

Original Research Paper

Genetic differentiation of populations of *Hoplobatrachus occipitalis* and *Xenopus* sp in Southwestern Nigeria

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Abstract

This study determined the level of genetic diversity within and between populations of *Hoplobatrachus occipitalis* and *Xenopus* spp., gene flow, and their genetic differentiation across vegetation zones in Southwestern Nigeria. DNA was extracted from 164 (100 *Hoplobatrachus* and 64 *Xenopus* spp) specimens from both species sampled from three locations each within the four vegetation zones (Guinea savanna, Derived savanna, Rain forest and Mangrove Forest) in Southwestern Nigeria. The CO1 gene fragments were amplified with the universal CO1 primers. The haplotype networks were analysed using a median-joining algorithm in PopART. Analysis of molecular variance (AMOVA) and pairwise genetic distances were performed using Arlequin 3.5. The Mantel test was carried out using R for 10,000 permutations. There were seven haplotypes observed in *Hoplobatrachus occipitalis* and nine haplotypes observed in *Xenopus* populations. Pairwise F_{ST} ranged from 0.0000 to 1.000 in *Hoplobatrachus occipitalis*, and 0.0992 to 0.9984 in *Xenopus*. AMOVA reveals little effect of vegetation on the differentiation of *Hoplobatrachus occipitalis* with a variation of 2.67% while 56.77% of the variation was due to the individuals. The Mantel test for *Hoplobatrachus* ($r = 0.01606$; $p = 0.3952 > 0.05$), suggested that isolation by distance did not limit gene flow. But there were indications that isolation by distance limited gene flow among the populations of *Xenopus* species ($r = 0.4462$; $p = 0.0000$). Despite the continual overexploitation of *Hoplobatrachus occipitalis* in the study areas, the IUCN status had remained “least concern” over the years. This may be due to their moderate genetic diversity and gene flow as revealed in this study. This cannot be said of *Xenopus* spp that are isolated by distance and may require a better conservation intervention for their survival.

Keywords: Biodiversity conservation, Isolation by distance, Genetic diversity, *Hoplobatrachus*, *Xenopus*.

Introduction

At least one third of amphibians are threatened with extinction (Stuart *et al.*, 2008). Overexploitation is one of the major causes of this decline (Gibbons *et al.*, 2000; Halliday, 2008). In Nigeria, just as in many other West African countries, edible frogs are used as an alternative source of animal protein (Mohneke *et al.*, 2009). Several tons of edible frogs (including *Hoplobatrachus occipitalis* and *Xenopus* sp) are unsustainably sourced from the wild in Nigeria to meet protein and international trade demands. This leads to loss of biodiversity and puts a lot of pressure on the ecosystem services they render (Mohneke and Rodel, 2009). According to Teixeira *et al.* (2001), frog now trade across international borders involving more than 30 countries, accounting for approximately USD 48.7 million in 1998 alone.

Ecological boundaries, which are being modified by climate change, have been found to influence dispersal (gene flow) in some species (Geffen *et al.*, 2004; Coker *et al.*, 2017). In Southwestern Nigeria, information on the dispersal of *H. occipitalis* and *Xenopus* sp across ecological boundaries is scarce. Amphibians, being an ecological indicator, can give insight into the way ecological boundaries influence gene flow and therefore help in their conservation efforts.

Genetic diversity is a key factor in biodiversity conservation efforts (Frankham *et al.*, 2010; Freeland *et*

al., 2011). Assessing the population genetics of these species can provide valuable information for conservation much more quickly than longitudinal demographic studies (Storfer *et al.*, 2009). Such studies have already been widely used to inform management strategies to halt or slow down amphibian decline (Emel and Storfer, 2012; Baguette *et al.*, 2013; Allentoft and O'Brien, 2010). Therefore, a wholesome and successful conservation of these species hinges on the provision of genetic information, which will guide management practices in their ranges. According to Arroyo-Lambaer *et al.* (2018), there is a paucity of genetic data for African amphibians. Blackburn (2010) opined that the diversity and ecology of amphibians in Nigeria remain understudied.

Standardized gene for species identification, referred to as DNA barcoding, has been recommended and reliably used to accelerate the rates of taxonomic discovery to meet or exceed rates of biodiversity loss. DNA barcode markers are now being applied to specific taxonomic groups of organisms and are proving invaluable for understanding species boundaries, community ecology, and the conservation of biodiversity (Hebert *et al.*, 2004; Willassen, 2005; Smith *et al.*, 2008; Kress *et al.*, 2015). DNA barcoding has been suggested for all organisms, and quite a few species have been barcoded globally, but very few African frog species have been characterized (Rockney *et al.*, 2015). Cytochrome oxidase subunit 1 (CO1) gene has been proposed as a universal marker for this purpose among animals (Vences *et al.*, 2005; Vences *et al.*, 2012).

