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COMPARATIVE CYTOGENETIC STUDY AMONG *PARACHANNA OBSCURA* POPULATIONS FROM SELECTED WATER BODIES IN SOUTH WEST, NIGERIA

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ABSTRACT

Parachanna obscura is a freshwater Perciform species that belong to the family Channidae. In contrast to many other fish species, the genetic information about *P. obscura* as a species is very scarce and its chromosome compliments in different confined populations all over remain undiscovered. In view of this, this study considered the cytogenetic variations among three populations of *P. obscura* collected from River Elemi, Ado-Ekiti, Ekiti State, Egbe-Ekiti Reservoir, Egbe, Ekiti State and Esa-Odo Reservoir, Esa-Odo, Osun State, Nigeria. Each sampled fish was injected with 0.05% Colchicines solutions (1.0ml/kg), metaphase chromosome spread were made from the head kidney of each sample. Slides were prepared using conventional Giemsa staining method while digital images of the metaphase spreads were taken. Study revealed that all *P. obscura* from the three different population has the same diploid chromosome number 2n=46. The chromosome length range from River Elemi, Ado-Ekiti (6.10-14.91 µm), Egbe water reservoir, Egbe-Ekiti (5.91-16.06 µm) and Esa-odo water reservoir, Esa-Odo (6.24-14.31 µm), were very close thus exhibiting homomorphic character with respect to total length. Karyotypic formula differs among populations of River Elemi, Ado-Ekiti (2n= 2sm + 44t), Egbe water reservoir, Egbe-Ekiti (2n=4sm + 2st + 40t) and Esa-odo water reservoir, Esa-Odo (2n=2st + 44t), this implies that the three populations were navigating different evolutionary course resulting from fragmentation and isolation of populations. In conclusion further molecular cytogenetic studies may add more to the view presented in this study.

Keywords: *P. obscura*, Chromosomes, Karyotype, Metaphase spread, Cytogenetics

INTRODUCTION

Parachanna obscura often referred to as African snakehead is a freshwater Perciform fish widely distributed in the African intertropical water. *Parachanna* and *Channa* are two extant genera belonging to the family Channidae (Courtenay and Williams, 2004). Genus *Parachanna* has three species which are confined to tropical Africa while more than forty species of *Channa* has been documented up to date and they are mainly distributed in the waters of southern Asia (Fricke *et al.*, 2019; Froese and Pauly, 2022)

The fish (*P. obscura*) which is of immense economic value is being faced with population decline resulting from climatic change, overfishing and anthropogenic threats (Lalèyè 2020; Amoutchi *et al*., 2021). *P. obscura* reproduces almost throughout the year, exhibit rapid growth and withstand stress (Isangedighi and Umoumoh, 2011; Olaosebikan and Raji, 1998). The local demand for *P. obscura* is beyond what natural continental water already overexploited can supply (Kpogue *et al.*, 2013). The way out is the cultivation of *P. obscura* in culturing systems that can help in preservation and improvement of natural stock in Africa (Azon *et al*., 2020).

The usefulness of cytogenetics in fish cannot be overemphasized as it is an important tool in the study of fish ploidy determination, evolution, mutagenesis and taxonomy of fish species (Foresti *et al.*, 1993; Demirok and Unlu 2001). Tropics holds large fish biodiversity yet cytogenetic information is still not available for several fish species (Dotti do Prado *et al.*, 2021). Khuda-Bukhsh *et al.*, (1986), Dhar and Chatterjee, (1984) reported that different populations of the same species can differ in chromosome number and morphology as observed in *C. orientalis*, likewise among individuals of the same population where variation can exist in chromosome numbers and morphology (Dhar and Chatterjee 1984)

