Preliminary Cytogenetic Study of *Ctenopoma kingsleyae* from Esa-Odo Water Reservoir, Osun State, Nigeria

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Abstract *Ctenopoma kingsleyae* belongs to the family Anabantidae covers a wide geographic region. It has been morphologically described by a number of authors, yet it is lacking in adequate cytogenetic information, which is a setback to understanding its karyotypic evolution. This study, therefore, describes the karyotype of *C. kingsleyae* (n = 10) from Esa-Odo, Reservoir, Osun State, Nigeria. Metaphase chromosome spread was obtained from the gills of fish after intraperitoneal injection with 0.05% colchicines. Slides prepared were stained with Giemsa stain, images of metaphase spread were taken digitally and a karyogram was prepared. The study revealed a karyotypic formula of 2n = 48t with uniarmed chromosomes. This study has provided needful information on *C. kingsleyae* karyotype and will serve as the basis for further study on its evolution, genetic resource management and production.

Keywords: Chromosomes, karyotypic formula, metaphase spread, Giemsa stain

Introduction

All over freshwater of the tropics of Africa and Asia, the Anabantoid fishes are well distributed, peculiar to Africa is the genus *Ctenopoma* Peters, 1844 (Anabantidae). They cover the Sahara and Cape Region (Norris & Douglas, 1992).
Ctenopoma kingsleyae Gunther, 1896 is a deep water species, often occurring in waters with low dissolved oxygen level such as marshes and swamps (Norris & Douglas, 1992). They describe *C. kingsleyae* as having a single multiple folded labyrinth organ; dorsal XVI-XVIII/10-13; anal VIII-IX/ 10-12; the presence of 11-16 scales on either opercula and scales below the upper lateral line as 7-8 (rarely 9). Also, it is characterized by a non-protrusible jaw, a laterally compressed body, translucent grey pelvic fins and a short caudal peduncle.

Ever since the study of the genus *Ctenopoma*, there has been classification and reclassification of the group. *Ctenopoma* was previously classified into three species: C. congicum, C. multispine and C. petherici (Norris & Teugel, 1990; Norris & Douglas, 1992); each of the three species was described by Norris and Douglas, 1991 as being likely monophyletic but the genus itself as potentially paraphyletic. Subsequently, another genus has been established. Extensive study of cytogenetics of fish as compared to the other vertebrates has not been done, this might be due to typical nature of fish chromosomes.

Kabir et al., 2012 reported karyotypes of *Anabas testudineus* as 2n=46, other members of family Anabantidae such as *Microctenopoma ansorgei, M. congicum and M. pekkolai* have chromosome numbers 2n=46, 2n=46 and 2n=48 respectively (Krysanov & Golubtsov, 2001). Karyotype of 2n=48 was reported as common to *Ctenopoma acutirostre*, *C. muriei*, *C. ocellantum*, and *C. petherici* (Krysanov & Golubtsov, 2001). The fish Ctenopoma kingsleyae is of commercial importance as an aquarium and food fish, but there is no documented plan concerning its conservation (Dankwa et al.,2020); hence, it appears that less information is available with respect to its karyotypic data and description; this unavailability of information is the main reason for the present study. This research sought to give the karyotpic

description of *C. kingsleyae* from Esa-Odo reservoir, Osun State, Nigeria.

Materials and methods

Fish Collection

A total of ten (10) specimens of *Ctenopoma kingsleyae* (Figure 1) were collected from local fishermen fishing in Esa-Odo reservoir, Esa-Odo, Osun State, Nigeria. The fish specimens were transported to the laboratory of University of Ilesa, Ilesa, Osun State, Nigeria. The fish were stocked in separate holding tanks in preparation for chromosomal analysis.

Procedure for Obtaining Metaphase Chromosome Spread

Specimen stocked into the holding tank was injected intraperitoneally with 0.05% colchicines solution at the rate of 0.1ml/kg. The fish was returned into the holding tank and then sacrificed after three hours. The fish were too small to access the anterior portion of the kidney, thus gill filaments were removed and placed in a beaker containing 0.56% potassium chloride for a period of 40 minutes. A 50 mL syringe without needle was used in squashing the filament to obtain homogenous cell suspension (Jegede et al., 2018). The supernatant was carefully poured into centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. The supernatant from the centrifuged content was then removed using Pasteur pipette leaving harvested cells at the bottom of the tube. Freshly prepared fixative (3:1 methanol: glacial acetic acid) was added (8 mL) to the centrifuge tubes and left for a period of 30 minutes before returning them back to the centrifuge. The content was centrifuged again at the rate of 1000 rpm for 10 minutes, the procedure of adding fixative and centrifuging was repeated twice. After the last centrifugation, supernatant was removed using Pasteur pipette and the harvested cells at the bottom of the centrifuge tube was re-suspended in 1ml of fixative.

