Genetic Diversity between *Chrysichthys nigrodigitatus* (Lacèpède, 1803) From Kainji and Asejire Dams Using Randomly Amplified Polymorphic DNA (RAPD) Markers

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ABSTRACT

Randomly amplified polymorphic DNA (RAPD) markers were used to assess the genetic variation in the silver catfish (Chrysichthys nigrodigitatus) sourced from two freshwater bodies; Kainji lake (North central) and Asejire dam (South-west) in Nigeria. Four primers OP-8, OPH- 19, OPK-19 and LC-71 were used to amplify RAPD markers on 10 experimental fish from each population. Genomic DNA extraction and purification from caudal fin tissue was performed using ZR Genomic DNA extraction kit. The amplified fragments were ran on gel electrophoresis and visualized as a single compact band of expected size under UV light and documented by gel documentation system (Sygene, INGENIUS). 54 bands were generated by the four RAPD primers from the two Chrysichthys nigrodigitatus populations of which 35 were polymorphic, accounting for 64.81%. The average percent of polymorphic loci for fish samples from Kainji was 5.7% while that of Asejire dam was 4% with percentage polymorphism of 78.57% and 49.76% respectively, indicating polymorphism in the two populations. Nei's genetic diversity was (Ne = 0.0270± 0.0714 and 0.3829± 0.1774) for the Kainji and Asejire populations respectively. Primer OPK-19 showed the best polymorphism out of the four primers with percentage polymorphism of 100 % for Kainji dam and 75 % for Asejire dam. Five (5) of the six (6) polymorphic loci showed significant difference at ($p<0.05$). A departure from homogeneity and both the relative genetic diversity (0.30740± 0.1503), genetic differentiation (Gst = 0.3334) and gene flow (Nm= 0.9996) as an average in the two populations which indicates some degree of genetic variation in the population pair. The Nei's genetic distance (0.1473) and genetic identity (0.8630) showed low genetic differentiation between the populations and no significant ($p>0.05$) correlation with geographical distances. The unweighted pair group method with averages (UPGMA) dendogram showed the Kainji population in one cluster, except two individuals which are found in one of the two sub clusters of the Asejire population thus, suggesting close genetic relationship in both populations and also reveals that the Asejire population are considerably more diverse. This findings could be use to make a future plan for conservation and management of C. nigrodigidatus in natural water bodies.

Keywords: *Chrysichthys nigrodigitatus*, genetic diversity, polymorphism, RAPD.

INTRODUCTION

The aquatic ecosystems have suffered aggressions like predatory fishing, introduction of exotic species, deforestation, pollution and hydroelectric dam implementations. They have caused deep modifications in environment dynamics, jeopardizing the rich fish varieties (Solé-Cava, 2001). The comprehension of genetic differences of native fish populations is fundamental to fisheries management. Information derived from molecular genetic techniques would contribute significantly to the preservation of aquatic genetic resources and sustainable development Almeida *et al.*, 2003; Martins *et al.*, 2003).

Biological diversity is the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystem with the ecological complexes. This includes diversity within species, and between species of ecosystems (Kushwaha and Kumar, 1999). In this way, biodiversity includes variety of all forms along with their genetic make-up and their possible assemblages. Species diversity is a property at the population level while the functional diversity concept is more strongly related to ecosystem stability and stresses, physical and chemical factors for determining population dynamics in the lentic ecosystem. Also, the various organisms including the planktons play a significant role in the dynamics of an ecosystem (Kar and Barbhuiya, 2004).

Molecular markers are realistic and useful tools for the investigation and

monitoring of genetic conditions both in native populations and in captive lots (Alam and Islam, 2005). Random Amplified Polymorphic DNA (RAPD) and microsatellite markers are among the molecular markers used to analyze genetic diversity of fish. Both of these markers may be analyzed by Polymerase chain reaction (PCR). RAPD is a low-cost, simple technique, which requires no previous sequence information and in which a large number or putative loci may be screened (Weising *et al.*, 1995). However, this technique has some disadvantages, associated mainly with dominance, reproducibility, homology inferences, and artifact fragments as reported. The RAPD method according to Williams *et al.* (1990) has been widely used in molecular biology laboratories and has been frequently applied to reveal population-genetic variation, divergence, and biogeography. The RAPD procedure has been instrumental to understand the genetic variability of fish populations (Prioli *et al.*, 2002) and (Almeida *et al.*, 2003). This technique consists of amplification by PCR of random segments of genomic DNA using a single short primer of arbitrary sequence. RAPD can detect high levels of polymorphism and produce genetic markers (Welsh and McClelland, 1990) and Williams *et al.*, 1990).

