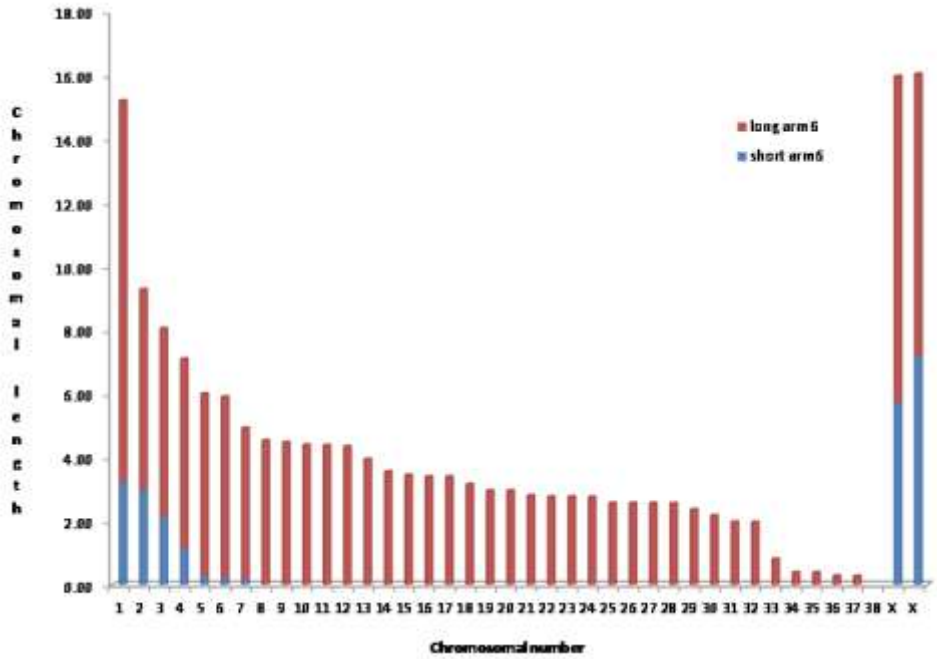

Figure 6: Ideograms of female *C. gambianus* showing the chromosomal arm lengths and number. The colour demarcated area presented the centromeric position

Figure 7 shows a combination of 18 groups of chromosomes forming a heterogenous pattern with first group having large chromosomes numbered 1-3, which were apparently clearly visible and identifiable on the metaphase spread, measuring 5.27 µm, 2.55 µm and 2.32 µm respectively. It was followed by other chromosomal groups appearing in decreasing order of size, 4, 5 to 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 to 16, 17 to 18, 19 to 23, 24 to 28, 29 to 32 and 33 to 37. The lengths were as follows: 5.27 µm, 2.55 µm, 2.32 µm, 2.23 µm, 2.12 µm, 2.02 µm, 1.92 µm, 1.72 µm, 1.52 µm, 1.21 µm, 1.01 µm 0.93 µm, 0.91 µm, 0.61 µm, 0.40 µm, 0.20 µm, 0.14 µm, and 0.10 µm, respectively. Both X chromosomes measured 10.63 µm and 4.60 µm, respectively.

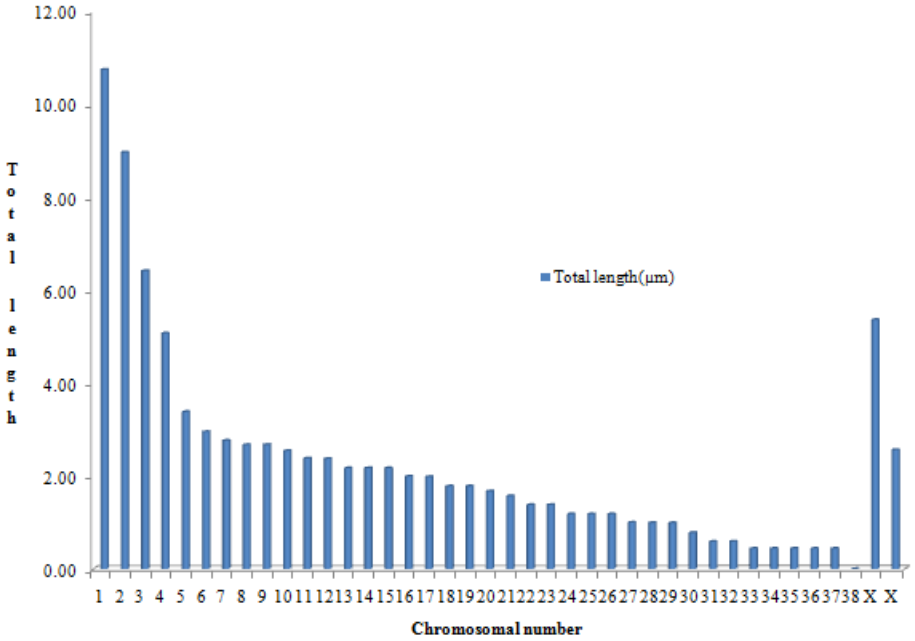

Figure 7: Total chromosomal length and number of a female *C. gambianus*

Figure 8 shows the morphology of the chromosome for *C. gambianus.* The sex chromosomes were the largest in the series, while the chromosomes 33 to 37 were the smallest within the complement.