During Eocene, snakefish went through multiple range expansion and repeated communication with lineages that had diverged in isolation, this reflected in their karyotypic diversity (Adamson *et al*., 2010; Rüber *et al.*, 2020). The cytogenetic study of *channa* species revealed a chromosome number ranging between 2n=32 and 2n=112 and chromosome arms between NF=46 and 116 (Kumar *et al*., 2019). This shows the level of evolutionary dynamism in the genus *channa*. Obviously, the genus *Parachanna* has not been well studied like the *channa* to expose its evolutionary dynamism. Prazdnikov (2023) explained that chromosome number and morphology of different isolated populations of extensive *channa* species remained unassessed and the course of karyotypic transformation is inadequately understood. It is with the lack of adequate information on *parachanna* and its karyotypic diversities that this study sought to elucidate possible variation in the chromosome morphology and karyoptypic fomular of *P. obscura* from three different populations. This will contribute to basics for the study of it's evolution, mutagenesis, conservation and ploidy manipulation.

MATERIALS AND METHODS

Collection of Fish Samples

Samples of *P. obscura* used were collected from River Elemi, Ado-Ekiti, Ekiti State, Egbe Water Reservoir, Egbe-Ekiti, Ekiti State and Esa-Odo Water Reservoir, Esa-Odo, Osun State. The water bodies are major ones and are geographically far apart from one another indicating separation of each of the water bodies from the other. It is imperative to compare *P. obscura* population in Egbe water reservoir with other

populations since it has not been recorded. At different landing site of River Elemi, Ado-Ekiti, Egbe water reservoir, Egbe-Ekiti and Esa-odo water reservoir, Esa-Odo, life samples of *P. obscura* were collected from fishermen and transported (using mobile holding tanks in vehicle) to the Laboratory of Department of Biological Sciences, University of Ilesa, Ilesa, Osun State, Nigeria. The fish collected were identified as specified by Kpogueet *et al.*, (2013).

Metaphase Chromosome Spread procedure

Each of the sample fish were injected intraperitoneally with 0.05% Colchicines solution to arrest the fish cells at metaphase. A dosage of 1ml/kg was given to each of the fish. Fish were left in holding tanks for a period of three hours before they were sacrificed. The fish were decapitated and the abdomen was cut opened while the fish remained with the dorsal part resting on dissecting tray. The head kidney of each fish was removed and placed in to separate mortal containing 0.56% Potassium Chloride solution. The head kidney were macerated in the Potassium Chloride solution and left for forty minutes. Supernatant from the macerated content were poured into centrifuge tubes and centrifuged at 1000rpm for 10minutes. The centrifuge tubes were removed from the centrifuge machine and supernatant from its content were removed using Pasteur pipette leaving harvested cells at the bottom of the tubes. Fixation of the harvested cells was done using freshly prepared fixative (3:1 Methanol: glacial Acetic acid). The tubes were filled with 8ml of fixative (3:1 Methanol: glacial Acetic acid) each and left for thirty minutes after which the tubes were centrifuge for 7minutes at 1000rpm. For the second time the supernatant from the tube were discarded using Pasteur pipette. Thereafter the procedure of refilling with fixative, centrifuging and remover of supernatant were done three times. Lastly the harvested cells at the bottom of the tubes were suspended by adding few ml of fixative (3:1 Methanol: glacial acetic acid).

Karyotyping

With the aid of Pasteur pipette, sample of suspended cell were taken from the tubes and dropped at a distance of 15cm on pre-warmed slides. The slides were warmed to dryness on slide warmer at 60°C for 24hours. The slides were stained in Giemsa stain (6%) between 20-22 minutes. The slides were washed in distilled water before drying on electronic slide warmer at 60°C for 24hours. The slides were viewed on an Olympus binocular microscope (CX23 made in China by Ningbo Movel Scientific Instrument Co Ltd.) in search of well spread metaphase chromosomes. Good metaphase spread pictures were captured using digital camera with model number SCMOS05000KPA. Chromosome morphology was classified following Levan *et al*. (1964). GIMP Corel draw professional XIII was used for measurement of total lengths and arms of chromosomes.