The genus *Chrysichthys* belongs to the family *Bagridae* and has been described by Reed *et al.* (1967) and Idodo-Umeh (2003). The genus has high economic importance and several aspects of its biology have been studied in terms of age and growth (Fagade 1980a, Fagade 1980b, condition factor, diet, reproductive biology and diseases Ajah *et al.* (2006), Atobatele and Ugwumba (2011). Morphometric studies on Nigerian fishes have been carried out to assess specific variation (Anyanwu and Ugwumba 2002), (Adedeji and Araoye, 2006) and congeneric variation (Anyanwu and Ugwumba (2003), Eyo (2003). Anyanwu and Ugwumba (2002) stated that variations in morphometric characters of a species from different localities are attributed to environmental factors which are temperature and water quality among others. It can also be attributed to the growth rate (Adedeji and Araoye 2006) which could depend on their genotype, availability of food and rate of reproduction. The objective of this study is to establish the genetic variation between *C. nigrodigitatus* in Kainji and Asejire dams located in different ecological zones of Nigeria.

MATERIALS AND METHOD

Sample collection

A total of ten fish samples each with average weight 250.00±0.01g were collected from Kainji and Asejire dams of Nigeria.

Molecular analysis using RAPD

The tissue from the caudal fin of each fish samples were separately collected to avoid gene pollution as a result of blood contamination and tissue mixing, Caudal fins were preserved in 95% ethanol, appropriately labeled and stored at 5 - 8^{0}C in a refrigerator and taken to the Plant Biology Laboratory of the University of Ilorin for Molecular analysis. Genomic DNA extraction and purification from caudal fin and blood sample was performed using ZR Genomic DNA tissue extraction kit (Zymo Research, California, USA) according to the manufacturer's protocols for solid tissue.

Caudal fins were placed in mortal and pounded with pestle to obtain soft tissue used for purification of DNA from up to 25 mg fresh tissue, the tissue yielded up to 3-5 µg DNA per mg tissues, each fish tissue sample (≤ 25 mg) was placed in a micro centrifuge tube and labeled. 95µl of both water and 2X Digestion Buffer were added followed by Proteinase 10µl to digest the protein Each tube was mixed and then incubated at 55°C for 3 hours until the tissue was completely disintegrated. 700 µl Genomic Lysis Buffer was added to the tube and mixed thoroughly by vortexing

and centrifuge at 10,000 x g for one minute to remove insoluble debris from the tail tissue used, the supernatant were transferred to a Zymo-spin ™ IIC Column in a collection tube and centrifuge at 10,000 x g for one minute. 200 µl of Pre-DNA wash buffer was added to the spin column in a new collection tube labeled in the same order for washing the DNA at 10,000 x g for one minute. 400 µl of g-DNA wash buffer was added to the spin column and still centrifuged at 10,000 x g for one minute. After been washed, the spin column were transferred to a clean micro centrifuge tube, and also labeled in the same order. About 30 µl DNA Elution buffer was added to the spin column and incubated for 5 minutes at room temperature, then centrifuged at top speed for 30 seconds to elute the DNA from RNA. The eluted DNA was used immediately for molecular based applications.

Agarose- gel electrophoresis of genomic DNA

The size of DNA fragment was estimated by gel electrophoresis. This technique separated fragments by charge, size (molecular weight) and shape. First, the agarose gel was made with slots (wells) in it and DNA samples were dispensed in the well, a buffer solution were placed in the apparatus, and an electric current was run through the gel. DNA molecules were negatively charged due to the phosphates in its backbone, and were placed in an electric field started at the negative (black) electrode and migrated towards the positive (red) electrode. The gel was a complex molecular network containing narrow passages. Smaller DNA molecules passed through more easily (less friction) and migrated faster through the gel than larger size fragments. Linear molecules also migrated faster through a gel, compared to globular forms.

Since the DNA fragments generated in this experiment were all negatively charged and linear in shape, the only variable observed during electrophoresis was sized. To determine the size of a molecule, a standard or positive control was run concurrently in the gel. The standard consists of fragments of DNA of known size.

Quality check for genomic DNA sample was performed on 0.8 % agarose gel electrophoresis. 0.8gm agarose was weighed and dissolved in 100 ml Tris base EDTA (TBE) buffer by heating them in microwave oven. This gel was allowed to cool for some time. 4 µl of Sybr safe (10 mg/ml) was added and mixed well. Melted gel was poured in gel caster. Samples were prepared for loading using 3 µg DNA premixed with plus loading dye (bromo phenol blue). The genomic DNA samples were loaded in the respective wells with external size standard DNA sample was diluted with appropriate amount of Tris-base EDTA buffer to yield a working concentration of 50 ng/µl and stored at 4°C till further uses.

