Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Heavy metal and genetic diversity studies in three populations of Snail (*Achatina achatina* Linnaeus, 1758) from Southwest, Nigeria

Estudos de heavy metal e diversidade genética em três populações de Snail (*Achatina achatina Linnaeus*, 1758) do sudoeste da Nigéria

I. O. Ayanda[a]* , J. O. Popoola[a] and S. Inyang[a]

[a]Covenant University, Department of Biological Sciences, Ota, Nigeria

Abstract

Environmental pollutants may often alter the genetic components of natural populations. In this study, heavy metals and genetic diversity in land snail (*Achatina achatina*) from three populations of south-western Nigeria were investigated, using the Atomic Absorption Spectrometry and DNA Sequencing technology respectively. Metal analysis revealed that the snails accumulated lead (Pb) and nickel (Ni) in high concentrations in two of the three states, while cadmium (Cd) was the least detected. Editing and alignment of the sequences of all snail accessions generated a range of 384bp to 419 bp. Analysis of Molecular Variance (AMOVA) in all 18 accessions was low at only 16%. The query coverage (QC) ranged between 96% and 100%, with 14 (77.8%) of the 18 accessions showing 100% identity. Pairwise comparison of the accessions studied also showed a high genetic similarity. The unweighted pair group method with arithmetic mean (UPGMA) generated two main clusters. Cluster I was unique and contain one sample (AaOy06) while the other cluster are very closely related and can be further sub-divided into sub-clusters. The similarity index of between the clusters is 0.5357. The close similarity among the accessions may be due to the geographical proximity of the three states. The uniqueness of accession AaOy06 in comparison to other accessions might be due to the negative influence of heavy metal, particularly lead. The determination of evolutionary relationships among snail populations may be useful towards the breeding efforts of the species in Nigeria.

Keywords: *Achatina achatina*, accessions, heavy metal, sequences, genetic.

Resumo

Os poluentes ambientais podem frequentemente alterar os componentes genéticos das populações naturais. Neste estudo, metais pesados e diversidade genética em caramujos terrestres (*Achatina achatina*) de três populações do sudoeste da Nigéria foram investigados, usando a tecnologia de espectrometria de absorção atômica e sequenciamento de DNA, respectivamente. A análise dos metais revelou que os caramujos acumularam chumbo (Pb) e níquel (Ni) em altas concentrações em dois dos três estados, enquanto o cádmio (Cd) foi o menos detectado. A edição e o alinhamento das sequências de todos os acessos de caramujos geraram uma faixa de 384pb a 419pb. A análise de variância molecular (AMOVA) em todos os 18 acessos foi baixa em apenas 16%. A cobertura da consulta (QC) variou entre 96% e 100%, com 14 (77,8%) dos 18 acessos apresentando 100% de identidade. A comparação pareada dos acessos estudados também mostrou alta similaridade genética. O método de grupo de pares não ponderados com média aritmética (UPGMA) gerou dois clusters principais. O cluster I era único e contém uma amostra (AaOy06), enquanto o outro cluster está intimamente relacionado e pode ser subdividido em subclusters. O índice de similaridade entre os clusters é 0,5357. A grande semelhança entre os acessos pode ser devido à proximidade geográfica dos três estados. A singularidade do acesso AaOy06 em comparação com outros acessos pode ser devido à influência negativa de metais pesados, particularmente chumbo. A determinação das relações evolutivas entre as populações de caramujos pode ser útil para os esforços de reprodução da espécie na Nigéria.

Palavras-chave: *Achatina achatina*, acessos, metal pesado, sequências, genética.

1. Introduction

The genetic structure of natural populations may gradually be facing the threat of genetic erosion. This is because natural occurrences such as natural selection, mutations, and even anthropogenic activities that bring about pollution in the environment are constantly modifying the genetic composition of natural populations, thereby severely disrupting the genetic structure of organisms (Ungherese et al., 2010). Of the different groups

*e-mail: opeyemi.ayanda@covenantuniversity.edu.ng
Received: February 20, 2021 – Accepted: July 17, 2021

of pollutants existing in the environment, heavy metals can be said to greatly affect genetic variability, whether directly through mutations or indirectly as a result of ecological and physiological effects. (Bickham et al., 2000, Belfiore and Anderson, 2001, De Wolf et al., 2004).

The mining and processing of metals, in addition to dumping of the mine wastes can lead to severe heavy metal pollution (Baker et al., 1994). Aside mining activities, wastewater, industries, agriculture, and runoffs also release these pollutants into different environmental media (Masindi and Muedi, 2018). Some important sources of anthropogenic heavy metal pollution in the environment are exhaust from automobile which releases lead; smelting and use of insecticides which releases zinc, copper, and arsenic; and burning of fossil fuels which releases mercury, nickel, tin etc. (He et al., 2005).

Animals that are constantly exposed to high levels of heavy metal-contaminated environment are prone to infertility problems amongst other adverse effects. Entering an organism's body by direct contact, ingestion through food or inhalation, they can bio accumulate and over time become poisonous to the organism, causing embryotoxicity, mutagenicity, carcinogenicity, nephrotoxicity, and hepatotoxicity. Heavy metals occur more frequently in the soil and aquatic ecosystem rather than atmosphere (Kennady et al., 2018).