RESULTS AND DISCUSSION

All the *P. obscura* from different populations of River Elemi, Ado-Ekiti, Egbe Water Reservoir, Egbe-Ekiti, and Esa-Odo Water Reservoir, Esa-Odo had diploid chromosome number of 2n=46. The diploid chromosome numbers were established from the metaphase spread of *P. Obscura* from River Elemi (Fig1), Egbe water reservoir (Fig 2) and Esa-Odo water reservoir (Fig 3). The chromosomal formula, total chromosomes length, average chromosomes length and chromosomes length range in each population of *P. obscura* from River Elemi, Egbe Water Reservoir and Esa-Odo Water Reservoir are described in Table 1. The karyotypes of *P. obscura* from River Elemi, Egbe Water Reservoir and Esa-Odo Water Reservoir are shown in fig 4,5 and 6 respectively.

Table 1: Comparative Giemsa analysis of *P. Obscura* from different populations

Collection site	2n Chromosomes	Karyotypic formula	Total length of chromosomes (µm)	Average length of chromosomes (µm)	Chromosome length range (µm)
River Elemi	2n=46	2n= 2sm + 44t	448.10	9.74	6.10-14.91
Egbe Water Reservoir	2n=46	2n=4sm + 2st + 40t	467.75	10.16	5.91-16.06
Esa-Odo Water Reservoir	2n=46	2n=2st + 44t	457.57	9.94	6.24-14.31

