Molecular characterization of wild and cultured species of *Clarias* Scopoli, 1777 and *Heterobranchus* Geoffroy Saint-Hilaire, 1808 in Zaria, Kaduna State, Nigeria

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Abstract

Genetic studies on wild and cultured species of *Clarias* and *Heterobranchus* were conducted from August 2012 to January 2013. A total of forty (40) specimens comprising *Clarias gariepinus* (10), *C. anguillaris* (5), *C. galmaensis* (5), *Heterobranchus bidorsalis* (5), *H. longifilis* (10) and *H. isopterus* (5) were obtained from River Galma, Zaria and Bagoma Reservoir, Birnin Gwari (wild) alongside Miracle Fish Farm, Zaria and National Institute for Freshwater Fisheries Research (NIFFR), New Bussa (cultured). A total of eighty (80) samples of muscle and fin tissue of fish were obtained and preserved at -20°C for molecular analysis. DNA was extracted from the tissues using genomic Quick-gDNA™ MiniPrep (50 Preps) w/ Zymo-Spin IIN Columns DNA extraction kits. The target gene cytochrome b gene (585bp) was amplified according to standard methods. All products were separated, purified and visualized under ultra violet light and documented. Amplification of cytochrome b gene in both fin and muscle tissues of the fish species from a portion of mtDNA of wild and cultured samples was observed. The phylogeny showed two groups, the genotypes clustered together are closely related while their neighbours were joined closely. Although genetic variation was observed between wild and cultured species, there is a close relationship between them, confirming that they are members of the same family Clariidae, with good prospects for culture.

Keywords: Genetics; *Clarias* species; *Heterobranchus* species; PCR; Cytochrome b gene.

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Introduction

Studies investigating the genetic composition of fish populations have been conducted for a number of decades, initially using protein coding allozyme loci (Utter *et al* 1987) and then, starting in the mid-1980's, using mitochondrial DNA (mtDNA) polymorphisms (Ng and Dodson, 1999; Avise, 2004). Recently, tandemly repeated microsatellite DNA markers have become the molecular markers of choice for determining intraspecific population genetic relationships (Koskinen *et al* 2002). In general, markers that are used today utilize the Polymerase Chain Reaction (PCR) as it enables analysis of archive materials such as scales.

Clariid catfishes (order Siluriformes) occur in Africa, Asia Minor, South-east Asia and the Indian sub-continent (Teugels and Adriaens, 2003). Although the bulk of *Clarias* species diversity is found in Africa (Teugels, 1986), 18 nominal species 12 of which are currently considered valid (Ng, 2001), are known from South East Asia. Two genera of the family Clariidae; *Clarias* and *Heterobranchus,* along with the cichlids are the most utilized in African aquaculture (Agnese *et al* 1995); this is due to their fast growth rate, resistance to diseases and capacity for high stocking density for clariids (FAO, 2000). Clariidae are known as air-breathing or labyrinth catfishes, possessing suprabranchial organs that enable them to respire (Teugels, 1996). Their extreme hardiness and ability to survive in poorly oxygenated water makes them popular for cultivation.

Clarias and *Heterobranchus* are the most culturable fish species in Nigeria (Tukura *et al* 2005) and the use of molecular markers in genetic studies have yielded tremendous success (Gao *et al* 2001; Fujii, 2002). Species for aquaculture must be properly identified and classified to preserve their germplasm and monitor genetic changes (Legendre *et al* 1992). One of the most important criteria for any efficient conservation and management programme is the taxonomic clarification of species complexes as well as the assessment of genetic biodiversity within and among populations. According to Paul-Michael *et al* (2004), species are the currency of biology.

Molecular marker which are genes with a known location or clear phenotypic expression that is detected by analytical methods or an identifiable DNA sequence facilitates the study of inheritance of a trait or a gene. Markers must be readily identifiable in the phenotype,

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for instance, by controlling an easily observable feature or by being readily detectable by molecular means, e.g. microsatellite marker (Williamson *et al* 2001). The rapid rate of evolution, the maternal mode of inheritance and the relatively small size of mtDNA make the Restriction Fragment Length Polymorphism (RFLP) analysis of this molecule one of the methods of choice for many population studies (Ferguson *et al* 1995).