Land snails, such as *Achatina achatina* are very important components of the ecosystem. In the food web, they are low eaters. Majority of land snails are defoliators, even consuming decomposing leaf litter and sometimes also feed directly on soil. They are very good sources of calcium, a very vital element needed to form shells and embryos. In the food chain, they are food for higher animals.

Nigeria is richly endowed with many species of snails such as *Archahatina marginata*, *Achatina achatina* and *Achatina fulica* (Nwankwo & Onwurah, 2015). The latter two, *Achatina achatina* and *Achatina fulica* have tremendous unexploited health, economic and genetic potentials (Okon et al., 2017). After *Archachatina marginata*, *Achatina achatina* is the next most popular reared species of snails in Nigeria (Okon et al., 2012). Its high nutritive value, large size and distinct markings are a few reasons why it is often sought after. In Nigeria, *Achatina achatina* is a delicacy and it commands a high market demand.

Differences in skin pigmentation and place of origin are two factors that have been reported to be responsible for the molecular differences that exist among snails of a given breed. The formula, P = G + E, is being popularly used to link trait(s) with the genotype and environment, where P is phenotype, G is the genotype and E is the environment (Etukudo et al., 2016). To ensure continuous preservation of the evolutionary potential of natural populations, an in-depth knowledge of the effect of pollutants on genetic variability is essential (Ungherese et al., 2010). According to Eeva et al. (2006), information on the contribution of pollution to genetic effects in terrestrial organisms is low. Furthermore, Awodiran et al. (2012) opined that there is need for more genetic studies to be carried out on snails in order to provide a wider phylogenetic relationship of achatinid snails in Nigeria, as they are only a few of them. This study was designed to investigate the genetic differences in snail populations within three southwestern states of Nigeria while also analyzing heavy metals in the snails for possible impact on the snail DNA profile.

2. Materials and Methods

2.1. Study Area and Sample Collection

A total of eighteen snail samples of six accessions per population per state were randomly collected from three states of Ekiti, Oyo, and Osun, South-west, Nigeria. The snails were collected from two different locations each in the three states. The sampling locations are presented in Figure 1. Codes were assigned to the snail

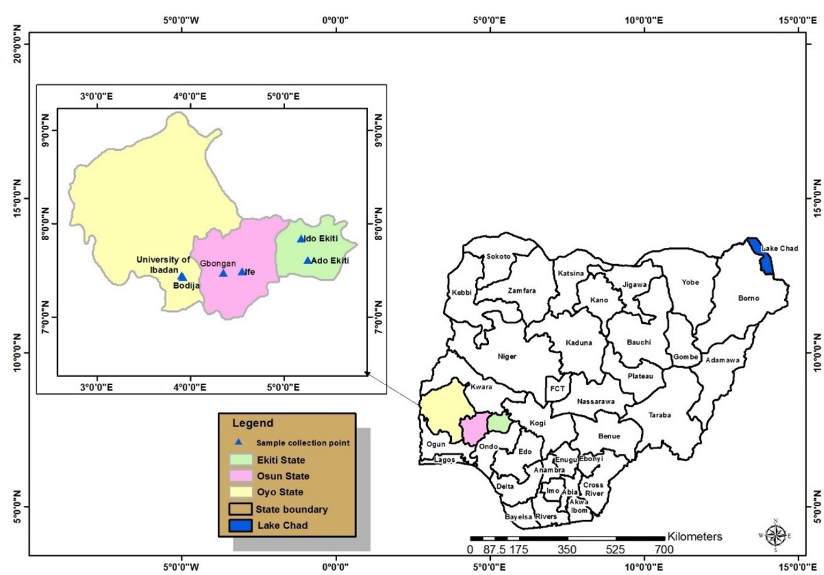

Figure 1. Map of Nigeria showing three south-western states of Oyo, Osun and Ekiti States.

samples according to the species name and their areas of the collection (Table 1). The samples were cleaned, shelled, and dissected at the Biology laboratory, Covenant University, Ota, Ogun State, Nigeria.

2.2. Digestion and analysis of samples

About 1g of flesh was cut from each snail sample, placed in a beaker, and digested using a combination of nitric and hydrochloric acid in the ratio 3:1 at a temperature of 100 °C until a clear solution was obtained. After allowing the solution time to cool, it was filtered to remove precipitates. Metal analysis in the digested samples was carried out using an atomic absorption spectrometer (Perkin Elmer Atomic Absorption Spectrometer PinAAcle 900T, USA). The metals analysed were Pb, Ni and Cd. Blank was prepared for each sample on which adjustment was made by reference to blank. Certified reference material was used for accuracy of analytical procedure. As a form of precaution, all solutions were prepared using de-ionized water. All the materials used were soaked in 10% nitric acid and rinsed in de-ionized water before being used as a form of precaution. Results were converted to mg kg^{-1} dry weight of samples. All analyses were done in triplicates. Average percentages of recovery between 85% and 95% were obtained for each of the metal, while limits of detection between 0.2 and 0.7 were detected for the metals.