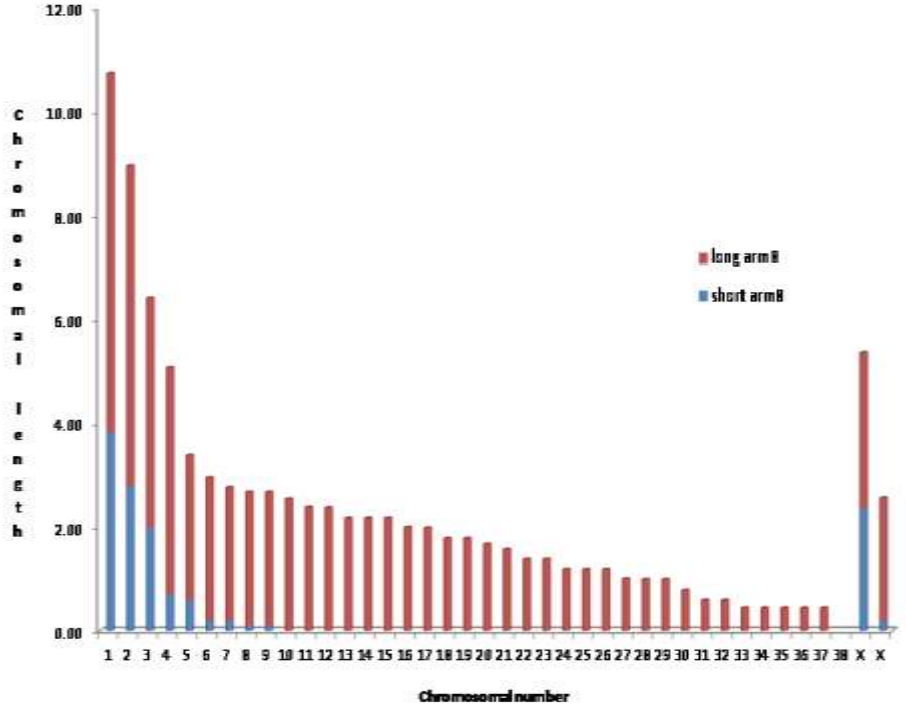

Figure 8: Ideograms of a female *C. gambianus* showing the chromosomal arm lengths and number. The colour demarcated area represents the centromeric position.

The mean chromosomal arm lengths of *Cricetomysgambianus*

Table 1 shows the chromosomal arm lengths of the male *C. gambianus* with their respective ranges. The long arms were higher than the short arms, and their mean and SEM were 0.04 ± 0.04 µm, 0.19 ± 0.12 µm for the short arms and 1.86 ± 0.45 µm, 3.05 ± 0.25 µmfor the long arms. The mean values of the long chromosomal arms shows a highly statistical significance ($P < 0.05$), but not significant for the short arms, while the mean lengths for the female rats were 0.58 ± 0.25 µm, 0.14 ± 0.10 µm for the short arms and 3.85 ± 0.40 µm, 1.15 ± 0.23 µm for the long arms.The mean values of the long chromosomal arms were statistically significant ($P < 0.05$), but not statistically significant ($P > 0.05$) among the short arms.

Table 1: Chromosomal arm lengths of *Cricetomysgambianus*

Pairs	Chromosomal Arm	Maxi-Mini	Mean ± SEM	P value
Male	Short arm1	1.78 - 0.00	0.04 ± 0.04	0.09
	Long arm1	15.02 – 0.19	1.86 ± 0.45	0.01*
Male	Short arm2	3.95 – 0.00	0.19 ± 0.12	0.12
	Long arm2	7.10 – 0.79	3.05 ± 0.25	0.00*
Female	Short arm6	7.18 – 0.00	0.58 ± 0.25	0.19
	Long arm6	12.01 – 0.28	3.85 ± 0.40	0.00*
Female	Short arm7	3.74 – 0.00	0.14 ± 0.10	0.17
	Long arm7	6.89 – 0.10	1.15 ± 0.23	0.00*

Data presented as Mean ± SEM

IV. Discussion

In the present study, the chromosomal arm lengths of the male *C. gambianus* with their respective ranges indicated that long arms were higher than the short arms, and their values were close to those found by Akintoye and Awopetu[46] for the genus *C. emni*, but and less than those obtained in the black rat by Amaka. [47] The chromosomal length was found to be in decreasing order of size, which is in line with the values reported by Levan*et al*. [48] In the present study, the total length of each chromosome in male rats showed that the mean total length of each chromosome was longer than in the females; this was similar to those found by Akintoye and Awopetu[46] but for the genus *C. emni*, as such finding was absent on the chromosomal measurement of the genus *C. gambianus*.

Comparing the chromosomal arm lengths of (both males and females) *C. gambianus*, based on the present findings, the mean chromosomal arm lengths of the male rats were significantly ($P < 0.05$) higher than those of their female counterparts. However, the chromosomal short arms were not significantly different. It has been obtained that chromosomal length is independent of sexes, and the total chromosomal lengths in the rats were different ($P < 0.05$) from what was found in other rodents within the same family. These findings are similar with results obtained by Akintoye and Awopetu[46] in *C. emni*, and Zhi-Ping *et al*. [49] in pea's tree rat (*Chiromyscuschiropus*), but no sexual dimorphism was reported in their study.

V. Conclusion

Cricetomysgambianus was found to have same diploid chromosomal number 2n = 80 and chromosomal lengths that are higher among the male counterparts.

The chromosomal lengths of *Cricetomysgambianus* showed a clear way of differentiating this genus from other genus within its family and from variable rodents within the genera.

Chromosomal analysis shows that males seem to have higher chromosomal arm lengths compared to their females counterparts. It has been noted to be independent of sexes, which shows a highly significant finding (P value < 0.05).

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CONFLICTS OF INTEREST

No conflict of interest

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