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Protein Profile Expression of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their Reciprocal Hybrids in South-West Nigeria

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Abstract: Proper genetic characterization would help in the selection of suitable strains for aquaculture that could lead to production of new varieties of fishes to alleviate the problem of short supply of fast growing quality fish seeds. The study was aimed at analyzing the muscle protein profiles of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids. Sixteen juveniles fish samples (comprising four samples from each mating combinations) artificially propagated and reared for sixteen weeks were analysed with the aid of electrophoresis. The separation of the different polypeptides of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrid were carried out using 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D SDS-PAGE). The relative concentration of individual protein bands were analysed using TotalLab™ 1D software. The individual protein bands in the electrophoregram were identified in relation to their molecular weights. The gel images obtained after electrophoresis were scored and subjected to cluster analysis. The 1st, 2nd, 3rd, 4th, 8th, 9th, 10th and 11th bands were detected across all mating combinations. The 5th band with molecular weight (78.58 kDa) distinguishes *C. gariepinus* from *H. bidorsalis* while the 6th band with molecular weight (54.41 kDa) distinguished the reciprocal hybrids Clariabanchus from Heteroclarias. The 7th and 12th bands distinguished the pure breeds from the hybrids. The 7th band was present in both hybrids-Clariabanchus (49.50 kDa) and Heteroclarias (49.77 kDa) species but absent in the pure breeds while 12th bands was present in the pure breeds *C. gariepinus* (19.92 kDa) and *H. bidorsalis* (20.29 kDa) but absent in the hybrids. The cluster analysis shows a high level of genetic similarity among the mating combinations which affirms the already established monophylogenetic relatedness among the species.

Key words: Electrophoregram, protein profile, *Clarias gariepinus*, *Heterobranchus bidorsalis*

INTRODUCTION

Aquaculture has been accepted over the world as a means for increasing fish production. In a developing country like Nigeria with its immense resources offer tremendous possibilities for fish culture. Catfish family Clariidae is very popular in Nigeria due to its culture characteristic which has endeared it to many fish farmers. *Clarias gariepinus* and *Heterobranchus bidorsalis* are species of high aquaculture importance in South-western Nigeria. They are widely cultured owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content (Idodo-Umeh, 2003).

Several efforts from the government and private sector, universities, research institutes in Nigeria, that have been directed towards increased fish production through various research activities in recent times, have

yielded positive impacts especially in the area of increased fish seed production through the establishment of fish hatcheries; development of hybrid catfish etc (Idodo-Umeh, 2003). Although, this effort has yielded some positive result, observation on the field has shown that there are problems of Clariid catfishes identification especially hybrids. This can jeopardize the future of aquaculture industry in Nigeria if something is not done quickly (Yisa and Olufeagba, 2005). For any successful catfish breeding and genetic improvement program, there is the need for identification of the parent stock, sexing to identify male and female broodstock and differentiation of reciprocal hybrids from the putative parent stock.

In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of whole muscle protein, sarcoplasmic proteins, serum proteins, liver proteins, salivary proteins

and a number of enzymes often have been used by some researchers as an aid in the species identification of fish (Pineiro *et al.*, 2001; Yilmaz *et al.*, 2007; Lamy *et al.*, 2008). Sexual dimorphism in electrophoretic patterns of blood serum proteins of a smelt (*Hypomesus nipponensis*) was investigated (Komagata *et al.*, 1991).

Most morphological traits are polygenic, quantitative or continuous characters and their expression is influenced by environmental conditions and are, therefore, not confirmatory enough (Omoniyi and Fagade, 2001). The traditional approach to characterization and evaluation is based on morphological features. However, biochemical analysis of total protein and isozyme markers reveal better diagnostic genetic variations and is usually free from genotype and environmental interactions (Torkpo *et al.*, 2006). Thus, gel electrophoresis has become common tool for studying variations at the genetic level. Therefore, the study aims at analyzing the muscle protein profiles of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids using the SDS-PAGE technique to establish the resemblances and differences between the species and their hybrids.