RAPD Assay

Polymerase Chain Reaction (PCR)

PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube in Applied Bio system Thermal Cycler (USA). PCR tubes containing the mixture were tapped gently and spin briefly at 10,000 rpm. The PCR tubes with all the components were transferred to thermal cycler. Twenty samples of the fish were analyzed using random decamer primers (RAPD). PCR was carried out in a volume of 25 µl with the following reaction set up. The PCR protocol was as shown below:

Selection of primers

For primer screening process, four (4) RAPD decamer primers were selected and the sequences were synthesised by Eurofins, Germany as shown in Table 2

Visualization of PCR Product

To confirm the targeted PCR amplification, 5 µl of PCR product from each tube was pre-mixed with 6X gel loading dye and electrophoresed on 1.5 % agarose gel containing SYBR safe (1 per cent solution at10 µl/100 ml) at constant 5V/cm for 30 min in 1 X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV

light and documented by gel documentation system (Sygene, INGENIUS). All the amplified PCR products were resolved on 1.5% agarose gel electrophoresis.

Statistical Analysis

Amplified fragment was scored as binary data, i.e. presence as 1 and absence as 0. Only data generated from reproducible bands were used for statistical analysis. The number of polymorphic loci, percentage of polymorphic loci (%P), observed number of alleles (ne) and Nei's gene diversity (H) were estimated using the AFLP-SURV v 1.0 software with Bayesian approach, this has non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999) gives an unbiased estimate of allele frequencies from RAPD data (Zhivotovsky, 1999). Genetic distance and identity was estimated using GenAlEx 6.41 software. The Tools for Population Genetic Analysis (TFPGA) software v. 1.3 (Miller, 1997) was applied for the estimation of population differentiation by the Reynolds' cancestry coefficient. It can also be used to construct an unweighted pair group method with average (UPGMA) dendograms based on the estimated genetic distances.

RESULTS

RAPD analysis

Rapid profiles and polymorphism generated by PCR

Among the 4 primers screened, OPK-19 yielded comparatively largest number of bands with good resolution and showed more discriminating bands than others though, LC-71 and OPH-19 showed better discriminating bands than OP-8 (Fig 1-4). Each primer produced a unique fragment pattern of amplified DNA with varied number of bands. The result in Table 3 shows that LC-71 have least % polymorphism (14.29%) as compared to other primers for Kainji dam but have the highest % polymorphism (85.71%) in Asejire dam.

Genetic variation in the populations

The proportion of polymorphic loci was higher in the *C. nigrodigidatus* species obtained from Kainji dam with three primers showing 100% polymorphism each while *C. nigrodigidatus* species from Asejire dam had low polymorphism. The gene diversity of *C. nigrodigidatus* species from Asejire dam was higher (0.3829± 0.177) than those of Kainji dam (0.0270± 0.0714). The effective number of alleles in the populations of *C. nigrodigidatus* from Kainji and Asejire dams was 1.0333± 0.0880 and 1.7062± 0.3546, respectively.

Genetic variation of Chrishththys nigrodigidatus

Genetic variation for all loci for species of *C. nigrodigidatus* from Kainji and Asejire dams for single population that is within each population are presented in Table 4 and 5 respectively using primer LC-71. AMOVA results revealed 14.29% polymorphism for Kainji as compared to Asejire which was 85.71%, the high value of percentage polymorphism from Asejire dam indicated high variation within and among population of *C. nigrodigidatus* as compared to Kainji dam.

The observed number of alleles (na) was low (1.1429±0.3780) from Kainji while that of Asejire was high (1.8571± 0.3780), likewise the effective number of alleles (ne) from Kainji dam was low1.0333±0.0880 as compared to 1.7062±0.3546 from Asejire dam, gene diversity (h) recorded from Kainji dam was low 0.0270±0.0714 when compared with 0.3829± 0.1774 as obtained from Asejire dam. Similarly, Shannon's Information index also revealed low value from Kainji dam (0.0482±0.1274) when compared to (0.54660±0.2477) from Asejire dam, In addition, only one polymorphic locus was recorded from Kainji dam while (6) polymorphic loci were obtained from Asejire dam.

Multi population descriptive statistics for the two populations combined Kainji and Asejire Dams, revealed number of alleles (na) 1.8571±0.3780 while the effective number of alleles was 1.4910±0.2769, Nei's gene diversity was

0.30740± 0.1503 and Shannon's Information index was 0.4648±0.2172 as presented in Table 6. Table 7 also revealed the estimate number of gene flow from Gst or Ges. E.g., Nm = 0.5(1 - Gst)/Gst; =0.9996, the number of polymorphic loci (6) and the percentage of polymorphic loci is: 85.71.

