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Genetic Differentiation of *Archachatina marginata* Populations from Three Vegetation Zones Using Radom Amplified Polymorphic DNA Polymerase Chain Reaction

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Abstract

The genetic differentiation of *Archachatina marginata* populations from three different zones of Nigeria was studied with a view to delimiting them into sub-species. One hundred and nineteen (119) snail specimens were collected, comprising of forty (40) specimens from Yenagoa (Mangrove forest) and from Kabba (Guinea Savanna) and thirty nine (39) specimens were from Ile-Ife (Rainforest). Eight parameters of the shell specimens of *A. marginata* which included height of shell, width of shell, aperture height, aperture width, spire length, spire width, penultimate whorl length and first whorl length were subjected to Principal Component Analysis (PCA) and Canonical Variates Analysis (CVA) to delimit the populations into sub-species. DNA of the various populations was extracted from the foot muscle using CTAB (Cetyl Trimethyl Ammonium Bromide) method, which was subjected to RAPD analysis. The RAPD studies employed five (5) oligonucleotide primers (OPB – 17, OPH – 12, OPH – 17, OPI – 06 and OPU – 14) to amplify DNA from 27 samples of *A. marginata* selected. All five primers produced different band patterns, and the number of fragments amplified per primer varied. Among them, OPB- 17 gave DNA profiles with more numerous bands than the others primers. Both PCA and CVA produced overlapped clusters of *A. marginata* specimens from the three vegetation zones. The height of shell was observed to be the most variable feature and preferably the most suitable parameter for population grouping. Analysis of the proportions of polymorphic loci and band sharing based on similarity indices for *A. marginata* samples indicated a relatively high level of genetic variation in the populations from the three areas.

Keywords: amplification, delimitation, genetics, measurements, shell morphology, sub-populations

Introduction

Archachatina marginata (Swainson, 1821) is a land snail of the family Achatinidae. It is a giant land snail native to Africa (Raut and Barker, 2002). Generally, classification within the family is based on conchological features (Bequaert, 1951) and the highly variable reproductive tract (Mead, 1991). However, Bequaert (1951) reported the presence of subspecies of *Archachatina marginata* in Nigeria. In his studies on the Achatininae, a group of African land snails, he stated that *A. marginata* covers a wide territory, extending at least from Dahomey to the Lower and middle Belgian Congo, and possibly in French Equitorial Africa to the Gribingui River (about 7°N., 19°W). As a result, it has produced several intra specific variations, sometimes well segregated geographically, but more overlapping, yet well worthy of varietal or subspecific names.

The races are well developed by colour peculiarities and in some cases also by the average size or the general shape. All of them have retained the characteristic microsculpture. As they frequently occur in the same territory with specimens of the typical race, although perhaps in a different type of environment, it is not surprising that connecting examples occur in some localities. Among those, Bequaert (1951) listed as available in West African sub region are *A. marginata ovum*, *A. marginata suturalis*, *A. marginata eduardi*, *A. marginata egregiella, A. marginata egregia, A. marginata clenchi, A. marginata icterica, A. marginata grevillei* and *A. marginata candefacta.*

There are sub-species of *A. marginata* in Nigeria that despite all efforts made are still not morphologically separated. Effort to use morphometric parameters to separate *A. marginata* into sub-populations across three zones of Nigeria could also not indicate the presence of sub-species (Awodiran, 2012). However, molecular phylogeny of four species of land snails and eight sub-populations of *A. marginata* showed potentiality to diverge into sub-species (Awodiran, 2012). There is dearth of information on the genetic variability of this species in Nigeria. Recently, Okon *et al.* (2013) genetically differentiated the black skinned and white skinned snails, which termed ecotypes of *A. marginata*, using RAPD and concluded that the genetic variability of the species from Cross River State in Nigeria is gradually eroding.

Though, *A. marginata* is not listed as a threatened species of land snails, but loss of natural habitat over exploitation for diverse uses and the effect of climate change are serious concerns for its

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Table 1. Locations and sample size (n) for *Archachatina marginata*

Vegetation Zone	Locality	Geographical coordinates	Sample size (n)
Rainforest	Ife (If)	7°28'N 4°34'E	39
Mangrove rainforest	Yenagoa (Yn)	4°50'N 6°15'E	40
Guinea savanna	Kabba (Kb)	7°50'N 6°4'E	40
Total			119