MATERIALS AND METHODS

Muscle sample collection: The experimental fish was produce through intergeneric hybridization between *C. gariepinus* and *H. bidorsalis* obtained from Hepa Fish Farm, Asero, Abeokuta, Nigeria. The following crosses were carried out for parental cross *C. gariepinus*×*C. gariepinus* and *H. bidorsalis*×*H. bidorsalis* and hybrids *C. gariepinus*×*H. bidorsalis* (*Clariabanchus*) and *H. bidorsalis*×*C. gariepinus* (*Heteroclarias*). Sixteen juveniles fish samples (comprising four samples from each mating combinations) were transported live in well open plastic rubber bowls to the Biotechnology Laboratory unit of the Animal Breeding and Genetics of the Federal University of Agriculture, Abeokuta. Muscles were isolated from the epiaxial muscle in the region below the cranial to the dorsal fin, above the lateral line of the experimental fish with the aid of a new surgical blade for each species (El-Serafy *et al.*, 2006; Reddish *et al.*, 2008) after fish killing by medullar transaction (El-Serafy *et al.*, 2006). Fish muscle protein extracts were prepared by quickly homogenizing 100 mg of muscle (on ice) in 1.5 mL of urea/thiourea buffer containing 80 M urea, 2 M thiourea, 75 mM DTT, 50 mM Tris, 0.004% bromphenol blue at pH 6.8. The extracts were centrifuged in a tube at 10,000 g for 10 min and stored at -20°C in the deep freezer.

Sample preparation for SDS-PAGE analysis, gel staining, image analysis and statistical analysis: Protein fractions were centrifuged for 5 min at 10,000×g at ambient temperature prior the electrophoretic analysis. Samples were loaded into (12.5% polyacrylamide gel with 3% stacking gel) 1 mm×12 cm×14 cm. First, gel capillary chamber was loaded with a broad range molecular weight standard (BioRad Laboratories, Hercules, CA). Proteins were separated by applying constant voltage of 300 V for 2 h, 30 min until the dye front reached the bottom of the gel. After electrophoretic separation, gels were stained overnight with gentle agitation on an orbital shaker. Staining buffer contains (400 mL methanol, 50 mL glacial acetic acid and Coomassie Brilliant Blue G250) and subsequently destained with 10% acetic acid. Images were digitized and acquired employing a CCD camera (Samsung ES25-Samsung Opto, Electronics Co. Ltd). Raw images were subsequently imported into TotalLab 1D software (Nonlinear Dynamics, Newsacastle upon Tyne, UK) and analyzed (band detection and molecular weight calculations). User defined parameters used were according to Agbebi *et al.* (2013). The images were scored to compare the degree of similarity of the hybrids with those of pure breeds. 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 statistics software PAST (Majolagbe *et al.*, 2012).

RESULTS

Figure 1 shows the calibration of Fig. 2. The blue diamonds denotes bands detected and calibrated under the calibrated image of 12.5% SDS whole Muscle Protein of artificially propagated *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids after analysis of raw image by TOTALLAB software. A total of twelve different protein bands were resolved across the entire gel. The 1st, 2nd, 3rd, 4th, 8th, 9th, 10th and 11th bands were detected across all mating combinations. The average number of protein bands resolved among the pure breeds; *C. gariepinus* and *H. bidorsalis* were eleven and ten, respectively in all examined species. In *C. gariepinus* the 5th band was present but absent in *H. bidorsalis*. The 12th band was present in the pure breeds but absent in the hybrids. In the hybrids, *Clariabanchus* and *Heteroclarias* the average number of protein bands resolved was ten and nine, respectively