The assessments of the patterns of genetic variation within and among species are important in understanding population structure among populations (Bos *et al.*, 2008; Marsden *et al.*, 2012). Several studies (Gow *et al.*, 2004; Geffen *et al.*, 2004; Pilot *et al.*, 2006; Coker *et al.*, 2017) have investigated the effect of ecological zone on the genetic distribution and structuring of organisms, but genetic structuring depends on dispersal mechanisms. This dispersal mechanism differs from species to species. Therefore, according to Nantongo *et al.* (2010), species must be considered individually to understand their genetic structure in relation to their habitat. Genetic information on these species will reveal how they utilize boundaries between vegetation zones. This study is aimed at: 1) determining the levels of genetic diversity and genetic structures within and among populations of *Hoplobatrachus occipitalis* and *Xenopus sp* in Southwestern Nigeria using mitochondrial cytochrome oxidase I (COI) gene loci, 2) examining the geographical pattern of genetic diversity and haplotypes of each species within and across the four vegetation zones in Southwestern Nigeria, and 3) assessing the correlations between the genetic distance and geographic distance of the populations.

Materials and Methods

Sampling

Four vegetation zones (Guinea savanna, Derived savanna, Rain forest and Mangrove forest) from Southwestern Nigeria were sampled for *Hoplobatrachus occipitalis* and *Xenopus* species. Three sampling sites/locations (Figure 1) within each vegetation zone were used. Toe clips were taken from a total of 169 individual frogs (100 *H. occipitalis* and 69 *Xenopus* species), with at most ten individuals per location per species. Toe clips/specimens were stored in sample bottles containing 70% ethanol in the field. These were taken to the laboratory for DNA extraction.

DNA extraction, PCR, Genotyping and Sequencing

Genomic DNA was extracted using the Zymo Research Quick-DNA Miniprep Plus Kit. Extracted DNA was quantified using the Nanodrop Spectrophotometer. The CO1 gene fragments were amplified with the universal CO1 primers: LCO1490: 5 – GGTCACAAATCATAAAGATATTGG–3/HCO2198:5–TAAACTTCAGGGTGACCAAAAAATCA – 3 (Folmer *et al.*, 1994) (for the *Xenopus* species) and Chmf4: TYTCWACWAAYCAYAAAGAYATCGG/ Chmr4: ACYTCRGGRTGRCCRAARAATCA (Che *et al.* 2012) (for *Hoplobatrachus* species).

For the LCO1490/HCO2198 primer pair, DNA was amplified using New England BioLabs PCR Protocol. Individual component reaction was mixed and assembled on ice prior to use. Each PCR reaction mixture comprised 12.5µl of master mix (1x), 1µl of COI forward and reverse primer each (0.1µM), 1µl of template DNA, and 9.5µl molecular grade water, totalling a reaction volume of 25µl. The reaction mixture was quickly spun in a centrifuge to collect the liquid at the bottom of the tube. The tubes were then transferred into the thermocycler for amplification to take place.

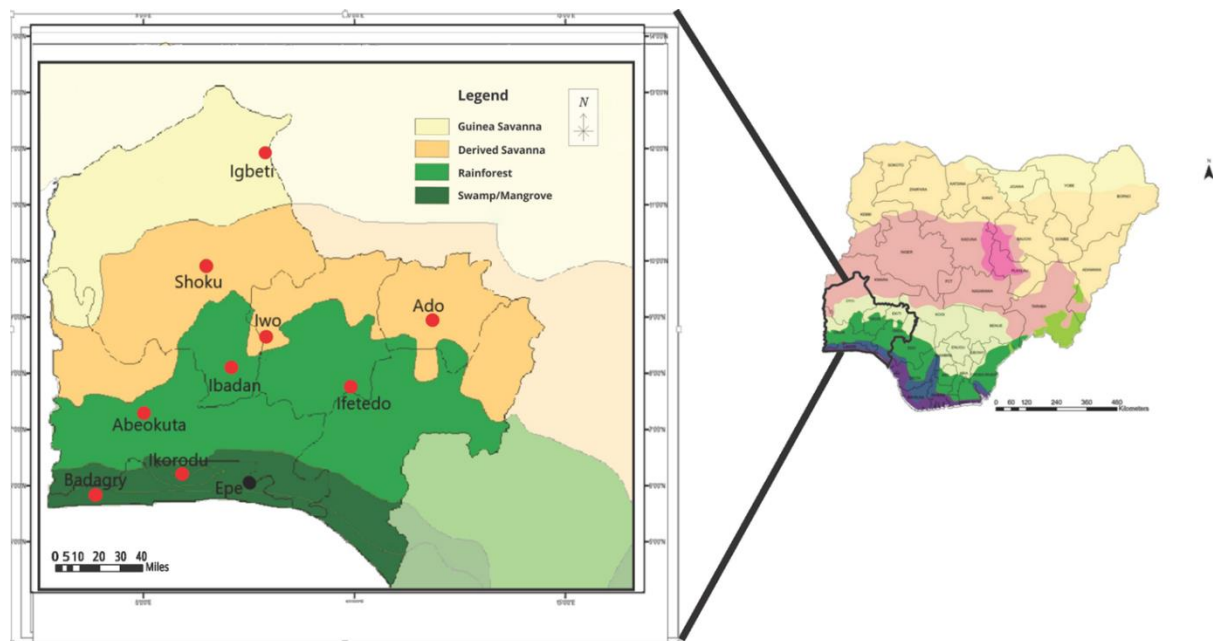


Figure 1. Southwestern Nigeria showing sampling locations

The PCR procedures were as follows: initial denaturation at 94°C for 2 mins; denaturation involving 40 cycles at 94°C for 1 min; primer annealing at 48°C for 45 secs; extension at 72°C for 30 secs; and final extension at 72°C for 10 mins. For the Chmf4/Chmr4 primer pair, a total of 20 µl volume containing NEB OneTaq 2X MasterMix with Standard Buffer (10 µl), Genomic DNA (1 µl), Forward primer (1 µl) and Reverse primer (1 µl), Nuclease free water (7 µl) was used. The PCR procedure was- initial denaturation at 94°C for 5 mins; denaturation involving 35 cycles at 94°C for 30 secs; primer annealing at 50°C for 30 secs; extension at 68°C for 1min; and final extension at 68°C for 10 mins.

The integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-Vision® Bluelight DNA Dye. High-quality amplicons showing single, clear bands of the expected size were selected and sent to Inqaba Biotec West Africa Ltd. for sequencing. This was done for a maximum of six samples per population. Raw sequence chromatograms were visually inspected for base-calling accuracy using *Geneious* software, and low-quality regions at the 5' and 3' ends were trimmed. Sequences containing ambiguous bases, poor signal strength, or overlapping peaks were excluded from further analyses.

Statistical Analysis

The sequences were BLASTed on the NCBI database to ascertain that they are for *Hoplobatrachus occipitalis* and *Xenopus Sp*, *Geneious* software was used to conduct multiple sequence alignment of the sequences. To complement newly generated mitochondrial COI sequences and provide a broader phylogenetic context, reference sequences were retrieved from the GenBank database. For *Hoplobatrachus occipitalis*, sequences from Mauritania (MT764386.1, MT764359.1) and additional reference sequences from the Republic of Congo (KY079610.1, KY079612.1) were included, while a COI sequence of *Hoplobatrachus litoralis* (MG935524.1) was used as an outgroup. For *Xenopus*, reference COI sequences included *Xenopus tropicalis* (EU566850.1), which served as the outgroup, *Xenopus laevis* from South Africa (GQ862280.1, GQ862300.1) and the Republic of Congo (GQ862287.1), *Xenopus borealis* (EU566842.1), and *Xenopus itombwensis* from the Republic of Congo (EU594660.1). All GenBank-derived sequences were combined with field-generated sequences, aligned, and analyzed under identical phylogenetic conditions.

The phylogenetic trees were constructed using IQTREE online platform and FIGTREE software. The bootstrap method, involving 1000 replications, was used to test the reliability of the final phylogenetic tree. The haplotype networks were analysed using a median-joining algorithm in PopART (Leigh and Bryant, 2015). Analysis of molecular variance (AMOVA) of the genetic sequences and pairwise genetic distances was performed using Arlequin 3.5 (Schneider et al., 2000),

Isolation by distance was tested using a Mantel test between pairwise genetic and geographic distance

matrices (Mantel, 1967). Genetic distances were calculated as uncorrected p-distances from mitochondrial COI sequences using the *dist.dna* function in the R package *ape*. Geographic distances were calculated as great-circle (Haversine) distances from latitude–longitude coordinates using the *geodist* package. No log transformation was applied to either distance matrix. Statistical significance was assessed using 10,000 permutations in the *vegan* package (Oksanen, 2015).

Mitochondrial COI sequence data were analyzed using DnaSP version 6 (Rozas et al., 2017) to estimate genetic diversity indices within populations. For each population, the number of sequences (n), number of segregating sites (S), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π) were calculated. Haplotype diversity was estimated as the probability that two randomly selected sequences differ, while nucleotide diversity was computed as the average number of nucleotide differences per site between sequences. Deviations from neutrality were assessed using Tajima's D and Fu's Fs statistics, with statistical significance evaluated at $\alpha = 0.05$. Neutrality tests were not performed for populations with fewer than four sequences, following standard recommendations.

Results

COI sequences submission to gene bank

COI sequences (accession numbers: SUB14118091:PP068372 - PP068440) generated from this study have been submitted to the gene bank and thereby available for further studies of the species.

Phylogenetic trees

The COI Maximum likelihood phylogenetic tree for the *Hoplobatrachus occipitalis* (figure 2) shows no pattern of local adaptation to specific habitat or vegetation zone in Southwestern Nigeria. There is no radiation of the species and are therefore suitable for the study of gene flow across the ecological zones in the study area. The *Xenopus* species on the other hand bifurcates (figure 3) into two branches belonging to the genus *Xenopus*. The sequences show that samples generated in this study cluster closely with corresponding reference sequences retrieved from GenBank. Study sequences of *Hoplobatrachus occipitalis* grouped together with published sequences from other geographic regions, while *Xenopus* species formed well-defined clades consistent with their taxonomic identities. The placement of GenBank reference sequences within these clades supports the accuracy of species identification and confirms the robustness of the inferred phylogenetic relationships.