Fishery management has largely been concerned with the abundance and size of fish available for harvesting, but in the long term this may cause extinction of the population. Concern with reduction in/of genetic resources of fish is part of a larger global concern for the genetic resource of the biosphere. Despite their importance, their biological diversity is being threatened by various anthropogenic activities such as urbanization, habitat fragmentation, over-exploitation and the expansion of aquaculture business. This study will enhance an understanding of the level of genetic variation that will provide management guidelines for commercial use and conservation of *Clarias* and *Heterobranchus* species. There is also a need to understand the genetic composition of natural population in order to evaluate the latent genetic effects induced by hatchery operations. *Clarias* and *Heterobranchus* are the most culturable fish species in Nigeria (Tukura *et al* 2005).

Materials and methods

Study area

Fish specimens and water samples were collected from Bagoma Reservoir, Birnin-Gwari (Latitude 11° 1′ 34′′ N, Longitude 6° 47′ 23′′ E) and River Galma, Zaria (Latitude 10° 27′N, 11° 24′N Longitude 7°23′E and 8° 45′E); representing the wild habitat. The National Institute for Freshwater Fisheries Research, New Bussa (Latitude 9° 52′ 19′′ N, Longitude 4° 30′ 53′′ E) and Miracle Fish Farm, Area BZ Residential Quarters, Ahmadu Bello University, Zaria (Latitude 11º3′ N and Longitude 7º40′ E) represented the culture habitat (Figure 1). The localities were selected based on the availability of fish species. Data were collected between August 2012 and January 2013.

Collection of wild and cultured fish specimens

Fish species collected and used in the study are shown in Table 1 and include:

A. ***Clarias:*** *Clarias gariepinus* (Bürchell, 1822), *Clarias anguillaris* (Linnaeus, 1758) and *Clarias galmaensis* Aken'Ova, 2007.
B. ***Heterobranchus:*** *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809*, Heterobranchus longifilis* Valenciennes, 1840, *Heterobranchus isopterus* Bleeker, 1863.

Identification: Keys/descriptions of Teugels (1986); Aken'Ova (2007) and Froese and Pauly (2015) were used to identify the different species.

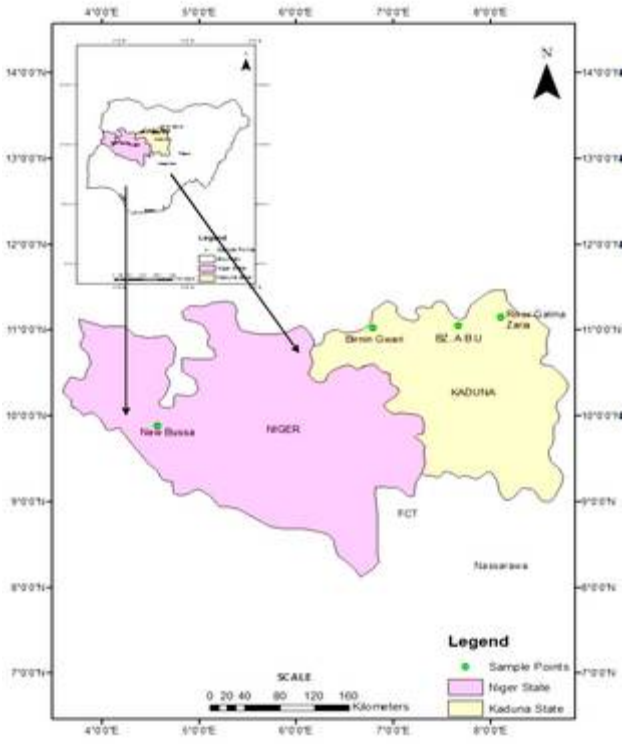

Figure 1. Map of Nigeria showing the study-area.
Source: Adapted and modified from Administrative Map of Nigeria.

Fin (rayed dorsal and adipose) and muscle tissues were obtained from 40 specimens of cultured and wild fish (Table 1) using a pair of forceps and dissecting scissors, then frozen at -20°C. The molecular studies were conducted in the Centre for Biotechnology, Ahmadu Bello University, Zaria.

Molecular analysis

Molecular characterization of species of the two genera (*Clarias* and *Heterobranchus*) was investigated by Polymerase Chain Reaction (PCR) amplification of cytochrome b gene.