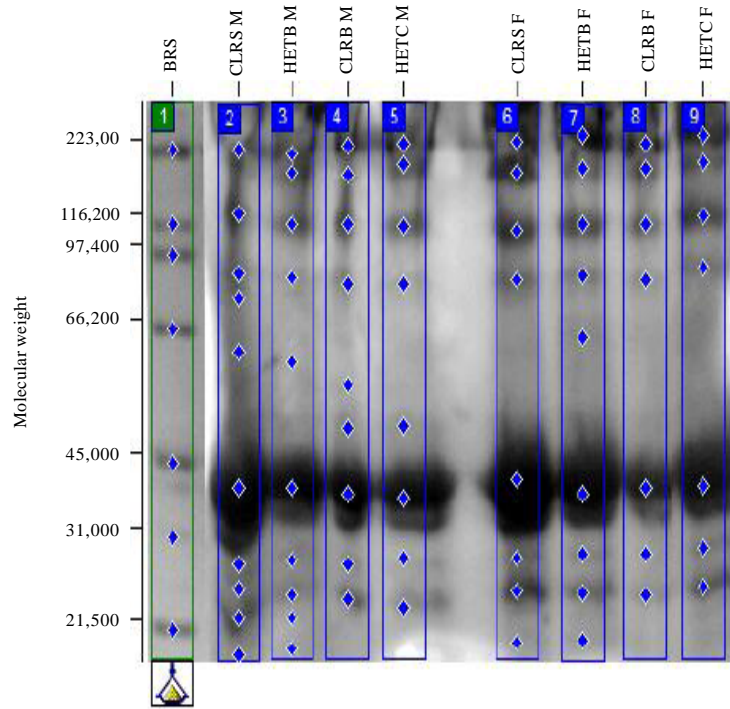


Fig. 1: Calibrated image of 12.5% SDS whole muscle protein of artificially propagated *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids, Lane 1: BRS (Bio Range Standard), Lane 2: CLRS M *Clarias gariepinus* ♂, Lane 3: HETB M *Heterobranchus bidorsalis* ♂, Lane 4: CLRB M “*Clariobranchus*” ♂, Lane 5: HETC M “*Heteroclarias*” ♂, Lane 6: CLRS F *Clarias gariepinus* ♀, Lane 7: HETB F *H. bidorsalis* ♀, Lane 8: CLRB F “*Clariobranchus*” ♀ Lane 9: HETC F “*Heteroclarias*” ♀

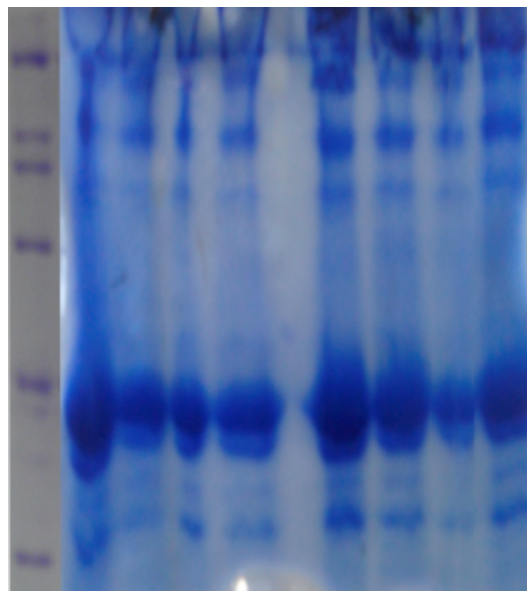


Fig. 2: Raw Image of 12.5% SDS whole muscle protein of artificially propagated *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids

Table 1: Molecular weights of bands detected for whole muscle protein of *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids

Ref band number	Bio range standard (kDa)	<i>C. gariepinus</i> (kDa)	<i>H. bidorsalis</i> (kDa)	Clariabanchus (kDa)	Heteroclarias (kDa)
1	223	234.13	234.65	232.79	239.91
2	-	188.11	191.77	188.15	202.21
3	116.2	121.53	117.58	116.47	119.63
4	97.4	87.99	88.12	85.13	88.025
5	-	78.58	-	-	-
6	66.2	60.94	61.49	54.41	-
7	-	-	-	49.50	49.77
8	45	41.61	40.05	39.98	39.60
9	31	27.83	28.19	27.89	28.70
10	-	24.63	24.31	24.15	24.90
11	-	22.61	22.39	23.71	23.10
12	21.5	19.92	20.29	-	-

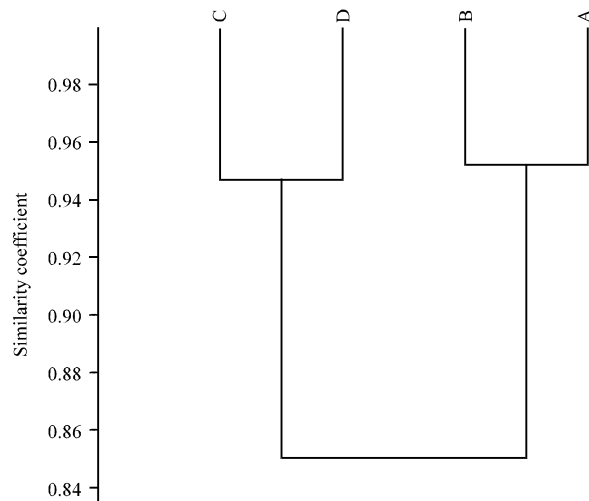


Fig. 3: Dendrogram from combined data on parents and juveniles of *C. gariepinus*, *H. bidorsalis* and their reciprocal hybrids. (a) *C. gariepinus*, (b) *H. bidorsalis*, (c) Clariabanchus and (d) Heteroclarias

in all examined species. The 7th band was present in both hybrids species but absent in the putative parents. The 6th band was present in Clariabanchus but absent in Heteroclarias, hence distinguishes the hybrids.

In the male pure breeds, ten protein bands were resolved for both *C. gariepinus* and *H. bidorsalis* while the male hybrids Clariabanchus and Heteroclarias nine and eight protein bands were resolved, respectively. In the female pure breeds, both hybrids resolved seven protein bands. Most female were observed to have less bands compared to male. The males had an average of nine bands while females had eight bands. Although, there were sexually dimorphic bands among individual species, no sexually dimorphic band can be satisfactorily reported.