Figure 1. *Ctenopoma kingsleyae* from Esa-Odo Reservoir, Nigeria

Slide Preparation and Microscopic Photographing

A few drops of the harvested cells were released on pre-warmed slides at a height of about 15 to 22 cm with the aid of a Pasteur pipette. Slides were dried on a slide warmer at a temperature of 60 0C for a period of 24 hours; thereafter, the slides were stained in 6% Giemsa stain in phosphate buffer pH 6.8 for 25 minutes. Slides were dried at 60 ^{0}C for 24 hours before being viewed under a binocular light microscope. Images of good spread were captured with a digital camera image view, model number SCMOS05000KPA.

Karyotyping

Chromosomal classification was done according to Levan et al. (1964), karyotyping

and chromosome length measurement were done electronically with the aid of GIMP corel draw professional XIII.

Results

Metaphase spreads (Figure 2) from the gill filaments were obtained from the slides, fish sex could not be ascertained because they were not matured and not morphologically distinguishable. Count from the spread revealed a modal diploid chromosome number of 2n = 48 mainly composed of uniarmed chromosome. Karyotype of 2n = 48 (48t), was recorded for *Ctenopoma kingsleyae* (Figure 3), sexually differentiated chromosome was not found among the chromosomes. Table 1 described the mean lengths of the chromosome.

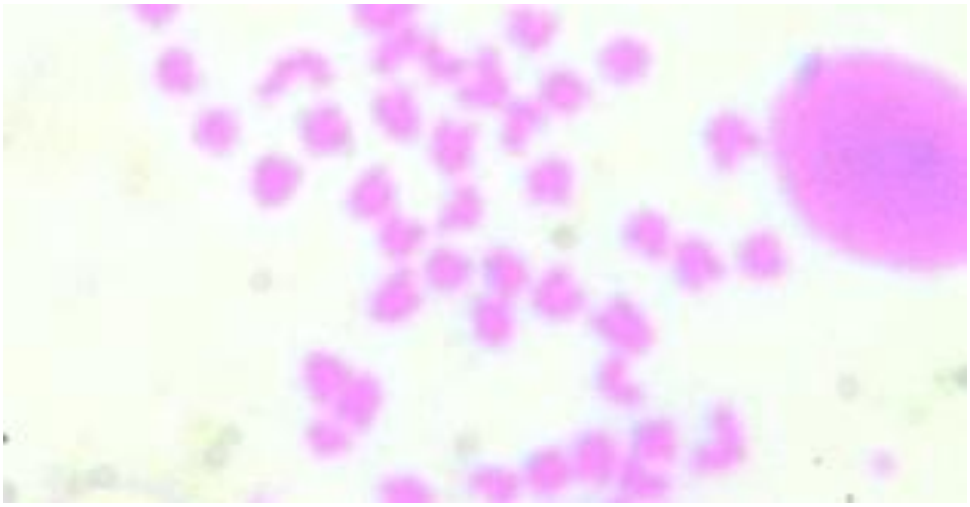

Figure 2. Metaphase Chromosome of *Ctenopoma kingsleyae* from Esa-Odo Reservoir, Nigeria

