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Comparative genetic and morphometric characterization of sympatric populations of *Heterobranchus bidorsalis* and *Heterobranchus longifilis*

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Abstract

African catfish species of Heterobranchus longifilis and H. bidorsalis are gaining breeding potentials and receiving great attention among catfish stakeholders. However, very few comparative research works have been done on the natural populations and cultured stocks of these economically important species. In this study, the genetic evaluation of the fish species populations obtained from Lake Kainji, New Bussa, Niger State, Nigeria, was conducted through Sodium Dodecyl Sulphate - Polyacylamide Gel Electrophoresis oftheir serum proteins, and morphometric evaluation. Morphometric data showed that adipose fin attributes [length; depth (cm), % standard length (SL)] of 23.4±1.1; 4.2±0.5 in the H. bidorsalis confirmed its hyper-development compared to H. longifilis of 25.7±3.2; 4.4±0.6 respectively. The pre-dorsal length gave higher value (%SL) in the H. longifilis (38.6±2.2) than H. bidorsalis (34.6±1.2); while H. bidorsalis further possessed more counts at dorsal and anal fin rays than H. longifilis. The comparative dendrogram of the PAST analysis showed a genetic distance of 4.2% that indicated the specificity of these species and, though significantly different, they had very close relationship. Therefore, proper identification of these species is highly necessary to maintain genetic purity in breeding programmes and biodiversity.

Keywords: African catfish species, *Heterobranchus bidorsalis, Heterobranchus longifilis,* electrophoresis, morphometric

Introduction

Heterobranchus bidorsalis Geoffroy Saint Hilaire, 1809and *H. longifilis* Valenciennes, 1840 are the two common species of Genus *Heterobranchus* Geoffroy Saint-Hilaire 1809 in Nigeria. The species belong to family *Clariidae* with four valid species been identified (Teugels *et al.*, 1990). They are economically important *Clariid* catfish due to their good performance; *H. longifilis* has been reported to be the fastest in growth performance compared to other African catfish (Legendre *et al.*, 1992). Legendre *et al.* (1992) further documented the doubled performance of *H. longifilis*compared to *C. gariepinus*.Owing to the performance of *Heterobranchus,* the species have been employed to improve breeding potentials within the genus (Legendre *et al.*, 1992; Akinwande *et al.*, 2009) and others such as *Clarias* (Aluko, 1995; Ataguba *et al.*, 2009). The breeding programs resulted to the evolving of similar or better products to either or both parents. Moreover, these congener species are closely related; hence, their proper recognition becomesnecessary.Previous work had indicated some basic morphometry for identification (Teugels *et al.*, 1990). The present study has employed molecular analysis using blood serum protein in addition to morphometry for proper characterization of the sympatric

Heterobranchus species.

Materials and Methods

Sample collection

Samples of *H. bidorsalis* and *H. longifilis* were obtained from Lake Kainji, Niger State, Nigeria; and transported live to Wet laboratory, Department of Animal Sciences, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. The samples were acclimatized in (2m × 1m × 1.5m) indoor plastic tanks and fed daily.

Morphometric evaluation

The following body measurements were carried out following the procedure of Teugels (1982, 1986), Teugels e*t al.* (1990) and Agnese *et al.* (1997) to determine TL, total length; SL, standard length; HL, head length; PAL, pre-anal length; PPL, pre-pelvic length; PPEL, pre-pectoral length; PDL, pre-dorsal length; DFL, dorsal fin length; ADFL, adipose fin length; ADFD, adipose fin depth; AFL, anal fin length; and PFL, pelvic fin length; while the following characters were counted for meristic data, viz: DFR, dorsal fin ray; and AFR, anal fin ray.

Serum preparation

Blood samples were drawn from the haemal arch of each sample using sterile hypodermic syringes. Physiological saline water (0.9% NaCl) was added at 3:2 blood samples; and left at ambient temperature for 1 h. The solution was then centrifuged at 3,000 rpm for 10 min. The supernatant (serum protein) was extracted and stored at -20°C for further analysis (Avtalion, 1984; Betiku and Omitogun, 2006).

Gel preparation

Sodium Dodecyl Sulphate-Polyacylamide Gel Electrophoresis (SDS-PAGE) gel preparation involved addition of SDS. SDS-PAGE analysis was carried out using the Bio-Rad Mini Protean II Cell kit of 10 mL capacity. A discontinuous buffer system analysis was employed. Solutions for 4% stacking gel, 12% resolving gel for SDS-PAGE were then prepared (Bio-Rad, 1995).

Sample preparation for SDS-PAGE

30-40 l of 7.5 % β-mercaptoethanol (Sigma) was added to 370 l of sample buffer. Therefore, to each of 10 l protein sample, 40 – 60 l of mixture of sample buffer plus β-mercaptoethanol was added at ratio 1:5. The prepared samples were heated at 95°C for 4 min for denaturation. Thereafter, after cooling, 10 l each was loaded in each well of the kit. The separation of protein was carried out with the aid of Bio-Rad Electrophoresis Power Supply Model 200/2.0 in the Bio-Rad Mini Protean II Cell at 150 V for about 45 min.

Staining and de-staining of gel

After the electrophoretic run, the gels were carefully removed from the kit and stained in 0.1% Coomassie blue in glacial acetic 1:4 methanol for about 1 h. Thereafter, the gels were destained with 60% glacial acetic 1:4 methanol solutionfor ~3hrs. The gel was then documented.

Data analysis

Each gel was scored both visually and observation of its scanned image for presence (1) or absence (0) of protein bands. The data were log transformed and analysed with PAlaeontological STatistics (PAST) software package to generate dendrograms (Hammer *et al.,* 2008). The mean value of each species was employed to generate distance indices data for comparative genetic distance evaluation choosing Dice option.