2.3. DNA extraction

The extraction protocols were performed at the Molecular Biology Laboratory, Department of Biological Sciences, Covenant University. DNA was extracted from tissue samples using the classical Phenol/chloroform/isoamyl alcohol-based DNA extraction method as described by Baker et al. (1998) and as modified by Javadi et al. (2014). DNA quantification was performed in a NanoDrop spectrophotometer to determine the concentration and purity of the extracted DNA.

2.4. Sources of Primers and DNA-PCR Amplification

The Primer sequences (from Whiting et al., 1997) 18SF: 5'-GCGAAAGCATTGCCAAGAA-3' and 18SR 5'-GCATCACAGACCTGTTATTGC -3' were adopted and used for this study. The primers were synthesized and supplied by Ahava Biotechnology and Forensic Services Limited (Nigeria) and optimized at the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. The PCR amplification was carried out in a total 10 µL reaction mixture consisting of 3 µL of DNA (100 ng / µl), 2.9 µL of distilled water, 1.0 µL of 10x PCR buffer, 0.4 µL of 50mM $MgCl_2$, 0.5 µL each of both forward and reverse primers, 0.8 µL of dimethyl sulphoxide (DMSO) solution, 0.8 µL of 2.5Mm dNTPs, and 0.1µL of Taq 5u/ul. The PCR reaction was carried out in a GeneAmp PCR system 9700 thermocycler (Applied Biosystem, USA). The reaction involved an initial denaturation temperature of 94^{o}C for 5 minutes next was a denaturation temperature of 94^{o}C for 15 seconds, an annealing temperature of 65^{o}C for 25 seconds, and an extension temperature of 72^{o}C for 30 seconds, it consisted of 9 cycles. It was then followed by another 35 cycles, it consisted of denaturation at 94.0°C for 15 seconds, annealing at 55°C for 20 seconds, extension at

Table 1. Codes and areas of collection of *Achatina achatina* used for this study.

S/N	Area of collection	State	Zone	Code
1	Ado Ekiti	Ekiti	Tropical rainforest	AaEk01
2	Ado Ekiti	Ekiti	Tropical rainforest	AaEk02
3	Iddo Ekiti	Ekiti	Tropical rainforest	AaEk03
4	Ado Ekiti	Ekiti	Tropical rainforest	AaEk04
5	Iddo Ekiti	Ekiti	Tropical rainforest	AaEk05
6	Ado Ekiti	Ekiti	Tropical rainforest	AaEk06
7	Gbongan	Osun	Rain forest	AaOs01
8	Gbongan	Osun	Rain forest	AaOs02
9	Gbongan	Osun	Rain forest	AaOs03
10	Gbongan	Osun	Rain forest	AaOs04
11	Ile Ife	Osun	Rain forest	AaOs05
12	Ile Ife	Osun	Rain forest	AaOs06
13	Bodija Market	Oyo	Savannah	AaOy01
14	Bodija Market	Oyo	Savannah	AaOy02
15	Bodija Market	Oyo	Savannah	AaOy03
16	Bodija Market	Oyo	Savannah	AaOy04
17	Bodija Market	Oyo	Savannah	AaOy05
18	University of Ibadan	Oyo	Savannah	AaOy06

AA – *Achatina achatina,* Ek – Ekiti, Os – Osun, Oy – Oyo.

72°C for 30 seconds, and a final extension stage of 72°C for 7 minutes. The PCR products (10 µL) were loaded on 1.5% agarose gel and measured using a 50bp ladder from New England Biolabs (USA).

2.5. DNA Sequencing reaction

Sanger method was used for the sequencing reaction which was performed in a 96-well plate with a volume of 20 µL which contained 4 µL of 2.5X ready reaction premix (purified PCR product), 2 µL of 5X Big Dye sequencing buffer, 3.2 pmol of primer, and 10.8 µL of sterile distilled water. The cycling reaction was performed using a MicroAmp® 96-Well Reaction Plate (Applied Biosystems, USA). The thermal profile was adjusted to 25 cycles as follows: 96°C for 1 minute, 96°C for 10 sec, annealing temperature at 50°C for 5 seconds, and an elongation temperature of 60°C for 4 minutes. Amplified products were precipitated using 60 µL of 100% (absolute) ethanol to each well and then washed twice with 70% ethanol. The products were resuspended in 15 µL formamide denaturing buffer after air-dried. The amplified products were accordingly sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems, USA) following standard procedures.