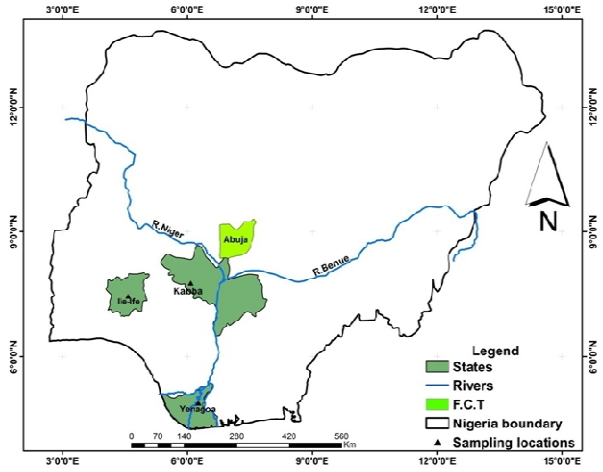

Fig. 1. Map of Nigeria showing sampling locations

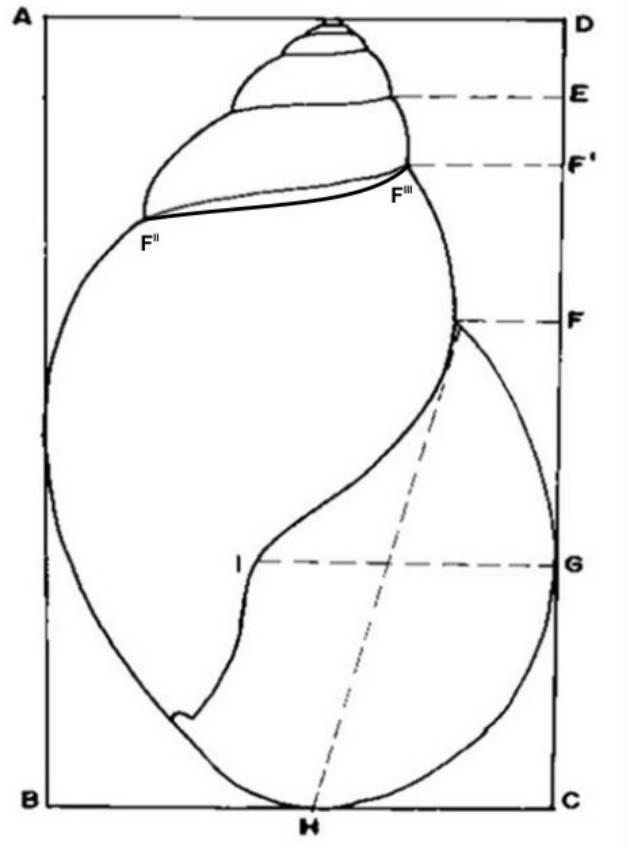

Fig. 2. Measurements of shell morphology used in multivariate analyses (AB = Height of Shell, HS; BC = Width of shell, WS; DF = Spire length, SL; $F^{II}F^{III}$ = Spire Width, SW; EF^{1} = 1st whorl length, 1WL; FH = Aperture height, AH and GI = Aperture Width, AW)

decline and drastic loss of populations in Nigeria (Osemeabo, 1992; Okon *et al.,* 2009). There is therefore a need to conserve the genetic resources of the species and its possible sub-species. Thus, an attempt was made in this study to employ the use of morphometrics and RAPD analysis to differentiate populations of *A. marginata* into sub-species.

Materials and Methods

Study areas and sample collection

Samples of *A. marginata* were collected from three different locations representing three different vegetation zones. The locations in Nigeria are Ile-Ife, Kabba and Yenagoa. Ile-Ife (Osun State) lies within the tropical zone and in the rainforest region of Nigeria; it has an average annual rainfall of 1,348 mm. Kabba (Kogi State) has vegetation consisting of mixed leguminous (Guinnea) woodland to forest Savanna, with annual rainfall ranging from 1,016 mm to 1,524 mm. Yenagoa (Bayelsa State) has a vegetation characterized by mangrove forest with heavy rainfall and a short dry season (usually from November to March) and has annual rainfall ranging from 2,000 mm to 4,000 mm. The various locations sampled were illustrated in Fig. 1, while the sample size and the geographical coordinates were listed in Table 1.

One hundred and nineteen (119) snail specimens were collected, comprising of forty (40) specimens from each location in Yenagoa and Kabba and thirty nine (39) specimens were from Ile-Ife. The samples were purchased fresh from local farmers. The samples were then taken to the Genetics Laboratory, Department of Zoology, Obafemi Awolowo University, Ile-Ife for morphometric analysis. From each location, nine (9) snail specimens were selected and taken to the Central Laboratory/Biotechnology Centre, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria for RAPD-PCR analysis.

Morphometric studies

To select characters for morphometric analyses, the available taxonomic descriptions from literature were reviewed (Brandt, 1974; Liu *et al*., 1979; Burch, 1980) and parameters of the shell that were of practical use in identification were adopted. The results of morphometric analyses might directly reflect the importance and effect of each character. A total of 8 shell measurements were taken with Vernier calipers on each of the snail shell. Shell length (HS) was measured along an axis passing through the apex to the bottom of the shell. Shell width (SW) is the maximum width perpendicular to the shell length distance. Aperture length (AL) is the length from the beginning of the 1st suture to the bottom of the aperture. Aperture width (AW) is the maximum diameter perpendicular to the aperture length. The penultimate whorl length (PWL) is the length between the beginnings of the 1st suture to the beginning of the 2nd suture. First whorl length was also measured (FWL). Spire length (SL) was measured from the beginning of the 1st suture to the apex of the shell. Spire width (SW) which is the maximum diameter of the spire from the first suture was also measured (Madec and Bellido, 2007). Fig. 2 shows measurements of shell morphology used in multivariate analyses.

Statistical analysis

Measurements of each morphometric character were transformed to shell width (SW) to remove size-effect by growth allometry using the method of Reist (1985) as described in

Table 2. Code sequences and nucleotide lengths used in the RAPD analysis

Primers	Primer Sequence (5'- 3')	Nucleotide Length
OPB-17	AGGGAACGAG	10-mer
OPH-12	ACGCGCATGT	10-mer
OPH-17	CACTCTCCTC	10-mer
OPI-06	AAGGCGGCAG	10-mer
OPU-14	TGGGTCCCTC	10-mer

Gunawickrama (2007). Width corrected data were then analyzed by multivariate statistical method. A multivariate Cluster Analysis was carried out on the morphometric data obtained to illustrate patterns of correlation among populations from various locations (Chiu *et al*., 2002; Madec *et al*., 2003). A Canonical Variates Analysis and Student's t-test were subsequently carried out to observe if species and populations from the various ecological zones were significantly different and to identify morphometric characters by which these operational taxonomic units could be diagnosed. The analysis was carried out with the aid of statistics software PAST (Hammer *et al*., 2006).

Genomic DNA extraction

The genomic DNA of the snail was isolated using CTAB (Cetyl Trimethyl Ammonium Bromide) method. The snail specimen preserved in ethanol was grinded in the mortar and 600 µl of extraction buffer was added to the sample, which was then incubated in the water bath at 65°C for 30 minutes. The sample was removed and allowed to cool to room temperature after which chloroform was added and the sample was mixed by gently inversion of the tube several times. Thereafter, the sample was spun at 14,000 rpm for 15 minutes and the supernatant was transferred into a new Eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. The sample was kept in the freezer for 1 hour later centrifugated at 14,000 rpm for 10 mins and the supernatant was discarded and the pellet was washed with 70% Ethanol; the obtained sample was air dried for 30 mins on the bench. The pellet was resuspended in 100 µl of sterile distilled water. DNA concentration of all the samples was measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined. The quality of DNA was detected by Agarose gel electrophoresis. The genomic DNA was used in PCR amplification using RAPD markers. Code sequences and nucleotide lengths used in RAPD studies are shown in Table 2.

DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% Agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g Agarose in 100 ml 0.5X TBE (Trisic Boric Acid) buffer solution. The gels were allowed to cool to about 45°C and 10 µl of 5 mg/ml Ethidium Bromide were added, mixed together before pouring the solution into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µl of the DNA with 5 µl sterile distilled water and 2 µl of 6X loading dye were mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 hours. The integrity of the DNA was visualized and photographed on UV (Ultra Violet) light source.

PCR reaction mix

10 µl of each DNA supernatant were taken into Eppendorf tube and 990 µl sterile distilled water was added to make the final volume of 1000 µl. The final concentration of the dilution of DNA for PCR became 20-50 ng/µl.

The reaction mix was carried out in 20 µl final volume containing 60-80 ng genomic DNA, 0.1 µM of the primers, 2 mM $MgCl_2$, 125 µM of each dNTP (di Nucleotide Triphosphate) and 1 unit of Taq DNA polymerase. The thermocycler profiles had an initial denaturation temperature of 94 °C for 3 mins, followed by 45 cycles of denaturation temperature of 94°C for 20 seconds, annealing temperature of 37°C for 40 seconds and primer extension temperature of 72°C for 40 seconds, followed by final extension temperature at 72°C for 5 minutes.

Gel electrophoresis

PCR amplicon electrophoresis was carried out by size fractionation on 1.4% Agarose gels. Agarose gels were prepared by dissolving and boiling 2.8 g agarose in 200 ml 0.5X TBE (Trisic Boric Ethylenediamine Tetra-acetic Acid) buffer solution. The gels were allowed to cool to about 50°C and 10 µl of 5 mg/ml Ethidium Bromide were added, mixed together before pouring into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100 V for 2 hours. The DNA was visualized and photographed on UV light source.

Band scoring and data analysis

Each gel was analyzed by scoring the present (1) or absent (0) polymorphic bands in individual lanes. The scoring was done based on the banding profiles which were clear and transparent. The scores were then pooled for constructing a single data matrix. Purity of the DNA was carried out using spectrophotometer by measuring absorbance at 260 nm and 280 nm. The RAPD banding profiles were visually scored for all the DNA samples and for each primer. Similarity coefficients were calculated across all the possible pair wise comparisons of snail samples among populations, using the formula below:

$$Sxy = \frac{2nxy}{nx + ny}$$

Where, nxy is the number of common bands shown in both individuals x and y, and nx and ny are the total numbers of bands observed in individuals x and y respectively.

As a mean of providing visual representation of genetic relationships, a dendrogram was constructed based on the similarity coefficient values (1 - Sxy) between pairs of snail samples. The NTSYS-PC software program was used to estimate genetic similarities with the Jaccard's coefficient (Rohlf *et al*., 2000) and a dendrogram was constructed using the Unweighted Pair Group Method of Arithmetic Averaging (UPGMA) employing the Sequential, Agglomerative, Hierarchical and Nested clustering module (SAHN).

Results

Morphometric studies

The average values, ranges and standard errors for all the shell variables measured are shown in Table 3. Wide range of sizes, especially on height of shell and width of shell, were found in the samples of Ile-Ife (Height of Shell SD 16.63 cm and 9.29 cm, respectively), revealing great heterogeneity in the population. The standard deviations for all Height of Shell,

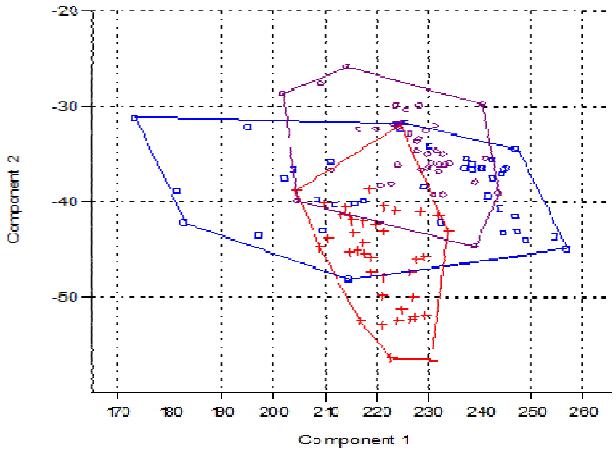

Fig. 3. Principal Components Analyses based on *Archachatina marginata* shell measurements of snails from Yenagoa (red +), Ile-Ife (blue □) and Kabba (purple o) showing overlap of the three populations under study

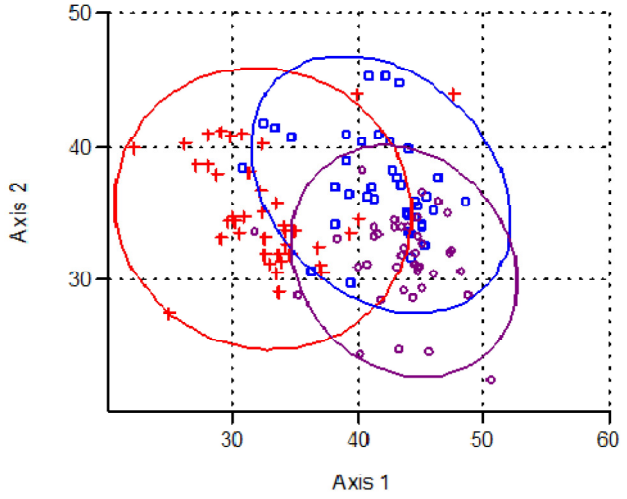

Fig. 4. Canonical Variates Analyses based on *Archachatina marginata* shell measurements of snails from Yenagoa (red +), Ile-Ife (blue □) and Kabba (purple o) showing overlap of the three populations under study

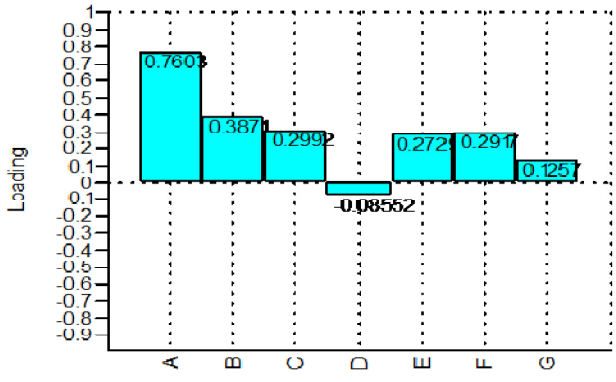

Fig. 5. Respective snail shell characters and their loadings on PC1 of the Principal Components Analysis (A = HS, B = AH, C = AW, D = SL, E = SW, F = 1WL, G = PWL)

for all the populations under study, were found to be higher than other parameters measured.