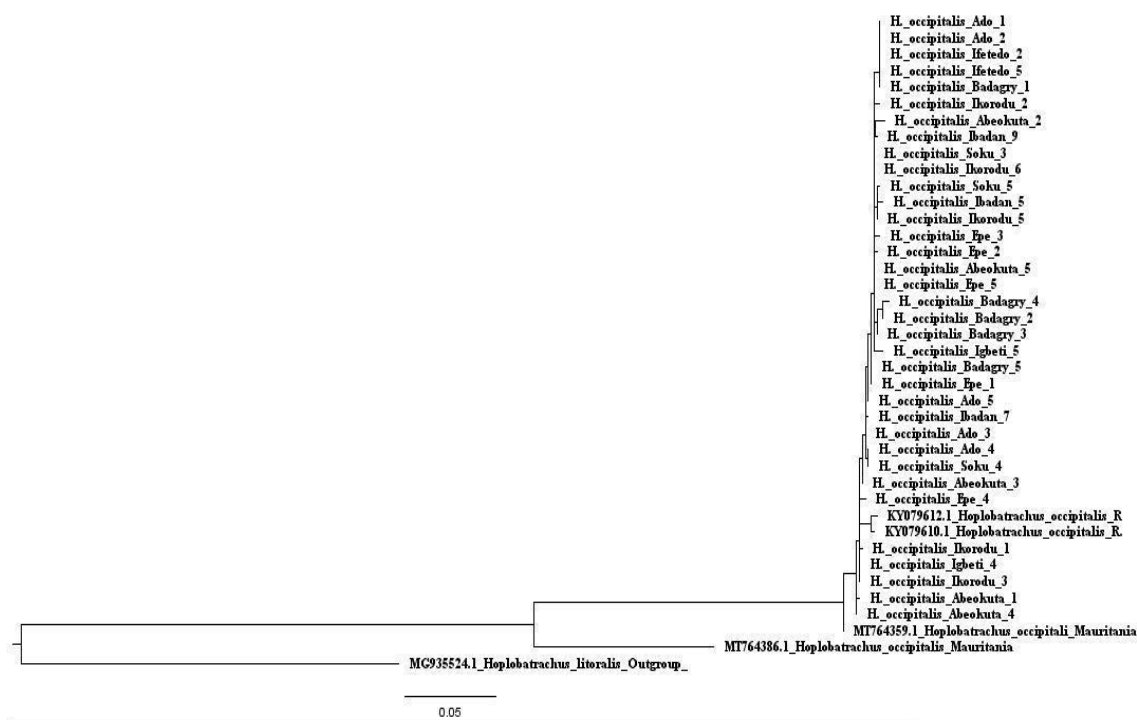


Figure 2. Maximum likelihood phylogram showing the relationships among COI haplotypes of *Hoplobatrachus occipitalis* from Southwestern Nigeria. *Hoplobatrachus litoralis* was used to root the tree.

Haplotype Network

There were seven haplotypes observed based on the COI locus of *Hoplobatrachus occipitalis* in Southwestern Nigeria (Figure 4). Savanna and mangrove ecological zones have haplotypes that are unique to them and can not be said of the rainforest ecological zone. The central and largest haplotype is suspected to be ancestral, having all the lineages and ecological zones represented (Bandelt *et al.*, 1999). The COI locus of the *Xenopus sp* from Southwestern Nigeria revealed nine haplotypes with five unique to the savanna ecological zone and only one unique to the rainforest zone (figure 5). The mangrove haplotype seems to be separated from others by several mutational steps, suggesting that the species is different.



Figure 3. Maximum likelihood phylogram showing the relationships among COI haplotypes of *Xenopus sp* from Southwestern Nigeria. *Xenopus tropicalis* was used to root the tree.

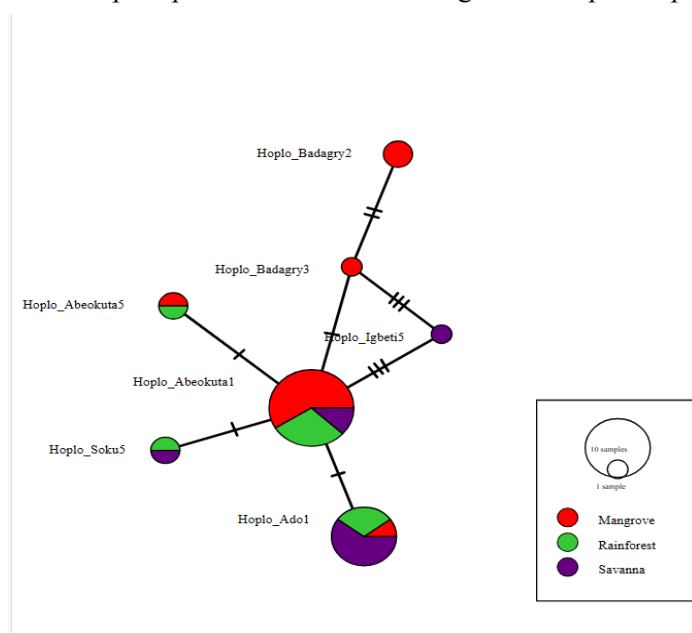


Figure 4. Median-joining haplotype network based on the COI loci of *Hoplobatrachus occipitalis* from Southwestern Nigeria. Each circle represents a haplotype, and the area of a circle is proportional to the number of observed individual. Colors within the nodes refer to the vegetation zones