2.6. Identification and sequence analysis

The forward and reverse sequences of each sample were visually identified, edited, and low quality trimmed using SnapGene Software (GSL Biotech, 2015) while alignment was performed using Geneious Prime 2020.0.4. The sequences were submitted to GenBank with the submission number SUB8190330 and all the eighteen sequences assigned an accession number. The percentage sequence identity/genetic similarity was evaluated using Geneious Prime 2020.0.4 while the number of nucleotide sites, polymorphic sites, and nucleotide diversity were estimated using DnaSP v5 (Rozas and Rozas, 1999). The Analysis of Molecular Variation (AMOVA) was performed using Arlequin V3 (Excoffier et al. 2005) while mean genetic diversity within the three subpopulations and diversity in the entire population was achieved using MEGA v5 (Tamura et al., 2011). Genetic differentiation among populations was determined using (i) the average number of nucleotide substitutions per site or Dxy (MEGA v5; Tamura et al. 2011); and (ii) corrected pairwise difference between populations or Pxy (Arlequin). The unweighted pair group method with arithmetic mean (UPGMA) method with genetic distance model of Tamura and Nei (1993) was used to generate a hierarchical cluster analysis.

3. Results

3.1. Aligned/consensus sequence characteristics

The edited and aligned sequences of the 18 snail accessions generated a range of 384 bp to 419 bp. The sequences were similar at 147 identical sites (32.5%) with a pairwise identity of 91.4%. The percentage GC and AT contents were highly similar with the total GC contents of 51.5% and AT contents of 48.5% (Table 2). The BLAST search

Table 2. Sequence length, percentage of nucleotide content, and BLAST search result of the 18SF/SR gene of *Achatina achatina* studied.

S/N	Accession	SL	%GC	%AT	Species Identity	QC	Identity	E-value	Accession Version
1.	AaEk01	414	51.7	48.3	*Achatina achatina*	100%	100.00%	0.0	MW029426.1
2.	AaEk02	409	51.6	48.4	*Achatina achatina*	97%	97.75%	0.0	MW029426.1
3.	AaEk03	418	51.7	48.3	*Achatina achatina*	99%	100.00%	0.0	MW029426.1
4.	AaEk04	413	51.6	48.4	*Achatina achatina*	100%	99.76%	0.0	MW029426.1
5.	AaEk05	398	51.3	48.7	*Achatina achatina*	100%	100%	0.0	MW029426.1
6.	AaEk06	413	51.6	48.4	*Achatina achatina*	100%	99.76%	0.0	MW029426.1
7.	AaOs01	**419**	51.8	48.2	*Achatina achatina*	100%	100%	0.0	MW029426.1
8.	AaOs02	414	51.7	48.3	*Achatina achatina*	99%	100%	0.0	MW029426.1
9.	AaOs03	416	51.4	48.6	*Achatina achatina*	100%	100%	0.0	MW029426.1
10.	AaOs04	410	51.5	48.5	*Achatina achatina*	99%	100%	0.0	MW029426.1
11.	AaOs05	406	51.5	48.5	*Achatina achatina*	98%	100%	0.0	MW029426.1
12.	AaOs06	398	51.5	48.5	*Achatina achatina*	99%	99.75%	0.0	MW029426.1
13.	AaOy01	414	51.7	48.3	*Achatina achatina*	99%	100%	0.0	MW029426.1
14.	AaOy02	396	51.6	48.4	*Achatina achatina*	99%	100%	0.0	MW029426.1
15.	AaOy03	387	50.9	49.1	*Achatina achatina*	96%	100%	0.0	MW029430.1
16.	AaOy04	**384**	51.0	49.0	*Achatina achatina*	99%	100%	0.0	MW029430.1
17.	AaOy05	397	51.4	48.6	*Achatina achatina*	96%	100%	0.0	MW029430.1
18.	AaOy06	387	50.9	49.1	*Achatina achatina*	96%	100%	0.0	MW029430.1
Mean			**51.5%**	**48.5%**					

SL – Sequence Length, QC – Query Coverage.

of the 18S small subunit ribosomal RNA gene sequence of *Achatina achatina* identified the 18 accessions of the species with a high percentage identity. The query coverage (QC) ranged from 96% to 100% with a total of 14 accessions (77.8%) out of the 18 accessions having 100% identity. The percentage identity ranged from 97.75% to 100% identity (Table 2). Overall, the analysis using DnaSP generated a total of 471 nucleotide sites, 149 invariable (monomorphic) sites, and 161 variable (polymorphic) sites with nuclear diversity (Pi = 0.061) while the number of polymorphic sites was 187. The aligned consensus sequences from the 18SRF gene for the 18 accessions of *A. achatina* and its associated consensus points of similarities and differences from positions 10 -470 are shown in Figure 2.

3.2. Genetic similarity of the snail samples studied

The pairwise comparison of the 18 samples of Snail studied showed a high genetic similarity which ranged from 0.306 to 1.000. Sample AaOy06 (with low identity values (0.306 – 0.329) was unique compared to other samples (with high identity values), it is less genetically similar to all other samples collected from different areas. The percentage bases and residues of sample AaOy01 are 100% genetically like that of AaOs02 (1.000) (Table 3).

