



Cytogenetic study and serum protein characterization of *Clarias gariepinus* (BURCHELL, 1822) and *Heterobranchus bidorsalis* (Geoffroy Saint-Hillaire, 1809) in South western Nigeria

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ABSTRACT

Specimens of *Clarias gariepinus* and *Heterobranchus bidorsalis* were cytologically analysed while their serum protein was employed to characterize the two species. The diploid chromosome numbers for *C. gariepinus* and *H. bidorsalis* were $2n=56$ and $2n=52$ respectively. The nombre fondamental (NF) of *C. gariepinus*, and *H. bidorsalis*, were 51 and 49 respectively. The electrophoretic banding pattern of the two species produced five common bands while the relative mobility of the bands studied showed that there are few slow moving bands, more fast moving bands but no intermediate bands. The occurrence of chromosome number around the modal value which occurs generally among the clariid fish may suggest an ongoing speciation while the presence of five common bands may also be used as a diagnostic marker for biochemical differentiation of the two fish species.

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INTRODUCTION

Catfishes are a major source of food protein for the teeming populations in West African sub-region. *Clarias gariepinus* and *Heterobranchus bidorsalis* are fish species which are widely cultured and are of great commercial importance in Nigeria. This is because they possess among other qualities high market price, fast growth and ability to tolerate adverse environmental conditions. The clariid fishes are also used in producing hybrid fishes which have been shown by

several authors to possess better qualities than the parents (Bartley et al., 2000; Sahoo et al., 2003; Odedeyi, 2007; Ataguba et al., 2010; Akinwande et al., 2012).

Cytogenetic study of these fish species will enhance improved production of their hybrids and help resolve some taxonomic problems that may be present in the family Clariidae. Several investigators have reported karyotypic and chromosomal studies of clariid fishes. Teugels et al. (1992) also reported the standard karyotype of *Clarias gariepinus*

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($2n=54$), *Heterobranchus longifilis* ($2n=52$) and hybrids between the two species ($2n=54$). The karyotype of *Clarias gariepinus* from two regions of Turkey (Goksu Delta and Orontes) was described using G-banding, C-banding, Q-banding and Ag NORs by Karahan and Ergene (2011). They reported the same diploid chromosome number of $2n=56$ and fundamental number $FN=100$ for the fish species. Shabeena et al. (2012) reported the diploid chromosome number of $2n=54$ for *Clarias batrachus* which was shown to have six pairs of metacentric chromosomes, nine pairs of sub metacentric chromosomes and seven pairs of telocentric chromosomes.

A better understanding of the mitotic chromosomes of these fish species of study may ensure better understanding of the mechanism of their hybridisation. Teugels et al. (1992) reported striking similarities in the karyotype of both species of study and that most pairs of chromosomes appear homologous. This has favoured their hybridisation.

Similarly, characterisation of fish species employing electrophoretic separation of their total protein and isozymes is a technique used in detecting genetic variations and also for delineating taxonomic relationships among them (Hauser, et al., 2003). Diyaware et al. (2012) reported the characterization of some clariid species using total protein and showed the possibility of their banding patterns being used as base line information to identify them and their hybrids in case of natural hybridisation which may be occasioned by their indiscriminate use in fish production.

This study was therefore designed to provide information on cytogenetic and electrophoresis of total protein of the fish species *Clarias gariepinus* and *Heterobranchus bidorsalis* which will form a basis for their genetic improvement through clearer understanding of their genetic variation and taxonomic relationship.

MATERIALS AND METHODS

Sampling sites and sample collection

Fish specimens used for this study were obtained from the Teaching and Research Farm, University of Agriculture and The Wet Laboratory of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife. The identification of two fish species was carried out according to Reed et al. (1967) and Teugels (1982, 1986).

Chromosome preparation

Metaphase chromosomes were freshly prepared from newly hatched larvae as described by Aluko and Awopetu (1995) though slightly modified by reducing the number of hours in Colchicine (BH15 1TD, England) solution (Olaniyi, 2008). One hour old embryos of the fish were put in 0.01% Colchicine solution for 3 h and for another 1 h in distilled water before fixing in 3:1 ethanol-acetic acid solution and kept in the refrigerator until use. The tails of the hatchlings containing mitotic cells were severed and minced in 50% acetic acid solution (freshly prepared) to form a cell suspension. Two drops of the cell suspension were put on a clean slide which had been dried on a slide dryer. Slides were stained with a drop of FLP-orcein solution for 10 minutes.