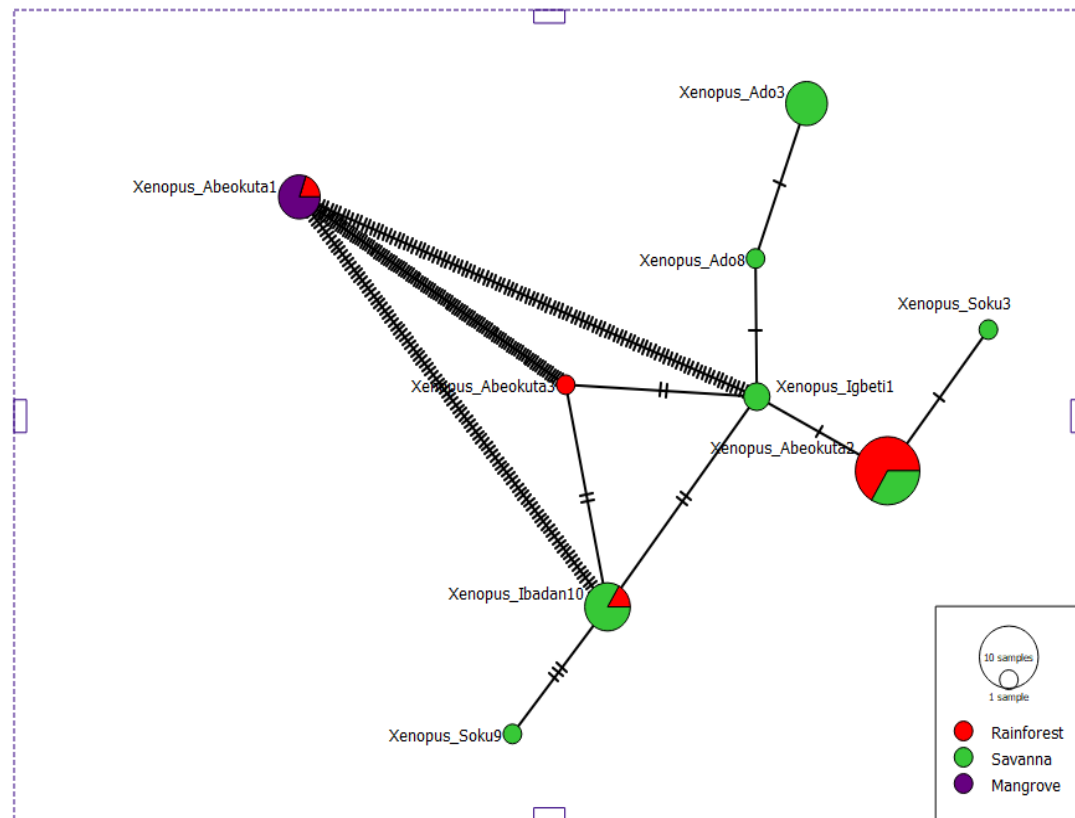


Figure 5. Median-joining haplotype network based on the COI loci of *Xenopus* species from Southwestern Nigeria. Each circle represents a haplotype, and the area of a circle is proportional to the number of observed individuals. Colors within the nodes refer to the vegetation zones.

Analysis of Haplotype, Nucleotide Diversity, Neutrality Test, and Mismatch Distribution of COI Gene in the Study Area

Genetic diversity indices based on mitochondrial COI sequences varied among populations of *Hoplobatrachus occipitalis* (Table 1). Sample sizes per location ranged from two to six individuals. Haplotype diversity (H_d) was generally high across most populations, ranging from 0.6667 in Ifetedo to 1.0000 in Badagry, Soku, Ibadan, and Igbeti. The number of haplotypes (H) ranged from two (Ifetedo and Igbeti) to six (Badagry), while the number of segregating sites (S) showed substantial variation among locations. Nucleotide diversity (π) was low in most populations, with values below 0.01 in Abeokuta, Ado, Epe, Ikorodu, Soku, Ibadan, and Igbeti, but markedly higher in Badagry (0.0809) and Ifetedo (0.1721). Tajima's D values were negative in several populations but statistically significant only in Badagry. Fu's F_s values were negative in most populations, with a significant value observed in Epe, while other populations showed non-significant departures from neutrality. Neutrality tests were not calculable for populations with fewer than four sequences (Ifetedo, Soku, Ibadan, and Igbeti).

Mitochondrial genetic diversity in *Xenopus* populations also varied among sampling locations (Table 2), with sample sizes ranging from four to six individuals. Haplotype diversity ranged from 0.0000 in Badagry, where a single haplotype was observed, to 1.0000 in Soku. Intermediate H_d values were recorded in Abeokuta, Igbeti, Iwo, Ibadan, and Ado. The number of haplotypes (H) ranged from one (Badagry) to five (Igbeti and Soku). Nucleotide diversity values were generally low across most populations, with the lowest value recorded in Ado (0.0007) and higher values observed in Abeokuta (0.0744) and Soku (0.1264). Tajima's D and Fu's F_s values were negative or positive across populations but were not statistically significant in any location. Neutrality tests were not calculable for Badagry due to the presence of a single haplotype.