The principal component analyses of the 8 morphometrics measurements of *A. marginata* shells from the three areas of study were shown in Fig. 3. All the three clusters produced by the Principal Components Analysis (PCA) overlapped. Canonical Variates Analyses (CVA) was conducted to see if the population of *A. marginata* collected from the three locations could be separated intra specifically (Fig. 4).

The CVA plots showed the overlapping of clusters of the specimens from the three different locations studied. Fig. 5 showed the respective snail shell characters and their loadings on PCI, which evidenced that the characteristic responsible for most of the variation among the populations of *A. marginata* was the shell height.

Data obtained for the analyzed characteristics were also compared in box plots as shown in Fig. 6. These box plots represent summaries of measurements of the three highest loading characters, which were Shell height, Aperture height and Aperture width in the Principal Component Analysis.

The clustering pattern of *A. marginata* specimens from across the three vegetation zones was revealed in a dendrogram (Fig. 7).

RAPD results

DNA profiles generated by RAPD primers

The Genomic DNA purity ranged between 1.8 and 2.0 for all the samples. The size of the DNA obtained was 25 kb for all the samples. Random Polymorphic DNA analysis was performed on genomic DNA samples of snails from three locations in the three zones using five 10 base pair-oligonucleotide random primers which showed different numbers of stable amplification and polymorphism bands. Fig. 8 shows the electrophoresis gel for DNA obtained for the 27 samples from the three locations (Samples 1 - 9 = Ile-Ife, Samples 10 - 18 = Kabba, Samples 19 - 27 = Yenagoa). Figs. 9, 10, 11, 12 and 13 show the electrophoresis gels for the five primers used for PCR amplifications namely OPB - 17, OPH - 12, OPH - 17, OPI - 06 and OPU - 14 respectively. All five primers produced different RAPD banding patterns and the number of fragments amplified per primer varied. No band was population specific and the average number of bands per primer was 14. Among the primers, OPB - 17 gave the DNA profiles with more numerous bands as shown in Table 3. The total number of fragments yielded from five primers were 71, of which 40 (56.3%) were polymorphic. The total number of RAPD bands produced in populations from Ile-Ife, Kabba and Yenagoa was of 71 bands, out of which 32, 13 and 19 bands were polymorphic for each population (45.1%, 18.3% and 26.8% respectively). Table 4 shows the list of the primers used, the number of polymorphic and monomorphic loci, total number of loci and the percentage polymorphism.

Dendrogram showing the cluster analysis of the individuals' genotype was presented in Fig. 14. Four major clusters were formed as per the dendrogram which consists of minor clusters at various degrees of coefficient of the phylogenetic analysis. The UPGMA (Unweighted Pair Group Method of Arithmetic Averaging) cluster diagram identified four major genotypic groups with inter and intra group relationships. At coefficient 1 (100%), a cluster was formed between samples from Kabba 3 and 8, and Ile-Ife 1, 2, 4 and 8, indicating a very high level of genetic similarity. The first cluster consisted of samples mainly

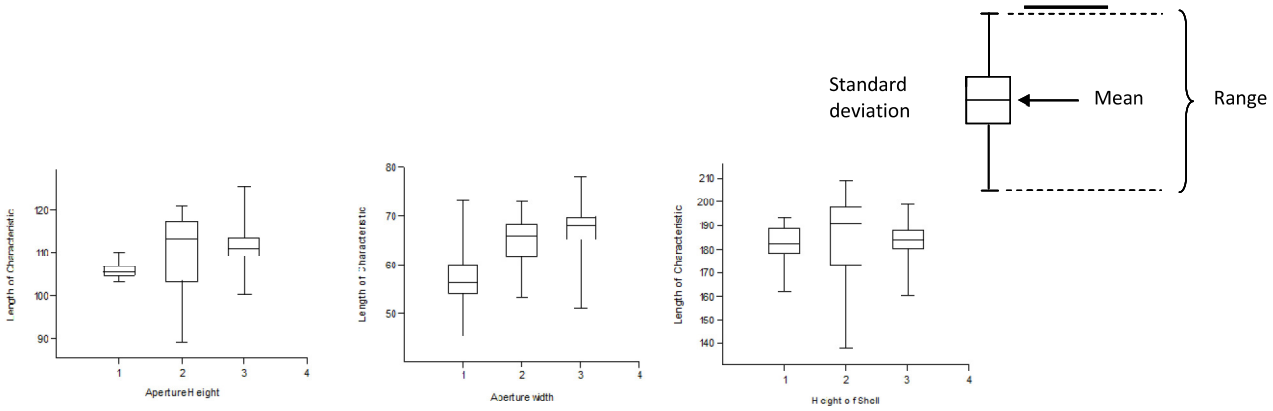

Fig. 6. Box plots of morphometric characteristics for *Archachatina marginata* from three different locations (Yenagoa = 1, Ile-Ife = 2 and Kabba = 3) for three different characteristics viz. Height of shell, Aperture Height and Aperture Width