The material was further squashed by laying a piece of filter paper on the cover slip and pressing firmly with the thumb to achieve a good spread of the cells and the chromosomes and also to remove excess stain which was absorbed by the filter paper. The slides were left on a slide warmer overnight to dry after which they were examined under $\times 10$, $\times 40$ and $\times 100$ objectives of the microscope (Olympus model). Cells that were adjudged to contain well spread metaphase chromosomes were selected for chromosome counting and then photographed. The photography was done under oil immersion using a photo microscope (PW-BK5000T, PROWAY OPTICS, CHINA).

The metaphase chromosomes of the two fish species were classified into four groups, namely metacentrics, sub-metacentrics, sub-telocentrics and acrocentrics, according to the method described by Aluko and Awopetu (1995).

Protein studies

The electrophoresis analysis was carried out in the Biotechnology laboratory, University of Agriculture, Abeokuta. The basic principle involves the separation of a mixture of proteins on size differences. From the caudal region of the fish, 3 ml of blood was withdrawn, diluted with 2 ml of 0.9% physiological salt solution and expelled from the syringe after removing the needle. Care was taken while allowing blood to run down the side of the centrifuge tube so as to avoid the inclusion of air bubbles that could be present in the syringe in the blood sample. The sample was left for one hour at room temperature and the serum obtained after centrifuging at 5000 rpm for 15 minutes was decanted and kept at about 5 °C until use.

Protein electrophoresis was then carried out according to standard methods using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Bromophenol blue was added to the sera to act as a tracer. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250, allowing visualisation of the separated proteins. The separation of protein was carried out with the use of Electrophoresis Power Supply Model 200/2.0 in Mini Protean 11 Cell (Bio-Rad) at 150 volts for 1 hour.

When electrophoresis was completed, the gel formed was removed from the electrode vessels and left in the stain overnight. After staining, the gels were removed and washed for about two minutes in distilled water. The gels were then rinsed several times with freshly prepared destaining

solution until the protein bands are distinct and are then kept in the destaining solution. The gels were scanned with a scanner and the images were stored in the computer for scoring to compare the degree of similarity of the hybrids with the parents.

The protein banding patterns obtained from electrophoretic profiles were subjected to cluster analysis to show the relationship in their clustering patterns using the Unweighted Pair Group Method with Arithmetic means for phenogram or dendrogram grouping (Sneath and Sokal, 1973) using PAST computer software (Hammer *et al.*, 2006).

RESULTS

Table 1 shows the range of diploid numbers observed, modal diploid number and the number of spread observed in each genetic group. A modal diploid chromosome number of $2n=56$ and $2n=52$ were recorded for *C. gariepinus* and *H. bidorsalis*; respectively (Table 1 and Figure 1). The parental chromosome numbers are relatively close to each other. Figure 2 and Table 2 show the karyotypes and nombre fondamental (NF) of the two species. The metaphase chromosomes of the fish species studied were metacentrics, sub-metacentrics, sub-telocentrics and acrocentrics. *C. gariepinus* consisted of 3 m + 6 sm + 14 st + 5a with NF=51; while that of *H. bidorsalis* were 6 m + 1sm + 16 st + 3a and NF =49.

The SDS-PAGE electrophoretic profiles of sera of the two fish species in the presence of SDS and β -mercaptoethanol are as shown in Figure 1. SDS-PAGE gels showed a high degree of qualitative and quantitative intergeneric variations in terms of the positions of the band in the protein profiles of the species studied. The serum protein banding patterns revealed 16 bands across the two species. The protein banding patterns of the species produced five similar bands.

Table 1: The Number of Metaphase Cells, the Range of Haploid, and the Modal Haploid and Diploid Chromosome Numbers of *Clarias gariepinus* and *Heterobranchus bidorsalis*.