Table 1: Parameters of genetic diversity and the neutral test based on mitochondrial sequence data of populations of *H. occipitalis*

Location	n	S	Hd	H	π	D	significance	Fs	significance
ABEOKUT									
A	5	5	0.9000	4	0.0042	-0.1909	ns	-2.3710	ns
ADO	5	3	0.9000	4	0.0034	1.5727	ns	-1.1950	ns
BADAGRY	6	125	1.0000	6	0.0809	-1.5109	s	1.1430	ns
EPE	5	3	0.9000	4	0.0027	-0.4102	ns	-3.3040	s
IFETEDO	3	134	0.6667	2	0.1721	n/c		n/c	
IKORODU	5	4	0.9000	4	0.0034	-0.4102	ns	-1.1950	ns
SOKU	3	4	1.0000	3	0.0051	n/c		n/c	
IBADAN	3	5	1.0000	3	0.0064	n/c		n/c	
IGBETI	2	4	1.0000	2	0.0077	n/c		n/c	

Legend: n = number of sequences, S = Number of segregating sites, Hd = Haplotype diversity, H = Number of haplotypes, π = nucleotide diversity, and D is the Tajima test statistic, Fs = Fu's Fs, s=statistical significance, ns= not significant, n/c=Not calculable (number of sequences less than 4)

Table 2: Parameters of genetic diversity and the neutral test based on mitochondrial sequence data of populations of *Xenopus* species

Location	n	S	Hd	H	π	D	significance	Fs	significance
ABEOKUTA	5	90	0.7000	3	0.0744	-1.2138	ns	7.8460	ns
ADO	6	1	0.3333	2	0.0007	-0.9330	ns	-0.0030	ns
BADAGRY	4	0	0.0000	1	0.0000	n/c		n/c	
IGBETI	6	15	0.9333	5	0.0108	-1.2596	ns	-0.0550	ns
SOKU	5	152	1.0000	5	0.1264	-1.1371	ns	1.8690	ns
IBADAN	6	3	0.3333	2	0.0020	-1.2331	ns	1.6090	ns
IWO	4	4	0.8333	3	0.0041	-0.7801	ns	0.1340	ns

Legend: n = number of sequences, S = Number of segregating sites, Hd = Haplotype diversity, H = Number of haplotypes, π = nucleotide diversity, and D is the Tajima test statistic, Fs = Fu's Fs, s=statistical significance, ns= not significant, n/c=Not calculable (number of sequences less than 4)

Analysis on the Genetic Diversity, Gene Flow, Genetic Differentiation, and Genetic Variation of Different Geographic Populations

The pairwise genetic distances among the populations of *Hoplobatrachus occipitalis* revealed a range of 0.0189 (IF/IK) to 1.0000 (IG/BD) (Table 3). For the *Xenopus* populations, pairwise genetic distances ranged from 0.1018 (IG/AD) to 0.9927 (SK/AB) (Table 4). The pairwise F_{ST} ranged from 0.0000 (AD/IF) to 1.000 (SK/IK) for the populations of *Hoplobatrachus occipitalis* (Table 3), while it ranged from 0.0992 (SK/AD) to 0.9984 (BD/AD) for the populations of *Xenopus sp* (Table 4).

Pairwise population differentiation based on mitochondrial COI sequences revealed a wide range of F_{ST} values, including several comparisons with $F_{ST} = 1.000$. These extreme values were observed between population pairs that shared no haplotypes and were characterized by fixed differences in mitochondrial sequences. Such comparisons typically involved populations with low within-population genetic variation, and in some cases relatively small sample sizes. Overall, the pattern indicates strong mitochondrial differentiation among certain population pairs.

The Analysis of Molecular Variance (AMOVA) reveals that vegetation zones tend to have little effect on the differentiation of the populations of *Hoplobatrachus occipitalis* with a variation of just 2.67% while 56.77% variation was due to the individuals (Table 5). The genetic differentiation of the populations of *Xenopus sp* reveals 15.64% variation was due to individuals (Table 6).

Table 3: Pairwise F_{ST} (above diagonal) and genetic distances (below diagonal) among populations of *Hoplobatrachus occipitalis* in Southwestern Nigeria

	AB	AD	BD	EP	IF	IG	IK	SK	IB
AB	0.0000	0.2593	0.2593	0.4203	0.4203	0.3052	0.2889	0.1471	0.1667
AD	0.2593	0.0000	0.5556	0.8519	0.0000	0.8122	1.0000	0.6875	0.4815
BD	0.2889	0.1111	0.0000	0.7884	0.8519	0.3381	0.4203	0.1111	0.1304
EP	0.2593	0.1111	0.1111	0.0000	0.7884	0.4203	0.4815	0.1111	0.1111
IF	0.1667	0.1304	0.2222	0.1304	0.0000	0.6667	0.4203	0.2222	0.1111
IG	0.4203	0.7884	1.0000	0.7884	0.3381	0.0000	0.5294	0.3103	0.1852
IK	0.1471	0.1852	0.3103	0.1852	0.0189	0.5294	0.0000	1.0000	0.5699
SK	0.5556	0.8519	1.0000	0.8519	0.4815	0.0000	0.6875	0.0000	0.6875
IB	0.3052	0.4203	0.5699	0.4203	0.2483	0.6667	0.3443	0.8122	0.0000

Legend: AB-Abeokuta, AD- Ado, BD- Badagry, EP- Epe, IF- Ifetedo, IG- Igbeti, IK- Ikorodu, SK- Shoku, IB- Ibadan.