Genetic Identity and Genetic Distance of *Chrishththys nigrodigidatus*

The genetic identity and genetic distance of *C. nigrodigidatus* was 0.7615 and 0.2725 respectively for Kainji and Asejire dams indicating low genetic distance 2725 and genetic identical 76.15% structure as shown in Table 8 while Based Nei's (1972) Genetic distance using UPGMA method for the populations from both Kainji and Asejire dams to be 13.63.

Dendogram

The dendogram between the *C. nigrodigidatus* from Kainji dam and Asejire dam was constructed using Unweighed paired group of arithmetic means (UPGMA) dendogram, based on Nei's genetic distance (Fig 5). The UPGMA dendogram separate the two populations into two main clusters. The first main cluster comprises four *C. nigrodigidatus* from Asejire dam and only one *C. nigrodigidatus* from Kainji dam while the second main cluster is divided into two sub cluster comprises nine *C. nigrodigidatus* from Kainji and six from Asejire. The UPGMA dendogram revealed a distant evolutionary relationship between *C. nigrodigidatus* from Kainji and Asejire dams.

DISCUSSION

The four RAPD primers used revealed a total number of 54 band loci that were generated for both Kainji and Asejire dams. Abdel-Kader *et al.* (2013) reported a total of 215 band loci using 15 primers and the size ranging from 118 to 2556bp. Suresh *et al.* (2013) observed a total number of 142 bands loci using 5 primers and band size ranging from 200 to 2,000bp. Danish *et al.* (2012) used 5 primers and obtained a total of 120 band loci, having band size ranging from 200 to 1360bp. Popoola *et al.* (2014) also reported a total of 425 band loci using 9 primers and size ranging from 250 to 2700bp. The different band loci and band size reported by these authors could be due to the fact that the number of bands loci and size depended on the number of primer, samples, type of species and the source of DNA.

The average percentage of polymorphic loci obtained in this study for Kainji and Asejire dams were 78.57% and 48.51% respectively. Barman *et al.* (2003) reported 45% average polymorphic loci in four Indian carp. Mustafa *et al.* (2009) also reported 57.69% polymorphic loci in wild and farmed *Labeo calbasu*. Garg *et al.* (2009) obtained 18.75% polymorphic loci. Higher polymorphic loci (93.49%) have been reported among species of tilapia. Abdel Kader (2013), Sultana *et al.* (2010) reported 83.87% polymorphic loci in *Heteropneustes fossilis*, the different percentage of polymorphic loci reported by authors are also dependent on the number of polymorphic band generated and the number of primers used which is expressed in percentage.

The percentage of polymorphic of *C. nigrodigitatus* from Kainji dam was higher than that of Asejire dam. However, *C. nigrodigitatus* from kainji dam revealed 100% polymorphism with primers OP-8, OPH-19, and OPK-19 while *C. nigrodigitatus* from Asejire were 0%, 33.33% and 80% respectively. This might be attributed to the environmental factors such as existence of another species of *Chrysichthys* like *Chrysichthys walker*, availability of food and water quality of the dam.

Primer LC-71 was the best primer because it showed monomorphism and polymorphism for the samples of *C. nigrodigitatus* obtained from both ecological zones. The mean value of genetic diversity for *C. nigrodigitatus* from Kainji and Asejire dams were 0.0270± 0.0714 and 0.3829±0.1774 respectively

which indicates low gene diversity from these two ecological zones, thus, poor fisheries management, this species needs urgent attention to improve its gene diversity, in order to avert all the negative effect of low gene diversity as well as guarding it from extinction. High gene diversity is very useful for adaptation, long term survival, ability to resist disease, fast growing and high reproduction as observed by (Pinsky and Palumbi, 2013).

There seem to be an inverse relationship between genetic identity and genetic distance. The gene distance and identity of *C. nigrodigitatus* obtained from both ecological zones were 0.2725 (27.3%) and 0.7615 (76.62%) respectively for Kainji and Asejire dams. The implication explicitly affirms to the fact that as the genetic distance between one species and another widens, their genetic identity narrows giving a good opportunity for selection to be made in the population with wide genetic distance, indicating a quite genetic distance and non identical. This agrees with the value of genetic identity standard given by Thrope and Sole-cava (1994) who stated that the values of genetic identity between species of the same genera should be considered high when it is above 0.85 (85%). This might be due to low polymorphic loci exhibited by *C. nigrodigitatus* from Kainji dam that was 14.29 as compared to 85.71 in Asejire dam. The genetic variation between *C. nigrodigitatus* from Kainji dam and Asejire dam were evaluated to present the genetic structure using non parametric analysis of molecular variance (AMOVA).