DNA was extracted from fish fins and muscle using genomic Quick-gDNA™ MiniPrep (50 Preps) w/ Zymo-Spin IIN Columns DNA extraction kits (Inqaba Biotech Laboratory, South Africa) following the manufacturer's guide. The PCR was performed using DreamTaq™ Green PCR Master Mix (2×), 200 reactions (Inqaba Biotech lab, South Africa) in appropriate volumes. Cytochrome *b* gene (585 bp) was amplified using primers L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' (Briolay *et al* 1998) of 7 min at 72˚C (Nwafili and Gao, 2007). The PCR cycling conditions were 2 min initial denaturation at 95°C and 35 cycles of 30s at 95°C for denaturation, 30s at 58.3°C for annealing, 1 min at 72°C for extension, and a final extension at 75°C for 10 min. Amplicons were loaded on 0.8-1.5% agarose gel and photographed under UV light.

Table 1. List of the clariids used in the study and their sources.

S/N	Species	Autopsy No.	Source	Specimens (*n*)	Habitat
1	*Clarias gariepinus*	CgaGAL	River Galma	3	Wild
		CgaBAG	Bagoma Reservior	2	Wild
		CgaMIR	Miracle Fish Farm	5	Culture
2	*Clarias anguillaris*	CanGAL	River Galma	3	Wild
		CanBAG	Bagoma Reservior	2	Wild
3	*Clarias galmaensis*	CgmGAL	River Galma	5	Wild
4	*Heterobranchus bidorsalis*	HbiGAL	River Galma	5	Wild
5	*Heterobranchus longifilis*	HloGAL	River Galma	5	Wild
		HloNFR	NIFFR	5	Culture
6	*Heterobranchus isopterus*	HisGAL	River Galma	5	Wild

Key: *n* = Number of specimens collected; CgaGAL = *Clarias gariepinus* from River Galma; CgaBAG *Clarias gariepinus* from Bagoma Reservior; CgaMIR = *Clarias gariepinus* from Miracle Fish Farm; CanGAL= *Clarias anguillaris* from River Galma; CanBAG = *Clarias anguillaris* from Bagoma Reservoir; CgmGAL= *Clarias galmaensis* from River Galma; HbiGAL = *Heterobranchus bidorsalis* from River Galma; HloGAL = *Heterobranchus longifilis* from River Galma; HloNFR = *Heterobranchus longifilis* from NIFFR; HisGAL = *Heterobranchus isopterus* from River Galma.

DARwin5 (version 5.0.157) software was used to illustrate the genetic variations observed.

Results

DNA was successfully extracted from preserved fin and muscle tissues. The amplification of cytochrome b gene (585bp) from a portion of mtDNA of wild and cultured *Clarias* and *Heterobranchus* species using primers L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' was observed. Amplification was however more pronounced in fin than in muscle tissue.

A pictorial representation of the amplicons showed some level of diversity in the genotypes of wild and cultured *Clarias* and *Heterobranchus* species. Basically, the phylogeny showed two groups, the genotypes clustered together are closely related while their neighbours were joined closely.

L B1 C1 D1 E1 F1 G1 H1 B2 C2 D2 E2 F2 G2 H2 L

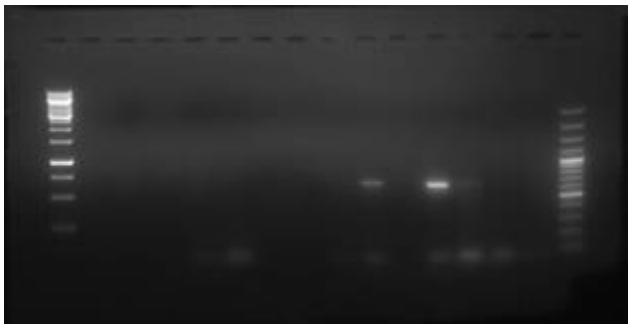

Plate 1. Agarose (1.2 %) gel electrophoretic profile of PCR amplification of cytochrome b gene (585bp). L is ladder (1kb), (B1 & B2) are muscle and fin samples of *Clarias gariepinus* (wild), (C1& C2) are muscle and fin samples of *Clarias gariepinus* (cultured), (D1 & D2) are muscle and fin samples of *Heterobranchus longifilis* (wild), (E1 & E2) are muscle and fin samples of *Heterobranchus longifilis* (cultured), (F1 & F2) are muscle and fin samples of *Clarias galmaensis* (wild), (G1 & G2) are muscle and fin samples of *Heterobranchus bidorsalis* (wild), (H1 & H2) are muscle and fin samples of *Heterobranchus isopterus* (wild) respectively.