Species	Number of Spreads	Range of Diploid Number	Modal Diploid Number
<i>Clarias gariepinus</i>	200	22 – 28	56
<i>Heterobranchus bidorsalis</i>	195	22 – 26	52

Table 2: Chromosome types of *Clarias gariepinus* and *Heterobranchus bidorsalis*.

Species	Metacentric chromosomes (m)	Sub-metacentric chromosomes (sm)	Sub-telocentric chromosomes (st)	Acrocentric chromosomes (a)	Nombre fundamental (NF)
<i>Clarias gariepinus</i>	1 – 3	4 – 9	10 – 23	24 – 28	51
<i>Heterobranchus bidorsalis</i>	1 – 6	7	8 – 23	24 – 26	49

Table 3: Relative Mobilities of protein bands of *Clarias gariepinus* and *Heterobranchus bidorsalis*.

Species	Total No of bands	Slow moving bands 1.0-2.9	Intermediate moving bands 3.0-4.9	Fast moving bands 5.0 and above	Peculiar bands (cm)
<i>Clarias gariepinus</i>	6	1	-	5	1
<i>Heterobranchus bidorsalis</i>	9	2	-	7	4

Table 4: Common band relationship of *Clarias gariepinus* and *Heterobranchus bidorsalis*.

	<i>Clarias gariepinus</i>	<i>Heterobranchus bidorsalis</i>
<i>Clarias gariepinus</i>	-	
<i>Heterobranchus bidorsalis</i>	5	-

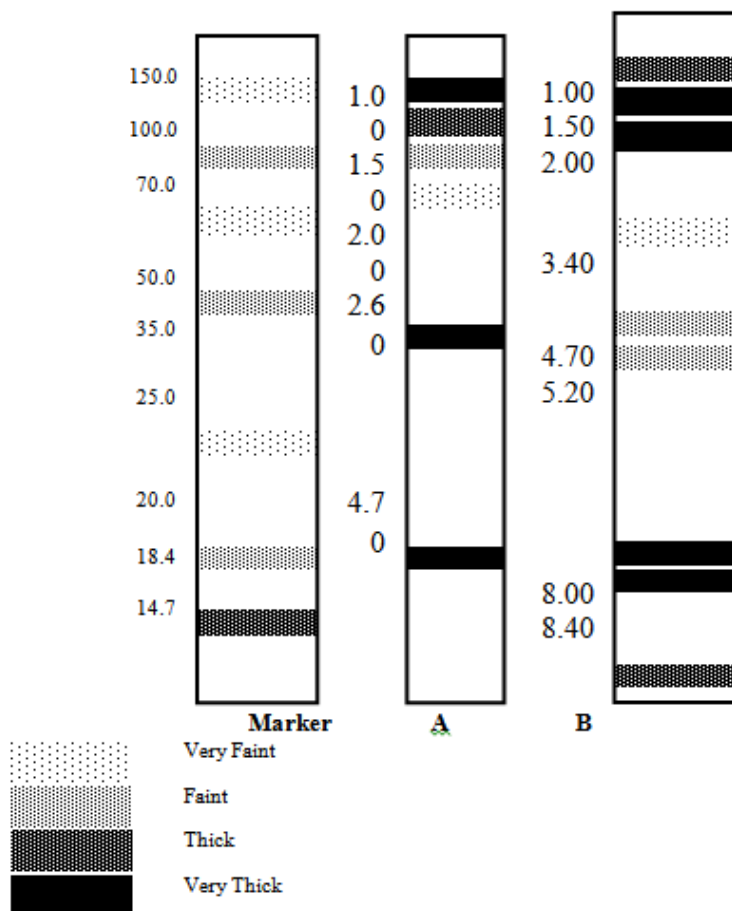


Figure 1: Schematic diagram of Coomassie-Blue stained SDS-PAGE gel showing serum banding patterns of the two fish species *Clarias gariepinus* and *Heterobranchus bidorsalis*. A: *Clarias gariepinus* (Cg); B: *Heterobranchus bidorsalis* (Hb).