Several authors had reported different values for Shannon's Information index gene diversity (uh) in different species. Abdel-Kadir *et al.* (2013) reported Shannon's Information index for *Oreochromis niloticus*, *O. aureus*, and *Tilapia zilli* as 0.318, 0.347, 0.363, respectively and 0.219, 0.238, 0.249, respectively. Suresh *et al.* (2013) observed genetic diversity in four Indian populations of *Mugil cephalus* such as Gujarat, Mahrashtra, Andhra, Pradesh to be 0.3717±0.1460, 0.316±0.1720, 0.4419±0.2112 and 0.4012±0.1310, respectively. Mustafa *et al.* (2009) also reported gene diversity and Shannon's Information index in hatchery population of *Labeo calbasu* to be 0.1224 and 0.1779, respectively and in wild population from Jamuna River to be 0.1726 and 0.2506 respectively while wild from Padma River were 0.1238 and 0.1756 respectively. Their findings were at variance with the result obtained in this study probably due to type of markers used and samples were taken from the wild, a different water body.

Various authors such as Mustafa *et al.* (2009); Pereirea *et al.* (2010) and (Popoola *et al.*, 2014) had reported that wild populations are more diverse, with high polymorphism and genetic variability than the cultured species of *C. nigrodigitatus*. The finding in this study was at variance with this assertion. The type of markers used, (LC-71 might have been responsible for low polymorphism in Kainji dam while other primers showed high polymorphism in Asejire dam. The decrease in population as a result of exposure to toxic chemicals can cause mutations (Taniguchi and Perez-Enriquez, 2000; Pinksy and Palumbi, 2013).

CONCLUSIONS

From the foregoing it could be concluded that primer LC-71 was the best among the primers used. There is wide genetic diversity in *C. nigrodigitatus* in the two ecological zones, thus encouraging selection of stocks from both zones.

The dendogram revealed distant genetic evolutionary relationship between *C. nigrodigitatus* from Kainji and Asejire dams which reaffirm placing of the two locations in different clusters which may be as a result of availability of food and water quality of the dams.

RECOMMENDATIONS

1. Further studies should be carried out on *C. nigrodigitatus* from other

ecological zones to ascertain the effect of ecological zones on this fish species in Nigeria.

2. Large sample size and other genetic markers such as Amplified Fragement Length Polymorphism (AFPL), which also does not requires prior knowledge of the genetic structure of the species that could be probe into and reveals more polymorphism than Random Amplified Polymorphic DNA (RAPD) should be used to investigate the genetic structure of cultured *C. nigrodigitatus* from this and other ecological zones.
3. Primer LC-71 is recommended out of the four primers used for further studies on genetic variability.

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Table 1: The PCR protocol, PCR reaction set up and amplification

Steps	Temperature	Time	Cycles
Initial Denaturation	94°C	2 min	1
Final Denaturation	94°C	30 Sec	
Annealing	50°C	30 Sec	
Extension	34°C	60 Sec	35
Final Extension	72°C	10 min	
Hold	4°C	5 min	

Table 2: List of RAPD primers used.

Sr /No	Primer Name	Primer sequence	T_m (°C)
1	OP-8	GTGTGCCCCA	34
2	OPH-19	CTGACCAGCC	34
3	OPK-19	CACAGGCGGA	34
4	LC-71	TGCCGAGCTG	34

Components	Quantity
Nuclease free water	9.5µl
DNA	2.0 µl (80 ng)
Primer (10pmole /ml)	1.0µl

Table 3: RAPD Primers Amplification Analysis for *Chrishththys nigrodigidatus* from Kainji and Asejire Dams.

	KAINJI DAM				ASEJIRE DAM			
Primers	TPB	TMB	TBL	% of P	TPB	TMB	TBL	% of P
OP-8	6	0	6	100	0	6	6	0
OPH-19	6	0	6	100	2	4	6	33.33
OPK-19	8	0	8	100	6	2	8	75
LC-71	1	6	7	14.29	6	1	7	85.71
TOTAL	21	6	27	314.29	14	13	27	194.04
AVERAGE	5.7	1.5	7.25	78.57	4	3.25	7.25	48.51

Keys:
TPB = Total polymorphic bands, TMB = Total Monomorphic band TBL = Total band loci, % of P = Percentage of Polymorphism