3.3. Analysis of molecular variance (AMOVA) and genetic differentiation

The mean diversity within subpopulations indicates a very low diversity between Ekiti and Osun States populations while Oyo and Osun States displayed a moderate/fair diversity of 0.42 and 0.55, respectively. The mean diversity in the entire 18 Snail samples was also low (0.16) – 16% (Table 4). There are no fixed differences among and between the populations as indicated by genetic parameters in Table 5. The mutation in population 1 but monomorphic in population 2 for Oyo/Osun States and Ekiti/Oyo States was 0 while it was 3 for Ekiti/Osun States. Mutation in population 2 but monomorphic in population 1 was 1 for Ekiti/Osun States while Oyo/Osun States and Ekiti/Oyo States had 174 (Table 5). The average number of nucleotide substitutions per site or Dxy values between subpopulation pairs were 0.00286 for Ekiti/Osun States, and 0.9236 for Osun/Oyo States and Ekiti/Oyo States, which indicate lack of diversity among Ekiti/Osun States populations (Table 5). The AMOVA results also indicate no divergence among/within groups and within populations (Table 6).

3.4. Cluster analysis

The unweighted pair group method with arithmetic mean (UPGMA) generated two major clusters at the similarity index of 0.5357. Cluster I was unique and contain one sample (AaOy06) while cluster II was further segregated into five sub-clusters (Figure 3). The sub-cluster IIA consisted of two snail samples that are genetically similar with a 100% similarity coefficient. Sub-cluster IIB consisted of one sample (AaOs06) while IIC consisted of two snail samples (AaEK04 and AaEK06) that are genetically the same and collected from similar areas. Sub-cluster IID consisted of one snail sample (AaOy05) while sub-cluster IIE was the most diverse and consisted of eleven snail samples of different sources (AaOy04, AaOy03, AaOy02, AaOy01, AaOs05, AaOs04, AaOs03, AaOs02, AaOs01, AaEk01, and AaEk03) (Figure 3).

3.5. Heavy metal studies

The concentrations of cadmium, lead and nickel in the different snail populations are presented in Table 7. Cadmium was the least detected of the three metals tested. None of the snail samples were positive for cadmium in Oyo State. It was detected in only one sample in Ekiti State while three of the samples from Osun State were positive for cadmium. In all cases where cadmium was detected, the concentrations were high in comparison with acceptable limits.

Lead was detected in two, four and all six samples in Osun, Ekiti and Oyo States, respectively. Samples from Oyo State had the highest concentrations of lead. The difference in average concentrations of the metal as shown in table two were statistically significant. These concentrations were also higher than acceptable limits for food.

Nickel was detected in all the snail samples and in all the three states. The concentrations of nickel in the snail samples in Osun and Ekiti States were similar, as the difference in the average concentration of this metal was not significant between the two states. As observed in lead analysis, the concentrations of nickel were higher in samples from Oyo State. The difference in the average concentration of nickel was statistically significant in all the three states.

4. Discussion

Gastropods can accumulate high levels of heavy metals in their bodies and, as such, are very useful sentinel organisms especially in the terrestrial environment (Abdel Kader et al., 2016). In the present study, variation in levels of heavy metals in snail populations in the study areas was observed. Cadmium was the least detected of all metals in all study sites. This may mean that activities like mining and refining that release cadmium into the environment are not operational in the study areas. Lead was the next frequently detected metal and was particularly in very high concentrations in all the samples from Oyo State. The samples were all collected in Ibadan, the state capital. This is the largest city in Western Africa, with very high vehicular traffic. Since lead is a product of vehicular emissions, the high concentrations of lead observed in Oyo may be attributed to this. All snail samples in the three states accumulated high levels of nickel, Oyo state having the highest average concentration of the metal. In comparison with cadmium and lead, there seems to be more activities that release nickel into the environment. This includes incineration of wastes and sewage, combustion of coal and even from phosphate fertilizers (Kabata-Pendias and Pendias, 1992). This may explain the high frequency of occurrence of nickel over the other metals. High concentrations of these metals were also reported in snails by Awharitoma et al (2016) in Edo State, Nigeria.

Figure 2. Aligned Consensus sequences from the 18SRF gene for the *Achatina achatina* and its associated consensus points of polymorphisms from positions 10-470. Note that the dotted line (…) in the sequence alignment indicates the similarity of the nucleotide to the nucleotide of AaEk01.

Table 3. Sequence Identity Matrix of the 18 samples of Snail (*Achatina achatina*) studied.