Wild species of *Clarias gariepinus* [3] and *C. galmaensis* [7], were found to be closely related while cultured *Clarias gariepinus* [4], wild and cultured *Heterobranchus longifilis* [5 and 6], wild *H. bidorsalis* [8] and *H. isopterus* [9] were also clustered together showing their close relatedness (Figure 2).

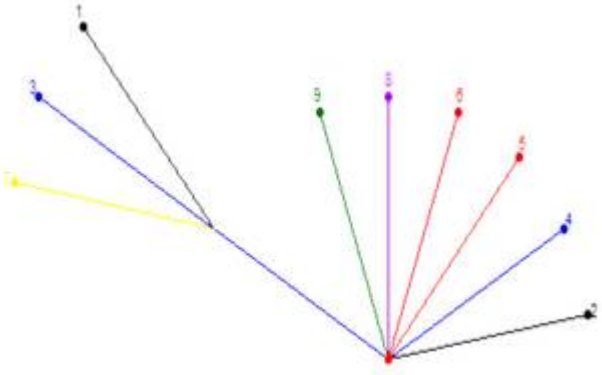

Figure 2. Radial phylogram (neighbour joining) representing genetic diversity of *Clarias* and *Heterobranchus* species

Key: Black = Indicator (1 and 2); Blue = *Clarias gariepinus* (3 and 4); Red = *Heterobranchus longifilis* (5 & 6); Yellow = *C. galmaensis* (7); Purple = *H. bidorsalis* (8); Green = *H. isopterus* (9).

Discussion

The PCR amplification of cytochrome b gene in *Clarias* and *Heterobranchus* species shows the presence of the

gene in these species. The clustered genotype of wild *C. gariepinus* and *C. galmaensis* reveals their close relationship which could be attributed to the fact that they were both obtained from the wild. Although there were some variations in the phenotype of wild and cultured *Heterobranchus* and *Clarias* species, the clustered groups of *H. longifilis*, *H. isopterus*, *H. bidorsalis* and *C. gariepinus* show a close relationship in their genotypes which is indicative of their phylogeny. Findings on serum protein pattern in interspecific and intergeneric hybrids of *H. longiflis*, *C. gariepinus* and *C. anguillaris* in Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) by Akinwande *et al* (2012) reported a high similarity coefficient between these species hinting at a very low genetic diversity. The close relationship between the *Heterobranchus* species and the species of the sub-genus *Clarias* to which *C. gariepinus* and *C anguillaris* belong has been emphasized by Teugels *et al* (1990) in a revisionary study using osteological features. Agnèse *et al* (1997) reported genetic variation at 25 protein and eight microsatellite loci, and two mitochondrial (mtDNA) segments in two sympatric clariids from the Senegal River.

The results obtained here concur to some extent with those of Rognon *et al* (1998) in a morphometric and the allozyme study of nine populations of *C. gariepinus* and seven populations of *C. anguillaris* to quantify their intra- and interspecific variation using *Clarias albopunctatus* and *Heterobranchus longifilis*, as outgroups. Morphometric and allozyme data were congruent for the Nilo-Sudanian populations of *C. gariepinus* and *C. anguillaris* in their study however, allozyme data suggested that *C. gariepinus* is not a monophyletic group and that *C. albopunctatus* was more divergent from *C. gariepinus* and *C. anguillaris* than it was from *H. longifilis*. They stressed the need for a revision of clariid systematics.

There are several rationales for summarizing the cytochrome b gene literature which was found to be inherent in *Clarias* and *Heterobranchus* species studied: First, cyto b is the gene that is perhaps most extensively sequenced to date for vertebrates (Lydeard and Roe 1997; Moore and De-Filippis 1997). Second, the evolutionary dynamics of the cyto b gene and the biochemistry of the protein product are better characterized than most other molecular systems (Esposti *et al* 1993). Third, levels of genetic divergence typically associated with sister species, congeners, and confamilial genera are usually in a range in which the cyto b gene is phylogenetically informative and unlikely to be severely compromised by superimposed nucleotide substitutions (Meyer, 1994).

Sequence variation of the maternally inherited mitochondrial DNA (cytochrome b gene) has been used for detecting genetic variation within and among populations, and among species. Sequence variation of coding nuclear genes, such as growth hormone and homeobox genes, are of phylogenetic interest which calls for further studies.

Conclusion

The variation in genotypes of wild and cultured *Clarias* and *Heterobranchus* species is low, indicating a close relationship between the species. The low variation is evidence of the fact that they are members of the same family Clariidae.

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