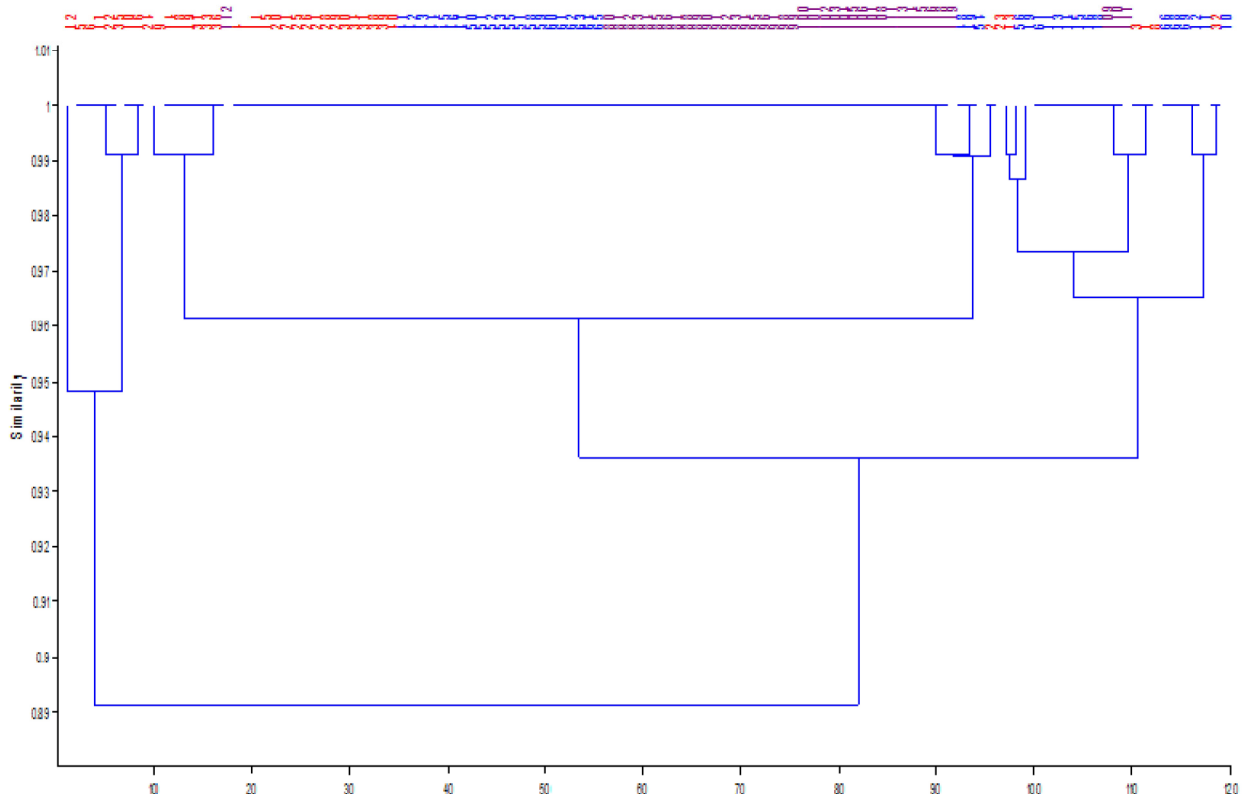

Fig. 7. Dendrogram showing relationship between the three populations of *A. marginata* from Ile-Ife: (Blue), Kabba: (Red) and Yenagoa: (purple)

from Kabba (Kabba 4, 5, 6 and 7). The second cluster was made of Yenagoa 3, 5, 9, 8, and Ile-Ife 5 and 9. The third cluster consisted of Yenagoa 2, Kabba 3, 8, 1, Ile-Ife 3, 1, 2, 4, 8, 7 and 6, and also Kabba 9, Yenagoa 6 and 7, while the fourth cluster consisted of Yenagoa 1, 4 and Kabba 2.

The dendrogram basically showed two major groups. The first group had a larger number mainly nine (9) samples from Yenagoa (Yenagoa 1, 4, 2, 6, 7, 3, 5, 9 and 8), nine (9) samples from Ile-Ife (Ile-Ife 3, 1, 2, 4, 8, 7, 6, 5 and 9) and five (5) samples from Kabba (Kabba 2, 3, 8, 1 and 9) making a total of 23 samples in the group. The second group had only four (4) samples from Kabba region (Kabba 4, 5, 6 and 7). The first group can be subdivided into two (2) major subgroups, the first subgroup comprising five (5) samples from Yenagoa (Yenagoa 1, 4, 2, 6,) seven (7) samples from Ile-Ife (Ile-Ife 3, 1, 2, 4, 8, 7, 6) and five (5) samples from Kabba (Kabba 2, 3, 8, 1 and 9). The second subgroup comprised of four (4) samples from Yenagoa (Yenagoa 3, 5, 9 and 8) and two (2) samples from Ile - Ife (Ile-Ife 5 and 9).

The values of pair-wise comparisons of Nei's (1978) unbiased genetic distances (D) between populations, computed from the combined data for the five primers,

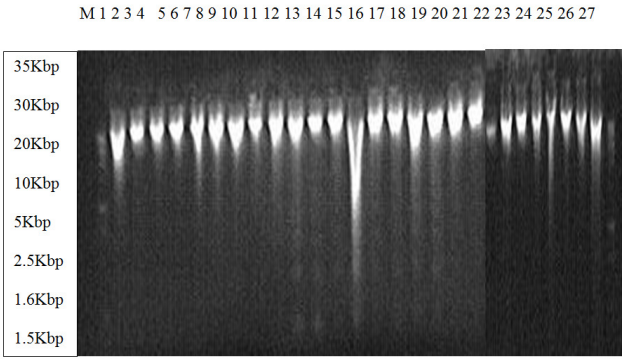

Fig. 8. Electrophoretic gel photograph of the 27 samples of *A. marginata* DNA; samples 1 - 9 are from Ile-Ife, samples 10 - 18 are from Yenagoa, while samples 19 - 27 are from Kabba; Kbp - kilo base pair and bp - base pair