The molecular weights of each protein fraction are presented below (Table 1) for artificially propagated

C. gariepinus, *H. bidorsalis* and their reciprocal hybrids in Kilo-Daltons (kDa).

The distinguishing 5th present in *C. gariepinus* but absent in *H. bidorsalis* had the molecular weight 78.58 kDa. The 12th band present in the pure breeds but absent in the hybrids had the molecular weights 19.92 and 20.29 kDa in *C. gariepinus* and *H. bidorsalis*, respectively. The 7th band present in both hybrids species but absent in the putative parents had the molecular weights 49.50 and 49.77 kDa while the 6th band which distinguishes the hybrids had the molecular weight 54.41 kDa.

The dendrogram obtained (Fig. 3) when the data from hybrids and the pure breeds was combined and is shown in two major clusters.

DISCUSSION

A total of twelve protein bands were resolved from the whole muscle protein bands of the Clariid. This varied slightly from the number of Clariid serum protein banding pattern of 16 and 15, respectively observed by Akinwande *et al.* (2012) and Majolagbe *et al.* (2012) but was similar to thirteen observed by Diyaware *et al.* (2011, 2012). This variation is of total number of protein bands, resolved in this study and the above, authors is as a result of the obvious use of muscle tissue and serum respectively because the structure of blood serum proteins, muscle proteins as well as enzymes in blood and some organs appear to be variable (Kirpichnikov, 1981). The pure species had more bands than the hybrids. This is contrary to study by most authors. There was also a slight decline of eleven to ten protein bands in the plasma and muscle of Tilapia, respectively as observed by El-Serafy *et al.* (2006).

In the identification of the protein pattern in the resulting hybrid and the pure parental strain of *Oreochromis* sp. using Iso-Electro Focusing method affirmed that banding patterns in electropherograms were found constant in each species and differ characteristically between species. The results reported in

the present study indicate species-specific patterns with common bands for all the studied species as well as specific bands characterizing each species. The whole muscle fraction obtained, in this study, showed a high number of polymorphic fractions, this proves the close monophylogenetic relationship of the parental genus hence successful hybridization between them. This is in agreement with Akinwande *et al.* (2012) on the serum protein pattern in interspecific and intergeneric hybrids of some Clariids. The discriminating protein bands obtained in this study between the pure strains: *C. gariepinus* and *H. bidorsalis* was low. Only the 5th band was present in *C. gariepinus* but absent in *H. bidorsalis*. This therefore can be used as diagnostic marker for the biochemical identification of the pure stock. The 7th bands present in the reciprocal hybrids and 12th bands present in the putative parent stock were the differentiating bands between them, hence can be used as diagnostic protein bands. The distinguishing band between both hybrid combinations is the 6th band in *Clariabranchnus*. Similar result was obtained by Diyaware *et al.* (2011) where the absence of the 12th band in the reciprocal hybrid *Heteroclarias* differentiated it from *Clariabranchnus*.

Sexual dimorphism in electrophoretic patterns of blood serum proteins of a smelt [*Hypomesus nipponensis*] was investigated (Komagata *et al.*, 1991). They found that in the electrophoretic analyses of blood serum proteins were different in the female and male *Hypomesus nipponensis*. Although, no sexually dimorphic band can be satisfactorily reported, male and female are easily distinguished from one another under field examination after they are about 10 cm long (Yisa and Olufeagba, 2005).

The high Similarity Coefficient (SC) value obtained (Fig. 3) further proves the monophylogeny of the two Clariids-*C. gariepinus* and *H. bidorsalis*. The monophylytic relationship between unidentical subfamilies of family Cyprinidae was proved in a study using allozyme electrophoretic technique. The close relationship between the genera *Heterobranchus* and the *Clarias* has been emphasized by Teugels *et al.* (1990) using morphological and osteological features. According to Teugels *et al.* (1992), the species from the subgenus *Clarias* are more closely related to *Heterobranchus* than to some other genus of Clariidae. High genetic similarities were reported in studies carried out for Clariids. However, the high SC reported in this study might also imply a low genetic diversity and therefore explains the high difficulty usually experienced in the field identification and differentiation of the hybrids and their parents based on morphometric and meristic data.

CONCLUSION

Protein fingerprinting presents a unique infallible method of fish identification via the protein profiles of the unknown species. This study will serve as a bridge between the existing gaps of information available on the muscle protein profile of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids.

It will enable aquaculturists to recognize or identify the right species to use in breeding and genetic studies for accurate results and profitability. It will also serve as an important tool in knowing species of catfish to be stocked in ponds and open water bodies, consequently solving the problem of poor growth performance and the genetic loss of the pure putative Clariid catfishes.

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