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Preliminary Identification and Phylogenetic Study of Chrysichthys Pisces: Claroteidae from Upper River Benue, Nigeria Using Microsatellite DNA

Duwal, S. D.
Lecturer, Department of Fisheries and Aquaculture, Adamawa State University, Mubi, Nigeria
Sanda, M. K.
Postgraduate Student, Department of Fisheries, Modibbo Adama University of Technology, Yola, Nigeria
Amadi, G. C.
Postgraduate Student, Department of Fisheries, Modibbo Adama University of Technology, Yola, Nigeria
Onyia, L. U.
Head of the Department, Department of Fisheries, Modibbo Adama University of Technology, Yola, Nigeria
Ja'afar, N. J.
HOD, Department of Biotechnology, Modibbo Adama University of Technology, Yola, Nigeria

Abstract:
This study was undertaken to identify the valid species at morphological and molecular levels and to illustrate the similarities and distances among the populations of Chrysichthys in Upper River Benue. The samples were purchase from fishers in the months of May through November, 2015 from Bakin Kogi landing site, Jimeta in Yola North Local Government Area of Adamawa State. Fifty-six (56) individuals of Chrysichthys were collected and morphologically identified into four species: Chrysichthys auratus longifilis, C. furcatus, C. nogrodigitatus and C. walkeri. DNA from alcohol preserved fin clips was extracted and purified using kit obtained from Zymo Research Corp. ZR Genomic DNA™. The DNA templates were PCR amplified using primer CN13 on Master Thermocycler PCR Machine. The PCR aplicons were run on 1% agarose gel and the bands visualized using a Digital UV Light Trans-illuminator. The photograph of the bands was saved and the amplified bands read and interpreted. Twenty-four samples gave a clear band for the CN13 primer used. The result showed that of the four species identified morphologically, two amplified 100% (C. nigrodigitatus and C. walkeri), C. auratus longifilis amplified 25% and C. furcatus amplified 14.3%. The Pearson Coefficient similarity matrix showed that there was a range from -0.083 (8.3%) to 1.000 (100%) and the distance matrix showed a range from 0.000 (0%) to 112.309%. The dendrogram also showed clades representing 3 levels of relationship between the four species studied. It was concluded that the CN13 locus amplified successfully for the 2 species C. nigrodigitatus and C. walkeri and could be used for molecular identification of the species.

Keywords: *Chrysichthys, identification, microsatellite marker, phylogeny, upper river benue*

1. Introduction

Chrysichthys spp is a Siluroidae belonging to the family Claroteidae (Teugels, 1996). The species of catfish *Chrysichthys* are widely distributed in fresh and brackish waters in West Africa where they are commercially important fish (Holden and Reed, 1991) with great potentials for aquaculture (Erondu, 1997; Nwafili *et al.*, 2014). In addition, the culture of the species of *Chrysichthys* is widely practiced in many countries of the West Africa sub region and constitutes one of the largest freshwater cultivated fish (Ouattara *et al.*, 2014). In Ivory Coast and Nigeria, *Chrysichthys* is highly valued food-fish and is among the wild commercial catches as well as cultural fish species (Otémé, 1993; Hem *et al.,* 1994). It plays a vital role in the aquatic ecology and fisheries of the Upper River Benue in Nigeria and other African water bodies. It makes significant contribution to the artisanal fisheries of rivers and lagoons, and its aquaculture potentials are great (Ezenwa *et al.*, 1986; Erondu, 1997). Owing to its economic importance and suitability for culture, considerable research has been devoted to the study of several aspects of the species in Nigerian waters (Ikusemiju and Olaniyan, 1977; Ezenwa, 1981; Ekanem, 2000).

Molecular genetic markers have powerful ability to detect genetic structures and genetic uniqueness of individuals, populations or species (Doveri *et al.,* 2008). These molecular markers combined with new statistical developments have revolutionized the analytical power, necessary to explore the genetic diversity. Various molecular markers, protein or DNA (mt-DNA or nuclear DNA) such as

microsatellites and other markers is now being used in fisheries management and aquaculture. The markers provide various scientific observations which have importance in aquaculture practice recently such as: species identification, genetic variation and population structure study in natural populations, comparison between wild and hatchery populations, assessment of demographic bottlenecks in natural population and propagation assisted rehabilitation programmes (Chauhan and Rajiv, 2010).