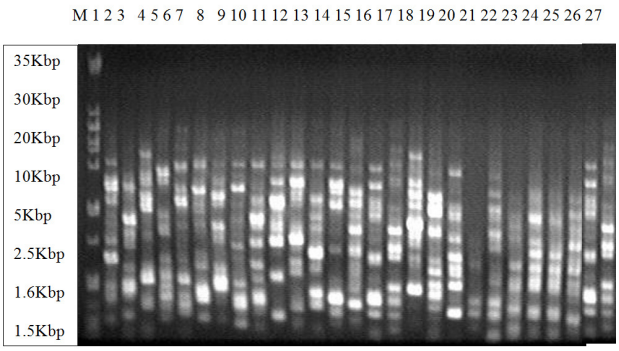

Fig. 9. RAPD banding profile of 27 samples of *A. marginata* population for Primer OPB – 17; samples 1 - 9 = Ile-Ife, samples 10 - 18 = Yenagoa, samples 19 - 27 = Kabba; Kbp - kilo base pair and bp - base pair

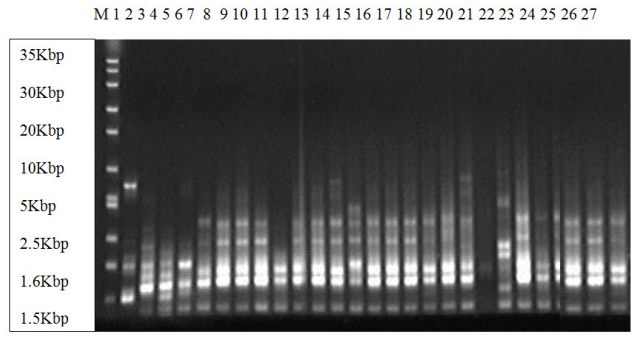

Fig. 10. RAPD banding profile of 27 samples of *A. marginata* using OPH – 12 primer; samples 1 - 9 = Ile-Ife, samples 10 - 18 = Yenagoa, samples 19 - 2=Kabba; Kbp - kilo base pair and bp - base pair

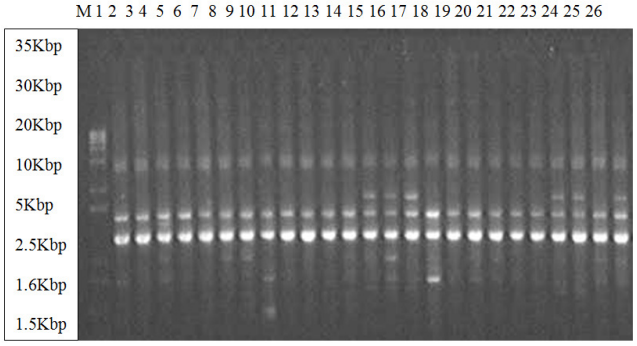

Fig. 11. RAPD banding profile of 27 samples of *A. marginata* using OPH – 17 primer; samples 1 - 9 = Ile-Ife, samples 10 - 18 = Yenagoa, samples 19 - 2 = Kabba; Kbp - kilo base pair and bp - base pair

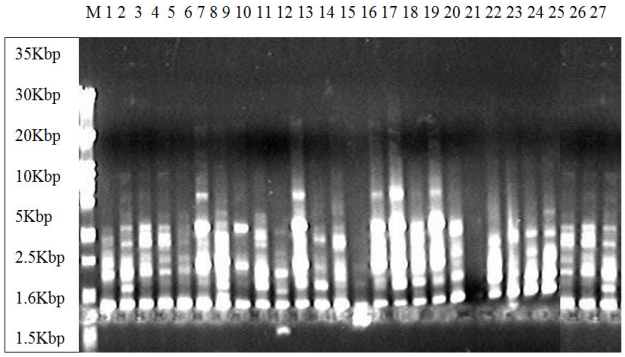

Fig. 12. RAPD banding profile of 27 samples of *A. marginata* using OPI – 06 Primer; samples 1 - 9 = Ile-Ife, samples 10 - 18 = Yenagoa, samples 19 - 27 Kabba; Kbp - kilo base pair and bp - base pair

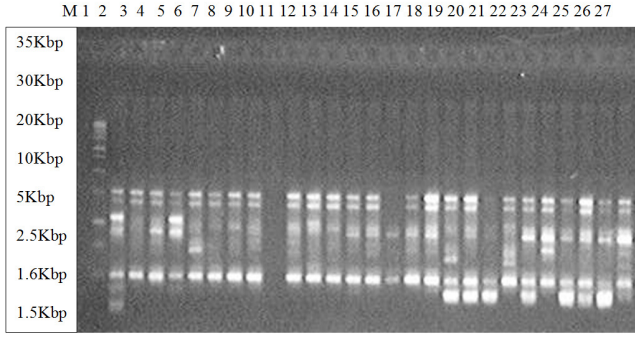

Fig. 13. RAPD banding profile of 27 samples *A. marginata* using OPU – 14 Primer; samples 1 - 9 = Ile-Ife, samples 10 - 18 = Yenagoa, samples 19 - 27 = Kabba; Kbp - kilo base pair and bp - base pair

ranged from 0.54 to 0.63. Samples from Ile-Ife were the most genetically distinct as population, which was segregated from Kabba population with the D value of 0.63 and from Yenagoa population with the D value of 0.58. Yenagoa and Kabba populations were separated from each other with the lowest genetic distance D value of 0.54. Genetic differences between populations exhibited a consistent geographical pattern clearly illustrated in the UPGMA dendrogram based on Nei's genetic distance as shown in Fig. 14. However, all the groups had various interrelationships showing highly heterogeneous populations. The cluster between *A. marginata* population from Kabba and Yenagoa implied that these populations are more similar genetically than to those of the Ile-Ife population (Fig. 15).