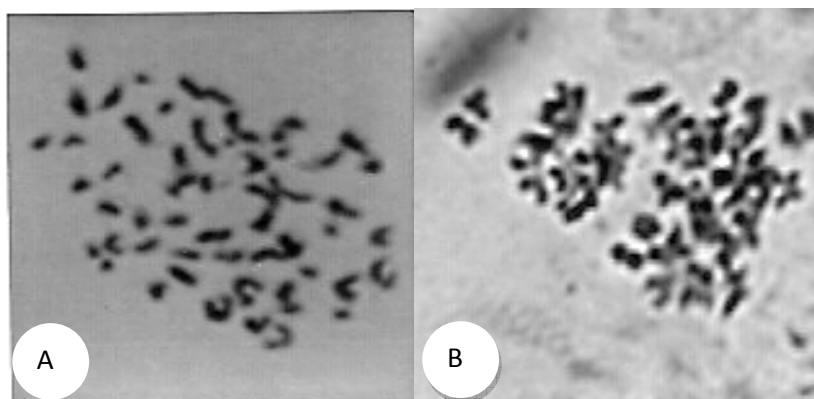


Figure 2: Chromosome spreads of the fish species. A = Metaphase chromosomes of *Clarias gariepinus* $2n=56$; B = Metaphase chromosomes of *Heterobranchus bidorsalis* $2n=52$.