Microsatellites have become the marker of choice for application in fish population genetic studies (Beckmann and Soller, 1990). They have multiple alleles which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana-Eguia *et al.,* 2004). In addition, microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations (Boris *et al*., 2011). High cost of developing species-specific markers has been the main challenge of microsatellite markers in the past (Castoe *et al*., 2010). Recently, molecular markers have been commonly used for population studies (Al-Atiyat *et al*., 2012). Microsatellites have been used to study the genetic diversity of farmed and wild populations of fishes (Norris *et al*., 1999; Boris *et al,* 2011). The objectives of this study are the morphological identification of species in the genus Chrysichthys and to determine the valid species at molecular level in Upper River Benue, Nigeria.

2. Materials and Methods

2.1. Study Site/ Location

The Study Site was Bakin Kogi Landing Site, Jimeta, Yola (latitude 9°17'15" N and longitude 12°28'00" East of the Greenwich Meridian (Figure 1).

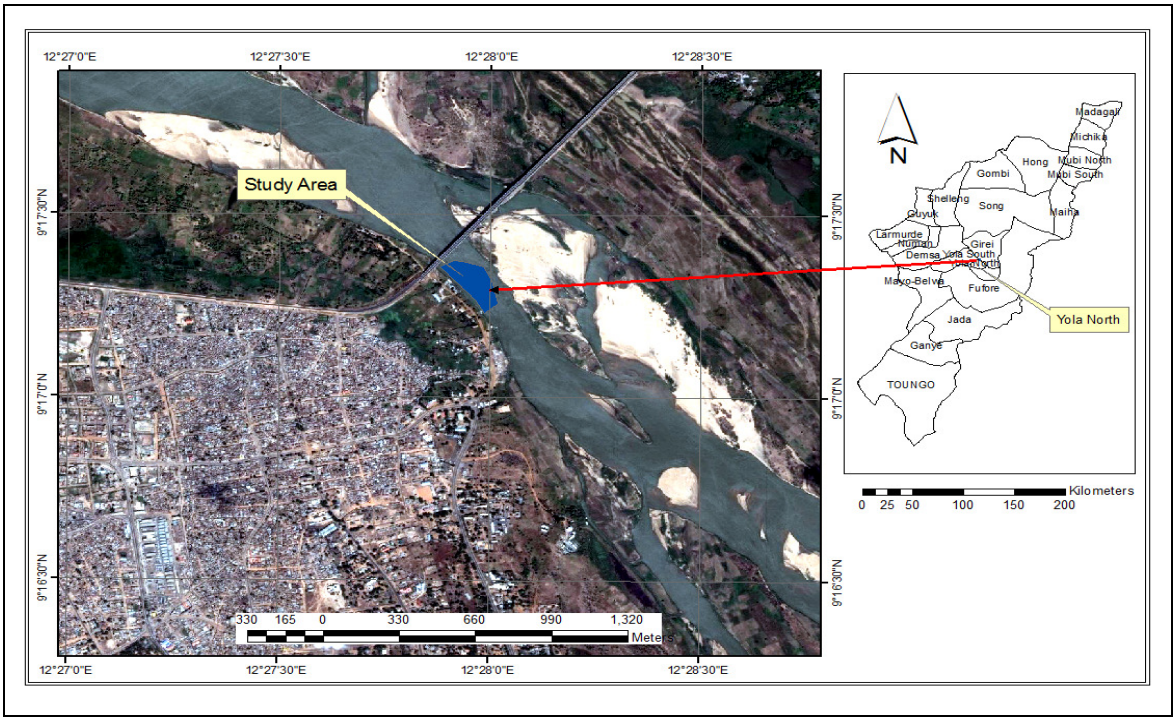

Figure 1: A Satellite Image of Bakin Kogi Landing Site.

2.2. Study Period

The period of study was between May, 2015 and November, 2015.

2.3. Sample Collection

Live fish samples were purchased from fishers at according to guidelines and standards recommended by APHA (1985) and World Health Organisation (1993).

2.4. Morphological Identification

The samples were identified morphologically according to Idodo-Umeh (2003) and Olaesebikan and Raji (2013). The clipped dorsal fins were kept individually per 5 ml sample bottle just before the DNA extraction. The length and weight of the samples were also taken and recorded.

2.5. DNA Extraction and Purification

The DNA extraction and purification kit used was obtained from Zymo Research Corp. ZR Genomic DNA™**.** The Water bath used for incubation of the samples was Cole Palmer Model 12501- 15. The incubation was carried outas recommended by ZR Genomic DNA™ Tissue MiniPrep Manual. The centrifuge used in the research was HERMLE Labnet Z233MK and was powered by electricity from the mains. Thermolyne Maxi Mix II Type 37600 Mixer was used for mixing of the samples during incubation and digestion. For accuracy of weight, Electronic Precision Balance METRA Model TL-300 was used to weigh samples for the research.