Table 3. The Range of Mean (R), Mean (X) and Standard Deviation (SD) of each morphometric variable of *A. Marginata* from the three different locations

Variables (cm)		Ile-Ife	Kabba	Yenagoa
N		39	40	40
HS	R	138-208.57	160.00-198.41	161.29-192.98
	X	184.66	182.70	182.04
	SD	16.63	8.06	6.56
WS	R	88.89-120.73	100.00-125.53	103.18-109.84
	X	109.43	111.03	105.62
	SD	9.29	4.32	1.72
AL	R	53.33-72.97	50.85-77.78	45.16-73.08
	X	64.68	67.10	57.20
	SD	5.18	4.79	5.38
AW	R	42.67-70.27	40.43-60.94	46.27-61.40
	X	52.59	48.05	52.76
	SD	6.43	3.81	3.67
PWL	R	40.24-65.7	37.93-65.96	41.82-63.16
	X	53.48	54.35	55.74
	SD	7.67	4.54	3.63
FWL	R	26.0-50.0	31.91-45.45	17.46-52.73
	X	40.63	37.89	34.67
	SD	6.30	3.11	5.32
SL	R	14.61-28.57	19.15-28.13	21.88-29.82
	X	32.07	23.70	25.21
	SD	3.94	1.78	1.67

Table 4. Total number of RAPD fragments and the percentage polymorphic fragments generated by five primers in *A. marginata* populations from Ile-Ife, Yenagoa and Kabba

Primers	No. of Polymorphic Loci	No. of Monomorphic Loci	Total no. of Loci	%Polymorphism
OPB – 17	24	2	26	92
OPH – 12	7	4	11	64
OPH – 17	5	4	9	56
OPI – 06	13	2	15	87
OPU –14	7	3	10	70
Total	56	15	71	

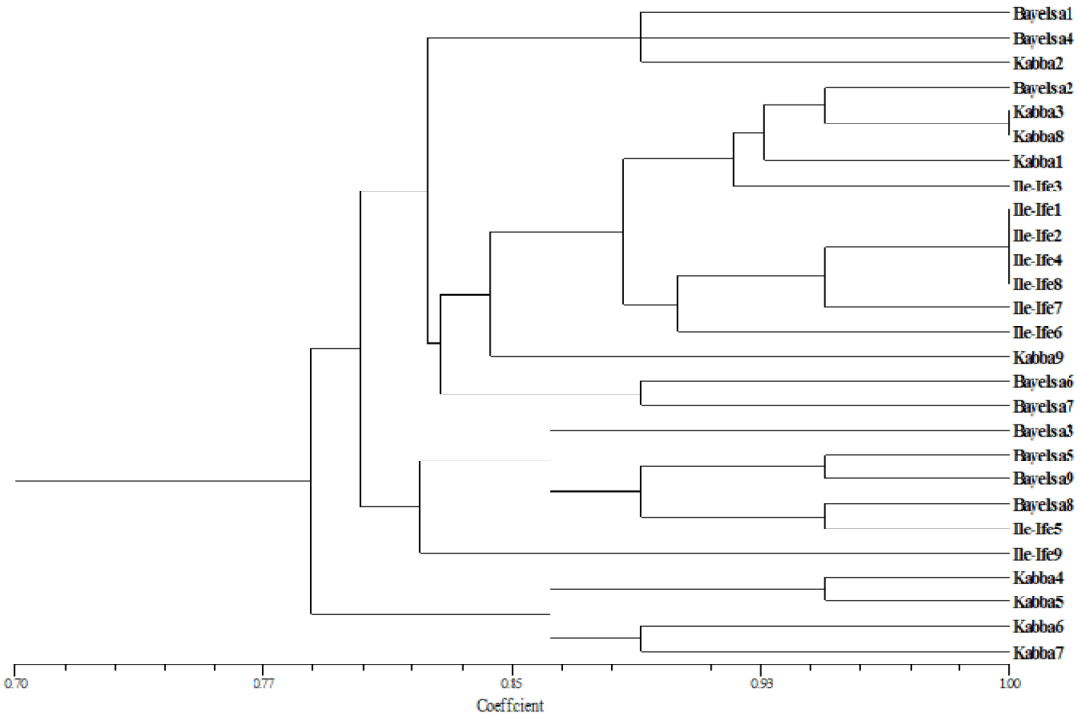

Fig. 14. Neigbour - joining dendrogram showing the genetic relationship between *A. marginata* from three vegetation zones of Nigeria: Rainforest (Ile-Ife), Mangrove forest (Yenagoa) and Guinea Savanna (Kabba)

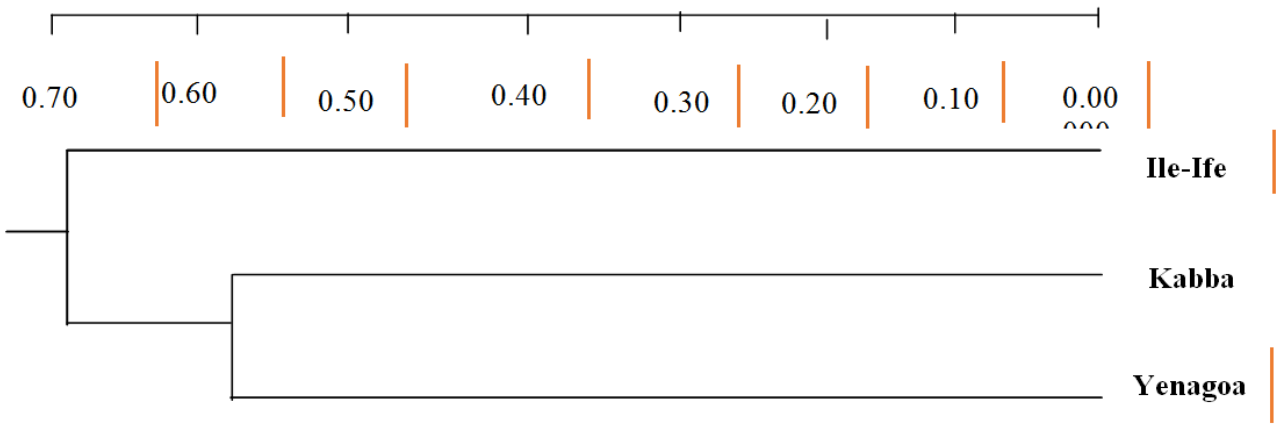

Fig. 15. UPGMA dendrogram based on Nei's (1978) unbiased genetic distance, summarizing the data on differentiation between *A. marginata* populations according to RAPD analysis