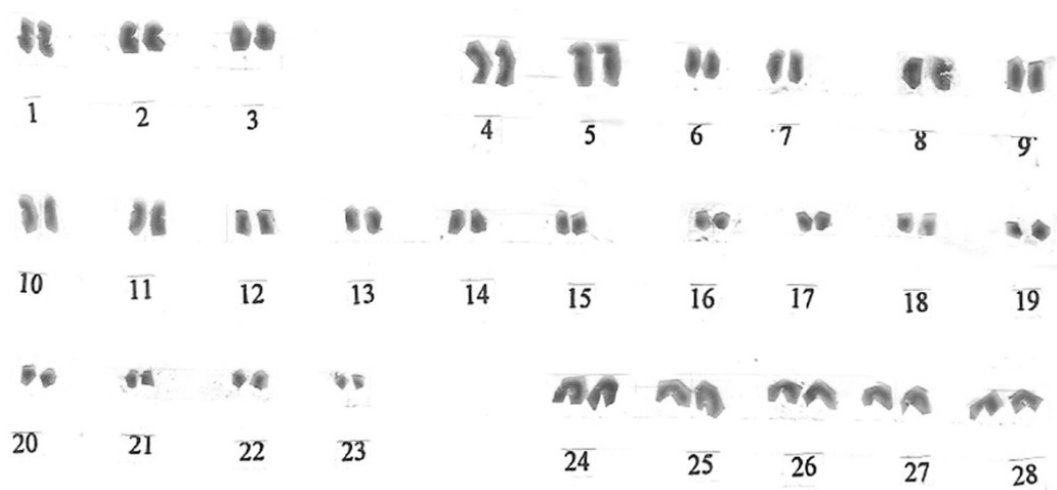


Figure 3: Karyotype of *Clarias gariepinus*

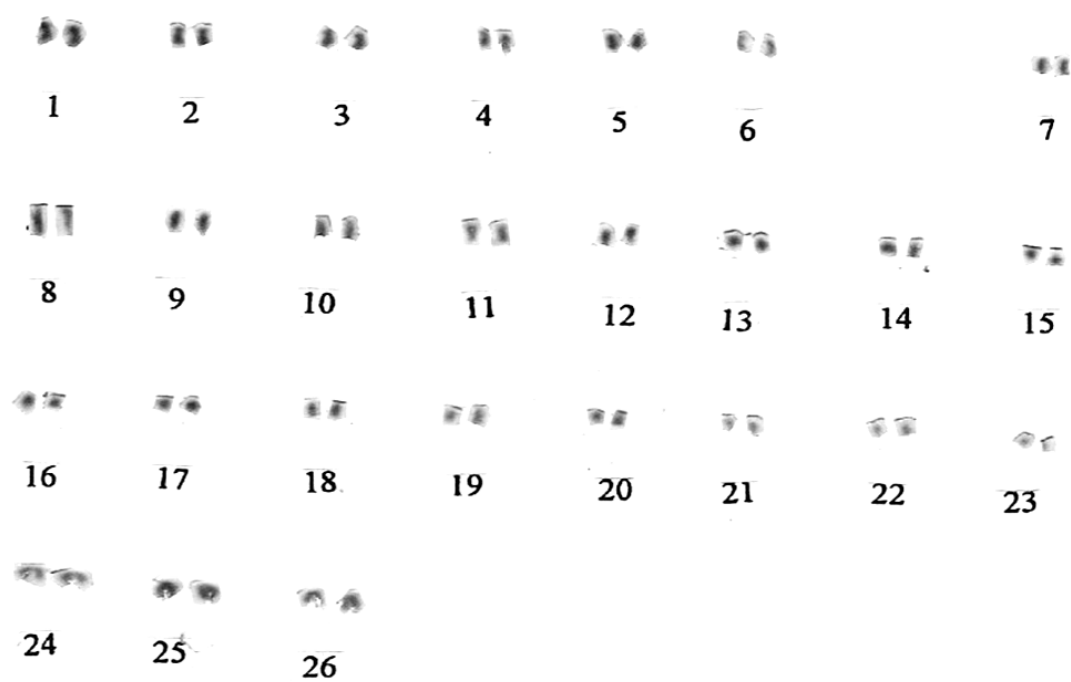


Figure 4: Karyotype of *Heterobranchus bidorsalis*.

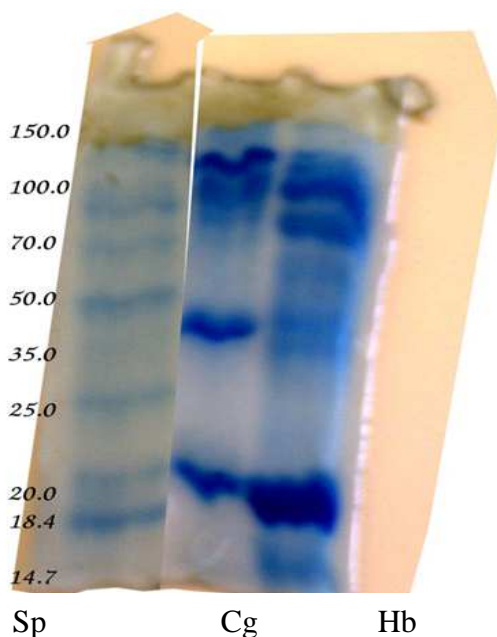


Figure 5: A representative Coomassie-Blue stained SDS-PAGE gel showing serum banding patterns of the two fish species, *Clarias gariepinus* (Cg) and *Heterobranchus bidorsalis* (Hb). Sp is the standard protein.

DISCUSSION

The results of the present cytogenetic study conform to the report by Fishbase (2004) that the siluriformes, most especially the family Clariidae has a range of diploid chromosome number of between 50 and 58, because *Clarias gariepinus* and *Heterobranchus bidorsalis* were found to have diploid chromosome numbers of 56 and 52 chromosomes respectively. The modal chromosome number of $2n=56$ was first reported by Teugels et al. (1992). More recently, Okonkwo and Obiakor (2010), Ifeoluwa et al. (2011) and Karahan and Ergene (2011) also reported $2n=56$ diploid chromosome number for *Clarias gariepinus* while Fagbuaro (2012) differed by reporting $2n=54$ for *Clarias gariepinus*. The difference may be due to method of chromosome preparation or reported chromosome polymorphism in several families of Siluriformes in course of evolution (Oliviera and Gosztonyi, 2000; Okonkwo and Obiakor, 2010; Fagbuaro, 2012). No report was found

on the modal chromosome number of *H. bidorsalis*. The chromosome complements of other members of the family Clariidae had been reported. Eyo (2005) reported that *C. anguillaris* and *C. gariepinus* have the same diploid chromosome number of 56 ($2n = 56$) and a nearly identical chromosome formula, and *H. longifilis* has diploid chromosome numbers of 52 chromosomes, $2n=52$. The chromosome complements of $2n=50$ for *H. longifilis* (Awodiran et al., 2000; Olufeagba and Moses, 2011) and $2n=54$ for *C. anguillaris* (Awodiran et al., 2000) have been reported.