Seq->	AaEk01	AaEk02	AaEk03	AaEk04	AaEk05	AaEk06	AaOs01	AaOs02	AaOs03	AaOs04	AaOs05	AaOs06	AaOy01	AaOy02	AaOy03	AaOy04	AaOy05	AaOy06
AaEk01																		
AaEk02	0.944																	
AaEk03	0.985	0.944																
AaEk04	0.985	0.956	0.971															
AaEk05	0.932	0.973	0.925	0.944														
AaEk06	0.985	0.956	0.971	1.000	0.944													
AaOs01	0.988	0.942	**0.997**	0.973	0.923	0.973												
AaOs02	0.990	0.949	**0.990**	0.975	0.934	0.975	0.988											
AaOs03	0.995	0.940	0.980	0.980	0.928	0.980	0.983	0.985										
AaOs04	0.990	0.951	0.980	0.980	0.939	0.980	0.978	0.990	0.985									
AaOs05	0.980	0.942	0.966	0.983	0.930	0.983	0.968	0.971	0.975	0.975								
AaOs06	0.942	0.948	0.933	0.941	0.969	0.941	0.930	0.942	0.937	0.951	0.944							
AaOy01	0.990	0.949	**0.990**	0.975	0.934	0.975	0.988	**1.000**	0.985	0.990	0.971	0.942						
AaOy02	0.956	0.949	0.947	0.958	0.936	0.958	0.945	0.956	0.951	0.965	0.975	0.945	0.956					
AaOy03	0.934	0.943	0.925	0.937	0.969	0.937	0.923	0.934	0.930	0.943	0.953	0.974	0.934	0.962				
AaOy04	0.927	0.938	0.918	0.929	0.964	0.929	0.916	0.927	0.923	0.936	0.945	0.969	0.927	0.969	0.992			
AaOy05	0.954	0.953	0.944	0.956	0.941	0.956	0.942	0.954	0.949	0.963	0.972	0.950	0.954	0.987	0.969	0.967		
AaOy06	0.325	0.316	0.327	0.316	0.317	0.316	0.329	0.323	0.329	0.321	0.314	0.312	0.323	0.305	0.308	**0.306**	0.308	

The significance of genetic diversity and make-up in conservation, breeding and utilization has been variously reported and studied (Nevo, 1998). The present study investigated the sequence diversity and homology available among the collected *Achatina achatina* populations from three states within South-Western, Nigeria. The various results including the pairwise comparison, analysis of molecular variance (AMOVA) and cluster analysis independently revealed a high genetic similarity and a very low genetic diversity among and within the three populations of the eighteen accessions of snails studied. This suggests a very high genetic closeness among all the accessions studied except accession AaOy06 which seems to be distinct from others (Figure 2). Generally, the high genetic relatedness may be due to low evolution among this species and absence of environmental heterogeneity (similarity in collection areas) (Awodiran et al. 2015). Furthermore, it is likely that over the years, this species is being bred and transited probably amongst market women around the three states seeing they are neighbouring states within southwestern Nigeria. The uniqueness of accession (AaOy06) might be due to the influence and accumulation of heavy metals in the area where the sample was collected. The sequence of the accession (AaOy06) is quite different from that of other accessions which also reflected in the cluster analysis (Figure 2). Looking at the concentration of heavy metals in this sample, it is not impossible that the high level of metal is responsible for this difference. Lead is a very toxic metal, which is introduced into the environment via vehicular emissions. This accession was obtained from University of Ibadan, where there is a lot of vehicular activities, as such might have been the reason why the metal levels in this accession is high compared to others. This high metal load may have brought about genetic changes in the accession hence, bringing about high sequence difference in comparison with other accessions. According to Ungherese et al. (2010) genotoxic agents can act as a selective force, eliminating sensitive genotypes within a population and leading to genetic variability.

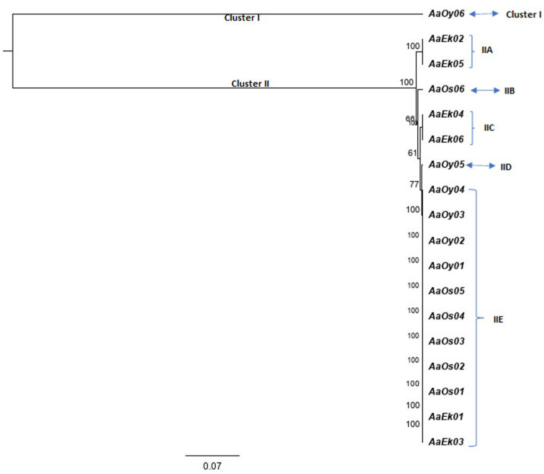

Figure 3. Cluster analysis of the 18 Snail samples studied using 18SRF gene. Two major clusters were identified.

Table 4. Mean Diversity Within Subpopulations of Snail Samples studied.

Populations	Osun	Oyo
Ekiti	0.01	0.42
Oyo	0.55	

Mean diversity in the entire population = 0.16.

Table 5. Divergence between Population.

Parameters	Ekiti/Osun	Osun/Oyo	Ekiti/Oyo
No of fixed Differences	0	0	0
Mutations in Pop 1 but monomorphic in Pop 2	3	0	0
Mutations in Pop 2 but monomorphic in Pop 1	1	174	174
Shared Mutations	0	0	0
Avr. No of nt difference bw pop	1.111	29.000	29.000
The average number of nuc. subs. per site between populations, Dxy	0.00286	0.9236	0.9236
Number of net nuc. subs. per site between populations, Da	0.00037	0.0000	0.0000

Key: Pop1/Pop2 – Population 1 and Population 2.

Table 6. AMOVA of Genetic differentiation of the Snail population studied.

Source of variation	df	Sum of Squares	Variance components	Percentage of variation	F-Statistics	p-value
Among groups	1	0.028	-0.00694 Va	-13.04	F_{ST} = -0.04348	1.00000
Within groups	1	0.083	0.00463 Vb	8.7		
Within populations	15	0.833	0.05556 Vc	104.35		
Total	17	0.944	0.05324			

Significance tests (1023 permutations).