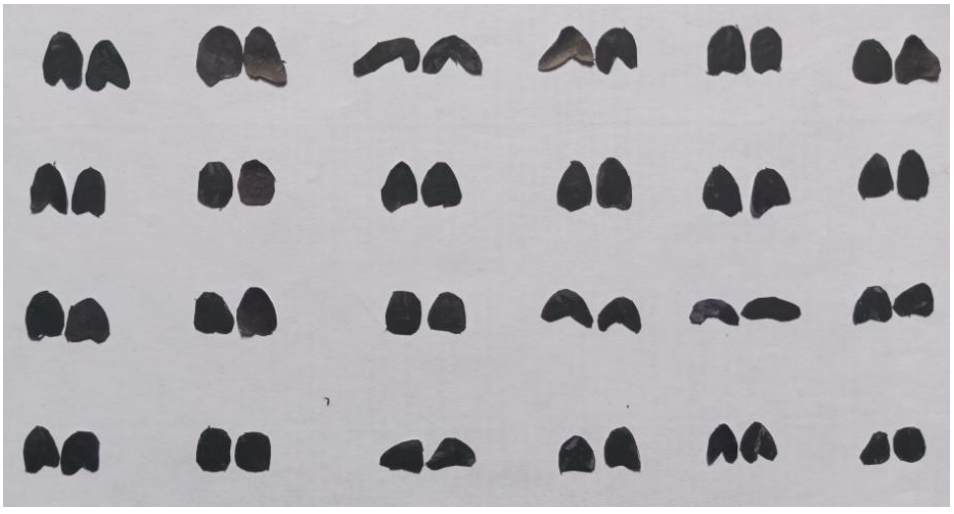

Figure 3: Karyogram of *Ctenopoma kingsleyae* from Esa-Odo Reservoir, Nigeria

Table 1: Mean length, long and short arm ratio of Mitotic chromosome of *Ctenopoma kingsleyae*

No of chromosome	Length of long arm µm	Length of short arm µm	Total length µm	Difference of long and short arm	Long arm: short arm ratio	Description of chromosome
1	1.28	0	1.28	1.28	0	T
2	0.9	0	0.9	0.9	0	T
3	0.87	0	0.87	0.87	0	T
4	0.87	0	0.87	0.87	0	T
5	0.87	0	0.87	0.87	0	T
6	0.85	0	0.85	0.85	0	T
7	0.85	0	0.85	0.85	0	T
8	0.83	0	0.83	0.83	0	T
9	0.8	0	0.8	0.8	0	T
10	0.78	0	0.78	0.78	0	T
11	0.78	0	0.78	0.78	0	T
12	0.76	0	0.76	0.76	0	T
13	0.76	0	0.76	0.76	0	T
14	0.73	0	0.73	0.73	0	T
15	0.73	0	0.73	0.73	0	T
16	0.72	0	0.72	0.72	0	T
17	0.72	0	0.72	0.72	0	T
18	0.72	0	0.72	0.72	0	T
19	0.72	0	0.72	0.72	0	T
20	0.7	0	0.7	0.7	0	T
21	0.7	0	0.7	0.7	0	T
22	0.68	0	0.68	0.68	0	T
23	0.67	0	0.67	0.67	0	T
24	0.64	0	0.64	0.64	0	T

Discussion

This study recorded a diploid chromosome number of 2n=48 for Ctenopoma kingsleyae, which corroborates the diploid number of 2n=48 reported for some other members of the genus Ctenopoma by Krysanov & Golubtson (2001); they reported 2n=48 for each of *C. acutirostre, C. muriei, C. ocellatum* and *C. petherici*. No biarmed chromosome was reported in this study, chromosomal formula is 48t (all uniarmed), this is in line with the report of Krysanov and Golubtson, (2001), they reported uniarmed chromosome in all the species of *Ctenopoma* studied but differ from this study by having 48A as chromosomal formula. The difference in the karyotypic formula might be species differences resulting from geographical isolation since both studies worked *Ctenopoma spp*. from different regions of the world. Krysanov & Golubtson (2001) in their study suggested the presence of 48 uniarmed chromosomes as being primitive for the Anabantidae, a characteristic shared by both *C. kingsleyae* in this study and *Ctenopoma* spp studied by them. This study showed homogenous karyotypic formula 48t for *C. kingsleyae* while some other members of Anabantidae exhibited heterogeneous karyotypic formula 15m + 9sm + 22t for Thai spotted-release form of *Anabas testudineus* (Kabir et al., 2012) and 6m + 40t for Thai spotted form (Tinni et al., 2007),

a condition that suggests primitivism in karyotype of *C. tenopoma* and its separation as separate genus. This study may likely be the first report of the karyotype of *C. tenopoma* as there is paucity of information on its karyotype.

Conclusion

The karyotype of fish is a valuable mechanism towards genetic improvement; further comprehensive research has to be conducted to establish C. tenotomy from another member of the genus that shares similar chromosomal numbers and formulas.

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Conflict of Interest

The authors of this article declare that there are no known competing financial interests or personal relationships that can affect the report of this study.

Author Contributions

Contribution to conception of the study: Ekundare O.V
Design of the study: Ekundare, O.V and Fagbuaro, O.
Collection of experimental sample: Ekundare O.V and Adeniran Idowu Isaac
Acquisition of data and data analysis: Ekundare, O.V and Adeniran Idowu Isaac
Laboratory work: Ekundare, O.V and Fagbuaro O.
Drafting and critical revision of the article: Ekundare, O.V and Adeniran Idowu Isaac
Contributions to final approval of the version to publish: Fagbuaro, O. and Ekundare, O.V

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