Fin clips tissues were weighed to approximately 25 mg from each labelled sample bottle and transferred into 1.5 ml micro centrifuge tube. All other protocol was followed according to ZR Genomic DNA™ Tissue MiniPrep Manual. The final volume of eluted DNA was 100 µl and was stored in cold chain before further analysis.

2.6. Preparation of PCR Cocktail

The PCR preparation was followed as recommended by New England Biolabs One *Taq* Quick-Load 2X Master Mix with Standard Buffer. The PCR Mix used for the research was pooled in a tube and is presented in Table 1. 23.5µl of the reaction cocktail (mixed with all ingredients except DNA template) which was added directly to each PCR tube. The amount of DNA template (eluted DNA) added to all PCR microtubes with labels was 5 µl. The PCR thermo cycler used was Master Cycler Personal, Eppendorf AG 22331, Hamburg and the PCR condition is presented in Table 1.

Step	Temperature	Time
Initial Denaturation	940C	30 seconds
	940C	30 seconds
35 Cycles	640C	60 seconds
	680C	60 seconds
Final Extension	680C	100 seconds
Hold	40C	

Table 1: Thermocycling Condition for the PCR Reaction

	Concentration
10XPCR Bufferd NTP Mix	1X 2mM
CN 13 Forward	0.2µm
CN 13 Reverse	0.2µm
DNA Template	<100ng
Distilled H2O	
Taq Polymerase	39.7µl 0.03U

Table 2: PCR Reaction Mixture in each Reaction Tube

2.7. Sequence of CN13 Primers Used

The sequence of the forward primer used was AAGCACAGATTTGGCCCTAC and the reverse primer was TTCGTGTGTACAGGCTTAG.

2.8. Data Analysis

The gel image was read and scored manually on MS PowerPoint. DNA weight were assigned according to a band's alignment with the DNA ladder used (50 bp). All the binary variables recorded were imputed in MS Excel. An online UPGMA statistical package at www.genomes.urv.cat/cgi-bin/UPGMAboot was used to analyse the data. The Pearson Coefficient was used to compare between the sets of variables to find the similarity and the distance between the species. The output dendrogram in Newick Format was constructed at http://bl.ocks.org/kueda/1036776 and using d3 and newick.js. 1000 bootstrap replicates were generated for the construction of the dendrogram.

3. Results

Chrysichthys Species (Bleeker, 1858)

Morphologically, four species of *Chrysichthys*as described by Idodo-Umeh (2003) were identified as *C. auratus longifilis* (Geoffrey St. Hilaire, 1809)*, C. furcatus* (Gűnther, 1864)*, C. nogrodigitatus* (Lacépède, 1803) and *C. walker* (Gűnther, 1899).

	CAL		CF		CN		CW	
	TL(cm)	WT(g)	TL(cm)	WT(g)	TL(cm)	WT(g)	TL(cm)	WT(g)
1	18.5	66.22	22.5	75.75	16.5	36.48	13.5	26.28
2	13.5	28.65	17.0	40.04	14.0	22.20	13.0	19.41
3	18.0	36.87	19.0	57.02	18.5	46.07	15.0	33.17
4	17.5	52.84	15.5	29.80	13.0	19.4	14.0	28.20
5	-	-	18.0	42.15	19.0	45.33	14.0	28.20
6	-	-	12.5	13.39	13.0	17.90	13.5	24.69
7	-	-	47.0	966.9	12.0	14.82	11.5	14.99
8	-	-	-	-	31.5	388.6	-	-
9	-	-	-	-	29.0	323.0	-	-

Table 3: Total Length and Weight of Chrysichthys Samples

Key: CAL- *Chrysichthys auratus longifilis*
CF – *Chrysichthys furcatus*
CN- *Chrysichthys nigrodigitatus*
CW- *Chrysichthys walkeri*
TL- Total Length (centimetres)
WT- Weight (grammes).