The karyotypic formula for *C. gariepinus* in this study is remarkably different from those reported by earlier workers. Eyo (2005) showed that *C. gariepinus* from Anambra River male had 8 metacentric, 24 sub metacentric and 24 acrocentric chromosomes while the female had 8 metacentric, 25 sub metacentric and 23 acrocentric chromosomes while Ifeoluwa et al. (2011) reported a different karyotypic form

of 25 metacentric, 14 sub metacentric, 14 sub telocentric and 3 telocentric chromosomes for *C. gariepinus*. The karyotypic constitution reported by Fagbuaro (2012) for the same species although from another population from New Bussa, Niger Statrent was 24 netacentric, 10 sub metacentric, and 10 sub terminal. These differences in karyotypic formulae of the same species of fish but of different populations agrees with the opinion of Ifeoluwa et al. (2011) that the different karyotypic forms exist from one population of *C. gariepinus* to another. This may be due to the fact that catfishes have been known to show great diversity in their karyotypes (Karahana and Ergene, 2011). In silurid families, chromosome or chromosome arm numbers exhibit great variability and one can assume that this quality can be used for species delimitation and characteristics (Fagbuaro, 2012).

One of the factors which influence the amount of genotypic diversity generated in a species is the number of chromosomes in the genome (Bailey et al., 2009). Genetic variation among catfishes capable of interbreeding determines their adaptive features and the cytological investigation revealed genetic variation in the fish species studied. Moreover, the chromosomes are variable numerically in the two fish species studied and this could explain the ability of the Clariids to adapt to different environmental conditions, while the autopolyploidy observed in the species and their hybrids could have adaptive significance on the hybrids in the subsequent generations. Eyo (2005) reported that in nature, the occurrence of chromosome number around modal values among the Clariids may suggest that chromosomal changes may be associated with the process of speciation within the group, possibly through high rate of hybridization. Karyological evidences have been employed in solving problems relating to chromosome number, functional arm, phyletic relationship, the taxonomic status as well as possibility of speciation among the studied

Clarias species. For instance, the wide dispersal of chromosome number around modal value ($2n = 56$) among the Clariids suggested possibilities of the species undergoing speciation (Eyo, 2005).

The variations in combination of protein bands at various distances is taxon specific in most species of animals as no two species have the same banding patterns (Adedeji and Adewale, 2006). The consistent presence of similar bands between the two different species shows high level of their genetic similarity which may be indicative of two things. First, that the two species have similar bands which are controlled by the same genes. Awodiran et al. (2013) reported that the appearance of a band in all individuals of a population, assumes that the gene, which codes for the enzyme or protein, does not vary. Second, the presence of common bands also may be indicative of evolutionary relationship of the two species belonging to the same family, Clariidae. This is confirmed by the finding of Akinwande et al. (2012) who reported the presence of five similar bands in the electrophoretic banding patterns of *Heterobranchus longifilis*, *Clarias gariepinus* and *Clarias anguillaris* and their intergeneric and interspecific hybrids. The five common bands may also be used as diagnostic markers for the biochemical differentiation of the two fish species. Though several works on the hybridization of clariid fish species have confirmed their closeness in ancestral relationships which has made successful hybridisation between them possible (Awodiran et al., 2000; Sahoo et al., 2003; Odedeyi, 2007; Ataguba et al., 2010; Diyaware et al., 2012), this study provides further biochemical evidence in support of the relatedness of the two fish species which enables their being used for hybridisation. Study on the biochemical characterisation of the two fish species using their serum protein which shows high level of genetic similarity also shows the very low genetic diversity.

Conclusion

The diversity of serum protein bands are indicative of genetic diversity and may be useful in characterising the clariid fish species studied. There is a strong correlation between protein band patterns and species differentiation of the two fish species studied. Electrophoretic differentiation of the serum protein and the karyotypic studies of the two fish species *C. gariepinus* and *H. bidorsalis* appear to demonstrate close relationship of two species and the differences between the two species of study biochemically and cytologically. However, further studies employing molecular methods may be needed to elucidate differences in different populations of *C. gariepinus* karyotypic forms.

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