Table 7. Heavy Metal Concentrations (mg/kg) in snail populations in the three states.

Metals	Osun	Ekiti	Oyo
Cadmium (mg/kg)	18.96±1.86	14.1±1.11	ND
	3.44±0.12	ND	ND
	1.92±0.20	ND	ND
	ND	ND	ND
	ND	ND	ND
	ND	ND	ND
Lead (mg/kg)	21.06±1.11	3.09±0.05	33.70±1.09
	13.35±1.05	12.36±0.65	59.66±1.54
	21.54±1.21	39.61±1.41	51.22±1.47
	15.88±0.58	15.29±1.13	43.02±1.33
	25.56±1.23	21.82±1.20	33.11±1.44
	21.05±1.04	14.69±1.08	84.66±2.43
Mean	**19.74±4.39[a]**	**17.81±12.28[a]**	**50.90±19.48[b]**
Nickel (mg/kg)	63.34±1.66	4.53±0.55	103.41±2.58
	ND	ND	99.11±1.22
	ND	ND	96.17±1.49
	ND	7.19±1.05	67.55±0.98
	33.20±1.25	55.28±1.33	92.13±1.79
	ND	56.81±1.64	179.07±1.53
Mean	**48.27±21.31[a]**	**31.20±29.00[b]**	**106.24±37.84[c]**

ND – Not detected. Mean values with the same superscript are not significantly different

5. Conclusion

The study provides preliminary sequencing information on some accessions of *Achatina achatina* obtained from three states with South-Western Nigeria. The sequencing length (bp) and content (GC%), were highly similar, hence, being the reason for the high similarity in clustering pattern among the 18 accessions sequenced. Though the number of accessions is low, the sequencing information provides ample opportunities towards genetic manipulation of the species. Further collections and characterizations are however recommended to enhance a clearer understanding of the genetic makeup and evolutionary diversity of the tiger snails which would be profitable the genetic manipulation of the species.

REFERENCES

ABDEL KADER, A., ISMAIL, N.M.M., ABDEL-MOTLEB, A., OSMAN, G.Y., MOHAMED, A.H. and GHARIEB, M.M., 2016. Bioaccumulation of heavy metals in freshwater snails in relation to lining of water courses in Egypt. *Journal of Bioscience and Applied Research*, vol. 2, no. 8, pp. 561-573. http://dx.doi.org/10.21608/jbaar.2016.108934.

AWHARITOMA, A.O., EWERE, E.E., ALARI, P.O., IDOWU, D.O. and OSOWE, K.A., 2016. Assessment of Heavy Metals in African Giant Snail (Archachatina marginata) and its Parasites Collected from three communities in Edo State, Nigeria. *International Journal of Scientific and Engineering Research*, vol. 7, no. 10, pp. 793-797.

AWODIRAN, M.O., ARTHUR, M.C. and IDOWU, A.J., 2015. Molecular phylogeny of three species of land snails (Stylommatophora and Achatinidae), *Archachatina marginata* (Swainson, 1821), *Achatina achatina* (Linnaeus, 1758), and *Achatina fulica* (Bowdich, 1822) in some southern states and north central states in Nigeria. *Annals of West University of Timisoara. Series of Biology*, vol. XVIII, no. 2, pp. 95-106.

AWODIRAN, M.O., AWOPETU, J.I. and AKINTOYE, M.A., 2012. Cytogenetic study of four species of land snails of the family achatinidae in South-Western Nigeria. *Ife Journal of Science*, vol. 14, no. 2, pp. 233-235.

BAKER, A.J.M., REEVES, R.D. and HAJAR, A.S.M., 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. and C. Presl (Brassicaceae). *The New Phytologist*, vol. 127, no. 1, pp. 61-68. http://dx.doi.org/10.1111/j.1469-8137.1994.tb04259.x. PMid:33874394.

BAKER, C.S., MEDRANO-GONZALEZ, L., CALAMBOKIDIS, J., PERRY, A., PICHLER, F., ROSENBAUM, H., STRALEY, J.M., URBAN-RAMIREZ, J., YAMAGUCHI, M. and VON ZIEGESAR, O., 1998. Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, vol. 7, pp. 695−707.

BELFIORE, N.N. and ANDERSON, S.L., 2001. Effects of contaminants on genetic patterns in aquatic organisms: a review. *Mutation Research*, vol. 489, no. 2-3, pp. 97-122. http://dx.doi.org/10.1016/S1383-5742(01)00065-5. PMid:11741031.

BICKHAM, J.W., SANDHU, S., HEBERT, P.D., CHIKHI, L. and ATHWAL, R., 2000. Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation Research*, vol. 463, no. 1, pp. 33-51. http://dx.doi.org/10.1016/S1383-5742(00)00004-1. PMid:10838208.

DE WOLF, H., BLUST, R. and BACKELJAU, T., 2004. The population genetic structure of *Littorina littorea* (Mollusca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. *The Science of the Total Environment*, vol. 325, no. 1-3, pp. 59-69. http://dx.doi.org/10.1016/j.scitotenv.2003.11.004. PMid:15144778.