Results and Discussion

Morphometric analysis

Table 1 shows the measurements and meristic counts for the sympatric population of *H. bidorsalis* and *H. longifilis*. The data revealed the presence of large adipose fin attributes [length; depth

Table 1. Measurements and meristic counts for population of H. bidorsalis and H. longifilis

	H. bidorsalis				*H. longifilis*	
Parameters	n	mean	SD	N	mean	SD
TL (cm)	32	51.7	4.1	35	53.7	4.6
SL(cm)	32	45.9	3.7	35	47.1	3.9
			%SL			
HL	32	29.9	0.9	35	31.2	1.4
PAL	32	58.4	3.3	35	63.2	4.3
PPL	32	48.0	1.6	35	49.4	2.3
PPEL	32	21.7	1.5	35	23.0	1.3
PDL	32	34.6	1.2	35	38.6	2.2
DFL	32	42.3	1.7	35	35.2	1.7
ADFL	32	23.4	1.1	35	25.7	3.2
ADFD	32	4.2	0.5	35	4.4	0.6
AFL	32	38.7	2.2	35	34.3	2.6
PFL	32	10.5	1.0	35	9.7	1.2
			Fin Rays			
		Min-Max			Min-Max	
DFR	32	40-45		35	26-32	
AFR	32	39-55		35	26-41	

TL, total length; SL, standard length; HL, head length; PAL, pre-anal length; PPL, pre-pelvic length; PPEL, pre-pectoral length; PDL, pre-dorsal length; DFL, dorsal fin length; ADFL, adipose fin length; ADFD, adipose fin depth; AFL, anal fin length; PFL, pelvic fin length; DFR, dorsal fin ray; AFR, anal fin ray

(cm)] (% standard length) of 23.4±1.1; 4.2±0.5 in the *H. bidorsalis* compared to *H. longifilis* of 25.7±3.2; 4.4±0.6 respectively. This confirmed that *H. longifilis*has longer adipose fin than *H. bidorsalis* (Teugels e*t al.*, 1990). The adipose hyper-development attributes have been documented as important feature of identification in *Heterobranchus* species (Teugel, 1990). However, the presence of adipose fin is not regarded being a unique feature of identification within *Clariidae*. This is due to the possession of adipose fin by some other members of *Clarias* (Teugel 1983, Teugel, 1990). Therefore, the molecular results of this study provide additional information on the relationship. The DFL revealed reversed data whereby the value (%SL) for *H. longifilis* (35.2±1.7) was lower compared to *H. bidorsalis* (42.3±1.7). Moreover, in relation to the major morphometric parameters, DFL and ADFL, the pre-dorsal length gave higher value (%SL) in the *H. longifilis* (38.6±2.2) than *H. bidorsalis* (34.6±1.2). This probably account for the low value of DFL and higher data in *H. longifilis* compared to *H. bidorsalis*. Furthermore, *H. bidorsalis*(40-45; 39-55) possess more counts than *H. longifilis* (26-32; 26-41) at dorsal and anal fin rays respectively, and this probably result from the longer length of their dorsal and anal fins.

Molecular analysis

SDS-PAGE representative gels of the samples are presented in gels A and B (Figure 1). Gels A and B respectively showed H. *longifilis* and *H. bidorsalis* sera protein in all their lanes. Clustered algorithm analysis of PAST software for similarity association between the samples was presented in Figure 2 ,showing the comparative dendrogram of *H. bidorsalis* and *H. longifilis*. The significant genetic

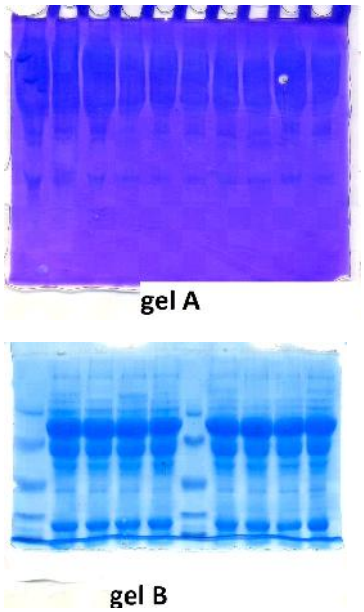

Figure 1.SDS-PAGE representative gels of the samples revealing sera protein bands; gel A, *H. longifilis*; and gel B, *H. bidorsalis*.

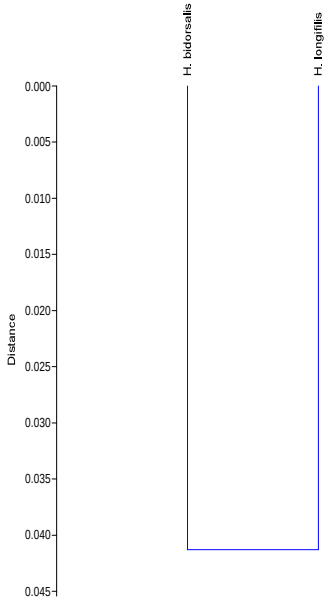

Figure 2. Dendrogram showing genetic relationships between *H. bidorsalis* species and *H. longifilis* species

difference between the two species revealed 4.2%. This indicates high level of proximity of these two species.

Conclusion

The study showed that *H. bidorsalis* and *H. longifilis* were closely related genetically but significantly different i.e. not the same. Therefore, they were very close substitutes for each other especially in breeding programs such as hybridization. The hyper-development of the adipose attributes quickly assists in their identification.

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