	CAL		CF		CN		CW	
1	-	-	-	-	200	180*	150	150
2	-	-	-	-	240	180*	150	150
3	-	-	-	-	240	240	150	150
4	230	230	-	-	240	180*	150	150
5			-	-	180	180	150	150
6			-	-	180	180	140	140
7			250	250	200	200	140	140
8					180	180		
9					200	200		

Table 4: Allele Sizes (in Base Pairs) at CN13 Locus

Key: CAL- *Chrysichthys auratus longifilis*
CF- *Chrysichthys furcatus*
CN- *Chrysichthys nigrodigitatus*
CW- *Chrysichthys walkeri*

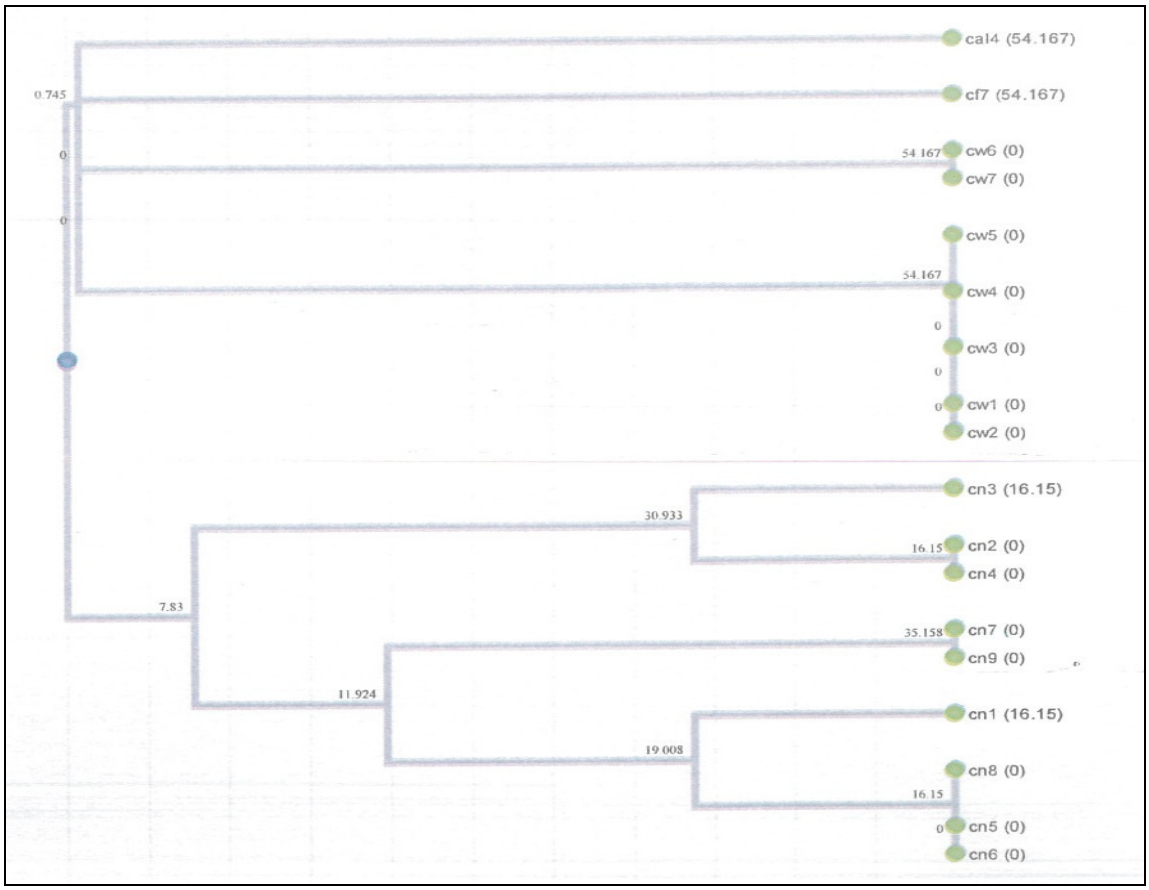

Figure 2: A Dendrogram of the Genus Chrysichthys

Similarities and distance coefficients between the species of *Chrysichthys* are shown in Tables 3 and 4 in a matrix arrangement. Binary similarity coefficients were used since only presence/absence data are available for the species that amplified at the CN13 locus, and are thus appropriate for the nominal scale of measurement. These similarity coefficients ranged from -0.083 (CAL4 and CF7, CW1 and CN5) to 1.000 (CN2 and CN4, CW5 and CW1). The distances also showed the ranges from 0.000 (CW1 and CW2) to 112.309 (CW3 and CN1).