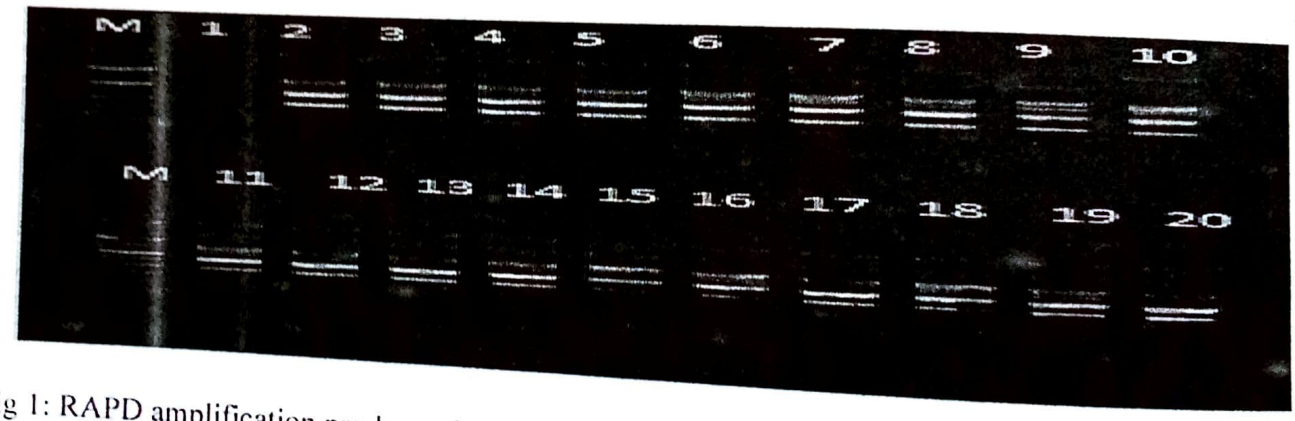

Fig 1: RAPD amplification product of DNA using primer OP-8 for Kainji sample.

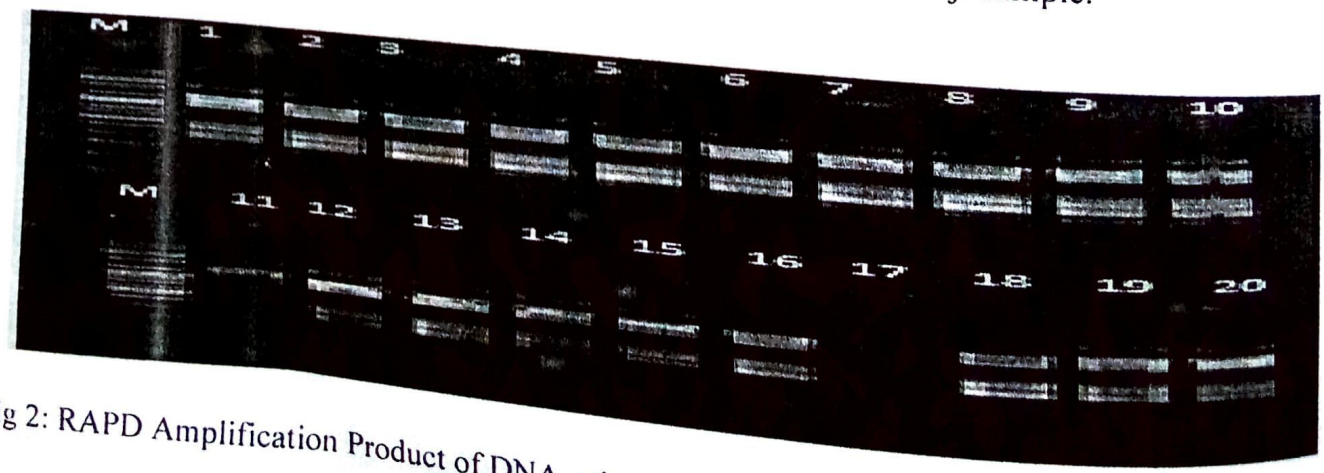

Fig 2: RAPD Amplification Product of DNA using Primer OPH-19 for Asejire sample.