Table 4: Pairwise F_{ST} (above diagonal) and genetic distances (below diagonal) among populations of *Xenopus sp* in Southwestern Nigeria

	AB	AD	BD	IW	IG	SK	IB
AB	0.0000	0.2091	0.7513	0.1018	0.1035	0.0992	0.1326
AD	0.7513	0.0000	0.9984	0.7131	0.6958	0.5886	0.7980
BD	0.9953	0.1326	0.0000	0.9891	0.9927	0.9760	0.9953
IW	0.9760	0.0992	0.3178	0.0000	0.2397	0.1502	0.4021
IG	0.9891	0.1018	0.4021	0.1502	0.0000	0.1714	0.1206
SK	0.9927	0.1035	0.1206	0.1714	0.2397	0.0000	0.3178
IB	0.9984	0.2091	0.7980	0.5886	0.7131	0.6958	0.0000

Legend: AB-Abeokuta, AD- Ado, BD- Badagry, IW- Iwo, IG- Igbeti, SK-Shoku, IB- Ibadan.

Table 5: Analysis of Molecular Variance (AMOVA) of *Hoplobatrachus occipitalis* populations in Southwestern Nigeria

Source of variation	D.f.	Sum of squares	Variance components	Percentage variation	p
Among Vegetation zones	2	4.803	0.01342 Va	2.67	0.2424
Among individuals within Vegetation zones	6	11.367	0.20371 Vb	40.56	0.001
Within individuals	65	18.533	0.28513 Vc	56.77	0.0000
Total	73	34.703	0.50225		

Table 6: Analysis of Molecular Variance (AMOVA) of *Xenopus sp* populations in Southwestern Nigeria

Source of variation	D.f.	Sum of squares	Variance components	Percentage variation	p
Among Vegetation zones	2	789.851	19.70307 Va	80.15	0.1212
Among individuals within Vegetation zones	4	55.859	1.03487 Vb	4.21	0.0000
Within individuals	61	234.467	3.84372 Vc	15.64	0.0000
Total	67	1080.176	24.58166		

Gene flow and Fixation Index (F_{ST})

The F_{ST} is 0.43 for *Hoplobatrachus* and 0.84 for *Xenopus*. Therefore, the populations of *Hoplobatrachus* experienced moderate Gene flow (0.72) while Gene flow is low (0.07) in the populations of *Xenopus sp* (Tables 7 and 8). Estimates of gene flow (N_m) derived from mitochondrial COI data indicated low to moderate values among several population pairs. These values reflect levels of maternal lineage connectivity inferred from mitochondrial haplotype distributions rather than overall population gene flow. Patterns of N_m were generally consistent with the observed mitochondrial population structure and differentiation indices.

Table 7: Gene flow and Fixation Index F_{ST} for *H. Occipitalis*

Species	Fixation index (F_{ST})	Gene flow (Nm)	P-value
<i>H. occipitalis</i>	0.43230	0.72	> 0.10

Table 8: Gene flow and Fixation Index F_{ST} for *Xenopus sp*

Species	Fixation index (F_{ST})	Gene flow (Nm)	P-value
<i>H. occipitalis</i>	0.84363	0.07	> 0.10

Isolation by Distance

The Mantel test for the *Hoplobatrachus* populations revealed no correlation between genetic distances and geographic distances ($r= 0.01606$; $p= 0.3952$), suggesting that isolation by distance did not limit gene flow. But there is an indication that isolation by distance limits Gene flow among the populations of *Xenopus* species ($r: 0.4462$; $p= 0.0000$).

Discussion

The adaptability of a species in the face of environmental change is contingent upon the genetic diversity inherent within its populations and this genetic diversity encompasses the assortment of genetic material present within a population or species (Freeland *et al.*, 2011). According to Sun *et al.* (2011), environmental homogeneity leads to low genetic diversity levels, whereas environmental heterogeneity in terms of geographic environment, climate, vegetation...results in high genetic diversity level.

There is a low representation of COI sequences for African herpetofauna in the GenBank and BOLD databases and it has been recommended that efforts should be put into building a comprehensive DNA libraries for African and Nigerian herpetofauna (Nneji *et al.*, 2019). Additional 69 (*Hoplobatrachus* (35), *Xenopus* (34) COI sequences ((accession numbers: SUB14118091:PP068372 - PP068440) has been added to the gene bank through this study. It is believed this will be available to researchers for further research.

Alam *et al.* (2008) suggested monophyly of Asian *Hoplobatrachus* species but was unable to clarify the position of African *Hoplobatrachus occipitalis*. This study reveals no evidence of polyphyly in the populations of *Hoplobatrachus occipitalis* studied in Southwestern Nigeria. The study covered a vast area of the distribution of the species, but their spatial distribution does not relate to genetic radiation. Further evidence of monophyly in *Hoplobatrachus* species were seen in the phylogeny studies of four Asian species (Sultana *et al.*, 2016). *Hoplobatrachus occipitalis* is the only species of the family Dicroglossidae in Africa, the other four are found in Asia (Channing and Rödel, 2019; Kosuch *et al.*, 2001; Frost, 2024). Despite the wide distribution of *H. occipitalis* across Africa, there has not been any evidence of radiation or speciation across their ranges. Gene flow across different populations seems to be high, which implies that they can defy potential barriers. It is a delicacy in most west African countries as they are used as an alternative source of protein and for international trade (Moneke *et al.*, 2009). This puts their conservation at risk since most of them are sourced from the wild and it is not impossible that their gene flow might have been aided by the trade process.