CAL4 and CF7 are distantly related (54.167) to CW6 and CW7, and CW5, CW4, CW3, CW1 and CW2 at the first clade. The second cluster was separated at 30.933 and had two species; CN3, CN2 and CN4. The three species were amplified as heterozygotes (having double bands). The third cluster was at 11.924 having CN7 and CN9 at 35.158. The other end of the clade had CN1, CN8, CN5 and CN6 all clustered at 19.008.

4. Discussion

In Nigeria, molecular marker based on PCR techniques has been used to determine population structure and genetic diversity of fish (Ahmad *et al*., 2012; Mojekwu *et al*., 2012) though there is little information in this area. Genetic variation in a species enhances the capability of organism to adapt to changing environment and is necessary for survival of the species. Genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups. The genetic diversity data has varied application in research on evolution, conservation and management of natural resources and genetic improvement programmes, etc.

The four species of African Silver Catfish identified in Upper River Benue were *C. aluensis longifilis*, *C. furcatus, C. nigrodigitatus* and *C. walkeri*. These species have been reported by Idodo-Umeh (2003) to inhabit rivers and euryhaline waters. Vanderpuye (1979) reported that *Chrysichthys* catfish have been found from practically all the river systems of tropical Africa within latitudes25°N and 25°S.Within this geographical range, 40 species have been identified (Vanderpuye,1979; Froese and Pauly, 2011). Most species of Chrysichthys are found in freshwaters but a few occur in both fresh and brackish waters of coastal lagoons (Chauvet, 1972). *C. auratus* (Geoffroy Saint-Hilaire, 1809) commonly known as Golden Nile Catfish or Long Fin Catfish has been reported by Akinyi *et al.* (2010) to exhibit pronounced intraspecific variability. They also reported that the populations of this species are found in the Nile, Chad, Senegal and the upper reaches of Niger River, Comoe, Benue and Volta.

The bibliography of African freshwater fish, published by the Food and Agriculture Organization (FAO) of the United Nations lists360 references under Tilapia and only 3 for *Chrysichthys*. More recently, some aspects of the biology of *C. walkeri, C. nigrodigitatus* and *C. filamentous* (*C. auratus longifilis*) in Lekki Lagoon, Nigeria (Ikusemiju, 1976; Ikusemiju and Olaniyan, 1977) and age and growth of *C. nigrodigitatus* in Ebrie Lagoon, Ivory Coast (Dia, 1975) have been published. *C. nigrodigitatus* and *C.*

walkeri are among 25 fish species considered by the FAO sponsored Committee on Inland Fisheries of Africa to be of high interest for future of fish culture in Africa (Micha and Frank, 1975).

Correlations between the different allelic distances were mostly close to 1 (Table 3), making it evident that the species are highly related. Similar findings were reported for RAPD markers in wild olives by Sesliand Yegenoglu (2010**).** The authors also observed that the high distance correlations seem to be constant for the different coefficients applied to dichotomic variables. Johns *et al*. (1997), in a study with RAPD markers in the common bean, found correlations on the order of 0.989, 0.972 and 0.979 between the genetic distances obtained by the complement of the simple matching coefficient, Jaccard and Nei-Li's coefficients and Rogers' modified distance, respectively.

Using un-weighted paired group method of arithmetic mean (UPGMA) analysis, Newick format was used to construct genetic distance dendrogram (Figure 2). The dendrogram segregated three distinct clusters of the *Chrysichthys* populations from the sample collection site. The estimation of genetic distance produced UPGMA dendrograms with similar clustering patterns. It also detected correlations between genetic distances and similarities for the population sunder study, with large genetic distance being found between distantly located populations. Similar report has been presented by Onyia (2012) on *Clarias anguillaris* and *Heterobranchus bidorsalis*. The four species identified a common ancestor from the dendrogram but with genetic variations which led to their separation into different clusters and subgroups. Onyia *et al*., (In Press) also reported this for the Clariids in three ecological zones of Nigeria.

5. Conclusion

The result showed that of the four species identified morphologically, two amplified 100% (*C. nigrodigitatus* and *C. walkeri*), *C. auratus longifilis* amplified 25% and *C. furcatus* amplified 14.3%. The Pearson Coefficient similarity matrix showed that there was a range from -0.083 (8.3%) to 1.000 (100%) and the distance matrix showed a range from 0.000 (0%) to 112.309%. The dendrogram also showed clades representing 3 levels of relationship between the four species studied. It was concluded that the CN13 locus amplified successfully for the 2 species *C. nigrodigitatus* and *C. walkeri* and could be used for molecular identification of the species.

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