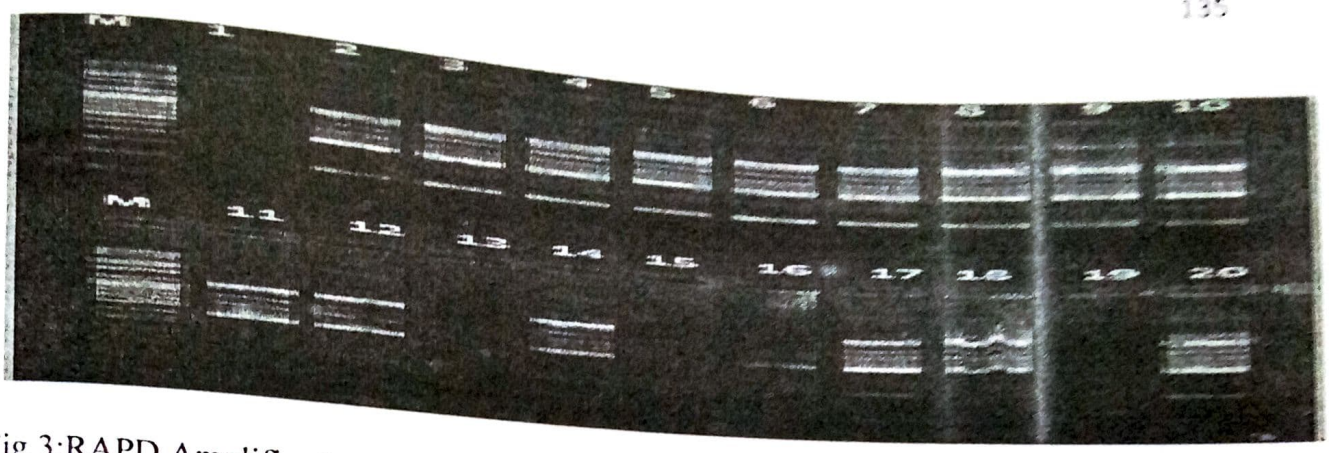

Fig 3:RAPD Amplification Product of DNA using Primer OPK-19 for Kainji sample

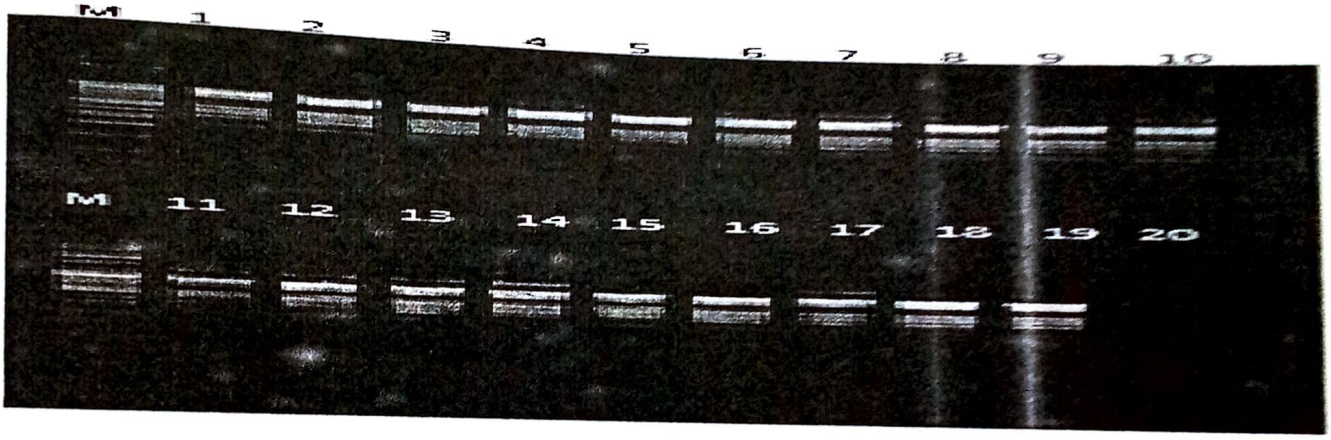

Fig 4:RAPD Amplification Product of DNA using Primer LC-71 for Asejire sample.

Table 4: Population 1- Kainji Dam (KCN1-KCN10)

Locus	Sample Size	na*	ne*	h*	I*
LC71-1	10	2.0000	1.2328	0.1889	0.3372
LC71-2	10	1.0000	1.0000	0.0000	0.0000
LC71-3	10	1.0000	1.0000	0.0000	0.0000
LC71-4	10	1.0000	1.0000	0.0000	0.0000
LC71-5	10	1.0000	1.0000	0.0000	0.0000
LC71-6	10	1.0000	1.0000	0.0000	0.0000
LC71-7	10	1.1429	1.0333	0.027	0.0482
Mean		1.1429	1.0333	0.0714	0.1274
st. Dev		0.378	0.088	0.0714	0.1274

Population size = 10
Gene frequency
* na = Observed number of alleles = 1.1429±0.3780
* ne = Effective number of alleles [Kimura and Crow (1964)]= 1.0333± 0.0880
* h = Nei's (1973) gene diversity=0.0270± 0.0714
* I = Shannon's Information index [Lewontin (1972)] =0.0482 ± 0.1274
The number of polymorphic loci is: 1
The percentage of polymorphic loci is: 14.29 %

Table 5: Population 2- Asejire dam (ACN1-ACN10)

Locus	Sample Size	na*	ne*	h*	I*
LC71-1	10	2.0000	1.7071	0.4142	0.6047
LC71-2	10	2.0000	1.5366	0.3492	0.5337
LC71-3	10	2.0000	1.9819	0.4954	0.6886
LC71-4	10	2.0000	1.9780	0.4954	0.6876
LC71-5	10	2.0000	1.9780	0.4944	0.6876
LC71-6	10	1.0000	1.0000	0.0000	0.0000
LC71-7	10	2.0000	1.7620	0.4325	0.6240
Mean	10	1.8571	1.7062	0.3829	0.5466
st. Dev		0.3780	0.3546	0.1774	0.2477