EEVA, T., BELSKII, E. and KURANOV, B., 2006. Environmental pollution affects genetic diversity in wild bird populations. *Mutation Research*, vol. 608, no. 1, pp. 8-15. http://dx.doi.org/10.1016/j.mrgentox.2006.04.021. PMid:16807076.

ETUKUDO, O.M., OKON, B., EKALUO, U.B. and IBOM, L.A., 2016. Genetic differentiation between black-skinned and white-skinned ectotypes of giant african land snails (Archachatina marginata) in Calabar, Nigeria. *Global Journal of Pure and Applied Sciences*, vol. 22, no. 2, pp. 135-139. http://dx.doi.org/10.4314/gjpas.v22i2.2.

EXCOFFIER, L., LAVAL, G. and SCHNEIDER, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, vol. 1, pp. 47-50. http://dx.doi.org/10.1177/117693430500100003. PMid:19325852.

GSL BIOTECH, 2015 [viewed 20 February 2021]. *SnapGene software* [online]. Available from: snapgene.com

HE, Z.L., YANG, X.E., STOFFELLA, P.J., 2005. Trace elements in agroecosystems and impacts on the environment. *Journal of Trace Elements in Medicine and Biology*, vol. 19, no. 2-3, pp. 125-140. https://doi.org/10.1016/j.jtemb.2005.02.010.

JAVADI, A., SHAMAEI, M., MOHAMMADI ZIAZI, L., POURABDOLLAH, M., DORUDINIA, A., SEYEDMEHDI, S.M. and KARIMI, S., 2014. Qualification study of two genomic DNA extraction methods in different clinical samples. *Tanaffos*, vol. 13, no. 4, pp. 41-47. PMid:25852760.

KABATA-PENDIAS, A. and PENDIAS, H., 1992. *Trace elements in soils and plants*. London: CRC Press.

KENNADY, V., VERMA, R. and CHAUDHIRY, V., 2018. Detrimental impacts of heavy metals on animal reproduction: a review. *Journal of Entomology and Zoology Studies*, vol. 06, no. 06, pp. 27-30.

MASINDI, V. and MUEDI, K.L., 2018. Heavy metals. In: SALEH, H. E-D. M. and AGLAN, R.F., editor *Environmental contamination by heavy metals*. Rijeka: IntechOpen. http://dx.doi.org/10.5772/intechopen.76082.

NEVO, E., 1998. Genetic diversity in wild cereals: regional and local studies and their bearing on conservation ex situ and in situ. *Genetic Resources and Crop Evolution*, vol. 45, no. 4, pp. 355-357. http://dx.doi.org/10.1023/A:1008689304103.

NWANKWO, C. and ONWURAH, F.B., 2015. Entrepreneurial snail farming: the way forward. In: *Proceedings of the 4th International Conference/Workshop on Giant African Land Snails (NetGALS)*, 2015, Akwa. Akwa: Nnamdi Azikiwe University, pp. 71-74.

OKON, B., IBOM, L.A., ETTAH, H.E. and UKPUHO, I.E., 2012. Effects of Genotype, dietary protein and energy on the reproductive and growth traits of parents and F1, Hatchlings of Achatina achatina (L) snails in Nigeria. *International Journal of Applied Science and Technology*, vol. 2, no. 1, pp. 179-185.

OKON, B., IBOM, L.A., HALILU, A. and ONWUKA, P.O., 2017. Comparative morphometric traits differentiation and phenotypic correlations between Achatina achatina (Linne, 1758) and Achatina fulica (Bowdich, 1822) snails with four and five whorls. *Journal of Animal Science and Veterinary Medicine*, vol. 2, no. 3, pp. 102-108. http://dx.doi.org/10.31248/JASVM2017.048.

ROZAS, J. and ROZAS, R., 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics (Oxford, England)*, vol. 15, no. 2, pp. 174-175. http://dx.doi.org/10.1093/bioinformatics/15.2.174. PMid:10089204.

TAMURA, K. and NEI, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, vol. 10, no. 3, pp. 512-526. https://doi.10.1093/oxfordjournals.molbev.a040023.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. and KUMAR, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731-2739. http://dx.doi.org/10.1093/molbev/msr121. PMid:21546353.

UNGHERESE, G., MENGONI, A., SOMIGLI, S., BARONI, D., FOCARDI, S. and UGOLINI, A., 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper Talitrus saltator (Montagu) (Crustacea, Amphipoda). *Environmental Pollution*, vol. 158, no. 5, pp. 1638-1643. http://dx.doi.org/10.1016/j.envpol.2009.12.007. PMid:20034716.

WHITING, M.F., CARPENTER, J.C., WHEELER, Q.D. and WHEELER, W.C., 1997. The strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18s and 28s Ribosomal DNA sequences and morphology. *Systematic Biology*, vol. 46, no. 1, pp. 1-68. http://dx.doi.org/10.1093/sysbio/46.1.1. PMid:11975347.