Discussion

All the clusters produced by the Principal Component Analysis on the parameters measured on *A. marginata* overlapped; also Canonical Variates Analysis plots that were produced showed overlapping of specimens clusters. Madec and Bellido (2007) reported that both genetic and environmental factors and the interplay of both are responsible for the development of shell characteristics. Chiu *et al.* (2002) also reported that extrinsic factors due to environmental conditions can induce variations in the shell shape and size. They also argued that environmental changes without genetic variation can create distinct non-genetic changes in shell morphology. Canonical Variates Analysis also did not separate the samples into distinct subpopulations due to the fact that there was a considerable overlap. *A. marginata* samples from Mangrove forest (Yenagoa) therefore showed a small cluster with lower values. Out of the measured parameters, shell height was found to be the most variable and thus the most suitable for populations' grouping. Awodiran *et al.* (2013) also reported the height of shell as the parameter most responsible for variation out of eight morphometric characters employed in the three populations of *A. marginata*. Results obtained from the morphometric studies hence showed that morphometric methods are effective to some reasonable extent in separating populations of land snails at interspecific level.

RAPD profiles are frequently used to analyze the genetic structure of populations of vertebrate and invertebrates (Gibbs *et al.*, 1994; Apostol *et al.*, 1996; Liao and Hsiao, 1998). RAPD technique is suggested to be more useful in closely related populations (Smith and Williams, 1994; Borowsky *et al.*, 1995). This technique has been used as a molecular tool for detecting genetic variation and genetic similarity in several snail species including Scallop *Chlamys farreri* (LIU *et al.*, 2004) species identification, Mollusca Clausiliidae *Balea biplicata* (Hille *et al.*, 2003) and *Littorina littorea* (Hans De Wolf *et al.,* 2013).

This study showed the effectiveness of RAPD in detecting polymorphism in different populations of *A. marginata*, and also in classifying different populations of the species into sub-populations, which could not be done by the application of morphometric analysis alone. Previous researchers have proved that RAPD is undoubtedly a powerful approach for the analysis of genetic variation and the identification of genetic markers (Barral *et al.*, 1993; Simpson *et al.*, 1993; Vidigal *et al.*, 1994; Leamon et *al.*, 2014). The results of this study also suggested OPB - 17 as an invaluable primer in detecting genetic polymorphism in populations of snail species due to the very high percentage of polymorphism recorded hereby. The OPA-17 marker was also used by various investigators on different organisms. For instance, Basavaraju *et al.* (2014) used the primer to determine the level of genetic heterogeneity between stocks of *Labeo fimbriatus*. The total number of fragments yielded from the five primers was 71, out of which 40 (56.3%) were polymorphic and the percentage of polymorphic loci was 45.1, 18.3 and 26.8% for populations of *A. marginata* in Ile-Ife, Kabba, and Yenagoa respectively. Analysis of the proportions of polymorphic loci and band sharing based similarity indices for *A. marginata* samples indicated that a relatively high level of genetic variation exists in the Ile-Ife population compared to Kabba and Yenagoa populations. The higher similarity and lower level of frequency of polymorphic loci and gene diversity estimates for Kabba and Yenagoa populations could be an indication of comparatively closer relationship among individuals in these populations. In other words, both populations were more homogenous than the Ile-Ife population.

Factors such as population size, bottlenecks and mutation, however may also influence genetic variability in populations. High level of polymorphism and genetic diversity is required for the adaptation of populations to the environmental changes. Bernardi *et al.* (2001) suggested that the genetic structure of organisms could be the result of habitat connectivity patterns, ecological

conditions and dispersal potential. The influence of environmental factors also plays a decisive role in the differentiation of populations. Similarly, Bowditch *et al.* (1993) reported that detection of genetic variation is essential to a wide range of comparative genetic research endeavors which include gene mapping, individual identification, parentage determination, population genetics and molecular phylogenetics. As expected, the band-sharing based on similarity indices were higher within samples than other sample comparisons. This implied that the *A. marginata* population of one location is more homogenous than the combined group of the three populations. Therefore, the maintenance of genetic variability is very important for the conservation of a species (Barroso *et al.,* 2005).

The UPGMA cluster diagram identified four major genotypic groups with inter and intra group relationships. All individuals in the first cluster are from Kabba region, while individuals from Yenagoa and Ile-Ife constitute a second cluster. The third cluster constitute individuals from Ile-Ife, Kabba and Yenagoa, while the forth cluster comprised individuals from Yenagoa and Kabba. However, all the groups had varied interrelationships, showing a highly heterogeneous population. The cluster between *A. marginata* populations from Ile-Ife and Yenagoa implied that these populations are more genetically similar than to those of Kabba population. Also, the cluster between Ile-Ife, Kabba and Yenagoa indicated that the three populations are genetically similar to some extent, while the cluster between Yenagoa and Kabba showed that the two populations are similar genetically. Dendrogram based on Nei's (1978) unbiased genetic distance showed that Ile-Ife and Yenagoa are closer genetically, while Kabba population is distant from them. The low level of genetic heterogeneity of both Yenagoa and Kabba samples may be because of recent origin of the populations. Both locations are geographically wide apart and the populations are therefore expected to be different genetically, hence it will be difficult to adduce reasons for the genetic homogeneity of the two populations. The high level of genetic homogeneity of the two populations may also be due to the limited number of individuals sample as the populations with similar genetic make-up needs large number of individuals and primers for precise differentiation of significant gene flow within each population (Basavaraju *et al*., 2014). Negel (1997) suggested that the high levels of migration and gene flow between populations increase the genetical similarity of populations.

Genetic drift and natural selection are the two primary evolutionary processes that cause population differentiation (Hufford and Mazer, 2003).

Conclusions

Out of the measured parameters, shell height was found to be the most variable and thus the most suitable for populations' grouping. Natural selection by ecological factors will result in development of ecological adaptation or ecotypes. It remains to be determined whether the observed population differentiation resulted from any natural selection. In addition, this genetic clustering prompted the question whether the four clusters of populations represent three different distribution zones of genetic diversity within the natural populations. If distribution zones do exist, additional questions would then be whether natural populations in each zone represent a regional ecotype, and what ecological factors contributed to this differentiation or adaptation.

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