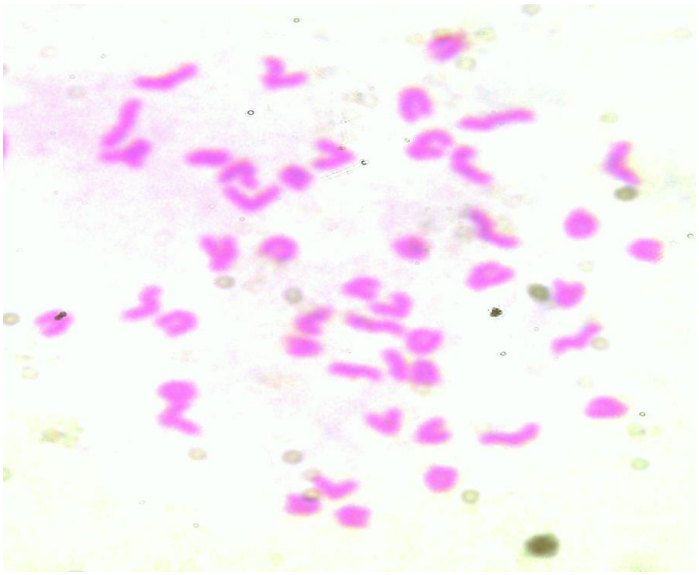

Figure 1: Metaphase chromosome spread of *P. Obscura* from River Elemi, Nigeria

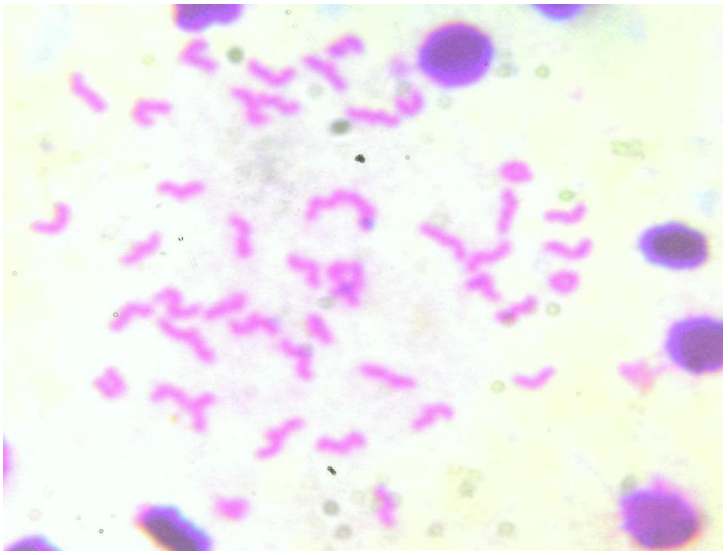

Figure 2: Metaphase chromosome spread of *P. Obscura* from Egbe water reservoir

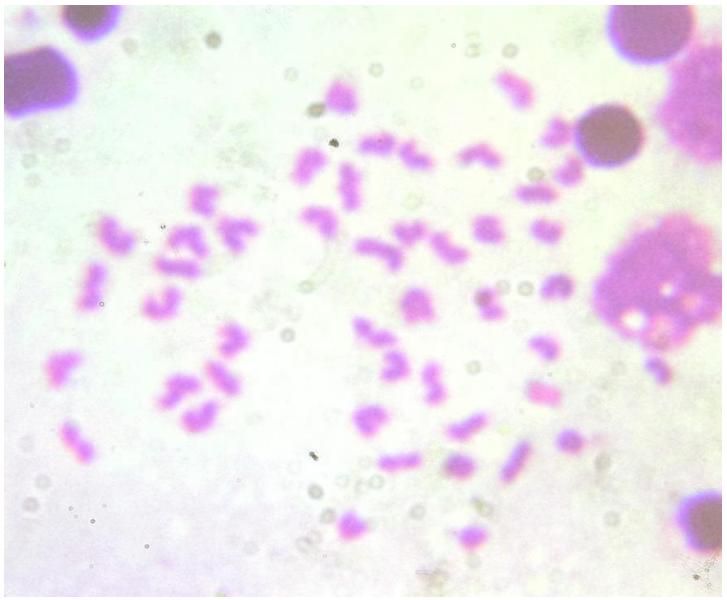

Figure 3: Metaphase chromosome spread of *P. Obscura* from Esa-Odo Water Reservoir