On the other hand, the phylogeny tree of the *Xenopus* species in this study bifurcates into two branches with the closest matches on NCBI as *Xenopus fraseri* and *Xenopus tropicalis*. The historical use of *Xenopus* species in the improvement of human health has been emphasized (Exner and Willsey, 2021) and recently *X. tropicalis*' annotated full genome sequence was flagged to contribute remarkably to the conservation of gene organization with mammals, as well as adding a new dimension to the study of immunity (Robert and Cohen, 2011). African claw frogs comprised of 21 known species divided into two clades ((*Xenopus* and *Silurana*), grouped based on their polyploidy (Evans et al., 2004).

Patterns of mitochondrial genetic diversity across populations of *Hoplobatrachus occipitalis* and *Xenopus* species suggest heterogeneous demographic histories. Populations exhibiting zero or very low haplotype and nucleotide diversity indicate limited mitochondrial variation, a pattern commonly associated with recent bottlenecks, founder effects, or strong genetic drift, though small sample sizes may contribute to this signal (Nei, 1987). In contrast, high haplotype diversity coupled with low nucleotide diversity, observed in several populations, is consistent with recent population expansion following a bottleneck, where new haplotypes arise rapidly with minimal sequence divergence (Grant

& Bowen, 1998). Predominantly negative but largely non-significant Tajima's D and Fu's Fs values further suggest departures from mutation–drift equilibrium without strong evidence of selection (Tajima, 1989; Fu, 1997). Populations showing elevated nucleotide diversity may reflect long-term persistence or admixture of divergent lineages. Overall, these results highlight spatial variation in genetic diversity and emphasize the need for increased sampling and complementary nuclear markers to refine demographic inferences.

Population differentiation occurs due to genetic and environmental factors that lead to variation in fitness and phenotypes (Mitchell-Olds *et al.*, 2007). Different landscapes controlled by different environmental conditions can confer appreciable differences leading to local adaptation. The occurrence of pairwise F_{ST} values of 1.000 reflects complete mitochondrial haplotype differentiation between some populations, a pattern that can arise in haploid, maternally inherited markers such as COI when populations share no haplotypes and exhibit fixed differences. High F_{ST} values are therefore not unexpected in situations involving strong geographic isolation or restricted gene flow. However, the magnitude of these estimates may be influenced by limited sample sizes in some populations, which can inflate measures of genetic differentiation in mtDNA-based analyses (Avice, 2000; Excoffier *et al.*, 2005). Consequently, while the results point to pronounced population structuring, F_{ST} values should be interpreted with caution, and complementary nuclear markers or increased sampling would help to further resolve population connectivity.

The Analysis of Molecular Variance (AMOVA) reveals that vegetation zones tend to have little effect on the differentiation of the populations of *Hoplobatrachus occipitalis* with a variation of just 2.67% while 56.77% variation were due to the individuals. The genetic differentiation of the populations of *Xenopus sp* reveals 15.64% variation were due to individuals. Wright (1978) categorized genetic differentiation as low for $F_{ST} < 0.05$, moderate for $0.05 < F_{ST} < 0.15$, high for $0.15 < F_{ST} < 0.25$, and very high for $F_{ST} > 0.25$ (Govindajuru 1989). The F_{ST} is therefore very high for *Hoplobatrachus* (0.43) and *Xenopus* (0.84)

Low levels of gene flow can result in increased genetic differentiation, reduced genetic diversity, elevated inbreeding, and diminished adaptive potential within populations (Pavlova *et al.*, 2017). These effects can have long-term implications for the genetic health and evolutionary trajectory of the populations. Sufficient gene flow between populations impedes the process of geographic differentiation by maintaining genetic similarity across large areas and preventing the establishment of distinct population structures (de Jong *et al.*, 2011). Interpretation of N_m values derived from mitochondrial DNA requires caution because COI is a haploid, maternally inherited marker and therefore reflects only female-mediated gene flow. Consequently, N_m estimates based on mtDNA do not represent total population connectivity and may underestimate gene flow when male-mediated dispersal occurs. The observed N_m values should therefore be interpreted as indicators of historical or maternal lineage exchange rather than contemporary, biparental gene flow. Taken together with the high F_{ST} values and strong haplotype structuring, the results suggest restricted maternal gene flow among populations, while acknowledging that patterns based on nuclear markers may reveal different levels of connectivity (Avice, 2000; Ballard and Whitlock, 2004).

Conclusion

Based on the COI locus examined in this study, genetic diversity among the two species of frogs in Southwestern Nigeria appear to be moderate. *Hoplobatrachus occipitalis* shows no evidence of genetic partitioning across the vegetation zones examined while *Xenopus* genus shows two clear lineages across the vegetation zones.

From the Analysis of Molecular Variation (AMOVA), populations of *Hoplobatrachus occipitalis* are not differentiated across the vegetation zones, while *Xenopus* populations are differentiated across the vegetation zones. There is an indication of moderate level of gene flow among *Hoplobatrachus* populations but it is hampered among *Xenopus* populations.

Despite the continual unsustainable persecution and harvesting of *Hoplobatrachus occipitalis* in the study area, the IUCN status had remained “least concern” over the years. This may be due to their moderate genetic diversity and Gene flow as revealed in this study. This can not be said of *Xenopus* which are isolated by distance and may require a better conservation intervention for their survival.

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Ethics

Ethical approval and clearance were obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC) to undertake this study, and all the ethical issues were strictly adhered to. Assigned number is UI-ACUREC/001-0422/4.

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