* na = Observed number of alleles= 1.8571± 0.3780
* ne = Effective number of alleles [Kimura and Crow (1964)]= 1.7062± 0.3546
* h = Nei's (1973) gene diversity=0.3829± 0.1774
* I = Shannon's Information index [Lewontin (1972)] =0.54660±0.2477

The number of polymorphic loci is: 6
The percentage of polymorphic loci is: 85.71 %

Table 6: Summary of Genetic Variation Statistics for All Loci

Locus	SampleSize	na*	ne*	h*	I*
LC71-1	20	2.0000	1.4686	0.3191	0.4993
LC71-2	20	2.0000	1.9033	0.4746	0.6675
LC71-3	20	2.0000	1.6604	0.3977	0.5871
LC71-4	20	2.0000	1.5319	0.3472	0.5314
LC71-5	20	2.0000	1.5319	0.3472	0.5314
LC71-6	20	1.0000	1.0000	0.0000	0.0000
LC71-7	20	2.0000	1.3628	0.2662	0.4365
Mean	20	1.8571	1.4941	0.3074	0.4648
st. Dev		0.3780	0.2769	0.1503	0.2172

* na = Observed number of alleles = 1.8571± 0.3780
* ne = Effective number of alleles [Kimura and Crow (1964)]= 1.4910±0.2769
* h = Nei's (1973) gene diversity=0.30740± 0.1503
* I = Shannon's Information index [Lewontin (1972)] = 0.4648±0.2172

Table 7: Nei's Analysis of Gene Diversity in Subdivided Populations

Locus	Sample Size	Ht	Hs	Gst	Nm*
LC71-1	20	0.3191	0.3015	0.0550	8.5934
LC71-2	20	0.4746	0.1746	0.6321	0.2910
LC71-3	20	0.3977	0.2477	0.3771	0.8257
LC71-4	20	0.3472	0.2472	0.2880	1.2361
LC71-5	20	0.3472	0.2472	0.2880	1.2361
LC71-6	20	0.0000	0.0000	****	****
LC71-7	20	0.2662	0.2162	0.1878	2.1623
Mean	20	0.3074	0.2049	0.3334	0.9996
st. Dev		0.0226	0.0096		

Ht= 0.3074±0.0226
Hs =0.2049±0.0096
Gst = 0.3334

* Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst; =0.9996

The number of polymorphic loci is: 6
The percentage of polymorphic loci is: 85.71

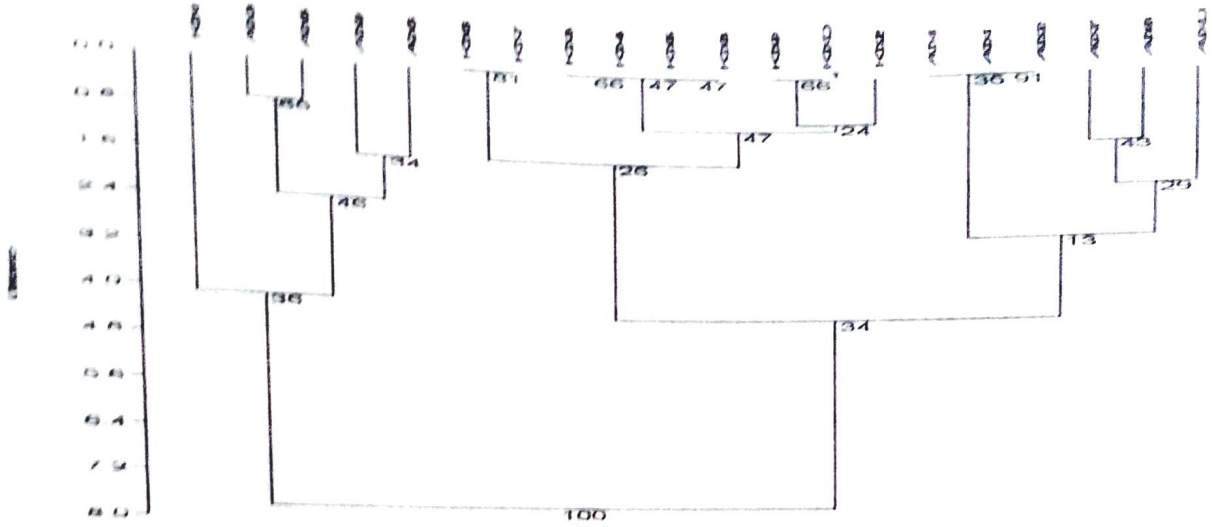

Fig 5 The Dendogram Relationship between the *C. nigrodigidatus* from Kainji and Asejire dams