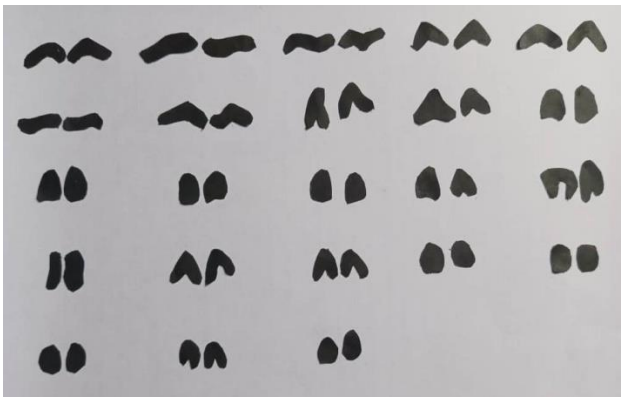

Figure 4: Karyotype of *P. Obscura* from River Elemi, Nigeria

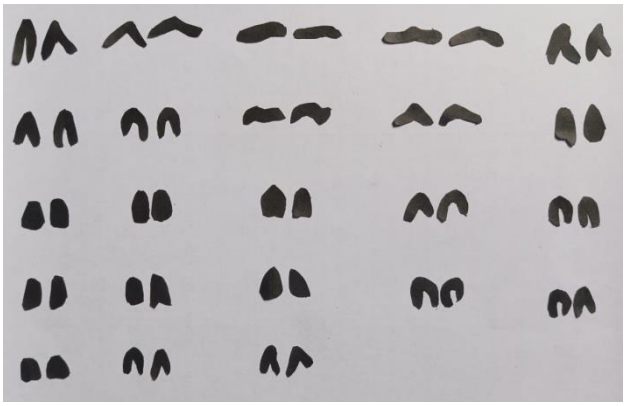

Figure 5: Karyotypes of *P.Obscura* from Egbe Reservoir, Nigeria

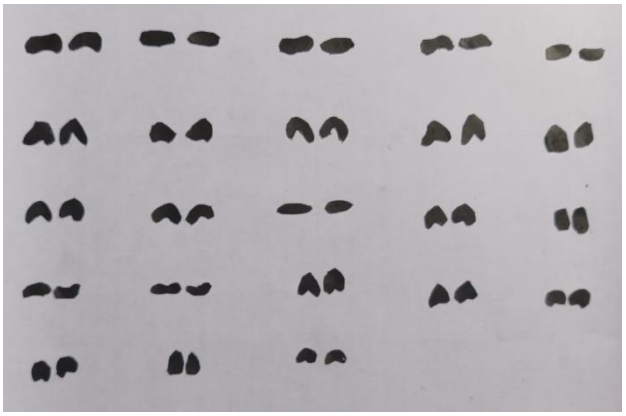

Figure 6: Karyotypes of *P. Obscura* from Esa-Odo Reservoir, Nigeria

P. Obscura from River Elemi, Ado-Ekiti, Ekiti State, Egbe Water Reservoir, Egbe-Ekiti, Ekiti State and Esa-Odo Water Reservoir, Esa-Odo, Osun State were found to possess same chromosome numbers of 2n =46 and chromosome morphology were not sexually differentiated. The only existing diploid chromosomal number of *P. obscura* available in record was described by Ola-Oladimeji *et al.* (2020), who stated that diploid chromosome number of 2n=44 and karyotypic formular 10m+12sm+20a+2t. The differences in their result and the present study might be associated with the fact that fish chromosome sizes are too small thus resulting in counting and identification problem. Neto *et al*. (2010) described both *Astyanax altiparanae* populations analysed (Feijão and Pântano streams) as being homomorphic with respect to karyotypic formular 6m+28sm+4st+12a and 6m+30sm+8st+6a. However the three populations analyzed in this study have variations with respect to the karyotypic formula (table 1). The differences observed in the karyotypic formula might have aroused due to chromosomal rearrangements resulting from population isolation in the course of evolution, as stated by Chu and Bender 1961, Campos and Cuevas 1997. These earlier study reported that chromosomal formula variation can be a product of chromosomal aberration or rearrangements due to population isolation during evolutionary process. Variation in karyotypic formular among populations of *P. obscura* might be synonymous to variation in *channa* species, their karyotypic variation was referred to as a product of life style and extensive geograpical distribution of the species, hydrographic and geographical activities in their habitat resulting from fragmentation and isolation of populations (Tan *et al.*, 2012; Robert *et al.*, 2019). The differences observed in the karyotypic formula indicated that each of the population towed different evolutionary course (Neto, 2010).

The uniformity in the diploid chromosome number 2n=46 among the *P. obscura* populations examined is evidence of possible success in hybridization as reported in the Clariid species by Eyo (2005).

Going by the chromosome length range of the three populations: 6.10 µm -14.91 µm, 6.24 µm -14.31 µm and 5.91 µm -16.06 µm, the ranges are very close thus the chromosomes of the three populations can be referred to as homomorphic in respect to chromosome length. Non of the karyotypes from the different populations shows advancement over the other since each exhibit relatively close number of telocentric and acrocentric chromosomes as reported by Stebbins (1971) they reported that presence of acrocentric and telocentric chromosomes indicates an advanced character that is they resulted from the fussion of metacentric chromosomes (Stebbins, 1971).

CONCLUSION

This study has revealed the basic information of the karyotypic features and karyotypic interpopulation similarities and difference among the cytologically yet undescribed three populations of *P. obscura*, from Elemi River, Ado-Ekiti, Ekiti State, Egbe Water Reservoir, Egbe-Ekiti, Ekiti State and Esa-Odo Water Reservoir, Esa-Odo, Osun State. It has established the constant diploid chromosome number of 2n=46 each of the populations which is good characteristics for identification and thus classification of P. obscura. The variation in the karyotypic formula is a reflection of different evolutionary course followed by each population. It is believed that further study in existing genetic diversities within and among the populations can be further revealed through molecular cytogenetics hence adding to the existing view on the comparative cytogenetics of *P. obscura* among populations.

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